THE TOTAL SYNTHESIS OF (±)-FRIEDELIN

Thesis by

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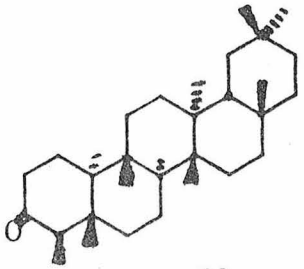
To my parents, my island brothers, and Holly. Man does not live on chemistry alone.

Acknowledgements

I wish to thank members of the Division of Chemistry and Chemical Engineering for making my graduate experience such an exciting and satisfying one. Special thanks go to Professor Robert E. Ireland for his guidance, encouragement, and friendship, and to members of The Group, past and present, for providing a most pleasant and stimulating environment.

Abstract

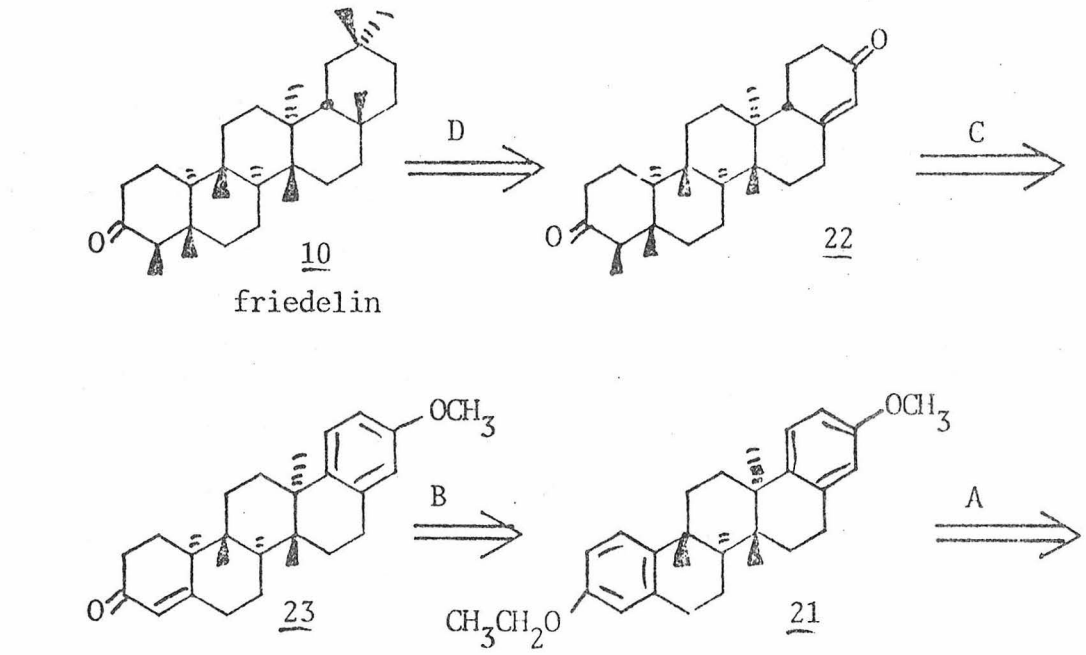
The total synthesis of the unsymmetrical pentacyclic triterpene friedelin (10) was completed in 31 steps in an overall

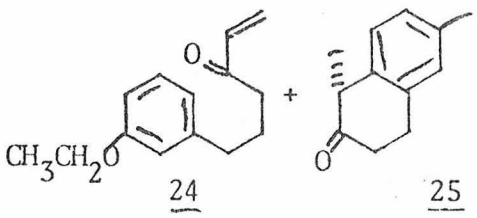


10

yield of 0.3%. The route, symbolized by the disconnections outlined in Chart 4, forms each of the 9 asymmetric centers of friedelin stereoselectively.

Chart 4


D
C
O
O
10
friedelin
22
OCH3
B
A
O
23
OCH3
CH3CH2O
21


O
+
CH3CH2O
O
24
25

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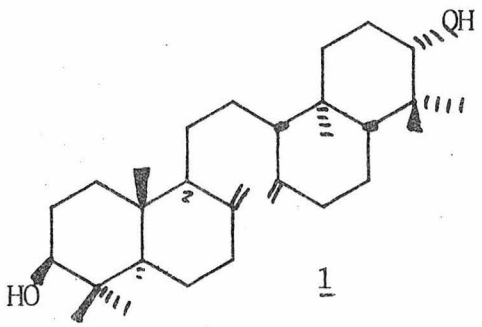
INTRODUCTION

The triterpenoids form a large class of structurally diverse naturally occurring compounds; at least 750 examples have been reported to date.[2,3] A large majority of these molecules are polycyclic species which fall into one of about 30 basic structural types[2] differing in the number and array of fused rings, the structure of various appended side chains, and the array of methyl groups appearing on the skeleton. As a result of the rigid skeletal framework associated with many members of the class these structures form an ideal substrate upon which the synthetic chemist may test and revise methods of stereoselective directed total synthesis first put to use in the steroid field.[4] Indeed, many new techniques and much understanding have evolved from past efforts in triterpene total synthesis which have been ably reviewed by ApSimon.[5]

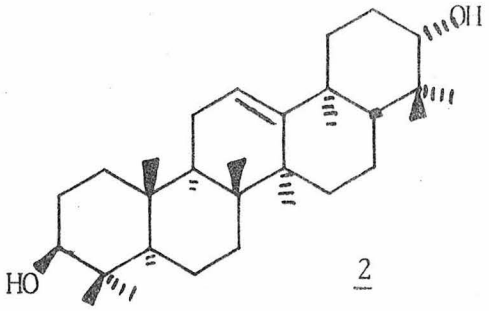
Several highly imaginative and elegant total syntheses of triterpenes have been completed,[3,5] and these may be divided into two basic types: (Chart 1) (1) syntheses in the onocerin (compounds 1,2,3,4) and amyrin (compound 5) structural groups which rely in essence upon attachment of an AB fragment to a DE fragment with formation of carbon-carbon bonds C-12a-C-13, C-13-C-14, or C-14-C-14a, followed by an acid catalyzed cyclization to form the C-6a-C-6b bond; and (2) the stereoselective directed total syntheses of germanicol[6a] (6) and lupeol[6b](7) of the amyrin group, and alnusenone[7](8) and shionone[8](9) of the friedelin group. Though the syntheses of the first type were of a pioneering nature, every example suffers from the fact that the formation of the C-6a-C-6b bond lacks regiospecificity, is stereochemically ambiguous, and proceeds in very low yield to give a complex mixture of products from which the

Chart 1: Triterpenes prepared by total synthesis of type 1



1

α-onocerin



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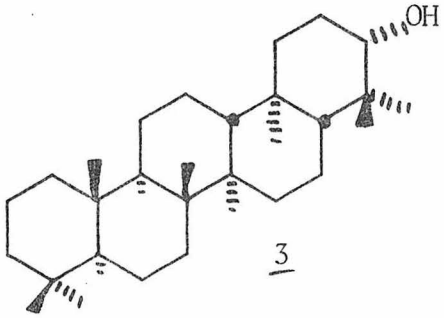
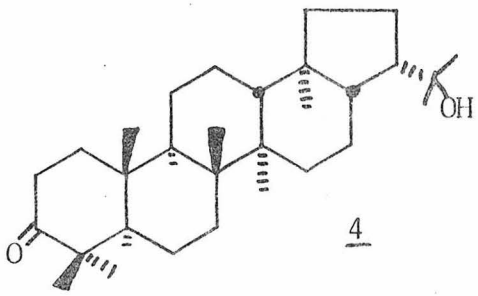
γ-onocerin



3

tetrahymanol



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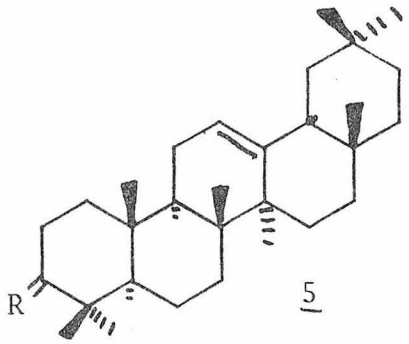
hydroxyhopanone



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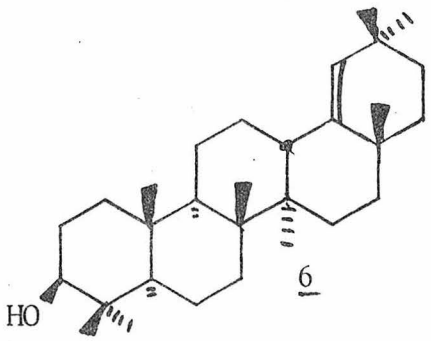
R=H_2, olean-13(18)-ene

R=β-OH, α-H, β-amyrin

Chart 1, continued: Triterpenes prepared by total synthesis of type 2



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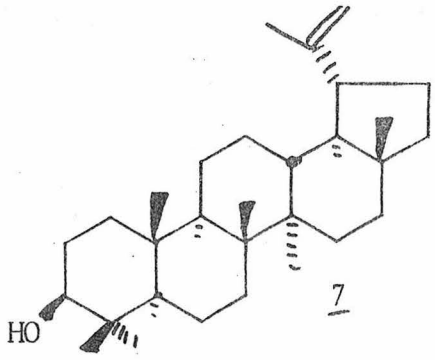
germanicol



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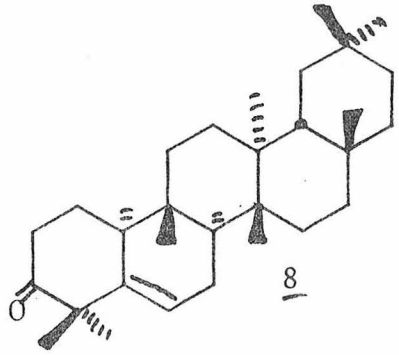
lupeol



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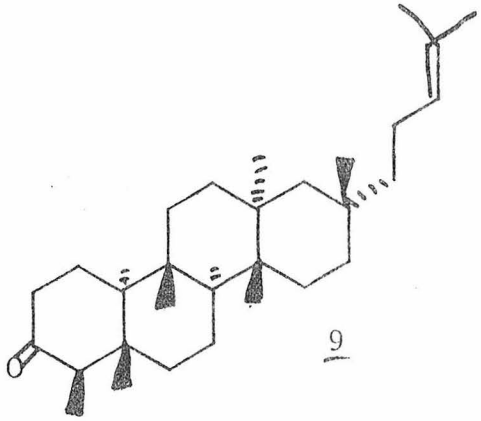
alnusenone

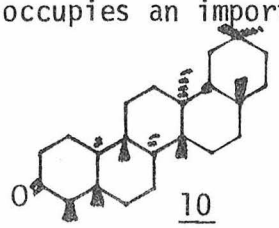


9

shionone

desired compound must be separated (excepting, of course, the synthesis of α-onocerin which has no C-6a-C-6b bond). Those of the second type, however, were completed via elegant routes designed with maximum efficiency in mind, including regioselectivity in all C-C bond forming steps and stereoselective introduction of each asymmetric center.

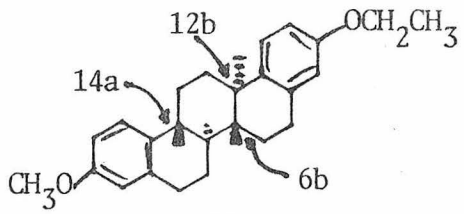
Friedelin[9] (10) occupies an important place in the lore of terpene



10

chemistry[9b] and its total synthesis has been a goal in these laboratories for almost a decade.[7,8,10,11] Friedelin is particularly interesting from the synthetic standpoint in that it contains, together with the difficultly accessible vicinal trans dimethyl grouping common to many pentacyclic triterpenes, other features unique to the friedelin group; specifically, the vicinal β-dimethyl grouping at C-4 and C-4a and the cis DE ring fusion with the synthetically challenging array of methyl groups on ring E. Because of its complexity friedelin was not chosen as the initial goal of total synthesis, rather two triterpenes in the same structural group, alnusenone (8) and shionone (9) were attacked first.

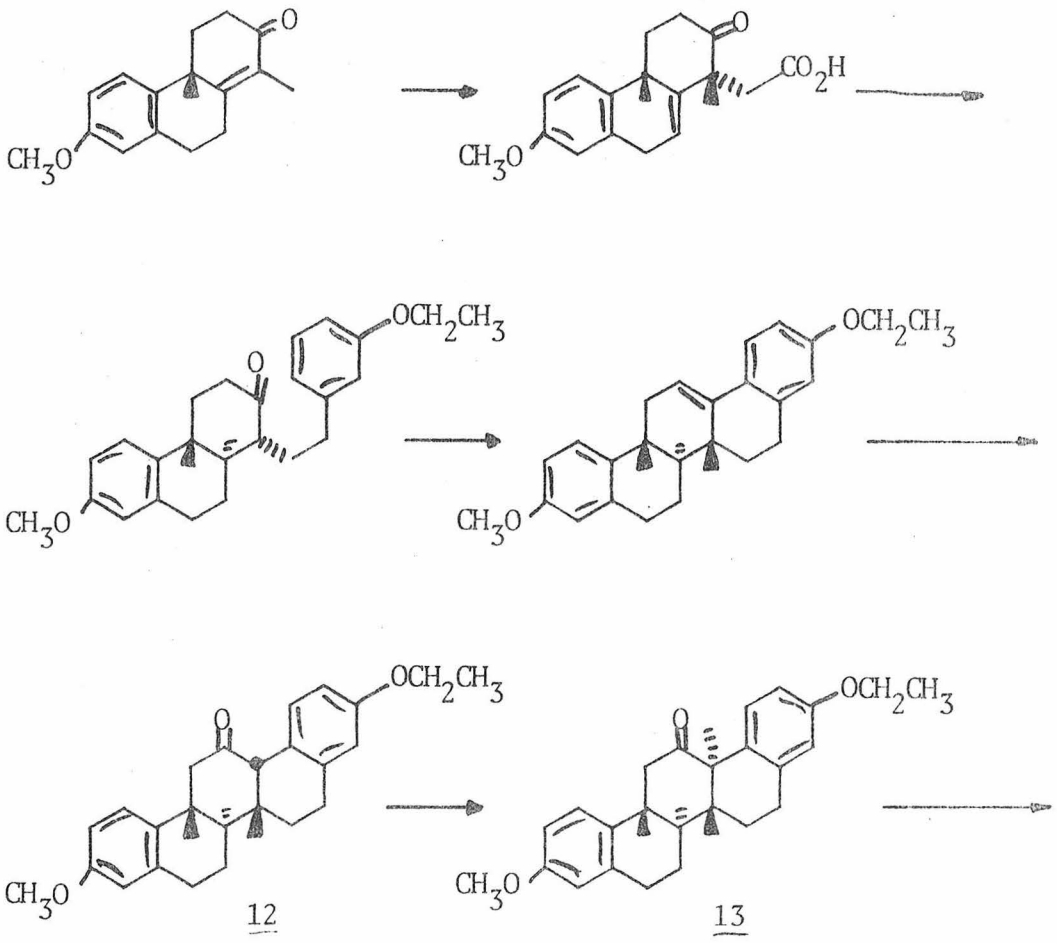
The initial phase of this work consisted of development of methods for the efficient synthesis of the pentacyclic diaromatic diether 11,



11

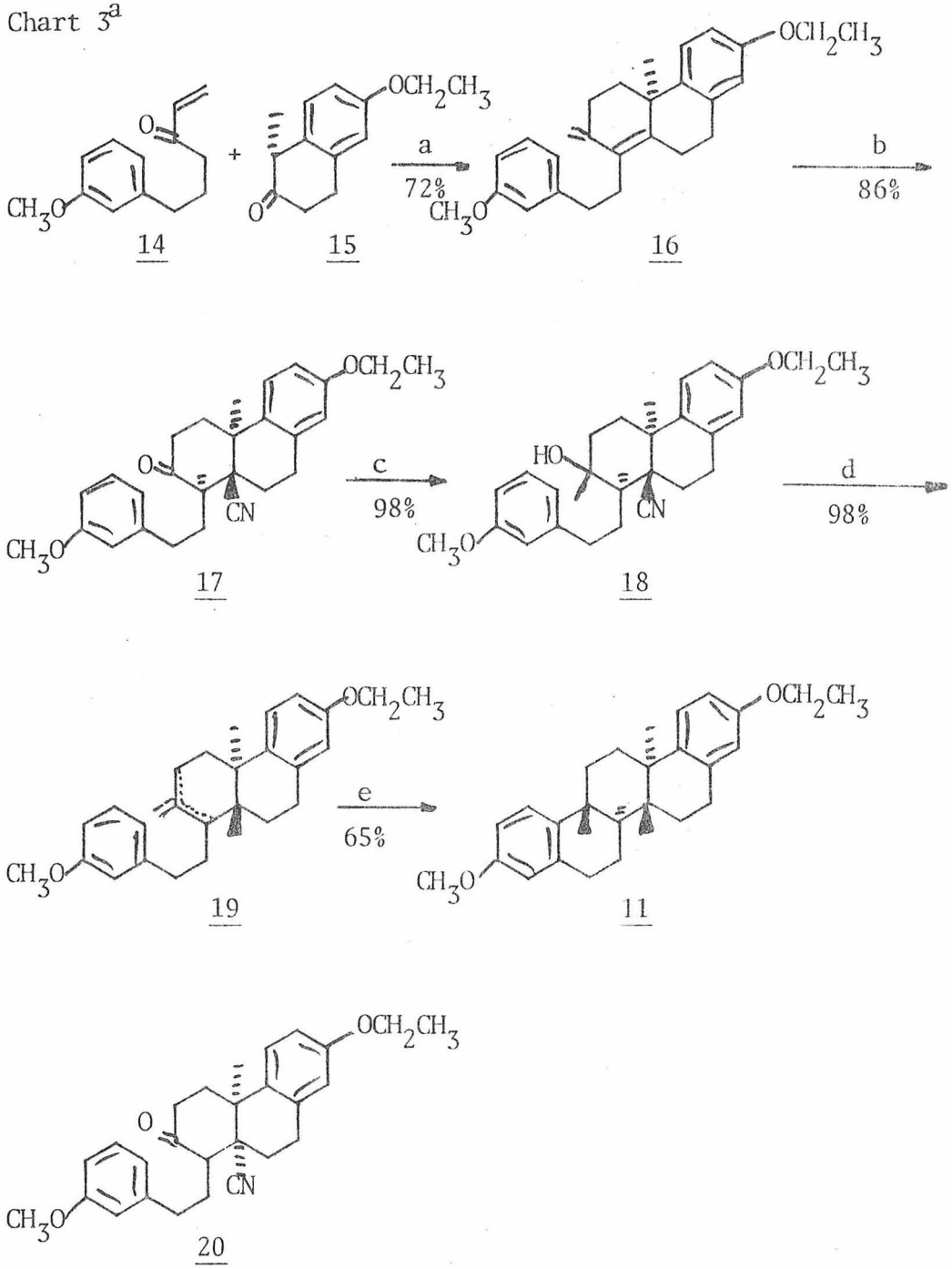
envisaged as a key intermediate for the synthesis of triterpenes in the friedelin group. Two routes to this compound were studied. The first[10] (Chart 2) was abandoned when the crucial alkylation of ketone 12 to give 13 with the vicinal-dimethyl grouping a C-6b and C-12b could not be performed in greater than 18% isolated yield. The second route (Chart 3) is based upon the observation in model systems that Friedel-Crafts cyclialkylation[11] of a tricyclic such as 19 should give predominantly the desired trans-anti-trans diether 11. Effort was then directed toward an efficient synthesis of a tricyclic of this nature starting with the enone 16, and using as the method of introduction of the crucial C-6bβ methyl group the Nagata procedure[12] for the stereoselective hydrocyanation of α,β-unsaturated ketones. The starting enone 16 was prepared from vinyl ketone 14 and β-tetralone 15 using methods developed previously.[10a] Hydrocyanation of 16 using conditions known to give the thermodynamically more stable product (Et_2ApCN,ϕ_H) produced the undesired cis-cyanoketone 20. However, when conditions of kinetic control were used (Et_3Al,HCN, THF) a dramatic increase in the amount of the desired trans cyanoketone 17 was observed (76% isolated yield), and by regenerating the enone by base catalyzed elimination of hydrogen cyanide from the cis isomer and recycling, a yield of 86% of 17 was realized. Thus the hydrocyanation route provides an elegant and efficient solution to the vicinal-trans-dimethyl problem.[13] Conversion of 17 to the desired olefins 19 was accomplished by selective grignard addition to give cyano-alcohol 18 followed by dehydration and reduction of the nitrile function. Finally, pTs OH catalyzed cyclization of 19 gave the diether 11 with the correct trans-anti-trans configuration as proven by single crystal X-ray analysis.

Chart 2



12 13

Chart 3[a]



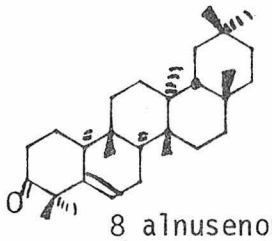
[a] a, aqueous KOH, CH_3OH; b, $(C_2H_5)_3Al$, HCN, THF (recycle cis-cyano ketone (20) by dehydrocyanation with base); c, CH_3MgI; d, $SOCl_2$, pyridine; e, p-CH_3-$C_6H_4SO_3H$, C_6H_5.

The overall yield of this route to 11 from the vinyl ketone 14 is 25%, quite sufficient for the purposes of this work.

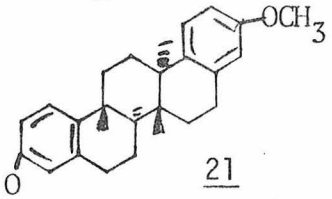
With an efficient synthesis of the diether 11 in hand, the total synthesis of alnusenone became fact with solution of the E ring problem (see discussion). This, coupled with methodology for construction of the friedelin A-ring obtained in connection with the synthesis of shionone (see Appendix) set the stage for completion of friedelin. Description of the conversion of a pentacyclic diaromatic diether similar to 11 to (±)-friedelin comprises the body of this thesis.

DISCUSSION

In previous reports from these laboratories[7,10,11] an efficient route to the pentacyclic diether 11 was described. A pentacyclic diaromatic compound of this type, with the trans-anti-trans BCD ring fusion



8 alnusenone



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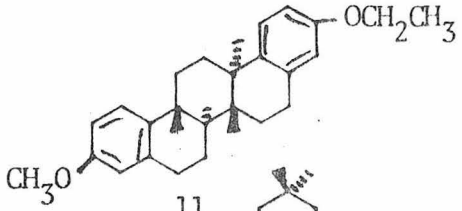
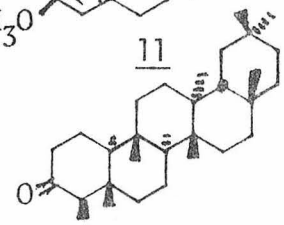


11

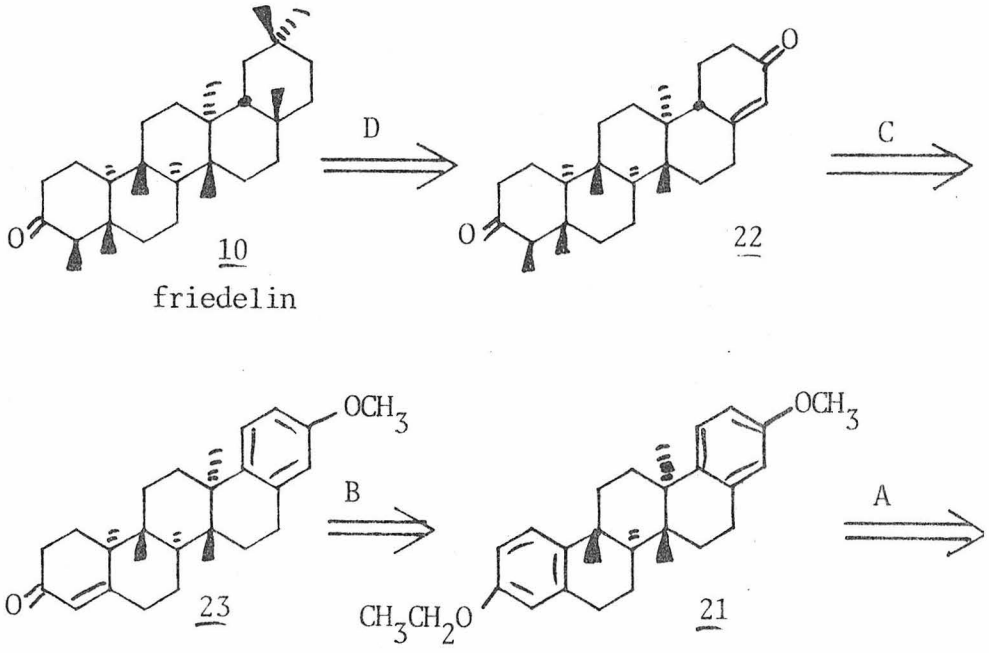


10 friedelin

and correct array of methyl groups, was envisaged as a useful key intermediate for synthesis of pentacyclic triterpenes in the friedelin structural group, and the successful completion of alnusenone[7] (8) proved the viability of this approach. The present report describes the conversion of a similar intermediate to the structurally slightly more complicated triterpene friedelin (10).

The route used for the synthesis of friedelin closely parallels that used for alnusenone in many respects, and is outlined by the series of disconnections presented in Chart 4. The basic philosophical steps A-D will be discussed in brief initially and will serve as a framework for discussion of the details of the synthesis: (A) the preparation of key intermediate 21 in a convergent manner from the vinyl ketone 24 and the β-tetralone 25 parallels exactly the synthesis of 11 from 14 and 15. Since these transformations are discussed in detail elsewhere (see

Chart 4

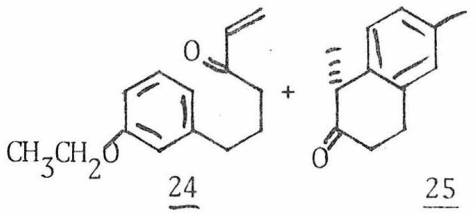


10 friedelin

22

23

21



24 + 25

Introduction and Ref. 7) they are not included here; (B) methodology for the differentiation of the A and E rings of similar diaromatic precursors had been developed in connection with the alnusenone work. In this case, the A ring was functionalized initially to give the enone 23; (C) construction of the friedelin A-ring system from the enone 23 represented at the beginning of this work the principal synthetic challenge. The novel methods developed for accomplishment of this difficult vicinal dialkylation have been reported in connection with the synthesis of shionone (9).[8] Once the A-ring problem was solved, simple Birch reduction[10a,14] afforded the required enone 22; (D) the completion of friedelin involved first differentiation of the carbonyl groups of 22 then completion of the E ring using methods reported for an analogous conversion in the alnusenone synthesis.

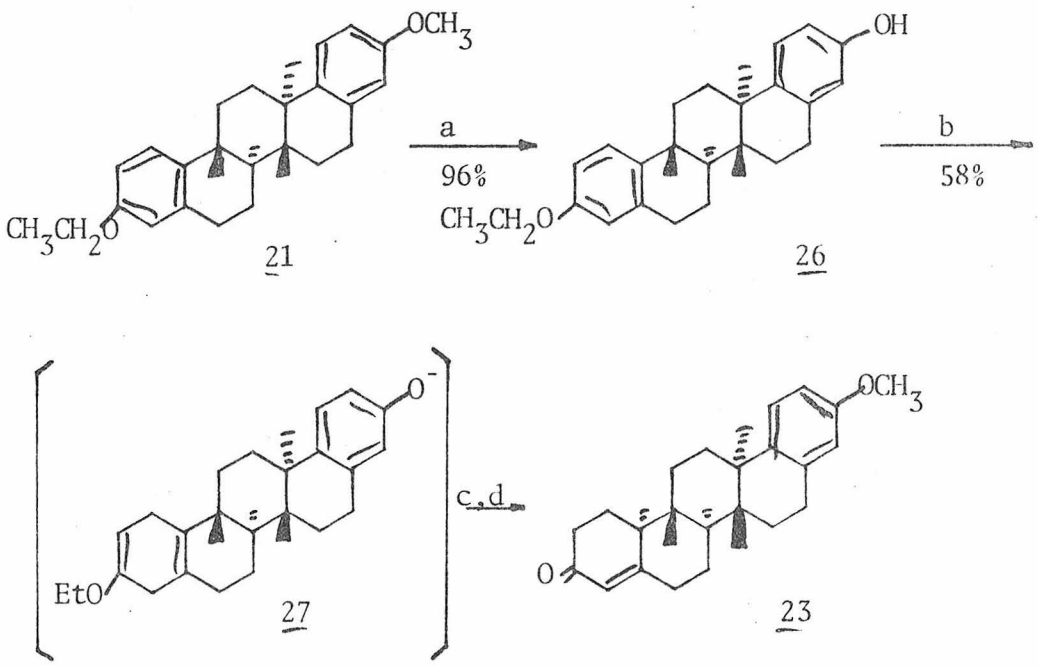
Step A; synthesis of the pentacyclic diether 21

The diether 21 was obtained in 25% overall yield from the vinyl ketone 24, or in 11% overall yield from m-hydroxycinnamic acid from which 24 was prepared, in a manner analogous to that used for the preparation of 11.

Step B; synthesis of the pentacyclic enone 23

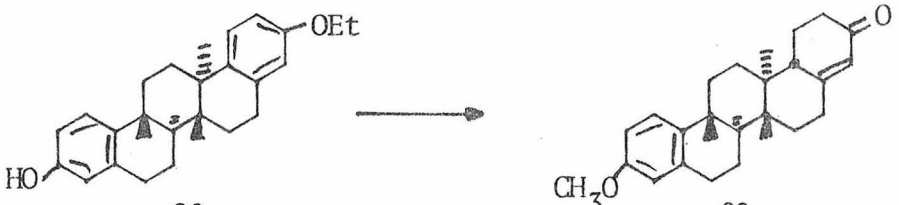
The crucial selective reduction of the A ring of 21 in the presence of an aromatic E ring is outlined in Chart 5, and is based upon the higher reduction potential of a phenoxide ion relative to that of a simple anisole.[7,15] Thus the phenol 26 became the initial goal. Treatment of diether 21 with lithium diphenylphosphide in refluxing THF[16,7] afforded the required phenol in high yield. However, selective Birch

Chart 5[a]



[a]a, $(C_6H_5)_2P^-Li^+$, THF; b, Li, NH_3, DME, EtOH; c, CH_3I, DME; d, 5 N HCl, ETOH, C_6H_6.

reduction of phenol 26 was complicated by the fact that the phenoxide ion generated to protect the E ring with respect to reduction also caused the molecule to be only slightly soluble in the solvent used (33% DME/ammonia) resulting in recovery of large amounts of starting ether 21 if normal concentrations and reaction times were used.[17] This same effect had been observed in the analogous reaction in the alnusenone series,[7] conversion of phenole 28 to enone 29a. In that system extensive

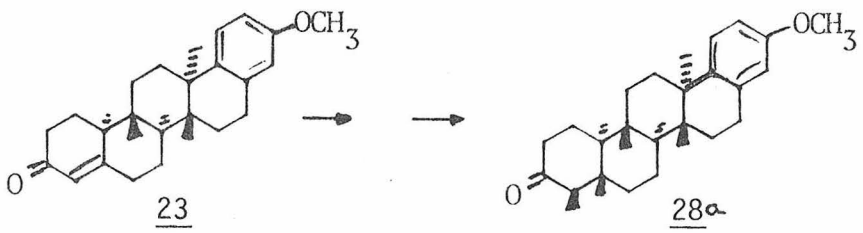


28 29a

experimentation with a variety of solvents and conditions had not improved the results obtained using simply high dilution, a large excess of lithium, and long reaction times. Preliminary studies on reduction of phenol 26 in the presence of one equivalent of dicyclohexyl-18-Crown-6 also indicated no improvement. Thus reduction of 26 as a 0.002 molar solution in 33% DME/ammonia, with 125 molar equivalents of lithium for 9 hours, followed by remethylation of the intermediate phenoxide 27 afforded enone 23 in 56% overall yield from diether 21.

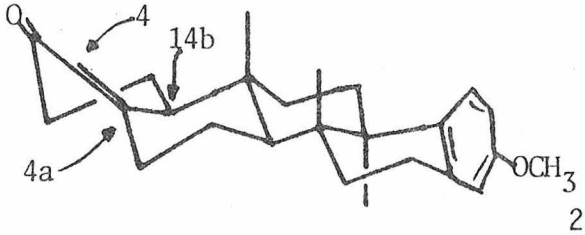
Step C; synthesis of the pentacyclic enedione 22

An enone of type 23 appears an ideal intermediate for conversion to a C-4β, C-4aβ dimethylated ketone of type 28a via conjugate addition



23 28a

followed by alkylation. However, previous experience in these laboratories[8,18] indicated that this transformation was quite difficult due to stereochemical problems which became apparent upon closer inspection. A perspective drawing of enone 23 indicates that the double bond



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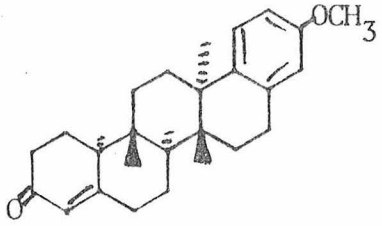
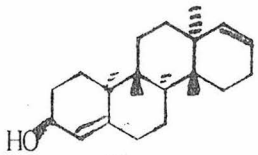


is quite hindered with respect to pseudo-axial (β) attack at C-4a as a result of the presence of the axial C-14aβ methyl group. Two approaches to solution of this problem were brought to fruition.[8] The first, based upon stereoselective Simmons-Smith methylenation[19] of the allylic alcohol 29 to give the cyclopropylcarbinyl alcohol 30



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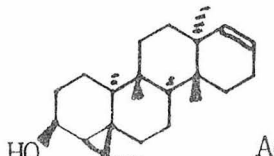

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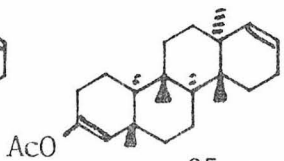

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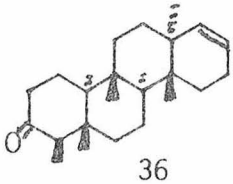
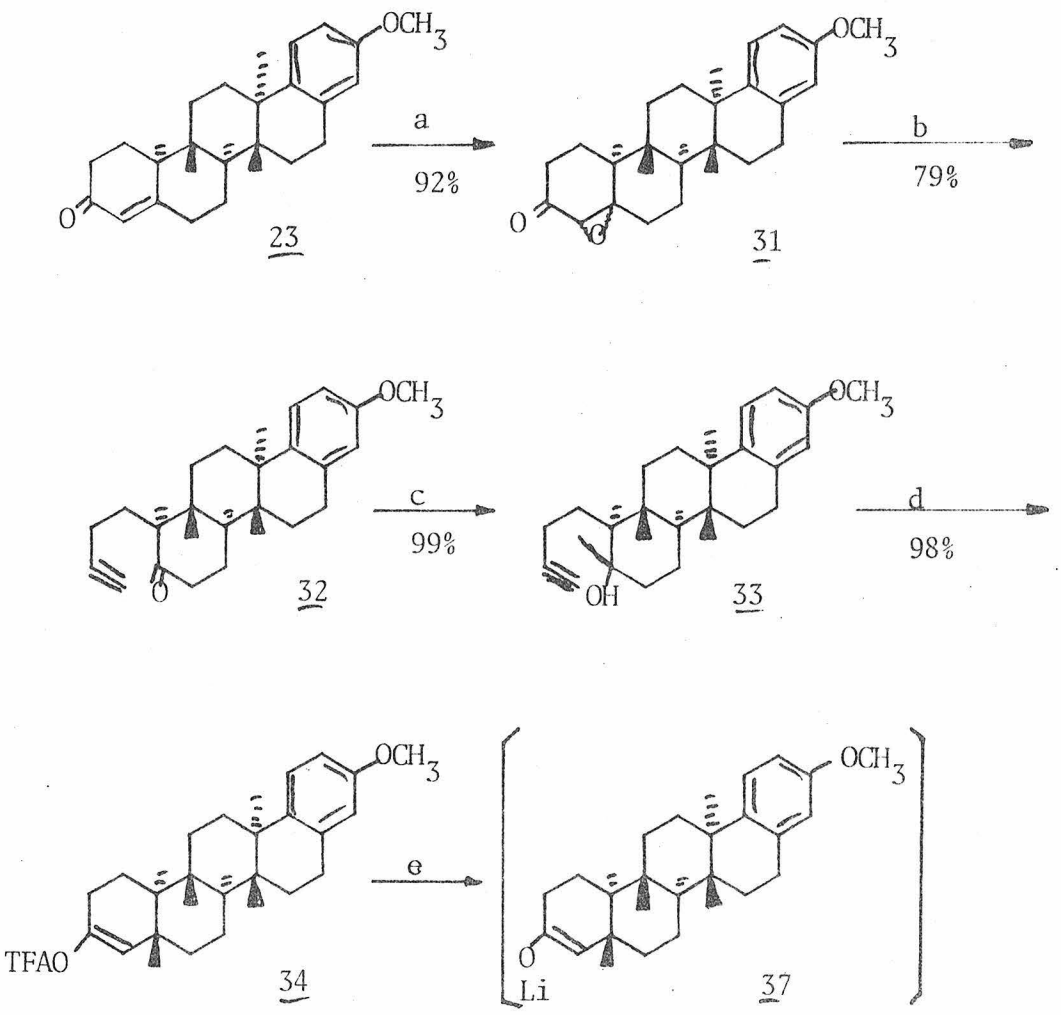


36

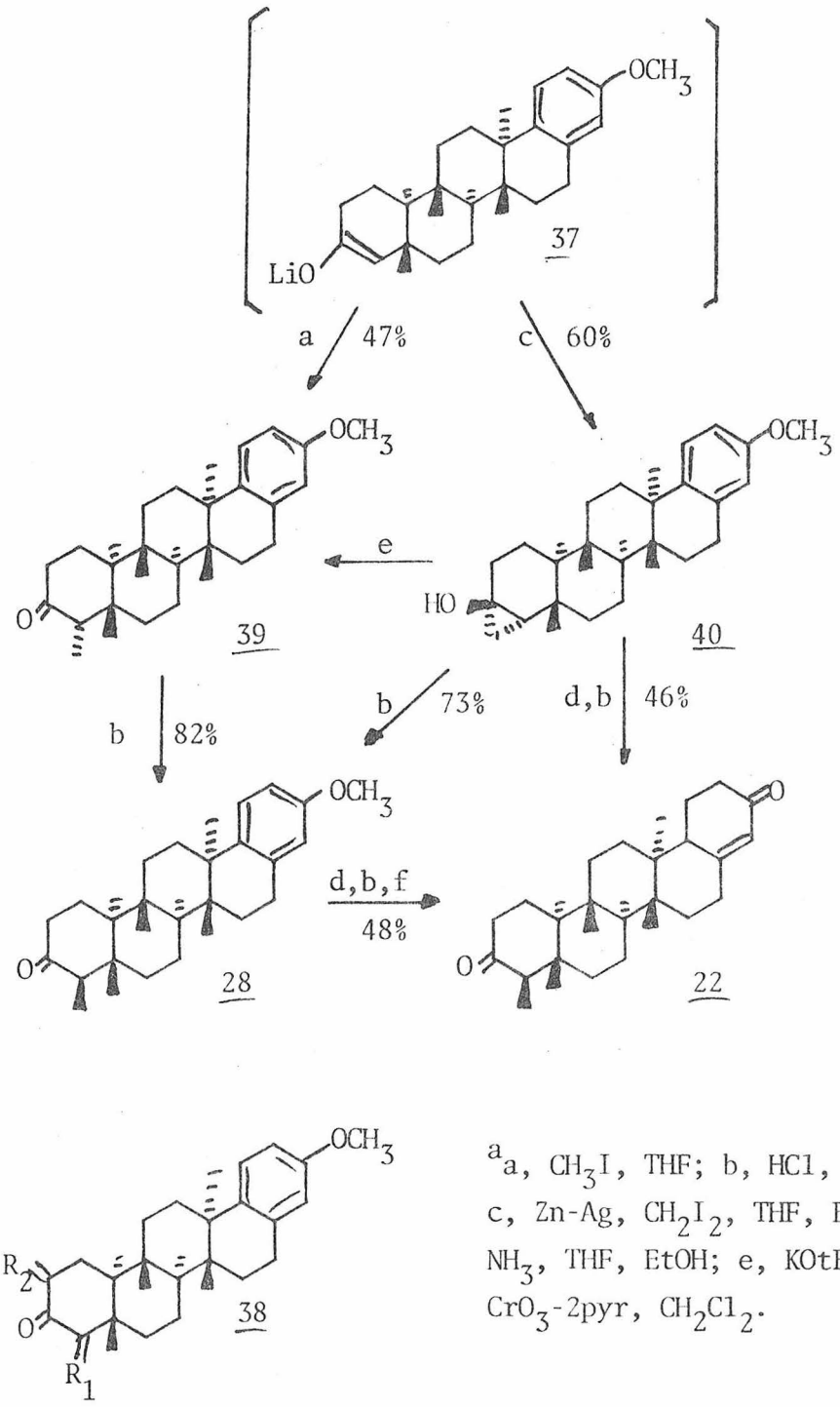
suffered from the difficulties observed in obtaining allylic alcohol 29 by reduction of an enone of type 23. The second approach, outlined in Chart 6, derives from the proposition that acid catalyzed cyclization of an acetylenic alcohol of type 33 would proceed from the α-face (directed by the C-14aβ methyl group) to yield the desired C-4aβ methyl group configuration,[8,13a] analogous to the Friedel-Crafts cyclialkylation[11] of the tricyclic olefins 19 to give the trans-anti-trans configuration in pentacyclic diether 11. This route seemed

Chart 6[a]

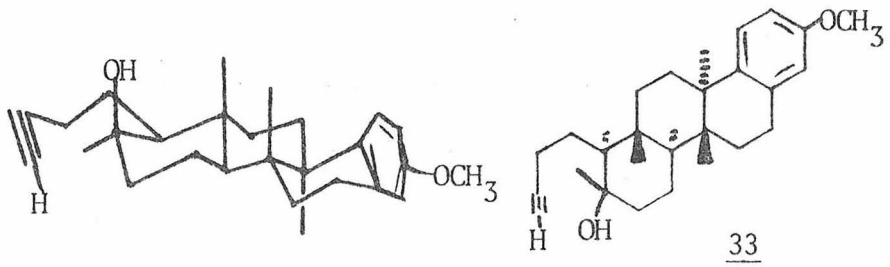


[a]a, H_2O_2, aq NaOH, CH_3OH; b, $pTsNHNH_2$, HOAc, CH_2Cl_2; c, CH_3Li, Et_2O; d, CF_3CO_2H-$(CF_3CO)_2O$; e, $LiN(CH(CH_3)_2)$, THF.

Chart 6, continued[a]



[a] a, CH_3I, THF; b, HCl, EtOH, C_6H_6; c, Zn-Ag, CH_2I_2, THF, Et_2O; d, Li, NH_3, THF, EtOH; e, KOtBu, Et_2O; f, CrO_3-2pyr, CH_2Cl_2.



33

particularly attractive in view of the fact that recent work[20] indicated that an enol trifluoroacetate of type 34 would result under appropriate conditions, and 34 appears an ideal intermediate for the regioselective introduction of the required C-4 methyl group. Indeed, good precedent existed for this conversion in that an intermediate in the above-mentioned but discarded scheme, starting with the allylic alcohol, the enol acetate 35, was converted via enolate formation[21] followed by methylenation[22] and subsequent acid catalyzed ring opening to the dimethylated ketone 36. Thus an efficient route to the alcohol 33 was required, and in fact, was available based upon the route of Eschenmoser et al.[23,13a] involving fragmentation of an α,β epoxy hydrazine derivative.

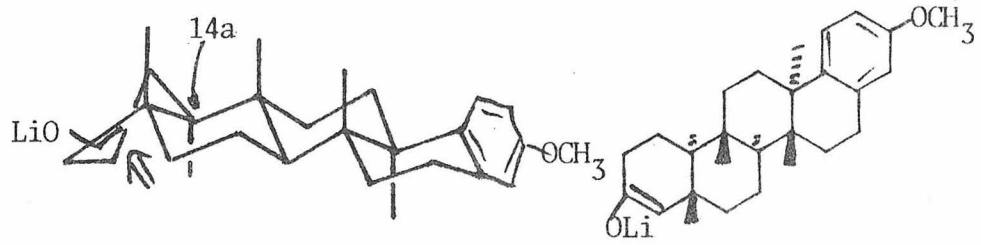
In execution, Eschenmoser cleavage of the derived epoxyketone 31 using p-toluene sulfonhydrazide in acetic acid-dichloromethane solution afforded, after careful choice of conditions, satisfactory yields of the acetylenic ketone 32. Cleavage using the recently reported modifications of Corey and Sachder[24] proceeded in comparable

yields, but gave 32 contaminated with a highly colored byproduct which could not be separated by chromatography or crystallization. The desired tert. alcohol 33 was formed in high yield upon treatment of 32 with methyllithium, and trifluoroacetic acid catalyzed ring closure indeed produced the expected enoltrifluoroacetate 34, also in high yield. It should be noted that the stereochemical assignment here rests on firm ground, as discussed in previous reports.[8,13a]

With the enoltrifluoroacetate 34 in hand, formation of the desired dimethylated system seemed a trivial extension of work carried out on the enol acetate 35. However, treatment of 34 with methyllithium in a fashion analogous to that used with 35 afforded none of the desired enolate 37 as evidenced by our inability to trap this species with Simmons-Smith reagent, tert. butyl dimethyl chlorosilane, or acetic anhydride. Experiments performed on model enoltrifluoroacetates indicated that this result was not an artifact of wet solvents or reagents, but apparently resulted from essentially quantitative protonation of the enolate by the initially formed trifluoromethyl methyl ketone. This problem was finally circumvented by use of lithium diisopropylamide to effect the required cleavage. This treatment presumably results in initial formation of trifluoro-N,N,dimethylacetamide which cannot protonate the enolate.

The method of alkylation of enolate anion 37 received much attention, and a detailed discussion of this conversion seems justified. Though inspection of the perspective drawing of enolate 37 indicates

that attack of an electrophile (e.g., iodomethane) from the α-face (axial) should not be abnormally hindered, studies done on a model system indicated that direct methylation of this enolate gave a mixture



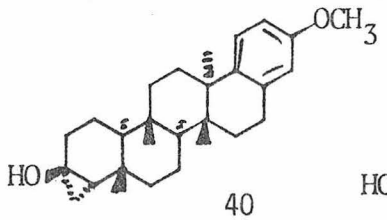
37

which always contained a substantial amount of unalkylated ketone, together with mono and polyalkylated products. Presumably alkylation of the enolate in this case is slow enough to allow exchange between starting enolate and mono-alkylated ketone to compete to an appreciable extent. In principle, if a dilute solution of the enolate 37 were to be added very slowly to a large excess of iodomethane, then a maximum yield of mono-alkylated ketone 39 should result. In practice, even under carefully controlled conditions, no better than a 50% yield of 39 was ever obtained, and the yields in the reaction were quite variable, with different amounts of unalkylated ketone 38 (R_1= R_2= H), and polyalkylated ketone 38 (R_1= R_2= H or CH_3) resulting. A possible explanation for this behavior becomes apparent when one observes that acid catalyzed epimerization of the C-4α methyl group of 4α-methyl ketone 39 proceeds to give

the C-4β epimer 28 with none of the C-4α epimer remaining at equilibrium. This effect has been explained by Eliel[25] as a consequence of puckering of the ring system due to the non-bonded interaction of the 3 methyl groups at C-4a, C-14a, and C-6b. This puckering causes the C-4α (axial) methyl group to interact with the C-14bα (axial) hydrogen to a much greater extent than is normal in conformationally rigid cyclohexanone systems. This same puckering could be the cause of the anomalous behavior of enolate 37 with respect to direct alkylation.

Though the desired ketone 28 was available via direct methylation, the only satisfactory yields obtained and the capricious nature of the reaction made the methylenation route used in the shionone work[8] seem the more attractive. Treatment of enolate 37 with the Simmons-Smith reagent[19,26] under carefully controlled conditions, followed by rapid workup and chromatography, afforded good yields of the cyclopropanol[27] 40. Though treatment of 40 with potassium tert. butoxide in refluxing ether for long periods afforded mainly 4α-methyl ketone 39 along with a small amount of 4β-methyl ketone 28, acid catalyzed rearrangement occurred with concomitant epimerization of the C-4 methyl group to give ketone 28 directly identical to that obtained by epimerization of 4α-methyl ketone 39. It is interesting to note that in spite of the expectation that rearrangement of cyclopropanol 40 to 4β-methyl ketone 28 should be nearly quantitative, only satisfactory yields (70%) were observed. Though no other products were isolated from this reaction, TLC analysis indicated the

presence of small amounts of numerous other products in the crude reaction mixture. It seems possible that since a cation such as 41 is formed



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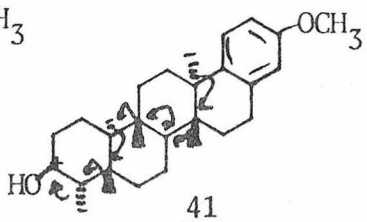

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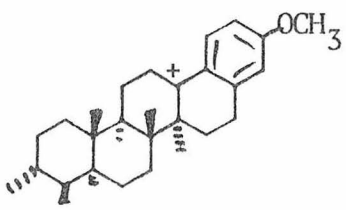
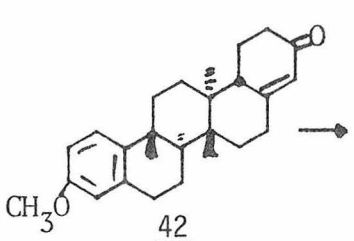


during the rearrangement, a series of 1,2 migrations up the backbone as indicated may account for the relatively low yield of this step. Rearrangements of this kind are well known in the friedelin group.[3,9]

With the ketone 28 in hand, completion of step C is straightforward. Birch reduction of 4β-methylketone to give a 3α-hydroxy enone followed by Collins oxidation afforded the enedione 22 in acceptable yield. However, since base catalyzed ring opening of cyclopropanols was known to require rather vigorous conditions (vide supra) direct Birch reduction of the cyclopropanol 40 was attempted. Indeed, treatment of 40 with lithium in DME-ammonia afforded a crude dihydroaromatic compound which upon treatment with acid gave enedione 22 in good yield. Thus the key enedione 22 was available from enone 23 in a respectable 19% overall yield in 6 steps.

Step D; synthesis of (±)-friedelin (10)

Conversion of the E-ring enone 42 to the enone 43 in 14% yield in 5 steps has been reported in connection with the synthesis of alusenone[7]



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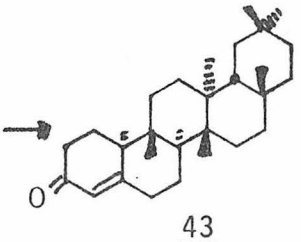

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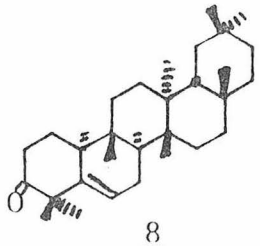
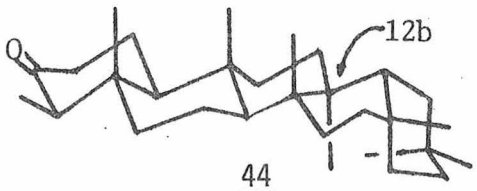


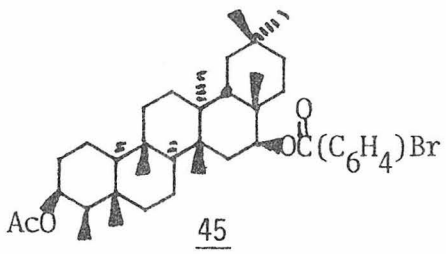
8

(8). In order to fully understand the magnitude of this achievement one must consider the structure of friedelin from a conformational standpoint. Inspection of the perspective drawing of friedelin in the all chair conformation (44) shows that the α-methyl group at C-11 is actually one C-C



44

bond distance from the α-methyl group at C-12b if no distortion is allowed; clearly this is an unacceptable situation. Though the actual conformation of friedelin even in the crystal is not yet known, X-ray structures of the two derivatives 45[28a] and 46[28b] have been solved. Though each of these derivatives contains features which could in principle distort its conformation relative to friedelin, both analyses gave the same general result, that is, a conformation in which both the D and E rings are in the boat form. Taken together, these studies indicate that friedelin itself exists mainly as the conformer 47.



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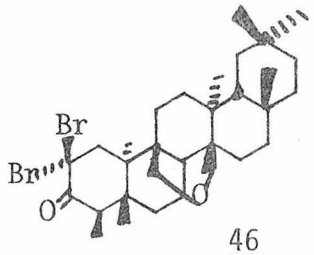

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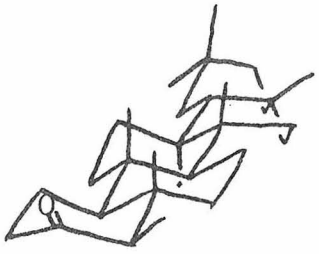
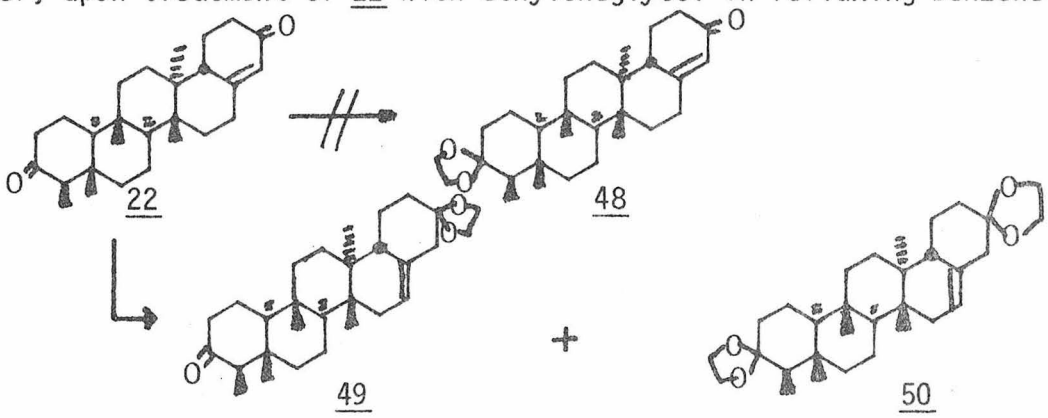


47

Regardless of its exact conformation, it is clear that the stereochemical situation in the DE ring system of friedelin and alnusenone is quite crowded, and a challenging synthetic target.

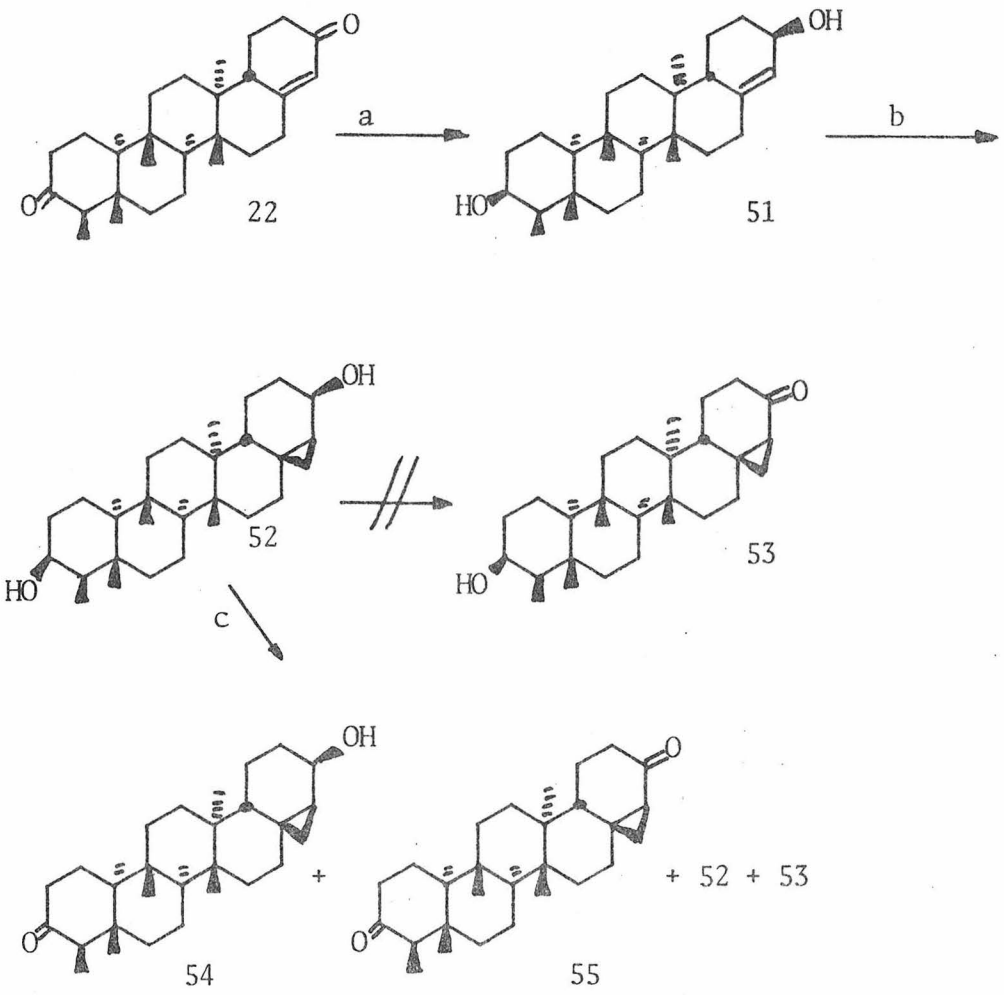
For conversion of the enedione 22 to friedelin, it is first necessary to differentiate the carbonyl groups at C-3 and C-10. Initially it was thought that a selective ketalization would serve this purpose. However, upon treatment of 22 with ethyleneglycol in refluxing benzene with



p-toluenesulfonic acid catalysis, none of the desired monoketal 48 could be detected. Rather, a mixture of monoketal 49 and bis ketal 50 was obtained. Apparently the hindered nature of the carbonyl at C-3, at least with respect to axial attack (see structure 47), relative to the sterically uncrowded environment at C-10 is enough to overcome the electronic preference for ketalization of a saturated carbonyl over an α,β-unsaturated one.[29]

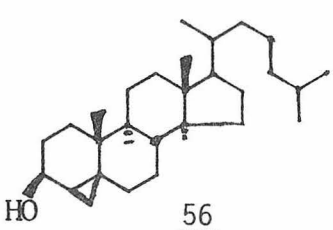
In view of this unsatisfactory result another route was attempted which delayed the necessary differentiation. This route, based on a selective oxidation, is outlined in Chart 7. Treatment of enedione 22 with lithium tri-tert. butoxyaluminum hydride[30] in refluxing THF resulted in reduction of both carbonyl groups to give the 3-β, 10-β dihydroxy olefin

Chart 7[a]



[a] a, $Li(OtBu)_3AlH$, THF, C_6H_6; b, Zn-Ag, CH_2I_2, Et_2O; c, MnO_2

51 in high yield, analogous to the initial step used in the alnusenone series. It is interesting to note that none of the epimaric alcohol products could be isolated from this reaction. Hydride attack on C-3 occurs almost exclusively from the α (equatorial) face, directed by the C-4aβ-methyl group, to give the β (axial) alcohol, while attack on C-10 occurs almost exclusively from the α (axial) face to give the β (equatorial) alcohol. Formation of the C-10 hydroxyl group here is critical since the next step, Simmons-Smith methylenation[17] of the diol 51, is directed by the C-10β hydroxyl to put the carbon atom in on the β-face, affording 52 with formation of the elements of the DE cis ring fusion. At this point, it was envisaged that conditions could be found to effect the necessary differentiation at C-3 and C-10 by selective oxidation of the cyclopropylcarbinyl hydroxyl group to give the hydroxy ketone 53. Abundant precedent for the selective oxidation of allylic alcohols in the presence of sec. hydroxyl groups exists[31] using a variety of reagents. However, treatment of the diol 52 with manganese dioxide, the most widely used reagent for such selective oxidations, under carefully controlled conditions, resulted in the formation of a mixture of starting diol 52, dione 55, and both mono-oxidation products 53 and 54. Subsequent studies using the cyclopropylcarbinyl alcohol 56 as a model for the E ring and natural friedelane-3β-ol (57) as a model for the A ring indicated that neither use of different grades of manganese



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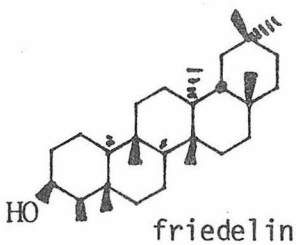
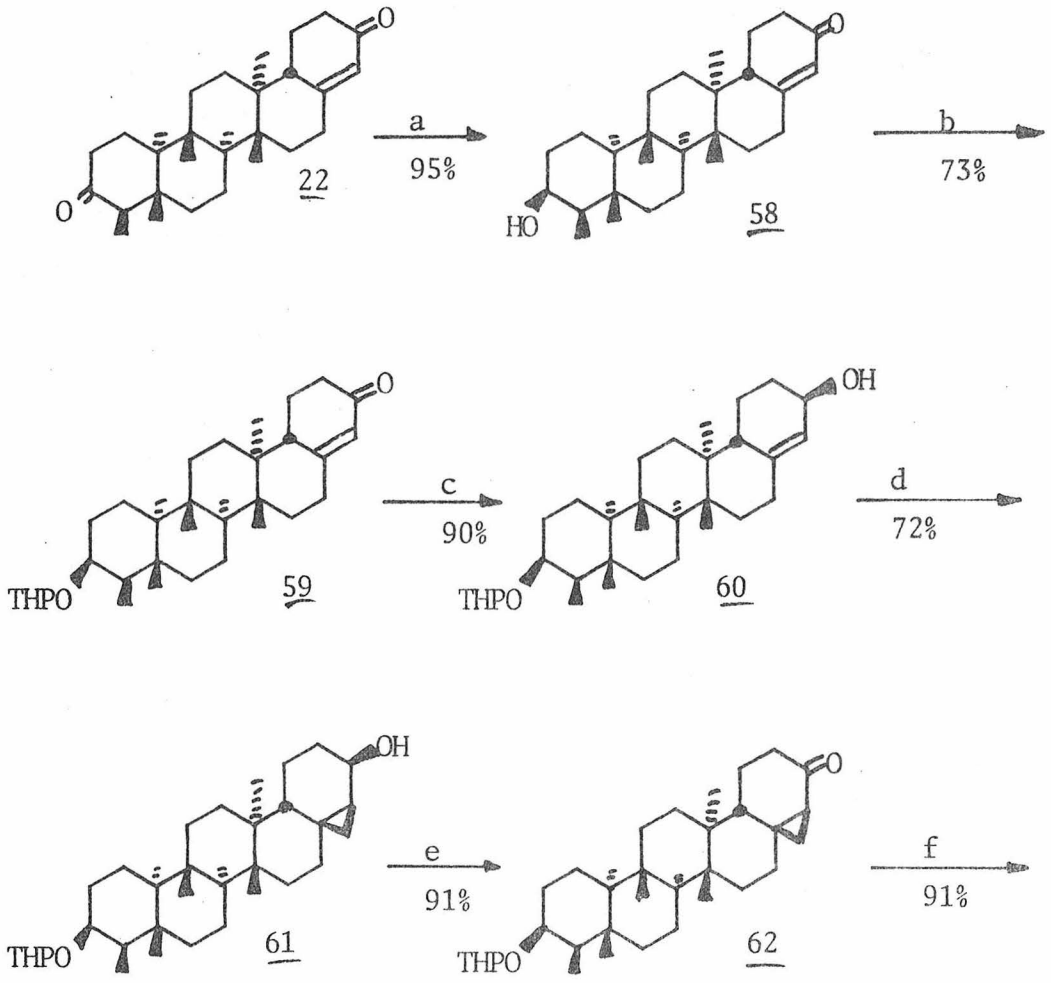


friedelinol

dioxide, dichlorodicyanoquin ne in benzene, nor aluminum isopropoxide in acetone would distinguish between the hydroxyl groups of 52 on electronic grounds. The diol 52 was also treated with Seloxette (CrO_3 on graphite) in refluxing toluene in the hope that the hydroxyl groups could be distinguished on steric grounds, but only a low yield of 54 was observed.

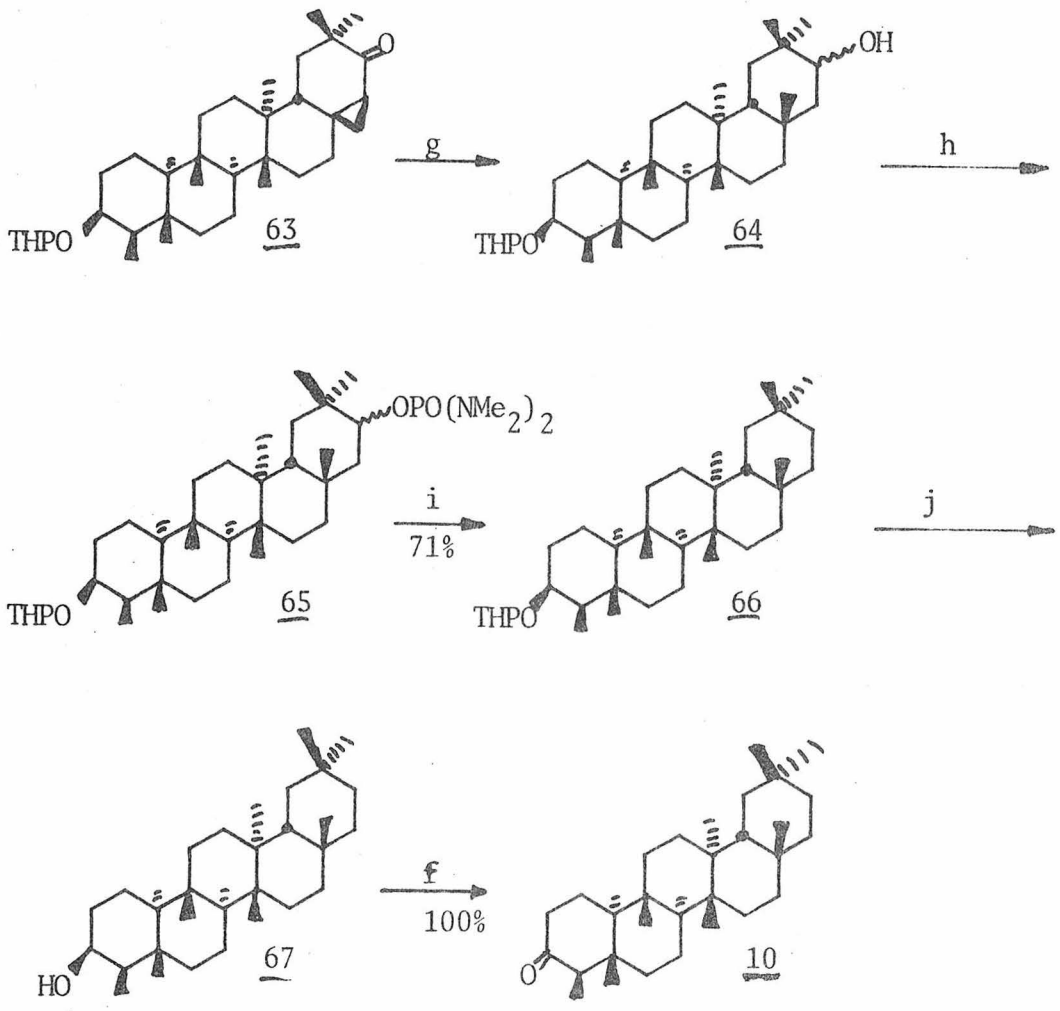
With the singular failure of the selective oxidation approach, a different tack was taken based upon the observation that treatment of the endione 22 with lithium tri-tert. butoxyaluminum hydride under more selective conditions (THF,0°,1 hour) afforded the hydroxy enone 58 in excellent yield. This successful route is outlined in Chart 8. With the differentiation of the carbonyl groups at C-3 and C-10 accomplished by selective reduction, efforts were directed toward a method of protection of the C-3 hydroxyl of 58. Work on the model alcohol 57 indicated that cyclopropylmethylation[32] with cyclopropylcarbinyl bromide in THF using sodium hydride or n-butyl lithium as base in the presence or or absence of HMPA was not satisfactory, affording only starting material and decomposition products. Formation of the tetrahydropyranylether (THP ether) of 58 occurred to us early on, but was not attempted initially because of supposed complications arising from the formation of diastereomers and the instability of THP ethers. However, upon treatment of 57 in dihydropyran-dichloromethane solution with either p-toluenesulfonic acid in benzene or a trace of phosphorylchloride, a good yield of the THP ether was isolated as a crystalline derivative stable to silica gel chromatography and comprising one spot on analytical TLC.

Chart 8[a]



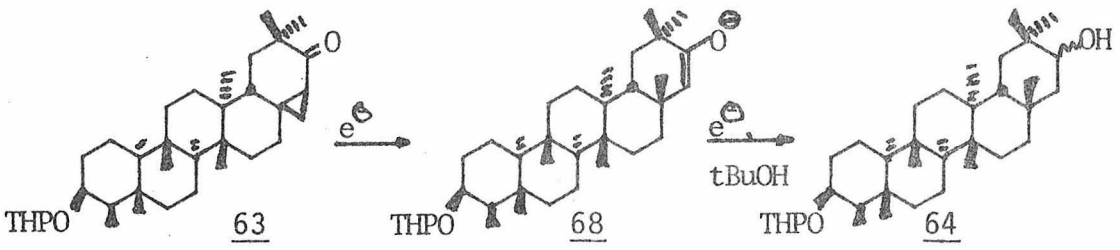
[a]a, $Li(OtBu)_3AlH$, THF, C_6H_6, 0°; b, DHP, $POCl_3$, CH_2Cl_2; c, $Li(OtBu)_3AlH$, THF, C_6H_6, refl.; d, Zn-Ag, CH_2I_2, Et_2O; e, CrO_3-2pyr, CH_2Cl_2; f, KOtBu, CH_3I, THF.

Chart 8, continued[a]



[a] g, Li, NH_3, THF, tBuOH; h, $ClPO(NMe_2)_2$, DME, HMPA, nBuLi; i, Li, $EtNH_2$, tBuOH; j, MeOH, THF, $pCH_3C_6H_4SO_3H$.

When attempted on the hydroxy enone 58 the THP formation reaction proved slightly capricious, but the protected hydroxy enone 59 could be obtained in fair yield. Reduction of 59 proceeded smoothly to give the allylic alcohol 60. Stereoselective methylenation to give cyclopropylcarbinyl alcohol 61 followed by Collins oxidation gave the desired cyclopropyl ketone 62 in good yield. Regioselective alkylation of 62 afforded the gem. dialkylcyclopropylketone 63, the cyclopropyl ring serving to protect C-9 from alkylation. Treatment of the alkylated cyclopropylketone 63 with lithium in THF-ammonia in the presence of tert.-butyl alcohol gave the mixture of alcohol epimers 64 as judged by TLC and spectral properties. Presumably the cyclopropane ring is cleaved initially to form the enolate anion 68. This enolate is then protonated to give the ketone 69 which undergoes further reduction to give 64. Normally dissolving metal reduction of a

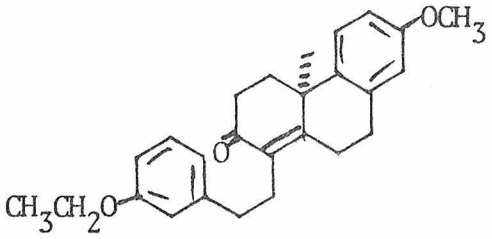


ketone would be expected to form the thermodynamically more favorable epimeric alcohol, though mixtures are reported to occur in the reduction of hindered or strained ketones.[33] In the present case, considering the conformation situation in the E ring, formation of a mixture is not surprising. The epimeric alcohols were not separated, but were trapped with the chlorophosphorodiamidate reagent[34] by a slight modification of the procedure used in the analogous reaction in the alnusenone

series to form the mixture of TMPDA derivatives 65 in good yield. Reductive removal of the TMPDA grouping gave the THP ether 66 in an overall yield of 71% from 63. Cleavage of the tetrahydropyranyl grouping followed by Collins oxidation of the resulting alcohol 67 afforded (±)-friedelin in high yield.

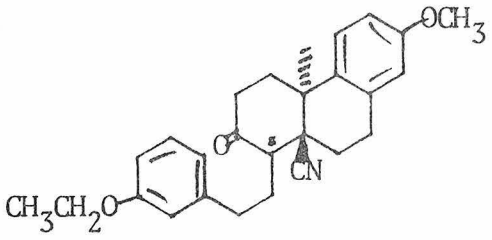
EXPERIMENTAL SECTION[1,35]

Since the experimental details for the preparation of diether 21 from the vinyl ketone 24 and the β-tetalone 25 are identical to those used for conversion of 14 and 15 to 11 (see Introduction), and the latter have been reported elsewhere,[7] they will not be included here. However, since the intermediates are new compounds, physical and spectral properties and analytical data are included.



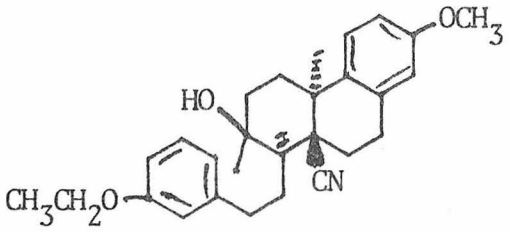
1-(2'-m-ethyoxyphenylethyl)-4,4a,9,10-tetrahydro-7-methoxy-4aα-methyl-2(3H)-phenanthrone: b.p. 216-225° (0.002 mm); ir($CHCl_3$) 1655 (C=O), 1610, 1580, and 1500 cm^{-1}(Ar); NMR($CDCl_3$) δ 1.37(t,3,J=7Hz, $ArOCH_2\underline{CH_3}$), 1.47(s,3,C-4aαCH_3), 3.77(s,3,$ArOCH_3$) and 4.00(q,2,J=7Hz, $ArO\underline{CH_2}CH_3$).

Anal. Calcd for $C_{26}H_{30}O_3$: C,79.96; H, 7.74. Found: C,79.98; H,7.63.



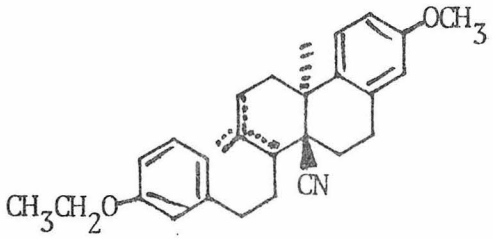
10aβ-Cyano-1-(2'-m-ethoxyphenylethyl)-1,4,4a,9,10,10a-hexahydro-7-methoxy-4aα-methyl-2(3H)-phenanthrone: mp 138.5-140° (from dichloromethane-ether-hexane); ir($CHCl_3$) 2240(C≡N), 1715(C=O), 1610 and 1580 cm^{-1}(Ar); NMR($CHCl_3$) δ 1.26(s,3,C-4aαCH_3), 1.33(t,3,J=7Hz,$ArOCH_2\underline{CH_3}$), 3.66(s,3,$ArOCH_3$), and 3.97(q,2,J=7Hz, $ArO\underline{CH_2}CH_3$).

Anal. Calcd for $C_{27}H_{31}O_3N$: C,77.66; H,7.48; N,3.35. Found: C, 77.60; H,7.43; N,3.39.



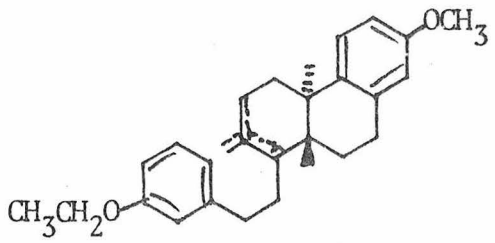
10aβ-Cyano-1-(2'-m-ethoxyphenylethyl)-1,2,3,4,4a,9,10,10a-octahydro-7-methoxy-2,4aα-dimethyl-2-phenanthrol: mp 102-104° (from ether-hexane); ir($CHCl_3$) 3600(OH), 2240(C≡N), 1610, 1580 and 1500 cm^{-1}(Ar); NMR ($CDCl_3$) δ 1.13(s,3,C4aαCH_3), 1.20(s,3,C-2CH_3), 1.40(t,3,J=7Hz, Ar$OCH_2\underline{CH_3}$), 3.76(s,3,ArOCH_3), and 4.06(q,2,J=7Hz, Ar$O\underline{CH_2}$,CH_3).

Anal. Calcd for $C_{28}H_{35}O_3N$: C, 77.56; H, 8.14; N, 3.23. Found C, 77.51; H, 8.17; N, 3.27.



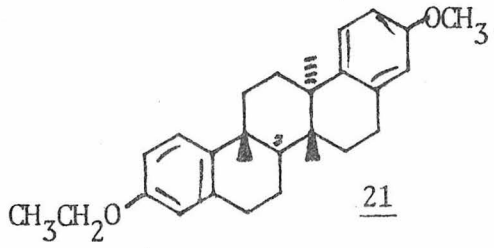
Cyano Olefin mixture: mp 105-107° (from ether-hexane); ir ($CHCl_3$) 2240(C≡N), 1610, 1580, and 1500 cm^{-1}(Ar); NMR($CDCl_3$) δ 1.08, 1.20 (2s, ∿2:1, C-4aαCH_3), 1.40(t,3,J=7Hz,Ar$OCH_2\underline{CH_3}$), 1.67(s, ∿2, $R_2C{=}CRCH_3$), 3.73(s,3,ArOCH_3), 4.03(q,2,J=7Hz, Ar$O\underline{CH_2}CH_3$), and 5.0-6.0(m, ∿1, $R_2C{=}CHR$). NMR indicative of a mixture of olefins.

Anal. Calcd for $C_{28}H_{33}O_2N$: C, 80.92; H, 8.00; N, 3.37. Found: C, 81.00; H, 8.01; N, 3.39.



Dimethyl Olefin mixture: mp 97-99° (from ether/hexane); ir ($CHCl_3$) 1610, 1580, and 1500 cm^{-1}(Ar); NMR ($CDCl_3$) δ 0.56, 0.70, 0.90, 1.06, 1.10, 1.20, 1.70 (7s, total 9H,C-2CH_3,C-4aCH_3, C-10aCH_3), 1.40(t,3,J=7Hz, $ArOCH_2\underline{CH_3}$), 3.73(s,3,$ArOCH_3$), 4.03(q,2,J=7Hz,$ArO\underline{CH_2}$ CH_3), and 4.6-6.4 (m, ∿1, $R_2C{=}CHR$).

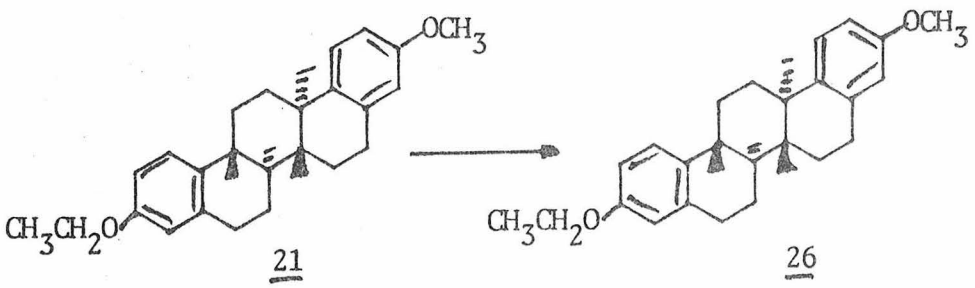
Anal. Calcd for $C_{28}H_{36}O_2$: C,83.12; H,8.97. Found: C, 83.16; H, 8.89.



21

3-Ethoxy-10-methoxy-6bβ,12bα,14aβ-trimethyl-5,6,6aα,6b,7,8,12b, 13,14,14a-decahydropicene(21): mp 152-154° (from dichloromethane-hexane); ir ($CHCl_3$) 1605, 1580, and 1495 cm^{-1}(Ar); NMR($CDCl_3$) δ 0.60, 1.06, 1.16 (3s,3 each, C-6bβCH_3, C-12bαCH_3, C-14aβCH_3), 1.36(t,3,J=7Hz, $ArOCH_2\underline{CH_3}$), 3.73(s,3,$ArOCH_3$), and 3.96(q,2,J=7Hz,$ArO\underline{CH_2}CH_3$).

Anal. Calcd for $C_{28}H_{36}O_2$: C, 83.12; H, 8.97. Found: C, 83.16; H, 8.92.



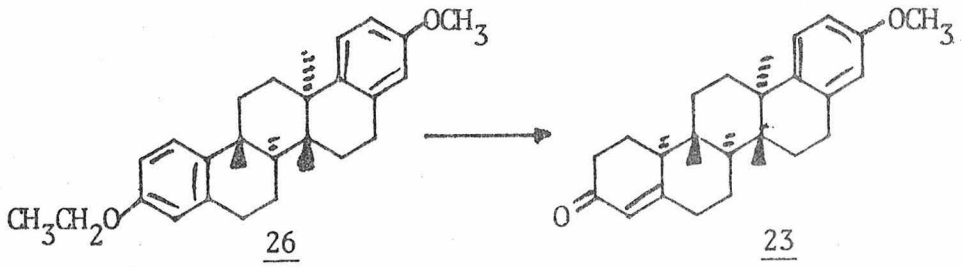
21 26

10-Ethoxy-5,6,6a,6bα,7,8,12b,13,14,14a-decahydro-6aβ,12bβ,14aα-trimethyl-2-picenol (26).

To a stirred and ice-cooled solution of 8.55 ml (49.4 mmoles) of diphenylphosphine in 100 ml of dry THF under argon were added dropwise 23.6 ml (51.9 mmoles) of a 2.2 m solution of nBuLi in hexane. After 10 min stirring without cooling 10.0 gms (24.7 mmoles) of the pentacyclic diether 21 was added via a powder funnel, and the resulting red solution was heated at reflux for 8-1/2 hours. After cooling again with an ice bath and addition of 4.27 ml (24.7 mmoles) of diphenylphosphine and 11.8 ml (25.9 mmoles) of nBuLi solution, reflux was continued for a further 12 hours. The aqueous solution was cooled, poured into 350 ml of 5% HCl, and the product isolated by ether extraction,[36] including five 10% aqueous hydrogen peroxide washes. When the crude product from this and a second identical run were combined and purified by silica gel chromatography (1:1 ether-petroleum ether) 18.6 gms (96%) of the phenol 26 resulted as a colorless foam. Crystallization of a portion of this foam from ether-petroleum ether afforded analytically pure material which melted at 202.5-204° (vacuum): ir ($CHCl_3$) 3580(ArOH), and 1600 cm^{-1}(Ar); NMR ($CDCl_3$) δ 0.63, 1.08, 1.18 (3s, 3 each, C-6bβCH_3,

C-12bα CH_3, C14aβCH_3), 1.38(t,3,J=7Hz,ArO$CH_2\underline{CH_3}$), 2.92 (broad t,4,J=7Hz, Ar$\underline{CH_2}$-), 4.02(q,2,J=7Hz,O$\underline{CH_2}CH_3$), and 4.67 (broad s,1,Ar$\underline{OH}$).

Anal. Calcd for $C_{27}H_{34}O_2$: C, 83.03; H, 8.77. Found: C, 82.81; H, 8.80.



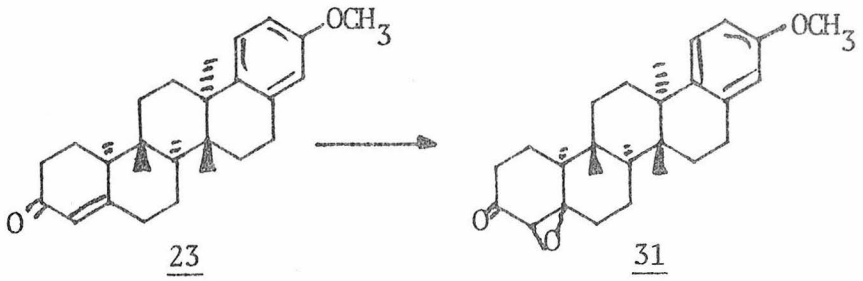
26 23

1,5,6,6aα,6b,7,8,12b,13,14,14a,14bα-Dodecahydro-10-methoxy-6bβ, 12bα,14aβ-trimethyl-3(2H)-picenone (23).

To a solution of 4.0 gms (10.24 mmoles) of the phenol 26 in 1800 ml of dry DME and 3600 ml of dry ammonia was added 7 cm (30 mg-atoms) of lithium wire with rapid stirring under argon. The solution went deep blue almost immediately. After 10 min, 46 cm (200 mg-atoms) of lithium wire was added in small pieces. The solution was stirred 10 min before 54 ml (922 mmoles) of dry 100% ethanol was added over a period of 10 min. During the next 8-1/2 hours one 46 cm portion of lithium wire was added whenever the blue color faded. After addition of the fifth portion of lithium another 54 ml portion of ethanol was added. A total of 280 cm (1260 mg-atoms) of lithium wire and 108 ml (1850 mmoles) of ethanol were used. After the solution had remained blue for 9 hours the excess lithium was quenched with 35 ml of methanol, and the ammonia was removed under a stream of argon with the aid of a hot air gun. To the resulting white suspension were added 1200 ml of dry DME and 260 ml of dry methyl iodide (4.2 moles). The resulting mixture was stirred under argon for

24 hours then filtered with suction through a celite pad. The clear yellow filtrate was concentrated at reduced pressure to a yellow paste which was diluted with one liter of 2% aqueous hydrochloric acid. The crude dihydroaromatic product was isolated by ether extraction,[36] including a 10% aqueous sodium thiosulfate wash. The resulting yellow solid was hydrolyzed by heating under reflux with 120 ml of benzene, 600 ml of 95% ethanol and 320 ml of 5N aqueous hydrochloric acid for 40 min. After cooling to room temperature the mixture was poured into 800 ml of 50% brine, and the crude enone 23 was isolated by ether extraction. Silica gel chromatography of this material (1:1 ether-petroleum ether, then ether) gave 2,260 mg (58%) of 23 as a slightly yellow foam. A portion of this material was crystallized from cyclohexane affording analytically pure material of mp 204-207° as very slightly yellow tiny crystals: ir ($CHCl_3$) 1660 (C=O), 1600 (C=C), and 1575 cm^{-1}(Ar); NMR ($CDCl_3$) δ 0.60, 0.85, 1.15 (3s, 3 each, C-6b CH_3, C-12b CH_3, C-14a CH_3), 3.77 (s,3,$ArOCH_3$), and 5.90 (broad s,1,R_2C=CHR).

Anal. Calcd for $C_{26}H_{34}O_2$: C, 82.49; H, 9.05. Found: C, 82.35; H, 9.17.



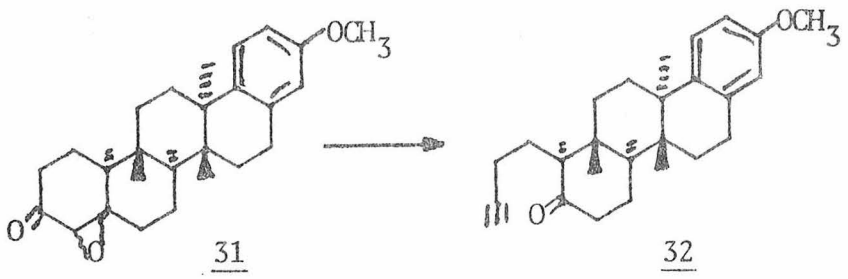
23 31

4,4a-Epoxy-1,5,6,6aα,6b,7,8,12b,13,14,14a,14bα-dodecahydro-10-methoxy-6bβ,12bα,14aβ-trimethyl-3(2H)-picenone (31).

To a solution of 1.097 gms (2.9 mmoles) of the enone 23 in 75 ml

of dichloromethane were added 100 ml of methanol, 18 ml of 30% aqueous hydrogen peroxide, and 9 ml of a 10% aqueous sodium hydroxide solution. After stirring under argon for 18 hours the mixture, which contained a white precipitate, was poured into 500 ml of brine, and the product was isolated by ether extraction.[36] Florisil chromatography of the crude product ($CHCl_3$) afforded 1.06 gms (92%)of the epoxy ketone 31 as a colorless foam. Crystallization of a portion of this foam from cyclohexane gave analytically pure material as colorless needles: mp 184-186° (vacuum); ir ($CHCl_3$) 1700 (C=O) and 1610 cm^{-1}(Ar); NMR($CDCl_3$) δ 0.55, 0.85, 1.15 (3s, 3 each, C-6bCH_3, C-12bCH_3, C-14aCH_3), 3.20(s,1,epoxy H), and 3.78 (s,3,ArOCH_3).

Anal. Calcd for $C_{26}H_{34}O_3$: C, 79.15; H, 8.69. Found: C, 79.20; H, 8.63.



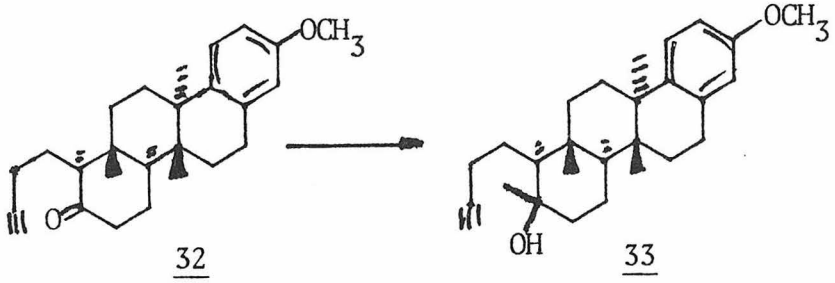
31 32

1β-(3'-Butynyl)-3,4,4aα,4b,5,6,10b,11,12,12a-decahydro-8-methoxy-4bβ-10bα,12aβ-trimethyl-2(1H)-chrysenone (32).

To a dry mixture of 790 mg (2.0 mmoles) of the epoxy ketone 31 and 410 mg (2.2 mmoles) of p-toluene-sulfonylhydrazid cooled with a -20° bath and protected by an argon atmosphere was added, with stirring, 17 ml of a -20° solution of 1:2 glacial acetic acid-dichloromethane which had been previously degassed by alternate evacuation and ebulition with argon. After 23 hours at -25°, then 13 hours at room temperature, the

light orange mixture was poured into 500 ml of H_2O and the product isolated by ether extraction,[36] including a base wash. Chromatography on Florisil ($CHCl_3$) afforded 595 mg (79%) of the acetylenic ketone 32 as a slightly yellow solid. One crystallization of a portion of this material from cyclohexane gave the analytical sample: mp 169-170° (vacuum); ir ($CHCl_3$) 3290 (C≡C-H), 2110 (weak, C≡C), and 1705 cm^{-1} (C=O); NMR ($CDCl_3$) δ 0.58, 0.75, 1.22 (3s, 3 each, C-4b CH_3, C10b CH_3, C-12a CH_s), and 3.75 s,3,$ArOCH_3$).

Anal. Calcd for $C_{26}H_{34}O_2$: C, 82.49; H, 9.05. Found: C, 82.37; H, 9.14.



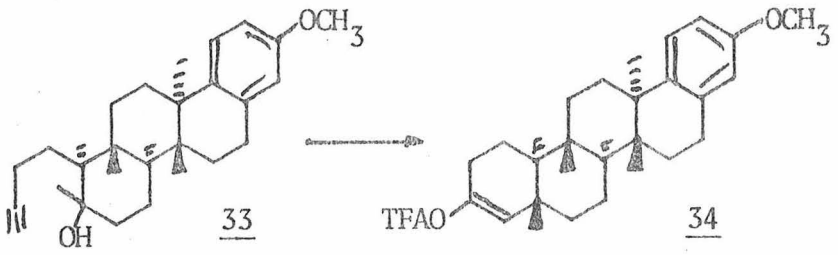
32 33

1β-(3'-Butynyl)-1,2,3,4,4aα,4b,5,6,10b,11,12,12a-dodecahydro-8-methoxy-2α,4bβ,10bα,12aβ-tetramethyl-2-chrysenol (33).

To a stirred and ice-cooled mixture of 11 ml (20 mmoles) of 1.8M ethereal methyllithium and 24 ml of dry THF under argon was added a solution of 974 mg (2.57 mmoles) of the acetylenic ketone 32 in 23 ml of dry THF over a period of 20 min. After 15 min stirring without cooling the excess methyllithium was carefully quenched with water. The resulting mixture was poured into 500 ml of brine, and the product was isolated by ether extraction.[36] Florisil chromatography of the crude product ($CHCl_3$) gave 1007 mg (99%) of the alcohol 33 as a colorless crystaline solid of mp 171-174° (vacuum). One recrystallization from n-hexane at

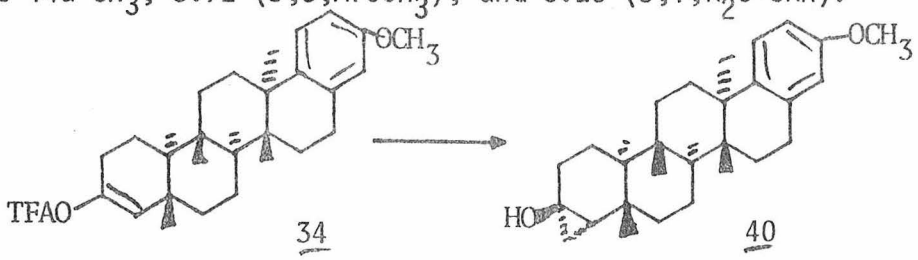
-20° gave analytically pure material as large milky prisms: mp 147-148° (vacuum); ir ($CHCl_3$) 3600 (OH), 3300 (C≡C-H), 1610, and 1575 cm^{-1}(Ar); NMR ($CDCl_3$) δ 0.53, 0.97, 1.10, 1.18 (4s, 3 each, C-2 CH_3, C-4b CH_3, C-10b CH_3, C-12a CH_3), 3.75 (s,3,$ArOCH_3$), and 6.7-7.2 (m,3,ArH).

Anal. Calcd for $C_{27}H_{38}O_2$: C, 82.18; H, 9.31. Found: C, 82.21; H, 9.61.



3-Trifluoroacetoxy-10-methoxy-4aβ,6bβ,12aα,14aβ-tetramethyl-1,2,4a,5,6,6aα,6b,7,8,12a,13,14,14a,14bα-tetradecahydropicene (34).

To 467 mg (1.184 mmoles) of the acetylenic alcohol 33 cooled to -20° under an argon atmosphere was added 80 ml of a -20° solution of 70:30 trifluoroacetic acid-trifluoroacetic anhydride. After 40 min of stirring at -20° the solvents were removed at reduced pressure (∿ 1 mm). The residue was taken up in 100 ml of ether, and after a base wash was isolated as usual.[36] The resulting slightly yellow foam, which amounted to 510 mg (98%), was used in subsequent steps without purification: ir ($CHCl_3$) 1785 (C=O), 1690 (C=C), 1600 and 1575 cm^{-1}(Ar); NMR ($CDCl_3$) δ 0.53, 0.87, 1.03, 1.10 (4s, 3 each, C-4a CH_3, C-6b CH_3, C-12b CH_3, C-14a CH_3, 3.72 (s,3,$ArOCH_3$), and 5.25 (s,1,R_2C=CRH).



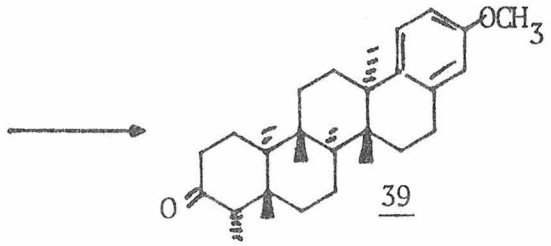
1,2,4a,5,6,6aα,6b,7,8,12b,13,14,14a,14bα-Tatradecahydro-3α, 4α-methano-10-methoxy-4aβ,6bβ,12aα,14aβ-tetramethyl-3β-picenol (40)

To a stirred solution of lithium diisopropylamide prepared from 20.2 ml (52.6 mmoles) of a 2.60M hexane solution of n-butyllithium and 9.5 ml (63 mmoles) of diisopropylamine in 85 ml of dry THF at -55° under an argon atmosphere was added a solution of 5.6 mg (1.05 mmoles) of the enoltrifluoroacetate 34 in 15 ml of dry THF over a period of 15 min. To the solution which resulted after 15 min of further stirring at -60° was added all at once 160 ml (100 mmoles) of the Simmons-Smith reagent prepared from the zinc-silver couple[26] [11.4 gms (175 mmoles) of granular zinc, 88 mg of silver acetate, and 88 ml of glacial acetic acid] and 13.9 ml (172 mmoles) of diiodomethane in 158 ml of dry ether in the presence of a few strands of silver wool after the procedure of Conia.[26]

After stirring at room temperature for 50 min, the solution was poured into 300 ml of ice cold 20% aqueous ammonium sulfate solution containing 0.2% concentrated ammonium hydroxide. This mixture was diluted with 300 ml of water, and the product was isolated by ether extraction[36] including washes with the above ammonium sulfate solution and 10% aqueous sodium thiosulfate solution. Immediate filtration of the oil which resulted after removal of most of the solvent with a rotary evaporator through 50 gms of basic III alumina (petroleum ether, then 75% ether-petroleum ether) afforded the crude alcohol as an orange oil. Purification of this oil by chromatography on silica gel (1:1 ether-petroleum ether) afforded 260 mg (60%) of the cyclopropanol 40 as a slightly yellow solid: mp-sintering at 92°, m 96° d(vacuum). Two

recrystallizations of a portion of this material from n-hexane gave the analytical sample as colorless microcrystals: mp-shrinking and discoloration at 105°, m 111-112° d(vacuum); ir ($CHCl_3$) 3590 (OH), 1605, and 1575 cm^{-1}(Ar); NMR ($CDCl_3$) δ 0.50, 0.82, 1.09, 1.15 (4s, 3 each, C-4a CH_3, C-6b CH_3, C-12b CH_3, C-14aCH_3) and 3.77 (s,3,ArOCH_3); Mass Spec. m/e 161(100%), 173(61), 187(47), 393(24), 408(15,M^+), mass measured M^+: Calcd for $C_{28}H_{40}O_2$: 408.302814. Found: 408.3029(±0.0004).

Anal. Calcd for $C_{28}H_{40}O_2$: C, 82.30; H, 9.87. Found: C, 82.24; H, 9.81.



39

1,4,4a,5,6,6aα,6b,7,8,12a,13,14,14a,14bα-Tetradecahydro-10-methoxy-4α,4aβ,6bβ,12aα,14aβ-pentamethyl-3-2H)-picenone (39).

A. By Direct Methylation of the Enolate 37.

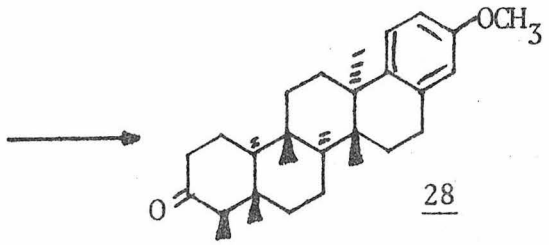
To a stirred and ice-cooled solution of lithium diisopropylamide prepared from 1.4 ml (3.1 mmoles) of a 2.2M hexane solution of n-butyllithium and 0.54 ml (3.54 mmoles) of dry diisopropylamine in 5 ml of dry THF under argon was added 61 mg (0.123 mmoles) of the enoltrifluoroacetate 34 in 3 ml of dry THF in one min. After 15 min at room temperature the resulting dark red solution was added dropwise via syringe to a stirred and ice-cooled solution of 5 ml of dry methyl iodide in 5 ml of dry THF to which 0.5 ml of the above n-butyllithium solution had been added to insure aprotic conditions.

After 30 min the reaction mixture was poured into 100 ml of water and the product isolated by ether extraction.[36] Silica gel chromatography (25% ether-petroleum ether) of the crude product gave 24 mg (47%) of the 4α-methyl ketone 39 as a colorless crystalline solid of mp 195-206° (vacuum): R_f on TLC (25% ether-petroleum ether) 0.23; ir ($CHCl_3$) 1700 cm^{-1}(C=O); NMR ($CDCl_3$) δ 0.55, 0.90, 0.95, 1.17 (4s,3 each, C-4a CH_3, C-6b CH_3, C-12b CH_3, C-14a CH_3), 1.14 (d,3,J=7Hz, C-4 CH_3), and 3.77 (s,3,AroCH_3); Mass measured molecular ion calcd. for $C_{28}H_{40}O_2$; 408.302814. Found: 408.3029 ± 0.0004.

Also isolated was 7.6 mg of material homogeneous by TLC (Rf 0.31, 25% ether-petroleum-ether) but identified as a mixture of polyalkylated products by GLC analysis (215°, at least 3 peaks), NMR spectrum, and mass spectrum.

B. By tert-Butoxide Catalyzed Ring Opening of the Cyclopropanol 40.

A stirred solution of the crude product (22 mg, 0.05 mmoles) from a preparation of the cyclopropanol 40 similar to that described above in 20 ml of dry ether was treated with 20 mg of potassium tert-butoxide at reflux under an argon atmosphere for 22 hours. The mixture was diluted with water and the product isolated by ether extraction.[36] NMR analysis of the crude material which resulted indicated the major component to be the 4α-methyl ketone 39 with about 10%-30% of the 4β-epimer 28 present. Treatment of this material with ethanolic hydrochloric acid as described below resulted in complete epimerization to the 4β-methyl ketone 28.



28

1,4,4a,5,6,6aα,6b,7,8,12a,13,14,14a,14bα-Tetradecahydro-10-methoxy-4β,4aβ,6bβ,12aα,14aβ-pentamethyl-3(2H)-picenone (28).

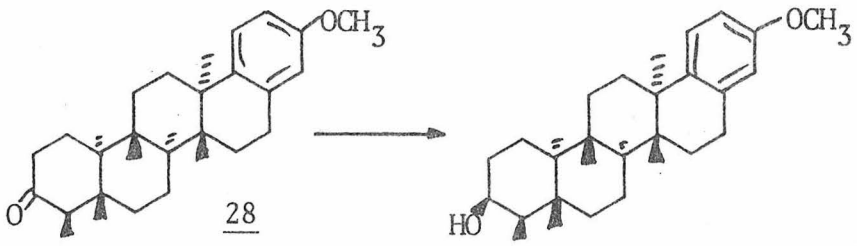
A. By Epimerization of the 4α-Methyl Ketone 39.

To a stirred solution of 43 mg (0.104 mmoles) of the 4α-methyl ketone 39 in 1.2 ml of benzene and 6.1 ml of 95% ethanol under argon was added 3.2 ml of 5N hydrochloric acid and the mixture was heated to reflux. After 20 min the mixture was cooled, poured into 50 ml of 50% brine, and the product isolated by ether extraction.[36] The crude product thus obtained was purified by silica gel chromatography (25% ether-petroleum ether) to afford 35 mg (82%) of the 4β-methyl ketone 28 as a colorless crystalline solid: ir ($CHCl_3$) 1700 (C=O), 1610, and 1580 cm^{-1}(Ar); NMR ($CDCl_3$) δ 0.55, 0.75, 0.92, 1.17 (4s,3 each, C-4a CH_3, C-6b CH_3, C-12b CH_3, C-14a CH_3), 0.91 (d,3,J=7Hz,C-4β CH_3), and 3.77 (s,3,$ArOCH_3$); Mass measured M^+: Calcd for $C_{28}H_{40}O_2$, 408.302814; Found, 408.3029 ± 0.0004.

B. By Acid Treatment of the Cyclopropanol 40.

To a stirred solution of 22 mg of the cyclopropanol 40 in 8 ml of 95% ethanol under argon was added 10 drops of concentrated hydrochloric acid. After 45 min at reflux the mixture was diluted with 40 ml of water, and the product isolated by ether extraction.[36] A slightly yellow oil resulted which amounted to 16 mg (73%). Ir and NMR spectra confirmed

the identity of this material as the 4β-methyl ketone 28, though some impurity peaks were present (notably in the RCH_2R region of the NMR). TLC analysis (25% ether-petroleum ether) showed one major component of Rf 0.25 with two minor components of Rf 0.18 and 0.09. GLC analysis at 250° showed one major product with a 3.7 min retention time, approximately 90% pure.

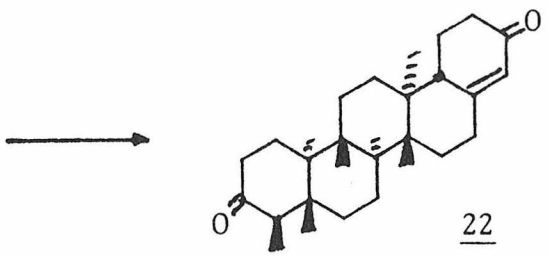


28

1,2,3,4,4a,5,6,6aα,6b,7,8,10,11,12,12aβ,12b,13,14,14a,14bα-Eicosahydro-4β,4aβ,6bβ,12bα,14aβ-pentamethyl-10-oxo-3α-picenol.

To a stirred solution of 105 mg (0.26 mmoles) of the ketone 28 in 112 ml of dry ammonia under an argon atmosphere were added 1.2 cm (5 mg atoms) of lithium wire and 1.5 ml (25 mmoles) of dry ethanol. During the course of the next 160 min 8 additional 1.2 cm portions of lithium wire and one additional 1.5 ml portion of dry ethanol were added. Thus a total of 10.4 cm (45 mg atoms) of lithium wire and 3 ml (51 mmoles)of ethanol were used. To the resulting solution was added 2.5 gms (47 mmoles) of ammonium chloride and the ammonia was evaporated with a stream of argon. The residue was diluted with 100 ml of water and the product isolated by ether extraction.[36] The resulting crude dihydro compound was hydrolyzed by refluxing with 3 ml of benzene, 15 ml of 95% ethanol,and 8 ml of 5N aqueous hydrochloric acid. After 40 min the cooled mixture was poured into 100 ml of 50% brine and the

product was isolated by ether extraction, including a base wash. The resulting crude material was purified by silica gel chromatography (40% ethylacetate-benzene) affording 59.7 mg (58%) of the hydroxy enone as a slightly yellow oil: ir ($CHCl_3$) 3600(OH), 1650 (C=O), and 1603 cm^{-1} (C=C); NMR ($CDCl_3$) δ 0.80, 0.98 (2s,3 each, angular CH_3), 0.88 (s,6, angular CH_3), 0.93 (s, $\sim$1.5, part of C-4β CH_3), 3.3 (broad S,1,$R_2\underline{CH}OH$), and 5.8 (R_2C CRH). This material was not further purified, but was used directly in subsequent steps.



22

1,4,4a,5,6,6aα,6b,7,8,12,12aβ,12b,13,14,14a,14bα-Hexadecahydro-4β,4aβ,4bβ,12aα,14aβ-pentamethyl-3,10(2H,11H)-picendione (22).

A. By Birch Reduction of the Cyclopropanol 40.

To a stirred solution of 270 mg (66 mmoles) of the cyclopropanol 40 in 116 ml of dry DME and 230 ml of dry ammonia protected by an argon atmosphere were added 2.96 cm (92 mg, 13.2 mg atoms) of lithium wire and 3.85 ml (66 mmoles) of dry ethanol. During the next 160 min one 2.96 cm portion of lithium wire was added whenever the solution decolorized. An additional 3.85 ml of ethanol was also added. A total of 18 cm (79 mg atoms) of lithium wire and 7.7 ml (132 mmoles) of dry ethanol were used. The reaction was quenched by addition of 5 gms (94 mmoles) of ammonium chloride and the ammonia was evaporated in a stream of argon with the aid of a heat gun. The resulting mixture was diluted

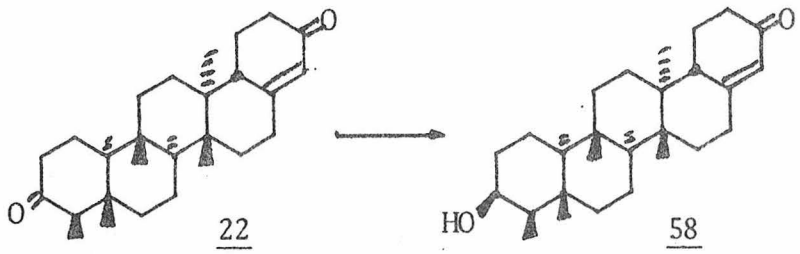
with 250 ml of water, and the product isolated by ether extraction.[36] The crude dihydro compound which resulted was hydrolyzed by refluxing with 8 ml of benzene, 40 ml of 95% ethanol, and 21 ml of 5N aqueous hydrochloric acid for 40 min. Dilution of the cooled reaction mixture with 125 ml of 50% brine and isolation of the product by ether extraction,[36] including a base wash afforded crude product which was purified by medium pressure silica gel chromatography (20% ethylacetate-benzene). Pure endione (22) amounting to 120 mg (46%) was obtained as a colorless crystalline solid of mp 247-250° (vacuum). Also obtained were 19 mg of a mixture containing approximately 70% of the endione 22 along with overreduced material. Analytically pure material was obtained after one recrystallization of a portion of the above pure fraction from cyclohexane: mp 247-250° (vacuum); ir ($CHCl_3$) 1700(C=O), 1650 (C=O), and 1610 cm^{-1} (C=C); NMR ($CDCl_3$) δ 0.73, 0.90, 0.93, 1.01 (45, 3 each, C-4a CH_3, C-5b CH_3, C-8a CH_3, C-12b CH_3), 0.87 (d,3,J=6Hz, C-4β CH_3), 5.88 (broad s,1, $R_2C{=}CHR$).

Anal. Calcd for $C_{27}H_{40}O_2$: C, 81.77; H, 10.17. Found: C, 81.68; H, 10.23.

B. From Collins Oxidation of the α-Hydroxy Enone

To a stirred solution of 60 mg (0.15 mmoles) of the α-hydroxy enone from above in 1 ml of dry dichloromethane under an argon atmosphere was added 7.5 ml (1.5 mmoles) of Collins reagent. (This reagent was prepared by dropwise addition of 0.48 ml of dry pyridine to a stirred suspension of 300 mg of anhydrous chromium trioxide in 15 ml of dry dichloromethane under argon. The supernatant liquid was used

after 20 min). After 10 min the dark solution was filtered through 10 gms of alumina, and the filter was washed with 75 ml of dichloromethane and 25 ml of ether. Removal of the solvents at reduced pressure afforded 49 mg (83%) of enedione 22 identical to that obtained above.



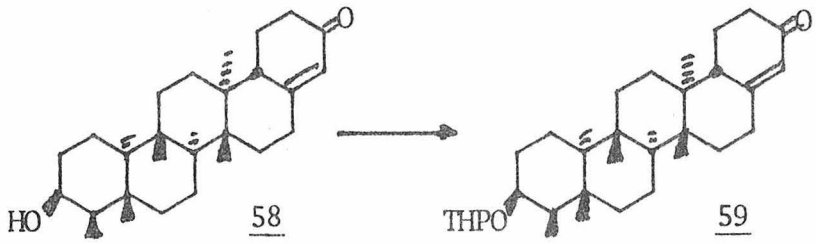
22 58

1,2,3,4,4a,5,6,6aα,6b,7,8,10,11,12,12aβ,12b,13,14,14a,14bα-Eicosahydro-4β,4aβ,6bβ,12aα,14aβ-pentamethyl-10-oxo-3β-picenol (58).

To a stirred and ice-cooled solution of 135 mg (0.34 mmoles) of the enedione 22 in 26 ml of dry THF and 5.3 ml of benzene protected by an argon atmosphere was added 430 mg (1.7 mmoles) of lithium tri-tert-butoxyaluminum hydride in 7 ml of ice-cold dry THF. After 2 hours at 0° the reaction was quenched by addition of 0.71 ml (1.79 mmoles) of a 10% aqueous solution of sodium hydroxide. After a further 15 min at 0° followed by 1 hour at ambient temperature the mixture was filtered through 10 gms of silica gel, with suction. The filter was washed with 20 ml of dichloromethane and 100 ml of 1:1 ethylacetate-benzene. Removal of the solvents followed by medium pressure silica gel chromatography of the crude product (20% ethylacetate-benzene) afforded 130 mg (95%) of the 3β-hydroxy enone 58 as colorless crystals of mp 249-252° (vacuum). Two recrystallizations of a portion of this material afforded

the analytical sample: mp 252-255° (vacuum); ir ($CHCl_3$) 3600 (OH), 1655 (C=O), and 1610 cm^{-1}(C=C); NMR ($CDCl_3$) δ 0.85, 0.91 (2s,3 each, angular CH_3), 0.98(s,6,angular CH_3), 3.74(s,1,$w_{1/2}$=8Hz, C-3α H), and 5.91(s,1, R_2C=CHR).

Anal. Calcd for $C_{27}H_{41}O_2$: C, 81.35; H, 10.62. Found: C, 81.46; H, 10.50.



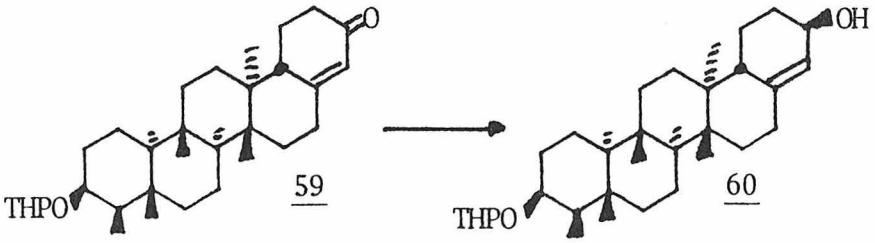
58 59

1,5,6,6a,6bα,7,8,8a,9,10,11,12,12aα,12b,13,14,14a,14bβ-Octahydro-6aβ,8aβ,9β,12bβ,14aα-pentamethyl-10β(tetrahydropyran-2'-yloxy)-3(2H)-picenone (59).

To a stirred solution of 12 mg (0.03 mmoles) of the hydroxyenone 58 in 2 ml of dry dichloromethane and 1 ml of dihydropyran (freshly distilled from sodium) under argon was added 5μl of distilled phosphorous oxychloride. After 1.25 hours another 5μl portion of phosphorous oxychloride was added. After 1.75 hours the reaction was quenched by addition of 3 drops of distilled triethylamine. Removal of the solvents at reduced pressure afforded 23 mg of the crude tetrahydropyranyl ether 59 as an oily yellow solid. Silica gel chromatography (1:1 ether-petroleum ether) gave 9.7 mg (67%) of the mixture of diastereomeric ethers 59 (a slightly broadened single spot by TLC, Rf 0.19 with 1:1 ether-petroleum ether) as a slightly yellow solid of mp 214-216° (vacuum). Subsequently 86 mg of the hydroxy enone were converted into 76 mg (73%)

of the ethers 59 with mp 216-220° (vacuum) in two runs, the crude products of which were combined and purified together. One recrystallization of a portion of this material from methanol-dichloromethane afforded the analytical sample as small colorless prisms: mp 226-227° (vacuum); ir($CHCl_3$) 1660 (C=O), and 1610 cm^{-1} (C=C); NMR ($CDCl_3$) δ 0.85, 0.91, 0.95, 0.98 (4s, complex, angular CH_3), 3.48, 3.69 (2s, 1/2 each, $w_{1/2}$= 6Hz, C-3α H), 3.50 (s,1,$w_{1/2}$=10Hz, C-6'H), 3.93 (broad m, 1,C-6'H), 4.54, 4.70 (2s, 1/2 each, $w_{1/2}$= 6Hz, C-2'H), 5.90 (s,1,R_2C=CHR). This NMR is indicative of the expected 50:50 mixture of diastereomers of the ether at C-2'.

Anal. Calcd for $C_{32}H_{50}O_3$: C, 79.62; H, 10.44. Found: C, 79.67; H, 10.43.

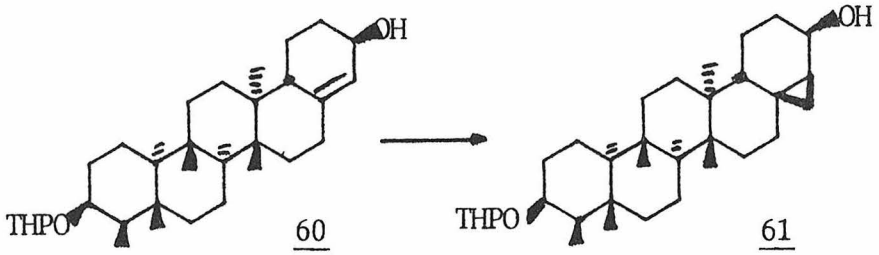


1,2,3,5,6,6a,6bα,7,8,8a,9,10,11,12,12aα,12b,13,14,14a,14bβ-Eicosahydro-6aβ,8aβ,9β,12bβ,14aα-pentamethyl-10β(tetrahydro-2'-yloxy)-3β-picenol (60).

To a stirred solution of 76.4 mg (0.158 mmoles) of the tetrahydropyranyloxy enone 59 in 2.5 ml of dry THF and 0.57 ml of benzene under argon was added 400 mg (1.58 mmoles) of lithium tri-tert-butoxyaluminum hydride in 2.5 ml of dry THF. The resulting homogeneous solution was heated under reflux for 1 hour then cooled and treated with 0.67 ml (1.66 mmoles) of aqueous 10% sodium hydroxide solution. After a further 1-1/2 hours the mixture was filtered through 5 gms of silica gel with

suction, and the filter was washed with 100 ml of ether. Removal of solvents from the combined filtrates at reduced pressure followed by silica gel chromatography of the crude product (1:1 ether-petroleum ether) afforded 69 mg (90%) of the allylic alcohol 60 as a colorless solid of mp 239-244° (vacuum). Analytically pure material was obtainec after one recrystallization of a portion of the above alcohol from methanol-dichloromethane: mp 249-252° (vacuum); ir ($CHCl_3$) 3600 (OH), and 1650 cm^{-1}(C=C); NMR ($CDCl_3$) δ 0.79 (s,3,angular CH_s), 0.89 (m, angular CH_3), 0.93 (s,6,angular CH_3), 3.44, 3.66 (2s, 1/2 each, $w_{1/2}$= 7Hz, C-3α H), 3.50(s,1,$w_{1/2}$=10Hz, C-6'H), 3.94(m,1,C-6'H), 4.14 (s,1, $w_{1/2}$=20Hz, C-10α H), 4.55, 4.67 (2s, 1/2 each, C-2'H), 5.42 (s,1, R_2C=CHR).

Anal. Calcd for $C_{32}H_{52}O_3$: C, 79.29; H, 10.81. Found: C, 79.22; H, 10.75.



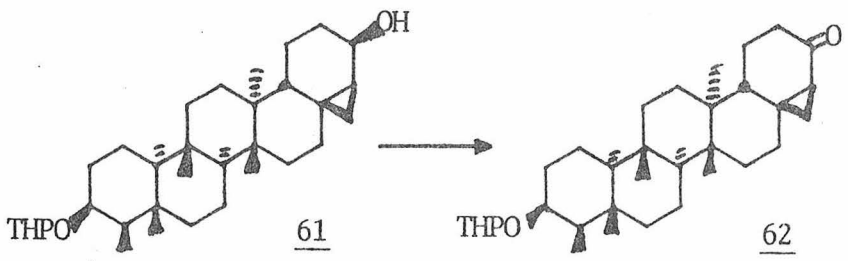
60 61

1,2,3,5,6,6a,6bα,7,8,8a,9,10,11,12,12aα,12b,13,14,14a,14bβ-Eicosahydro-4β,4aβ-methano-6aβ,8aβ,9β,12bβ,14aα-pentamethyl-10β(tetrahydropyran-2'yloxy)-3β-picenol (61).

To a stirred solution of 38 mg (0.079 mmoles) of the allylic alcohol 60 in 4 ml of dry THF under argon was added 4.3 ml of the Simmons-Smith reagent prepared from the zinc-silver couple[32] [850 mg (13 mmoles) of granular zinc, 6.5 mg of silver acetate, and 6.5 ml of

glacial acetic acid] and 0.81 ml (10 mmoles) of diiodomethane in 9.2 ml of dry ether in the presence of a few strands of silver wool after the procedure of Conia.[32] The colorless homogeneous mixture was heated at reflux for 1 hour then cooled and diluted with 50 ml of 20% aqueous ammonium sulfate solution containing 0.2% of ammonium hydroxide. Isolation of the product by ether extraction[36] including 20% buffered ammonium sulfate and sodium thiosulfate washes afforded, after removal of most of the solvents at reduced pressure, a colorless oil which was immediately chromatographed on alumina (petroleum ether, then 1:1 ether-petroleum ether). The yield of cyclopropylcarbinyl alcohol 61 was 28 mg (72%) as a slightly yellow solid of mp 252-257° (vacuum). One recrystallization of a portion of this material from methanol-dichloromethane gave analytically pure material as white microcrystals: mp 253-255° (vacuum); ir ($CHCl_3$) 3600 cm^{-1}(OH); NMR 0.42, 0.59, (2m,3,cyclopropyl H), 0.86, 0.94, 0.99, 1.09 (4s,complex,angular CH_3), 3.47, 3.65 (2s,1/2 each, C-3α H), 3.49 (s,1,C-6'H), 3.93 (m,1,C-6'H), 4.17 (complex m,1,C-10α H), 4.54, 4.67 (2s,1/2 each,C-2'H).

Anal. Calcd for $C_{33}H_{54}O_3$: C, 79.46; H, 10.91. Found: C, 79.36; H, 11.00.

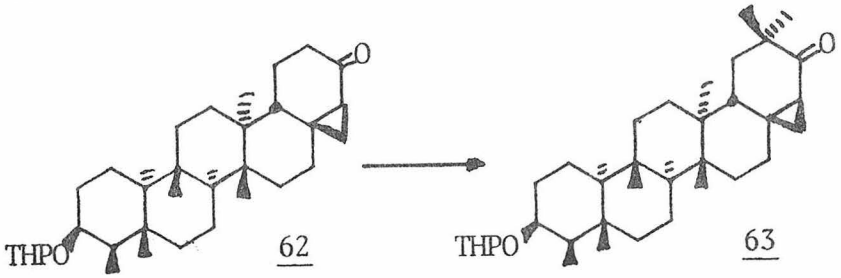


61 62

1,5,6,6a,6bα,7,8,8a,9,10,11,12,14aα,12b,13,14,14a,14bβ-Octadecahydro-4β,4aβ-methano-6aβ,8aβ,9β,12bβ,14aα-pentamethyl-10β(tetrahydropyran-2'-yloxy)-3(2H)-picenone (62).

To a stirred solution of 37 mg (0.075 mmoles) of the cyclopropylcarbinyl alcohol 61 in 1.3 ml of dry dichloromethane under argon was added 3.7 ml (0.746 mmoles) of Collins reagent. (This reagent was prepared by dropwise addition of 0.48 ml of dry pyridine to a stirred suspension of 300 mg of anhydrous chromium trioxide in 15 ml of dry dichloromethane under argon. The supernatant liquid was used after 20 min.) After 10 min at room temperature the dark brown mixture was filtered with the aid of suction through 10 gms of alumina, and the filter was rinsed with 100 ml of ether and 10 ml of dichloromethane. Removal of the solvents from the filtrate at reduced pressure afforded 33.7 mg (91%) of the ketone 62 as a slightly yellow solid of mp 265.5-268° (vacuum) which was homogeneous by TLC (1:1 ether-petroleum ether, R_f 0.18). One recrystallization of a portion of this material from cyclohexane afforded the analytical sample: mp 268.5-270° (vacuum); ir ($CHCl_3$) 1675 cm^{-1}(C=O); NMR ($CDCl_3$) δ 0.88, 0.94, 1.01 (3s,complex,angular CH_3), 3.47, 3.66 (2s,1/2 each, C-3α H), 3.51 (s,1,C-6'H), 3.92 (m,1,C-6'H), 4.55, 4.67 (2s,1/2 each, C-2'H).

Anal. Calcd for $C_{33}H_{52}O_3$: C, 79.79; H, 10.55. Found: C, 79.75; H, 10.51.

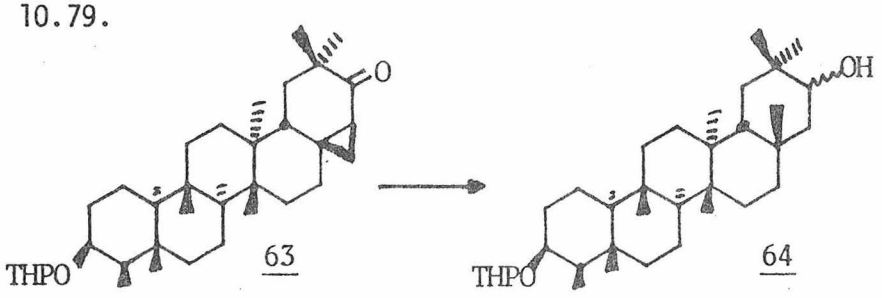


62 63

1,5,6,6a,6bα,7,8,8a,9,10,11,12,12aα,12b,13,14,14a,14bβ-Octadecahydro-4β,4aβ-methano-2α,2β,6aβ,8aβ,9β,12bβ,14aα-heptamethyl-10β-(tetrahydropyran-2'-yloxy)-3(2H)-picenone (63).

To a stirred solution of 13 mg (0.026 mmoles) of the ketone 62 and 56 mg (0.49 mmoles) of potassium tert-butoxide in 3 ml of dry THF under argon was added 0.03 ml (0.52 mmoles) of dry iodomethane, resulting in the immediate formation of a white precipitate. After 19 hours an additional 0.01 ml (0.16 mmoles) of dry iodomethane was added. After one hour the reaction mixture was poured into 50 ml of water, and the product isolated by ether extraction,[36] affording 13.3 mg (97%) of the crude ketone 63 as a slightly yellow solid. Silica chromatography of this material (25% ether-petroleum ether) gave 12.4 mg (91%) of 63 as a slightly yellow solid which was homogeneous by TLC (same solvent, R_f 0.28) of mp 239-243° (vacuum). One recrystallization of a portion of this material from methanol-dichloromethane gave the analytical sample as small colorless prisms: mp 254.5-256° (vacuum); ir ($CHCl_3$) 1675 cm^{-1} (C=O); NMR ($CDCl_3$) δ 0.88, 0.96, 1.11, 1.15 (4s,3 each,angular CH_3), 1.06 (s,6,angular CH_3), 3.47, 3.66 (2s,1/2 each, C-3α H), 3.51 (s,1, C-6'H), 3.95 (m,1,C-6'H), 4.57, and 4.70 (2s,1/2 each,C-2'H).

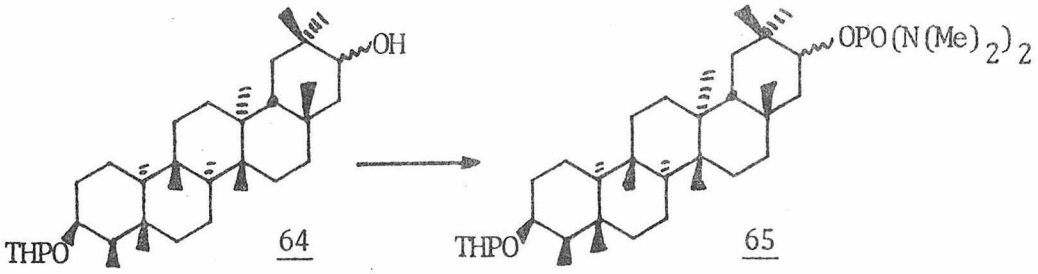
Anal. Calcd for $C_{35}H_{56}O_3$: C, 80.10; H, 10.76. Found: C, 80.15; H, 10.79.



Mixture of Alcohols 64.

To a stirred solution of 16.8 mg (0.032 mmoles) of the ketone 63 in 6.6 ml of dry THF and 13 ml of dry ammonia under argon was added 1.4 mm (0.062 mmoles) of lithium wire. The deep blue solution which resulted

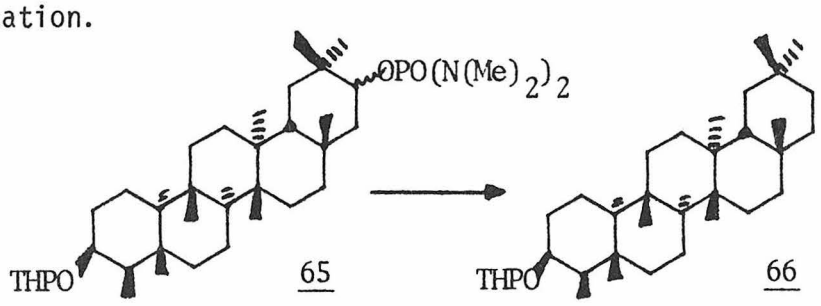
was stirred for 20 min before 0.061 ml (0.66 mmoles) of dry tert-butyl alcohol and another 1.4 mm portion of lithium wire were added. After 40 min the excess lithium was quenched by addition of 0.5 ml of anhydrous methanol, and the ammonia was evaporated in a stream of argon. The residue was diluted with 50 ml of water and the product isolated by ether extraction.[36] The resulting crude mixture of alcohols 64 amounted to 15.3 mg (91%) of a slightly yellow solid which by TLC (25% ether-petroleum ether) appeared to be a mixture of epimers, R_f 0.09, and 0.17. The ir spectrum of this crude mixture showed no carbonyl absorption, with the expected hydroxyl absorption at 3600 cm^{-1}. The NMR showed many signals in the angular methyl region, and some signals in the δ-3-5 region besides those due to the THP ether. The spectrum is indicative of a mixture of alcohol epimers. Material from a similar reaction was purified by silica gel chromatography (25% ether-petroleum ether) to give a 94% yield of the mixture of alcohols, homogeneous by TLC. However, normally the crude product was used without purification.



Mixture of Phosphoradiamidates 65.

To a stirred solution of 15.3 mg (0.029 mmoles) of the crude mixture of alcohols 64 from above in 3.5 ml of dry DME, 0.86 ml of dry HMPA, and 0.87 ml (4.34 mmoles) of the chlorophosphoridiamidate reagent,[34] and a trace of 1,10 phenanthroline indicator under argon was

added a 2.32M hexane solution of n-butyllithium dropwise until the dark red color persisted for 5 min (0.12 ml total, 0.28 mmoles). After 15 min the color faded to a light yellow. After a further 15 min the reaction was quenched by addition of a few drops of water, and the mixture was diluted with 50 ml of ether. Isolation of the product by ether extraction[36] including a 2% aqueous sodium hydroxide wash afforded 22 mg of the mixture of TMPDA derivatives 65 as a yellow semi-solid. The infrared spectrum of this crude mixture showed no alcohol absorption at 3600 cm^{-1}. TLC analysis (5% methanol-ether) showed one broad spot at R_f 0.38. Silica gel chromatography of the crude mixture from a similar run starting with chromatographed alcohols 64 afforded a 76% yield of the mixture of diastereomers as a white solid. Normally, however, the crude product was used without further purification.



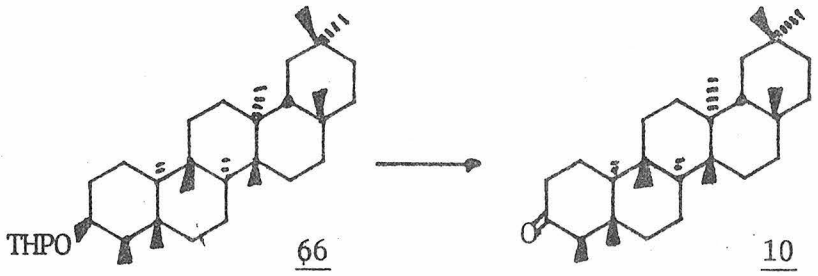
3β-(tetrahydropyran-2'-yloxy)friedelane (66).

To the deep blue solution resulting from addition of 12 mm (5.8 mmoles) of lithium wire to 30 ml of dry ethylamine, then vigorous stirring for 1 hour under argon, was added a solution of the crude mixture of esters from above in 3 ml of dry DME and 0.536 ml (5.78 mmoles) of dry tert-butanol. After 2 min the solution decolorized and an additional 12 mm (5.8 mmoles) of lithium wire was added. The resulting deep blue solution was stirred for an additional 30 min before the

excess lithium was destroyed by the addition of a few ml of concentrated aqueous ammonium chloride solution. The ethylamine was evaporated under a stream of argon, and the resulting residue was diluted with 50 ml of water. The crude THP ether 66 resulting from isolation by ether extraction[36] was purified by silica gel chromatography (5% ether-petroleum ether) to give 10.8 mg (73%) of a colorless solid of mp 235-238° (vacuum) which was homogeneous by TLC (same solvent, R_f 0.28). The same reaction carried out on chromatographed phosphorodiamidates 65 gave a 90% yield of THP ether 66 of mp 237.5-239.5° (vacuum). The overall yield from the ketone 63 without purification of the intermediates 64 and 65 is 69% and with purification is 64%. A portion of the above THP ether was recrystallized from methanol-dichloromethane to give analytically pure material: mp 243.5-245.5° (vacuum); ir ($CHCl_3$) 2920 cm^{-1} (broad, RH); NMR ($CDCl_3$) δ 0.86, 1.16 (2s, 3 each, angular CH_3), 0.94 (s,6,angular CH_3), 0.99 (s,9,angular CH_3), 3.48, 3.68 (2s, 1/2 each, C-3 2H), 3.52 (s,1,C-6'H), 3.95 (m,1,C-6'H), 4.57, 4.70 (2s, 1/2 each, C-2'H).

Anal. Calcd for $C_{35}H_6O_2$: C, 81.97; H, 11.79. Found: C, 81.96; H, 11.73.

Mass measured M^+: Calcd for $C_{35}H_{60}O_2$; 512.45930. Found: 512.4593 ± 0.0005.



(±)-Friedelin (10).

To a stirred solution of 10.8 mg (0.021 mmoles) of the tetrahydropyranyloxy friedelane 66 in 6 ml of dry THF and 3 ml of methanol under argon was added 2 mg of p-toluenesulfonic acid enonohydrate. After 20 hours at ambient temperature the reaction mixture was poured into 50 ml of 1/2 saturated sodium bicarbonate solution and the product isolated by chloroform extraction.[36] The crude alcohol 67 which resulted was oxidized directly without further purification.

To a stirred solution of the crude alcohol 67 in 5 ml of dry dichloromethane under argon was added 1 ml of Collins reagent. (This reagent was prepared by dropwise addition of 0.48 ml of dry pyridine to a stirred suspension of 300 mg of anhydrous chromium trioxide in 15 ml of dry dichloromethane under argon. The supernatant liquid was used after 20 min.) After 10 min at room temperature the brown suspension was filtered through 5 gms of alumina with suction, and the filter was washed with 50 ml of ether. Removal of the solvents at reduced pressure afforded 10.6 mg of a yellow solid. Silicagel chromatography of this material (10% E/PE) gave 9 mg (100%) of a slightly yellow solid of mp 236-240° (vacuum). Recrystallization of this material from methanol-dichloromethane afforded 7.1 mg (79%) of analytically pure (±)-friedelin (10) as fluffy tiny white rods: mp 246.5-248° (vacuum); ir ($CHCl_3$)

1700 (C=O); NMR ($CDCl_3$) δ 0.72, 0.89, 0.95, 1.04, 1.17 (5s,3 each, angular CH_3), 1.00 (s,6,angular CH_3), 0.90 (d,3,J=6Hz, C-4 CH_3).

Anal. Calcd for $C_{30}H_{50}O$: C, 84.44; H, 11.81. Found: C, 84.48; H, 11.77.

Mass measured M^+: Calcd for $C_{30}H_{50}O$; 426.386147. Found: 426.3860 ± 0.0005.

APPENDIX

[Reprinted from the Journal of Organic Chemistry, 40, 990 (1975).]

Experiments Directed toward the Total Synthesis of Terpenes. XX. Total Synthesis of (±)-Shionone, a Tetracyclic Triterpene[1]

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Received October 18, 1974

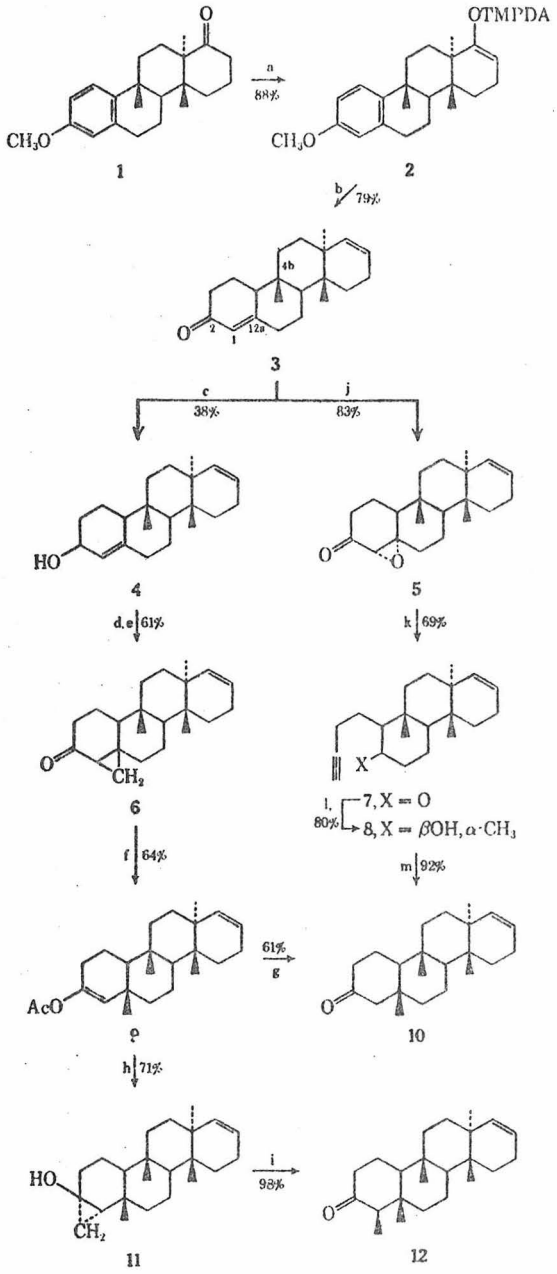
The conversion of the tetracyclic ketone 1 to the triterpene shionone (23) is explored by two alternative sequences. Both approaches rely on the introduction of the more or less completely formed side chain and then modification of the aromatic A ring. One unsuccessful approach entails incorporation of the intact side chain and then cleavage and recyclization of the enone 18. Acid-catalyzed recyclization of the A ring results in hydration of the side-chain double bond. This problem was overcome and the synthesis of (±)-shionone achieved through postponement of the introduction of the side-chain unsaturation until the A ring sequence was complete.

In the preceding paper[5] in this series the development of a practical and efficient synthesis of the tetracyclic ketone 1 is described. This material, as well as some of the intermediates used in its synthesis, were envisaged as key intermediates for synthesis of both penta- and tetracyclic triterpenes. In this report the successful conversion of the ketone 1 to the tetracyclic triterpene shionone (23)[6] is described.[7] For this synthesis it was necessary to devise two mutually compatible schemes for the remaining operations, namely, the introduction of the side chain in ring D and the modification of the aromatic A ring to that of the natural product. The investigation of the latter problem was undertaken first (Chart I).

A convenient system—the enone 3—with which to explore means for the A ring conversion was obtained by first transformation of the tetracyclic ketone 1 to the enol phosphorodiamidate (TMPDA) 2[8] and then Birch reduction to remove the TMPDA as well as reduce the aromatic ring. This two-stage transformation afforded the enone 3 in 70% overall yield; during the course of optimizing this yield, it was observed that if a proton source, such as alcohol, was omitted from the Birch reduction step, the TMPDA grouping was still reductively removed in high yield, but the aromatic ring remained intact. Of course, the corresponding aromatic olefin could be subsequently reduced to the enone 3 under standard Birch reduction conditions, and this two-step reduction sequence primarily serves to demonstrate the functional selectivity possible during the reductive removal[8] of the TMPDA grouping.

The α,β-unsaturated ketone system of the enone 3 offers an ideal substrate for the regioselective introduction of the two remaining methyl groups at C-12a and C-1 through conjugate addition and then α-methylation. The stereochemical situation is, however, somewhat less satisfactory.

Chart I
Ring A Modification of Tetracyclic Ketone 1[a]



[a] a, $LiN[CH(CH_3)_2]_2$, THF; $ClPO[N(CH_3)_2]_2$; b, Li, NH_3, THF, t-BuOH; 5 *N* HCl, EtOH; c, LiR_3BH, THF; d, Zn–Cu, CH_2I_2, Et_2O; e, $CrO_3 \cdot 2Py$, CH_2Cl_2; f, Li, NH_3, THF; Ac_2O; g, KOH, EtOH; h, CH_3Li, DME; Zn–Cu, CH_2I_2, Et_2O; i, HCl, H_2O, EtOH; j, H_2O_2, aq NaOH, CH_3OH; k, *p*-$TsNHNH_2$, HOAc, CH_2Cl_2; l, CH_3Li, Et_2O; m, CF_3CO_2H–$(CF_3CO)_2O$, CH_3COCH_3, CH_3OH, aq HCl.

The C-4bβ angular methyl group severely shields the C-12a carbon from attack by a reagent from the desired β face of the molecule. Thus, while conjugate addition of a methyl group per se $[LiCu(CH_3)_2]$ to this enone system would be expected to lead to a cis-fused product, even a reagent known to produce trans-fused rings systems in other molecules[9] ($AlEt_3$–HCN[10]) gave no reaction or predominantly a low yield of cis-fused product here.[11] To overcome this stereochemical situation a method was sought that relied on the *intra*molecular orientation of carbon–carbon bond formation at C-12a, and two such schemes were investigated.

One method relies on the orientation[12] of the Simmons–Smith methylenation reaction[13] by the alcohol function in an allylic alcohol system, and here requires the generation of a C-2β (axial) hydroxyl group. The formation of the desired allylic alcohol 4 proved itself to be a thorny problem, for standard hydride reductions ($LiAlH_4$, $NaBH_4$) produced little, if any, of the β (axial) alcohol. The only satisfactory method for reduction of this enone system was through the use of lithium perhydro-9b-boraphenalylhydride recently developed by Brown and Dickason[14] and utilized effectively in an earlier stage[5] in the synthesis. Unfortunately, probably owing to the flat, unhindered character of the enone system, the yield of the desired β (axial) alcohol 4 was not as high as the yields experienced elsewhere when saturated ketones were reduced.[14] It was possible, however, after a rather tedious and inefficient chromatographic sequence, to realize a fair yield of the desired allylic alcohol 4 and pursue the sequence further, as shown in Chart I.

These remaining stages resulted in quite satisfactory yields of the respective intermediate products. A useful consequence, of course, of the methylenation process for the formation of the C-12a bond in the β (axial) orientation is that lithium–ammonia reduction[15] of the cyclopropyl ketone 6 generates the enolate anion necessary for the introduction of the C-1 methyl group by methylation. In spite of the fact that this methylation would be expected to take place through the unhindered, α (axial) approach to the tetracyclic enolate, direct methylation[16] of the enolate generated during reduction of the cyclopropyl ketone 6 or methylation[17] of the enolate regenerated in dimethoxyethane from the intermediate enol acetate 9 were singularly unsuccessful. The desired monomethylated ketone in low yield was always accompanied by unmethylated material in much higher yield. While the reasons for this behavior are unclear, a convenient solution to the problem was found in the Simmons–Smith methylenation[13] of this same enolate—a procedure suggested by the work of Whitlock and Overman.[18] While it was possible to achieve the desired end result by removal of the ammonia and then addition of the Simmons–Smith reagent directly to the enolate formed from reduction of the cyclopropyl ketone 6, a cleaner product was obtained in more reproducible yields if this enolate was regenerated in dimethoxyethane with methyllithium from the initially trapped enol acetate 9. Contrary to the results reported by Whitlock and Overman,[18] there is no question but that the expected cyclopropyl alcohol is the primary product of this process. By rapid and careful chromatography of the crude product, it is possible to remove all the iodide–iodine formed and isolate the cyclopropyl alcohol 11 in good yield. This material is quite labile to traces of iodide ion in hydroxylic solvents and is rapidly cleaved to the corresponding methylated ketone. This lability and the failure to remove these by-products probably accounts for the fact that Whitlock and Overman[18] did not observe the formation of a cyclopropyl alcohol in their investigations. For preparative purposes a more convenient means of cyclopropyl alcohol cleavage is the use of mineral acid, which not only provides for the cleavage but also isomerizes the initially α (axial) methyl group.

The overall yield of the ketone 12 from the enone 3 by this sequence is only 10.3%, and the route suffers primarily

from the only fair yield of the allylic alcohol 4 and the tedious procedure necessary to achieve even that result. As a consequence of this experience another sequence was investigated in which the stereochemical outcome of the formation of a carbon–carbon bond at C-12a is controlled *in a desirable fashion* by the C-4bβ methyl group. For this result to pertain it is necessary to plan for the formation of the C-1–C-12a ring bond which is α (equatorial) to the B ring. Such a plan implies the prior introduction of the potential C-12a methyl group, as well as the cleavage and reformation of the C-1–C-12a bond which already exists in the enone 3. A sequence which involved just such a process is outlined in Chart I, and in spite of what at first sight seems inefficiency owing to the necessity of ring cleavage, this route is significantly more efficient than that just described.

Utilization of the sequence developed by Eschenmoser and coworkers[19] provided an excellent means for cleavage of the A ring of the enone 3 without the loss of any carbon atoms. Owing to the diversity of the functionality that results from the Eschenmoser cleavage, it was now possible to incorporate the potential C-12a angular methyl group through the direct addition of methyllithium to the acetylenic ketone 7 without the necessity of incorporating blocking groups in the sequence. With the acetylenic alcohol 8 in hand the stage was set for the re-formation of the C-1–C-12a ring bond through cyclization. The pioneering work of Peterson[20] and the extensive work of Johnson and Lansbury and their coworkers[21] provided the basis for the selection of the reaction conditions. Confidence that the stereochemistry of the molecule that would result from this cyclization would be that with the C-12a methyl group in the desired β (axial) orientation stemmed from the extensive previous experience[22] in these laboratories that demonstrated the stereochemical control provided by the axial C-4bβ methyl group during similar cationic ring closures. It was nevertheless gratifying to find that cyclization of the acetylenic alcohol 8 in trifluoroacetic acid led to an enol trifluoroacetate in excellent yield and that hydrolysis of this intermediate provided the same saturated ketone 10 that was obtained on saponification of the corresponding enol acetate 9 from the previously described route. The convergence of these two routes at this point serves to confirm the β (axial) orientation of the C-12a methyl group, for the β (quasi-axial) assignment of the configuration of the C-2 hydroxyl group in the allylic alcohol 4—and hence the orientation of the Simmons–Smith methylenation reaction—rests on firm ground. In view of the ease with which the enol trifluoroacetate could be isolated from this cyclization and the already proven utility of the enol acetate 9 for the incorporation of the remaining methyl group at C-1, the present route seemed well suited to the construction of the shionone A ring, and attention was turned to the introduction of the side chain in ring D.

Since the general plan for the total synthesis of shionone (23) entailed the incorporation of the ring D side chain and then modification of the aromatic A ring by the process discussed above, the tetracyclic ketone 1 again became the starting point. While the ketone functionality in the D ring of this material would obviously serve to introduce the two required alkyl groups in the adjacent α position, the efficiency and stereochemical outcome of these alkylation reactions were circumspect. In addition such a plan incorporates the potential difficulties that would be associated with the ultimate necessary removal of what would then be a very hindered ketone function. In order to circumvent these anticipated chemical problems, as well as have a sound basis for the stereochemical results, means were sought to remove the existing ring D ketone and at the same time introduce activating functionality external to the ring system. This plan was effectively accomplished (Chart II) by a two-step process that led from the tetracyclic ketone 1 through the chloro aldehyde 13 from the Vilsmeier reaction[23] and then by lithium–ammonia reduction–methylation[16] to the aldehyde 14. The yields in this process were quite satisfactory, and the efficiency of the structural changes that attend the reduction–methylation step is noteworthy. The stereochemical outcome of the methylation of the enolate from the lithium–ammonia reduction of the chloro aldehyde 13 is well precedented[24] in similar systems, but the preparative use of α,β-unsaturated aldehydes in such reductions to generate useful aldehyde enolates appears[25] to be novel. As might be expected, the conditions for the reduction stage had to be carefully controlled (see Experimental Section) in order to prevent dimerization and overreduction.

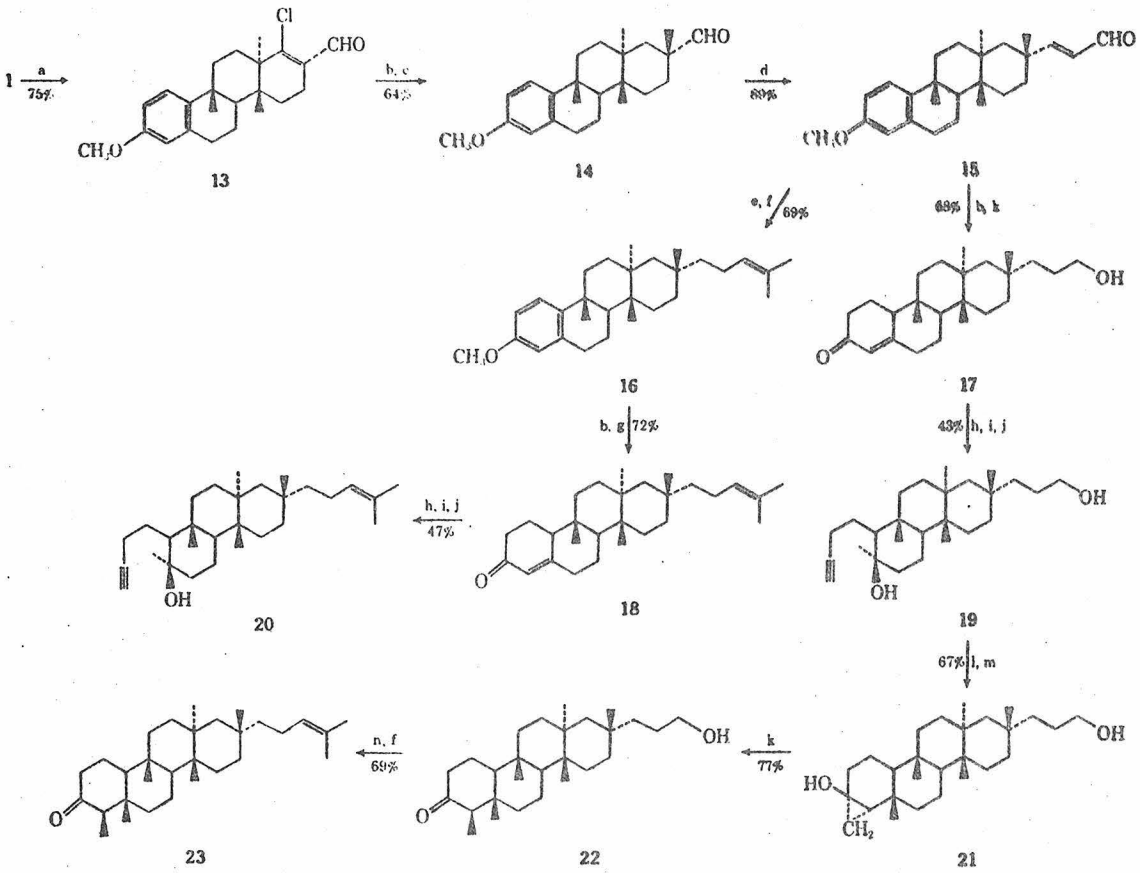
With an eye toward the rapid completion of the side chain, the aldehyde 14 was quantitatively reduced to the corresponding primary alcohol with lithium aluminum hydride and efforts were made to convert this alcohol to the iodide preparative to a coupling reaction[26] with π-(1,1-dimethylallyl)–nickel bromide complex. Unfortunately, both the neopentyl character and the severe steric congestion about this axial hydroxymethyl grouping thwarted all attempts to prepare the iodide or other halides. Reactions of the alcohol with triphenyl phosphite–methyl iodide,[27] triphenylphosphine, carbon tetrabromide, and carbon tetrachloride,[28] and thionyl chloride–quinoline led either to recovered alcohol after no reaction or a plethora of products that resulted from the intervention of cationic species that led to backbone rearrangements.

To overcome these difficulties an alternate scheme was developed for the addition of the remainder of the side chain through the use of two successive Wittig-type condensations. The aldehyde 14 was first converted to the unsaturated aldehyde 15 by the efficient formylolefination procedure of Nagata and Hayase,[29] and after reduction[30] of the unsaturated aldehyde 15 with triethylsilane in the presence of tris(triphenylphosphine)rhodium chloride, the process was completed in 30% overall yield from the tetracyclic ketone 1 by the condensation of the saturated aldehyde with isopropylidenephosphorane.

With the aromatic olefin 16 thus in hand, modification of the A ring by the method developed above was projected to complete the synthesis. Indeed this process proceeded well (Chart II) up to the stage of final reformation of the A ring from the acetylenic alcohol 20. This approach irreversibly broke down at this point, for the acidic conditions necessary to effect the cyclization invariably resulted in acid-catalyzed hydration of the side-chain trisubstituted double bond. When modifications were made in the reaction conditions in order to avoid this addition reaction by reducing the acidity, lowering the temperature, and/or changing the acid catalyst, it was found that the sequence of events involved initial rapid addition to the side-chain double bond. In experiments where the conditions were vigorous enough, cyclization of the acetylenic alcohol was a subsequent step. Indeed, it was possible to hydrate the side-chain double bond without affecting the acetylenic alcohol system. The lability of this side-chain unsaturation was a surprise, particularly when the model system used to explore this sequence—the acetylenic alcohol 8—was specifically chosen with this side chain in mind and itself contains an isolated (albeit disubstituted) double bond.

The solution to this last problem dictated a change in methodology for either the A ring modifications or the

Chart II
Conversion of Tetracyclic Ketone 1 to (±)-Shionone (23)[a]



[a] a, $POCl_3$, DMF; b, Li, NH_3, THF, *t*-BuOH; c, $NaO_2CC_6H_5$, CH_3I; d, NaH, $(EtO)_2POCH_2CH{=}NC_6H_{11}$, THF, aq $(CO_2H)_2$, C_6H_6; e, Et_3SiH, $[(C_6H_5)_3P]_3RhCl$, C_6H_6, CH_3COCH_3, aq HCl; f, $(C_6H_5)_3PCH(CH_3)_2{}^+I^-$, C_6H_5Li, THF; g, $(CO_2H)_2$, aq EtOH, NaOH, aq EtOH; h, H_2O_2, aq NaOH, CH_3OH-CH_2Cl_2; i, *p*-$TsNHNH_2$, HOAc-CH_2Cl_2; j, CH_3Li, THF; k, aq HCl, EtOH; l, CF_3CO_2H-$(CF_3CO)_2O$; m, $LiN[CH(CH_3)_2]$, THF, Zn-Ag, CH_2I_2, Et_2O; n, $CrO_3 \cdot 2Py$, CH_2Cl_2.

side-chain construction. Rather than tamper with the more intricate procedures in the former process, a reshuffling of the steps in the side-chain construction seemed advisable. Since the offending functionality in the side chain was the double bond that resulted from the last stage in the process, this reaction was deferred until the completion of the A ring. Thus, complete reduction of the unsaturated aldehyde 15 led in good yield to the hydroxyenone 17, which could be carried through the A ring synthesis without major incident (Chart II). The result of the trifluoroacetic acid catalyzed cyclization of the acetylenic alcohol 19 was the expected bis trifluoroacetate, but this posed no significant experimental problem in the subsequent stages that completed the shionone (23) synthesis.

A noteworthy point did come to light when the bis trifluoroacetate was used to generate the enolate in ring A. Under the conditions used earlier for the generation and methylenation of the enolate from the enol acetate 9, none of the expected cyclopropyl alcohol was observed, and the product was the C-1 demethyl keto alcohol. Model studies showed that this was not the result of hydrolysis or protonation of the enolate by traces of moisture, nor was it the result of the failure of the methylenation reaction. Reasoning that the enolate generated by methyllithium addition[17] was being rapidly protonated by the initially formed 1,1,1-trifluoroacetone, an aminolysis reaction was substituted for the Grignard reaction with salutory results. The enol trifluoroacetate was readily cleaved by lithium diisopropylamide, and the resulting enolate behaved as expected in the methylenation reaction. This observation should render enol trifluoroacetates generally useful for the formation of ketone enolates, and coupled with the addition of trifluoroacetic acid to acetylenes, the overall process is an interesting ketone synthesis.

Experimental Section[31]

8-Methoxy-4aβ,10bβ,12aα-trimethyl-3,4,4a,4bα,5,6,10b,-11,12,12a-decahydrochrysen-1-yl Tetramethylphosphorodiamidate (2). To a solution of lithium diisopropylamide prepared from 8 ml (57 mmol) of diisopropylamine and 13 ml of a 2.84 *M* hexane solution of *n*-butyllithium in 150 ml of dry ether under an argon atmosphere was added over a 10-min period a solution of 2.27 g (7 mmol) of the ketone 1 in 20 ml of dry tetrahydrofuran and 8 ml of *N,N,N',N'*-tetramethylethylenediamine. The mixture was then cooled in an ice bath, and 15 ml (81 mmol) of tetramethyldiamidophosphorochloridate[32] was added dropwise. After the resulting yellow solution was allowed to warm to room temperature and then stirred for 1.5 hr, the mixture was poured into ice and 400 ml of 10% aqueous hydrochloric acid, and the product was isolated by ether extraction[33] including a base wash. On chromatography of

the crude product on 200 g of silica gel, 2.81 g (88%) of the phosphorodiamidate **2**, mp 108–111° (vacuum), was eluted with 1800 ml of 10% acetone–ethyl acetate after an initial wash with 800 ml of ethyl acetate and then 600 ml of 5% acetone–ethyl acetate. The analytical sample, obtained after cystallization of a portion of this material from ether–heptane, also melted at 108–111° (vacuum): ir ($CHCl_3$) 1670 (C=C), 1605, 1500 (Ar), 1305 (P–N), and 980 cm^{-1} (P–O–C); NMR ($CDCl_3$) δ 1.02 (s, 3, C-4a CH_3), 1.22 (s, 2 × 3, C-10b and C-12a CH_3), 2.70 (d, 12, J = 10 Hz, NCH_3), 3.75 (s, 3, OCH_3), and 5.20 (m, 1, C=CH).

Anal. Calcd for $C_{26}H_{41}O_3N_2P$: C, 67.80; H, 8.97; N, 6.08; P, 6.73. Found: C, 67.96; H, 8.86; N, 6.16; P, 6.64.

8-Methoxy-4aβ,10bβ,12aα-trimethyl-3,4,4a,4bα,5,6,10b,-11,12,12a-decahydrochrysene. To a solution of 37 mg (5.3 mg-atoms) of lithium in 50 ml of dry ammonia and 10 ml of dry tetrahydrofuran under an argon atmosphere was added a solution of 210 mg (0.45 mmol) of the phosphorodiamidate **2** in 6 ml of dry tetrahydrofuran. After 1.5 hr the blue color faded, and an additional 37 mg (5.3 mg-atoms) of lithium was added. After stirring for 3.5 hr longer, the reaction mixture was treated with 400 mg of sodium benzoate and then 200 mg of solid ammonium chloride. After the ammonia was evaporated in a stream of argon, the residue was dissolved in 50 ml of water, and the product was isolated by ether extraction[33] including an acid and base wash. On preparative TLC (30% ether–petroleum ether) of the crude product there was obtained 115 mg (82%) of the tetracyclic olefin (R_f 0.7) as a colorless oil. The analytical sample was obtained after further preparative TLC (30% ether–petroleum ether) and then evaporative distillation (120°, 0.01 mm) of a portion of this material: ir ($CHCl_3$) 1605 and 1500 cm^{-1} (Ar); NMR ($CDCl_3$) δ 0.82 (s, 3, C-4a CH_3), 1.00 (s, 3, C-12a CH_3), 1.22 (s, 3, C-10b CH_3), 3.77 (s, 3, $ArOCH_3$), 5.52 (m, 2, CH=CH), and 6.50–7.17 (m, 3, ArH).

Anal. Calcd for $C_{22}H_{30}O$: C, 85.11; H, 9.74. Found: C, 84.96; H, 9.64.

4bβ,6aα,10aβ-Trimethyl-4,4aα,4b,5,6,6a,9,10,10a,10bα,11,12-dodecahydro-2(3*H*)-chrysenone (3). A. From the Phosphorodiamidate 2. A solution of 370 mg (53 mmol) of lithium wire in 550 ml of dry ammonia and 140 ml of dry tetrahydrofuran was stirred for 30 min, and then a solution of 1.53 g (3.32 mmol) of the phosphorodiamidate **2** in 30 ml of dry tetrahydrofuran was injected all at once with a syringe. After 5 hr an additional 960 mg (139 mmol) of lithium and 85 ml of dry *tert*-butyl alcohol were added. After the reaction had stirred for an additional 2 hr, the excess lithium was decomposed with 20 ml of methanol, and the ammonia was allowed to evaporate overnight. The gray residue was treated with 500 ml of water, and the product was isolated by ether extraction.[33] A solution of the resulting residue in 200 ml of ethanol and 130 ml of 5 *N* aqueous hydrochloric acid was heated at 65–70° for 40 min in an argon atmosphere. The cooled reaction mixture was then poured into 500 ml of water, and the product was isolated by ether extraction,[33] including a base wash. On chromatography of the dark yellow, oily residue on 100 g of silica gel there was obtained 782 mg (79%) of the enone **3**, mp 88–92°, by elution with 600 ml of 50% ether–petroleum ether. Crystallization (ethanol–water) and then sublimation (120°, 0.01 mm) of a portion of this material gave the analytical sample: mp 94–97°; ir ($CHCl_3$) 1665 (C=O) and 1620 cm^{-1} (C=C); NMR ($CDCl_3$), δ 0.87 (s, 2 × 3, C-4b and C-10a CH_3), 1.07 (s, 3, C-6a CH_3), 5.47 (m, 2, CH=CH), and 5.92 (s, 1, O=C–CH=C).

Anal. Calcd for $C_{21}H_{30}O$: C, 84.54; H, 10.13. Found: C, 84.51; H, 10.22.

B. From Tetracyclic Olefin. A stirred solution of 182 mg (0.58 mmol) of the above olefin in 60 ml of dry ammonia, 20 ml of dry tetrahydrofuran, and 10 ml of dry *tert*-butyl alcohol under an argon atmosphere was treated with 111 mg (16 mg-atoms) of lithium. After 2 hr the excess lithium was decomposed with 3 ml of methanol, and the ammonia was evaporated in a stream of argon. The gray residue was dissolved in 150 ml of water, and the product was isolated by ether extraction.[33] A solution of the crude product in 30 ml of ethanol and 20 ml of 5 *N* aqueous hydrochloric acid was heated at 65–70° for 40 min under an argon atmosphere, and after dilution of the cooled reaction mixture with 100 ml of water, the product was isolated by ether extraction,[33] including a base wash. The crude product was chromatographed on 22 g of silica gel, and elution with 175 ml of 50% ether–petroleum ether afforded 153 mg (78%) of the enone **3**, mp 91–94°, that was identical (mixture melting point, ir, NMR) with the material prepared above in part A.

2β-Hydroxy-4bβ,6aα,10aβ-trimethyl-2α,3,4,4aα,4b,5,6,6a,9,-10,10a,10bα,11,12-tetradecahydrochrysene (4). Following the general procedure of Brown and Dickason,[14] an ice-cold solution of 781 mg (2.62 mmol) of the enone **3** in 15 ml of dry tetrahydrofuran under an argon atmosphere was treated with 6.0 ml (5.1 mmol) of a 0.85 *M* tetrahydrofuran solution of the trialkylborohydride. After 30 min the organoborane was decomposed by the sequential addition of 1.0 ml of 3 *N* aqueous sodium hydroxide solution and 2.0 ml of 30% hydrogen peroxide. The reaction mixture was immediately poured into 50 ml of saturated aqueous sodium carbonate solution and the product isolated by ether–benzene (4:1) extraction. The crude product was chromatographed on 100 g of Florisil which was eluted with 59% ether–petroleum ether. The first 300 ml eluted 75 mg of a mixture of nonpolar products that was discarded. The next 200 ml afforded 183 mg (23%) of the axial alcohol **4**, mp 130–133°. Further elution with 400 ml of the same solvent gave 316 mg of a mixture of the two alcohols which on further separation by preparative TLC (10% ether–chloroform) gave 188 mg (24%) of the equatorial alcohol (R_f 0.4) and 114 mg (15%) of the axial alcohol **4** (R_f 0.5). Finally, washing the column with 500 ml of ether gave 206 mg (26%) of the equatorial alcohol, mp 119–120° (vacuum). The total yield of axial alcohol **4** was 296 mg (38%) and that of the equatorial alcohol was 394 mg (50%).

The analytical sample of the axial alcohol **4**, prepared by crystallization of a portion of similar material from another reduction experiment from ethyl acetate–heptane and then ethanol–water, melted at 131–134° (vacuum): ir 3600, 3450 (OH), and 1650 cm^{-1} (C=C); NMR ($CDCl_3$) δ 0.85 (s, 2 × 3, C-4b and C-10a CH_3), and 1.05 (s, 3, C-6a CH_3), 4.00–4.20 (m, 1, CHO), 5.48 (m, 2, CH=CH), and 5.50–5.77 (m, 1, OCCH=C).

Anal. Calcd for $C_{21}H_{32}O$: C, 83.94; H, 10.73. Found: C, 83.87; H, 10.78.

The analytical sample of the equatorial alcohol was prepared in the same fashion and melted at 114–116° (vacuum): ir ($CHCl_3$) 3605, 3450 (OH), and 1655 cm^{-1} (C=C); NMR ($CDCl_3$) δ 0.77, 0.82, and 1.05 (s, 3 each, C-4b, C-6a, and C-10a CH_3), 3.95–4.30 (m, 1, CHO), and 5.37–5.53 (m, 3, CH=C).

Anal. Calcd for $C_{21}H_{32}O$: C, 83.94; H, 10.73. Found: C, 83.93; H, 10.87.

1β,12aβ-Methano-4bβ,6aα,10aβ-trimethyl-1α,4,4aα,4b,5,6,-6a,9,10,10a,10bα,11,12,12a-tetradecahydro-2(3*H*)-chrysenone (6). To a suspension of 4.0 g (57 mmol) of zinc–copper couple[34] in 4.6 ml (57 mmol) of diiodomethane and 60 ml of dry ether was added a solution of 638 mg (2.21 mmol) of the axial alcohol **4** in 10 ml of dry ether, and the resulting mixture was heated at reflux under an argon atmosphere for 4 hr. After cooling, the reaction mixture was poured into 100 ml of saturated aqueous sodium carbonate, and the product was isolated by ether–benzene (4:1) extraction.[33] On chromatography of the product on 250 g of grade III alumina, elution with 600 ml of 3% methanol–ether gave 512 mg (80%) of the corresponding cyclopropyl alcohol, mp 135–139° (vacuum): ir ($CHCl_3$) 3600, 3450 cm^{-1} (OH); NMR ($CDCl_3$) δ 0.85, 0.95, 1.05 (3 s, 3 each, C-4b, C-6a, and C-10a CH_3), 4.07–4.43 (m, 1, CHO), and 5.47 (s, 2-CH=CH–).

After the procedure of Radcliffe and Rodehorst,[35] a solution of 512 mg (1.69 mmol) of the above cyclopropyl alcohol in 8 ml of dry dichloromethane was added under an argon atmosphere to a solution of 1.62 ml (20 mmol) of dry pyridine and 1.00 g (10 mmol) of anhydrous chromium trioxide in 50 ml of dry dichloromethane, and the red solution was stirred for 10 min. The dark mixture was then filtered through a pad of grade III alumina with the aid of 200 ml of ether. Evaporation of the solvents from the filtrate at reduced pressure afforded 490 mg (77%, 61% overall) of the ketone **6**, mp 149–152° (vacuum). The analytical sample, prepared from a portion of this material by preparative TLC (50% ether–petroleum ether) and then crystallization from ether–hexane, melted at 150–153° (vacuum): ir ($CHCl_3$) 1670 cm^{-1} (C=O); NMR ($CDCl_3$) δ 0.90, 1.02, 107 (3 s, 3 each, C-4b, C-6a, and C-10a CH_3), and 5.47 (s, 2, CH=CH).

Anal. Calcd for $C_{22}H_{32}O$: C, 84.56; H, 10.32. Found: C, 84.49; H, 10.39.

2-Acetoxy-4bβ,6aα,10aβ,12aβ-tetramethyl-3,4,4aα,4b,5,6,6a,-9,10,10a,10bα,11,12,12a-tetradecahydrochrysene (9). To an argon-protected solution of 18 mg (2.6 mg-atoms) of lithium in 60 ml of dry ammonia and 20 ml of dry tetrahydrofuran was added a solution of 203 mg (0.65 mmol) of the cyclopropyl ketone **6** in 5 ml of dry tetrahydrofuran. After stirring for 1.5 hr, the blue color faded; an additional 18 mg (2.6 mg-atoms) of lithium was then added, and the mixture was stirred for 2.5 hr. Most of the ammonia was then removed by evaporation in a stream of argon through a mercury bubbler, and the resulting gray suspension was treated

with 5 ml (53 mmol) of dry acetic anhydride at room temperature. After stirring for 6 hr, the reaction mixture was poured into a mixture of ice and 70 ml of 10% aqueous potassium hydroxide solution, and the product was isolated by ether–benzene (1:1) extraction.[33] On chromatography of the crude product (285 mg) on 30 g of silica gel, elution with 150 ml of 20% ether–petroleum ether gave 146 mg (64%) of the enol acetate 9, mp 119–121° (vacuum). The analytical sample, prepared from a portion of this material by preparative TLC (20% ether–petroleum ether) and crystallization from ether–hexane, melted at 121–123° (vacuum): ir ($CHCl_3$) 1755 (C=O), 1690 (C=C), and 1220 cm^{-1} (C–O–C); NMR (CCl_4) δ 0.78, 0.88 (2 s, 3 each, C-4b, and C-10b CH_3), 1.02 (s, 2 × 3, C-6a and C-12a CH_3), 2.00 (s, 3, CH_3CO), 4.97 (s, 1, C-1 H), and 5.40 (s, 2, CH=CH).

Anal. Calcd for $C_{24}H_{36}O_2$: C, 80.85; H, 10.18. Found: C, 81.05; H, 10.23.

Further elution of the column with 25 ml of the same solvent mixture gave 37 mg of a mixture that consisted of approximately equal parts of the enol acetate 9 and the Δ^2-enol acetate of the starting ketone 6 (**2-acetoxy-1β,12aβ-methano-4bβ,6aα,10aβ-trimethyl-1,4,4aα,4b,5,6,6a,9,10,10a,10bα,11,12,12a-tetradecahydrochrysene**) on the basis of the comparative integration of the acetyl methyl signals at δ 2.00 and 2.03 in the NMR spectrum. An analytically pure sample of the latter Δ^2-enol acetate was obtained from another similar experiment after preparative TLC (20% ether–petroleum ether) and then crystallization of the material with R_f 0.4 from hexane and melted at 110–112° (vacuum): ir (CCl_4) 1755 (C=O), 1685 (C=C), and 1220 cm^{-1} (C–O–C); NMR (CCl_4) δ 0.85, 0.98, and 1.03 (3 s, 3 each, C-4b, C-6a, and C-10a CH_3), 2.03 (s, 3, CH_3CO), 4.80–5.05 (m, 1, C-3 H), and 5.40 (s, 2, CH=CH).

Anal. Calcd for $C_{24}H_{34}O_2$: C, 81.31; H, 9.67. Found: C, 81.46; H, 9.75.

Saponification of this Δ^2-enol acetate in aqueous, alcoholic potassium hydroxide solution afforded a 70% yield of the cyclopropyl ketone 6, mp 123–126°, alone or in admixture with authentic material of the same melting range.

Finally, continued elution of the column with 100 ml of the same solvent mixture afforded 10 mg (5%) of the ketone 10, mp 150–158°. Further purification of this material by preparative TLC (40% ether–petroleum ether) and then crystallization from hexane–dichloromethane gave material that melted at 172–176°, alone or in admixture with authentic ketone 10, mp 172–176°, prepared below by hydrolysis of the enol acetate 9.

4bβ,6aα,10aβ,12aβ-Tetramethyl-3,4,4aα,4b,5,6,6a,9,10,10a,-10bα,11,12,12a-tetradecahydro-2(1*H*)-chrysenone (10). A. From Enol Acetate 9. A solution of 90 mg (0.25 mmol) of the enol acetate 9 and 180 mg (2.7 mmol) of potassium hydroxide in 5 ml of ethanol was stirred at room temperature for 14 hr under argon atmosphere. The mixture was then diluted with water, and the product was isolated by ether–benzene (1:1) extraction.[33] Purification of the crude product by preparative TLC (40% ether–petroleum), then crystallization from hexane–dichloromethane, and finally sublimation at 160–170° and 0.025 mm gave 46 mg (61%) of analytically pure ketone 10: mp 172–176° (vacuum); ir ($CHCl_3$) 1705 cm^{-1} (C=O); NMR ($CDCl_3$) δ 0.82 (s, 3), 0.90 (s, 6), and 1.06 (s, 3) (C-4b, C-6a, C-10a, and C-12a CH_3), and 5.48 (s, 2, CH=CH).

Anal. Calcd for $C_{22}H_{34}O$: C, 84.02; H, 10.90. Found: C, 83.90; H, 10.99.

B. From Acetylenic Alcohol 8. A mixture of 2.0 ml of trifluoroacetic acid and 0.6 ml of trifluoroacetic anhydride was cooled in a −18° bath; 1.6 ml of this cold solution was then added to 16.0 mg (0.051 mmol) of the acetylenic alcohol 8 at −18°, and the mixture was stirred at this temperature for 20 min. The pressure in the system was then reduced with a vacuum pump, and the cooling bath was then removed to facilitate evaporation of the solvents. Most of the liquid was gone within 5 min, but the residual oil was dried at room temperature for 20 min at ca. 0.05 mm pressure. This oil was handled so as to avoid prolonged contact with moist air and appeared to be the desired enol trifluoroacetate: ir ($CHCl_3$) 1790 (CF_3CO), 1695 (–OC=C–), 1385 (CH_3), and 1220, 1170, 1140 cm^{-1} (C–O–C and CF_3); no remaining 3300 cm^{-1} (–C≡CH); NMR ($CDCl_3$) δ 0.81, 0.90, 1.04 (3 s, 3, 3, and 6, respectively, C-4a, C-6a, C-10a, and C-12a CH_3), 5.28 (m, 1, CF_3CO_2C=CH), and 5.44 (s, 2, HC=CH); analysis by GLC (250°) showed only one peak at retention time 1.2 min.

A solution of this crude enol trifluoroacetate in 1 ml of acetone and 1 ml of methanol was treated with 5 drops of water and 5 drops of 10% aqueous hydrochloric acid and then stirred at room temperature for 75 min. After neutralization of this solution with solid sodium bicarbonate, the product was isolated by ether extraction.[33] Purification of the crude product (17.5 mg) by preparative TLC (40% ether–petroleum ether) afforded 14.7 mg (92%) of the tetracyclic ketone 10 as a white solid, mp 166–172° (vacuum); the ir and NMR spectra of this material were identical with those of purified ketone 10 prepared in part A above. Crystallization of this solid from dichloromethane–hexane afforded white crystals, mp 170–174° (vacuum), alone or in admixture with material prepared above, mp 172–176° (vacuum), in part A.

2β-Hydroxy-1α,2α-methano-4bβ,6aα,10aβ,12aβ-tetramethyl-1β,2,3,4,4aα,4b,5,6,6a,9,10,10a,10bα,11,12,12a-hexadecahydrochrysene (11). A solution of 153 mg (0.43 mmol) of the enol acetate 9 in 5 ml of dry dimethoxyethane was added to an argon-protected solution of methyllithium (0.7 ml, 1.2 mmol), and the mixture was stirred at room temperature for 30 min. To this solution was added by syringe the supernatant solution from the preparation of the Simmons–Smith reagent from 1.20 g (17 mmol) of zinc–copper couple[34] and 1.40 ml (1.20 mmol) of diiodomethane in 17 ml of dry ether. After stirring in an ice bath for 1 hr, the reaction mixture was poured into 50 ml of saturated aqueous sodium carbonate solution, and the product was isolated by ether–benzene (1:1) extraction[33] including a base and 10% aqueous sodium thiosulfate solution wash. On chromatography of the crude material on 70 g of grade III alumina, elution with 200 ml of ether gave 100 mg (71%) of the cyclopropyl alcohol 11, mp 161–165° (vacuum). The analytical sample, obtained after crystallization of a portion of this material from dichloromethane–hexane, melted at 166–168° (vacuum): ir ($CHCl_3$) 3600, 3450 (OH), and 1180 cm^{-1} (C–O–C); NMR ($CDCl_3$) δ 0.80, 0.87, 1.02, and 1.15 (4 s, 3 each, C-4b, C-6a, C-10a, and C-12a CH_3), and 5.42 (s, 2, CH=CH).

Anal. Calcd for $C_{23}H_{36}O$: C, 84.09; H, 11.04. Found: C, 83.85; H, 11.13.

1β,4bβ,6aα,10aβ,12aβ-Pentamethyl-3,4,4aα,4b,5,6,6a,9,10,-10a,10bα,11,12,12a-tetradecahydro-2(1*H*)-chrysenone (12). A solution of 161 mg (0.49 mmol) of the cyclopropyl alcohol 11 and 1 ml of concentrated hydrochloric acid in 12 ml of ethanol was heated under reflux in an argon atmosphere for 1 hr. After cooling, the solution was diluted with 50 ml of water, and the product was isolated by ether extraction,[33] including a base wash. The resulting material amounted to 157 mg (98%) of the ketone 12, mp 169–176° (vacuum), from which the analytical sample, mp 178–182° (vacuum), was prepared by preparative TLC (40% ether–petroleum ether) and then sublimation at 150–155° and 0.7 mm: ir ($CHCl_3$) 1705 cm^{-1} (C=O); NMR ($CDCl_3$) δ 0.73 (s, 3), 0.83 (s, 4.5), 0.95, (s, 4.5), and 1.05 (s, 3) (C-1, C-4b, C-6a, C-10a, and C-12a CH_3), and 5.47 (s, 2, CH=CH).

Anal. Calcd for $C_{23}H_{36}O$: C, 84.09; H, 11.04. Found: C, 84.16; H, 11.16.

1α,12aα-Epoxy-4bβ,6aα,10aβ-trimethyl-3,4,4aα,4b,5,6,6a,9,-10,10a,10bα,11,12,12a-tetradecahydro-2(1*H*)-chrysenone (5). To a stirred solution of 125 mg (0.42 mmol) of the enone 3 in 10 ml of methanol at room temperature was added 1 ml (*ca.* 300 mg, 16 mmol) of 30% aqueous hydrogen peroxide solution and 0.5 ml of 10% aqueous sodium hydroxide solution, and the mixture was stirred at room temperature for 1 hr. The solution was then diluted with ether and water, and the product was isolated by ether extraction.[33] Purification of the resulting semicrystalline solid (126 mg) by preparative TLC (30% ether–petroleum ether) afforded 109 mg (83%) of the epoxy ketone 5 (R_f 0.45), mp 98–100° (vacuum). The analytical sample, mp 102.5–103.5° (vacuum), was obtained after two crystallizations of this material from methanol–dichloromethane: ir ($CHCl_3$) 1700 (C=O), 1450 (CH_2), and 1385 cm^{-1} (CH_3); NMR ($CDCl_3$) δ 0.82, 0.87, and 1.05 (3 s, 3 each, C-4b, C-6a, and C-10a CH_3), 3.16 (s, 1 C-1 H), and 5.49 (s, 2, CH=CH).

Anal. Calcd for $C_{21}H_{30}O_2$: C, 80.21; H, 9.62. Found: C, 80.44, H, 9.69.

1β-(3′-Butynyl)-4bβ,8aα,10aβ-trimethyl-3,4,4aα,4b,5,6,8a,9,-10,10a-decahydrophenanthren-2(1*H*)-one (7). A slight modification of the general procedure of Eschenmoser and coworkers[19] was employed. To a dry mixture of 77.0 mg (0.244 mmol) of the epoxy ketone 5 and 48.8 mg (0.261 mmol) of *p*-toluenesulfonylhydrazine at −20° was added with stirring and swirling 1.5 ml of −20° acetic acid–dichloromethane (1:1). After stirring for 5 min at −20°, the solution was stored at −20° for 15 hr. The mixture was then stirred at room temperature for an additional 4 hr (during which time it turned red) and then the product was isolated by ether extraction,[33] including a base wash. Purification of the crude product (81 mg) by preparative TLC (30% ether–petroleum ether)

afforded 50.5 mg (69%) of the acetylenic ketone 7 as a yellow oil (R_f 0.48) which was suitable for analysis: ir ($CHCl_3$) 3300 (–C≡CH), 2120 (–C≡C–), 1700 (C=O), and 1390 cm^{-1} (CH_3); NMR ($CDCl_3$) δ 0.77, 0.85, 1.13 (3 s, 3 each, C-4b, C-8a, and C-10a CH_3), and 5.49 (s, 2, HC=CH).

Anal. Calcd for $C_{21}H_{30}O$: C, 84.51; H, 10.13. Found: C, 84.25; H, 10.02.

1β-(3′-Butynyl)-2α,4bβ,8aα,10aβ-tetramethyl-1,2,3,4,4aα,4b,-5,6,8a,9,10,10a-dodecahydro-2β-phenanthrol (8). To a stirred and ice-cooled mixture of 0.36 ml (0.68 mmol) of 1.9 *M* ethereal methyllithium solution and 2.0 ml of dry ether was added over a 2-min period a solution of 19.0 mg (0.064 mmol) of the acetylenic ketone 7 in 1.2 ml of dry ether. After stirring for 10 min longer at 0°, and for 5 min without cooling, the reaction mixture was cautiously quenched with 0.5 ml of water and then the product was isolated by ether extraction.[33] Purification of the crude product (18.8 mg) by preparative TLC (50% ether–petroleum ether) afforded 16.0 mg (80%) of the alcohol 8 as a white solid, mp 84–88° (vacuum). The analytical sample, obtained after two crystallizations of a portion of this material from ether–hexane, melted at 91.0–92.5° (vacuum): ir ($CHCl_3$) 3600 (OH), 3300 (–C≡CH), 2115 (–C≡C–), and 1385, 1370 cm^{-1} (CH_3); NMR ($CDCl_3$) δ 0.81, 1.00, 1.03, 1.17 (4 s, 3 each, C-2, C-4b, C-8a, and C-10a CH_3), and 5.45 (s, 2, HC=CH).

Anal. Calcd for $C_{22}H_{34}O$: C, 84.02; H, 10.90. Found: C, 84.01; H, 11.04.

1-Chloro-2-formyl-8-methoxy-4aβ,10bβ,12aα-trimethyl-3,-4,4a,4bα,5,6,10b,11,12,12a-decahydrochrysene (13). Following a modification of the procedure of Moersch and Neuklis,[23] ice-cooled phosphoryl chloride (12 ml, 20.1 g, 0.131 mmol) was stirred and treated over a 1-min period with 13.6 ml (12.8 g, 0.176 mmol) of dimethylformamide. After stirring for 30 min without cooling, the viscous solution of reagent was added at room temperature to a stirred solution of 1.158 g (3.54 mmol) of the tetracyclic ketone 1 in 24 ml of dimethylformamide. The stirred reaction mixture was then heated with a preheated, 60° oil bath for 6 hr so that the internal temperature rose to a constant 55–56°. After cooling with an ice bath, the solution was poured onto 350 g of ice and 40 ml of 40% aqueous sodium hydroxide solution, and the product was isolated by dichloromethane extraction.[33] The crude residue (1.310 g) was chromatographed on 200 g of silica gel in a medium-pressure column with dichloromethane. After the first 400 ml of eluent was discarded, evaporation of the next 600 ml of eluent at reduced pressure provided 854 mg (65%) of the chloroaldehyde 13 as a white solid, mp 196–198° (vacuum). The analytical sample, obtained after crystallization of a portion of this material from acetone–dichloromethane–water, melted at 198.5–199° (vacuum): ir ($CHCl_3$) 2750 (–CHO), 1665 (C=C–CHO) 1605, 1575, 1500 (ArH), 1385 (CH_3), and 1150, 1040 cm^{-1} ($ArOCH_3$); NMR ($CDCl_3$) δ 0.92, 1.21 (2 s, 3 and 6, C-4a, C-10b, and C-12a CH_3), 3.77 (s, 3, $ArOCH_3$), 6.6–7.3 (m, 3. ArH), and 10.30 (s, 1, CHO).

Anal. Calcd for $C_{23}H_{29}O_2Cl$: C, 74.08; H, 7.84; Cl, 9.51. Found: C, 74.12; H, 7.96; Cl, 9.49.

No material was eluted from the column by additional 350 ml of dichloromethane, but evaporation of the following 600 ml of eluent afforded 154 mg (13%) of starting ketone as a white solid; ir and NMR spectra are the same as those of a purified sample of ketone 1.

Further elution with 500 ml of 5% methanol–ether gave 147 mg of a white solid, which on crystallization from acetone–dichloromethane–water afforded **2,9-bisformyl-1-chloro-8-methoxy-4aβ,10bβ,12aα-trimethyl-3,4,4a,4bα,5,6,10b,11,12,12a-decahydrochrysene:** mp 265–266° dec (vacuum); ir ($CHCl_3$) 2770 (CHO), 1670 (C=O), 1605, 1570, 1495 (ArH), and 1150, 1055 cm^{-1} ($ArOCH_3$); NMR ($CDCl_3$) δ 0.92, 1.21 (2 s, 3 and 6, C-4a, C-10b, and C-12a CH_3), 3.90 (s, 3, $ArOCH_3$), 6.70 (s, 1, C-7 H), 7.80 (s, 1, C-10 H), 10.30 (s, 1, C-2 CHO), and 10.50 (s, 1, C-9 CHO).

Anal. Calcd for $C_{24}H_{29}O_3Cl$: C, 71.90; H, 7.29; Cl, 8.84. Found: C, 71.82; H, 7.24; Cl, 8.90.

The yield of the desired chloro aldehyde 13 based upon recovered starting material was 75%. In a similar experiment, in which the reaction mixture was heated with a 60° bath for 4.5 hr, the yield of purified aldehyde 13 (without recovery of starting material) was 71%.

2α-Formyl-8-methoxy-2β,4aβ,10bβ,12aα-tetramethyl-1,2,3,4,-4a,4bα,5,6,10b,11,12,12a-dodecahydrochrysene (14). To an argon-protected solution of 260 mg (38 mg-atoms) of lithium in 120 ml of dry ammonia and 50 ml of dry tetrahydrofuran was slowly added over a 50-min period with vigorous stirring a solution of 253 mg (0.68 mmol) of the chloro aldehyde 13 and 128 μl (100 mg, 1.36 mmol) of dry *tert*-butyl alcohol in 60 ml of dry tetrahydrofuran. After stirring for an additional 15 min the blue color of the reaction mixture was discharged by the portionwise addition of dry, powdered sodium benzoate, and the ammonia was evaporated through a mercury bubbler by heating the mixture with a hot air gun. After the addition of 40 ml of dry tetrahydrofuran, the reaction mixture was stirred with ice cooling and treated with 5 ml (11.4 g, 80 mmol) of iodomethane. After stirring without cooling for 2 hr, the resulting white suspension was diluted with 200 ml of ether and the product was isolated by ether extraction,[33] including both an acid and a base wash. Purification of the crude product (281 mg) by preparative TLC (15% ether–petroleum ether, double development) afforded 154 mg (64%) of the aldehyde 14 (R_f 0.45): mp 111–116° dec (vacuum); ir ($CHCl_3$) 2805, 2705 (CHO), 1720 (C=O), 1605, 1575, 1500 (ArH), 1385 (CH_3), and 1245, 1040 cm^{-1} ($ArOCH_3$); NMR ($CDCl_3$) δ 0.76 (s, 3, C-12a CH_3), 0.93, 1.00 (2 s, 3 each, C-2 and C-4a CH_3), 1.21 (s, 3, C-10b CH_3), 3.76 (s, 3, $ArOCH_3$), 6.6–7.3 (m, 3, ArH), and 9.45 (s, 1, CHO). The same spectral properties were observed for the analytical sample which was prepared by crystallization of a portion of this material from ether–hexane and also melted over the range 111–116° dec (vacuum).

Anal. Calcd for $C_{24}H_{34}O_2$: C, 81.31; H, 9.67. Found: C, 81.43; H, 9.67.

Reduction of 143 mg (0.403 mmol) of this aldehyde 14 in 10 ml of dry tetrahydrofuran with 110 mg (2.9 mmol) of lithium aluminum hydride afforded 139 mg (97%) of the corresponding primary alcohol as a white foam: ir ($CHCl_3$) 3625, 3470 (OH), 1608, 1575, 1495 (ArH), and 1385 cm^{-1} (CH_3); NMR ($CDCl_3$) δ 0.97, 1.00 (2 s, 6 and 3, C-2, C-4a, and C-12a CH_3), 1.21 (s, 3, C-10b CH_3), 3.43, 3.55 (2 s, 1 each, $–CH_2O–$), 3.67 (s, 3, $ArOCH_3$), and 6.6–7.3 (m, 3, ArH). The same spectral properties were observed for the analytical sample which was prepared by crystallization of a portion of this material from methanol and melted at 123.5–125.5° (vacuum).

Anal. Calcd for $C_{24}H_{36}O_2$: C, 80.85; H, 10.18. Found: C, 80.73; H, 10.25.

Attempts to convert this primary alcohol to the corresponding primary halide with triphenyl phosphite–iodomethane,[27] thionyl chloride–quinoline, and triphenylphosphine–carbon tetrachloride[28] led either to no observable reaction or a mixture of numerous products, judged to be the result of deep-seated rearrangements by the ir and NMR spectra.

8-Methoxy-2β,4aβ,10bβ,12aα-tetramethyl-2α-(3′-oxo-1′-propenyl)-1,2,3,4,4a,4bα,5,6,10b,11,12,12a-dodecahydrochrysene (15). Following an adaptation of the general procedure of Nagata and Hayase,[29] a stirred suspension of 880 mg (21 mmol) of 57% sodium hydride–mineral oil dispersion in 13.5 ml of dry tetrahydrofuran was cooled with an ice bath and treated over a 5-min period with a solution of 5.46 g (21 mmol) of diethyl 2-(cyclohexylimino)ethylphosphonate in 25 ml of dry tetrahydrofuran. After 15 min, a solution of 1.100 g (3.10 mmol) of the aldehyde 14 in 20 ml of dry tetrahydrofuran was then added over a 1-min period. This stirred mixture was heated with a preheated 60° oil bath for 80 min, cooled with an ice bath, and then poured onto 150 ml of ice and water. After isolation of the crude product by ether extraction[33] there was obtained 6.3 g of a yellow-brown oil that contained the corresponding aldimine.

Hydrolysis of the aldimine was accomplished by treatment of this crude product in 150 ml of benzene with 500 ml of 1% aqueous oxalic acid solution. This two-phase system was stirred at room temperature for 19 hr. The organic layer was separated and the aqueous layer was then extracted with three 200-ml portions of ether. The combined organic phases were washed with 2% aqueous hydrochloric acid (200 ml), 2% aqueous sodium hydroxide solution (two 200-ml portions), and saturated brine (200 ml), and then dried ($MgSO_4$). After removal of the drying agent and evaporation of the solvent at reduced pressure, 1.64 g of a yellow oil was obtained. Purification of this oil on 200 g of silica gel in a medium-pressure column was accomplished by elution with 40% ether–petroleum ether. When the second 200 ml of eluent from the column was evaporated at reduced pressure, there was obtained 1.066 g (89%) of unsaturated aldehyde 15 as a white solid. Crystallization of a portion of this material from ether–petroleum ether afforded analytically pure material that melted at 131–133° (vacuum): ir ($CHCl_3$) 2735 (CHO), 1675 (C=O), 1625 (C=C), 1605, 1575, 1495 (ArH), 1385 (CH_3), and 1035 cm^{-1} ($ArOCH_3$); NMR ($CDCl_3$) δ 0.87, 1.04 (2 s, 3 and 6, C-2, C-4a, and C-12a CH_3), 1.21 (s, 3, C-10b CH_3), 3.76 (s, 3, $ArOCH_3$), 6.05 (dd, 1, J = 16 and 7.5 Hz, C-2′ C=CH, 6.6–7.3 (m, 4, ArH and C-1′ C=CH), and 9.52 (d, 1, J = 8 Hz, CHO).

Anal. Calcd for $C_{26}H_{36}O_2$: C, 82.06; H, 9.53. Found: C, 82.07; H, 11.41.

8-Methoxy-2β,4aβ,10bβ,12aα-tetramethyl-2α-(3′-oxopropyl)-1,2,3,4,4a,4bα,5,6,10b,11,12,12a-dodecahydrochrysene. After the procedure of Nagai and coworkers,[30] a solution of 135 mg (0.355 mmol) of the unsaturated aldehyde 15 and 1.25 ml of triethylsilane in 1 ml of benzene was treated with 4.5 mg (4.9 μmol) of tris(triphenylphosphine) rhodium chloride, and the mixture was heated at 50° for 1.25 hr. While heating was continued for an additional 1.50 hr, two 2-mg (2.2 μmol) portions of the rhodium catalyst were added at 0.5-hr intervals. After dilution with 25 ml of ether and then filtration, evaporation of the solvents from the filtrate at reduced pressure afforded a yellow oil that contained the corresponding silyl enol ether.

A solution of this oil in 5 ml of acetone was treated with 0.5 ml of 5% aqueous hydrochloric acid, and the mixture was stirred at room temperature for 20 min. Isolation of the product by ether extraction,[33] including a base wash, afforded 200 mg of a yellow, semicrystalline solid which on purification by preparative TLC (35% ether–petroleum ether) gave 113 mg (83%) of the saturated aldehyde (R_f 0.42) as a white, amorphous solid: ir ($CHCl_3$) 2735 (CHO), 1720 (C=O), 1605, 1575, 1495 (ArH), 1380 (CH_3), and 1035 cm^{-1} ($ArOCH_3$); NMR ($CDCl_3$) δ 0.90, 1.00, 1.05 (3 s, 3 each, C-2, C-4a, and C-12a CH_3), 1.22 (s, 3, C-10b CH_3), 3.77 (s, 3, $ArOCH_3$), 6.6–7.3 (m, 3, $ArOCH_3$), and 9.80 (m, 1, CHO). This material was not further purified but used directly in the following experiment.

8-Methoxy-2β,4aβ,10bβ,12aα-tetramethyl-2α-(4′-methyl-3′-pentenyl)-1,2,3,4,4a,4bα,5,6,10b,11,12,12a-dodecahydrochrysene (16). A stirred suspension of 4.62 g (10.7 mmol) of isopropyltriphenylphosphonium iodide in 30 ml of dry tetrahydrofuran at room temperature was treated dropwise over a 3-min period with 4.03 ml (8.55 mmol) of a 2.12 *M* solution of phenyllithium in 30% ether–benzene. The red suspension was stirred for 2.25 hr, and then a solution of 819 mg (2.14 mmol) of the above saturated aldehyde in 13 ml of dry tetrahydrofuran was added over a 5-min period. After stirring at room temperature for 50 min longer the product was isolated by ether extraction,[33] including a 10% hydrogen peroxide wash and a 10% sodium thiosulfate wash. After removal of the desiccant and evaporation of the solvent at reduced pressure, a semisolid mixture was obtained. This material was filtered through a glass wool plug with the aid of 100 ml of petroleum ether to remove most of the relatively insoluble triphenylphosphine oxide. Concentration of the filtrate at reduced pressure afforded 1.2 g of a yellow oil which was purified by chromatography on 120 g of silica gel with 4% ether–petroleum ether. After the first 150 ml of eluent was discarded, the next 25 ml contained 101 mg of a mixture which on preparative TLC (4% ether–petroleum ether) afforded 69 mg (8%) of the olefin 16 which was combined with the bulk of the product obtained later. Concentration of the following 250 ml at reduced pressure afforded 651 mg (75%) of the olefin 16 as a white solid: ir ($CHCl_3$) 1605, 1575, 1495 (ArH), 1385 (CH_3), and 1035 cm^{-1} ($ArOCH_3$); NMR ($CDCl_3$) δ 0.92, 0.98, 1.05 (3, s, 3 each, C-2, C-4a, and C-12a CH_3), 1.21 (s, 3, C-10b CH_3), 1.59, 1.66 [2 s, $C=C(CH_3)_2$], 3.76 (s, 3, $ArOCH_3$), 4.85–5.35 (m, 1, C=CH), and 6.6–7.3 (m, 3, ArH). The combined yield of the olefin 16 was thus 720 mg (83%). Crystallization of a portion of this material from methanol with a trace of methylene chloride afforded the analytical sample as fluffy, white crystals, mp 78–80° (vacuum), and with the same ir and NMR spectra as those recorded above.

Anal. Calcd for $C_{29}H_{44}O$: C, 85.23; H, 10.85. Found: C, 85.06; H, 10.66.

4bβ,6aα,8β,10aβ-Tetramethyl-8α-(4′-methyl-3′-pentenyl)-4,4aα,4b,5,6,6a,7,8,9,10,10a,10bα,11,12-tetradecahydro-2(3*H*)-chysenone (18). To a solution of 50 mg (0.127 mmol) of the olefin 16 and 2.1 ml (1.66 g, 22.5 mmol) of dry *tert*-butyl alcohol in 5 ml of dry tetrahydrofuran and 15 ml of dry ammonia under an argon atmosphere was added with stirring 24 mg (3.5 g-atoms) of lithium, and the mixture was allowed to reflux for 2 hr. The excess lithium was then quenched with 0.6 ml of methanol, and after evaporation of the ammonia, the crude product (56 mg) was isolated by ether extraction.[33]

To a stirred solution of this crude dihydroaromatic system in 1 ml of dichloromethane at room temperature was added sequentially 2 ml of ethanol, 0.5 ml of water, and 50 mg of oxalic acid. After stirring for 2 hr, the reaction mixture was diluted with 100 ml of ether, and the product (50 mg) was isolated by ether extraction,[33] including a base wash.

Conjugation of the double bond of this β,γ-unsaturated ketone [ir ($CHCl_3$) 1710 cm^{-1} (C=O)] was effected by stirring at room temperature a solution of the crude material with 1 ml of ethanol, 0.15 ml of water, and 0.30 ml of 10% aqueous sodium hydroxide in 5 ml of dichloromethane. After isolation of the crude product (50 mg) by ether extraction[33] and purification of that material by preparative TLC (40% ether–petroleum ether), there was obtained 35 mg (72%) of the desired enone 18 as a pale yellow solid: ir ($CHCl_3$) 1655 (C=O), 1615 (C=C), and 1390, 1375 cm^{-1} (CH_3); NMR ($CDCl_3$) δ 0.88, 0.90, 0.93, 1.15 (4 s, 3 each, C-4b, C-6a, C-8, and C-10a CH_3), 1.61, 1.67 [2 s, 3 each, $C=C(CH_3)_2$], 5.08 (m, 1, $RCH=CR_2'$), and 5.87 (br s, 1, O=C-CH=C); analysis by GLC (300°)[31] indicated the presence of a single volatile component to the extent of >99% with retention time of 3.8 min. This material was used directly in subsequent experiments without further purification.

1,12a-Epoxy-4bβ,6aα,8β,10aβ-tetramethyl-8α-(4′-methyl-3′-pentenyl)-1,4,4aα,4b,5,6,6a,7,8,9,10,10a,10bα,11,12,12a-hexadecahydro-2(3*H*)-chrysenone. To a stirred solution of 392 mg (1.0 mmol) of the enone 18 in 9 ml of dichloromethane at room temperature was added sequentially 20 ml of methanol, 5.3 ml of 30% aqueous hydrogen peroxide solution, and 1.2 ml of 10% aqueous sodium hydroxide solution. After stirring in a closed flask for 21 hr, the crude product was isolated by ether extraction[33] and on crystallization from methanol–dichloromethane afforded 310 mg (76%) of the epoxy ketone: mp 103.5–105.5° dec (vacuum); ir ($CHCl_3$) 1700 (C=O) and 1375 cm^{-1} (CH_3); NMR ($CDCl_3$) δ 0.90, 1.12 (2 s, 9 and 3, C-4b, C-6a, C-8, and C-10a CH_3), 1.59, 1.67, [2 s, 3 each, $C=C(CH_3)_2$], 3.11 (s, 1, epoxy H), and 5.07 (m, 1, C=CH). The analytical sample, obtained after two further crystallizations of a portion of this material from methanol–dichloromethane, had the same spectal properties and melted at 107–108.5° (vacuum).

Anal. Calcd for $C_{28}H_{44}O_2$: C, 81.50; H, 10.75. Found: C, 81.49; H, 10.73.

1β-(3′-Butynyl)-4bβ,7β,8aα,10aβ-tetramethyl-7α-(4′-methyl-3′-pentenyl)-3,4,4aα,4b,5,6,7,8,8a,9,10,10a-dodecahydro-2(1*H*)-phenanthrenone. According to the procedure described above for the cleavage[19] of the epoxy ketone 5, a solution of 51 mg (0.124 mmol) of the epoxy ketone above and 24.5 mg (0.131 mmol) of *p*-toluenesulfonylhydrazine in 2.5 ml of 1:2 acetic acid–dichloromethane was stored for 30 hr at −20° and then stirred for 13 hr at room temperature. After isolation of the product by ether extraction,[33] including a base wash, and then purification of the residue by preparative TLC (15% ether–petroleum ether), 31 mg (63%) of the acetylenic ketone (R_f 0.32) was obtained as a white solid: ir ($CHCl_3$) 3300 (C≡CH), 2120 (C≡C), 1700 (C=O), and 1390, 1380 cm^{-1} (CH_3); NMR ($CDCl_3$) δ 0.76 (s, 3), 0.91 (s, 6), 1.21 (s, 3) (C-4b, C-7, C-8a, and C-10a CH_3), 1.62 and 1.68 [2 br s, 3 each, $C=C(CH_3)_2$], and 5.1 (m, 1, RCH=C). The analytical sample, obtained after two crystallizations of this material from methanol, had the same spectral properties and melted at 90.5–91.5° (vacuum).

Anal. Calcd for $C_{28}H_{44}O$: C, 84.79; H, 11.18. Found: C, 84.72; H, 11.21.

1β-(3′-Butynyl)-2α,4bβ,7β,8aα,10aβ-pentamethyl-7α-(4′-methyl-3′-pentenyl)-1,2,3,4,4aα,4b,5,6,7,8,8a,9,10,10a-tetradecahydro-2β-phenanthrol (20). To a stirred and ice-cooled solution of 1.1 ml (1.81 mmol) of 1.65 *M* ethereal methyllithium in 2.2 ml of dry tetrahydrofuran was added over a 7-min period a solution of 55 mg (0.139 mmol) of the acetylenic ketone above in 4.5 ml of dry tetrahydrofuran, and the mixture was allowed to stir at room temperature for an additional 25 min. After the reaction was quenched with 1 ml of water, the product was isolated by ether extraction[33] and amounted to 56 mg (99%) of a white foam: ir ($CHCl_3$) 3600 (OH), 3300 (C≡CH), 2115 (C≡C), and 1385, 1375 cm^{-1} (CH_3); NMR ($CDCl_3$) δ 0.89 (s, 6), 0.97, 1.06, 1.13 (3 s, 3 each) (C-2, C-4b, C-7, C-8a, and C-10a CH_3), 1.62 and 1.68 [2 br s, 3 each, $C=C(CH_3)_2$], and 5.1 (m, 1, RCH=C); analytical TLC (50% ether–petroleum ether) showed a one-component system with R_f 0.50. This material was not further purified but used directly in numerous acid-catalyzed cyclization reactions, all of which resulted in hydration of the terminal double bond with or without cyclization of the acetylenic side chain.

8α-(3′-Hydroxypropyl)-4,4aα,4b,5,6,6a,7,8,9,10,10a,10bα,11,12-tetradecahydro-4bβ,6aα,8β,10aβ-tetramethyl-2(3*H*)-chrysenone (17). To a solution of 19 ml of dry dimethoxyethane and 2.33 ml of ethanol in 65 ml of dry ammonia containing 56 mg (8 mg-atoms) of lithium was added dropwise with stirring a solution of 76 mg (0.2 mmol) of the aldehyde 15 in 19 ml of dry dimethoxyethane and 2.3 ml of ethanol. The blue color of the solution was maintained over a 2-hr period by the portionwise addition of 497 mg (71 mg-atoms) of lithium, and then the excess lithium was destroyed by the addition of 3 ml of methanol. After evaporation of

the ammonia in a stream of argon and then treatment of the residue with 150 ml of 5% aqueous hydrochloric acid, the product was isolated by ether extraction,[33] including a base wash. The residual, light yellow oil was dissolved in a mixture of 9 ml of ethanol and 6 ml of 5 *N* aqueous hydrochloric acid, and the resulting solution was refluxed under an argon atmosphere for 1 hr. After dilution of the solution with 50 ml of water, the product was isolated by ether extraction,[33] including a base wash, and then purified by preparative TLC (ether). The resulting clear, colorless oil amounted to 51 mg (68%) of the hydroxyenone 17. Crystallization of a portion of this oil from *n*-hexane–dichloromethane afforded analytically pure material: mp 144–146.5° (vacuum); ir ($CHCl_3$) 3610, 3400 (OH), 1655 (C=O), and 1610 cm^{-1} (C=C); NMR ($CDCl_3$) δ 0.90 (s, 6) 0.95, 1.15 (2 s, 3 each) (C-4b, C-6a, C-8, and C-10a CH_3), and 3.62 (t, 2, J = 6 Hz, $-CH_2OH$).

Anal. Calcd for $C_{25}H_{40}O_2$: C, 80.59; H, 10.82. Found: C, 80.86; H, 10.80.

1,12a-Epoxy-1,4,4aα,4b,5,6,6a,7,8,9,10,10a,10bα,11,12,12a-hexadecahydro-8α-(3′-hydroxypropyl)-4bβ,6aα,8β,10aβ-tetramethyl-2(3*H*)-chrysenone. To a stirred solution of 52 mg (0.15 mmol) of the hydroxyenone 17 in 3.6 ml of dichloromethane were added 5.4 ml of methanol, 0.9 ml of 30% aqueous hydrogen peroxide, and 0.44 ml of 10% aqueous sodium hydroxide solution. After stirring under argon for 8 hr, the reaction mixture was poured into 50 ml of brine, and the product was isolated by ether extraction.[33] Purification by medium-pressure chromatography (ether) afforded 34 mg (64%) of the epoxy ketone and 13 mg (25%) of recovered hydroxyenone 17. Crystallization of a portion of the epoxy ketone from ether afforded analytically pure material: mp 165–166° (vacuum); ir ($CHCl_3$) 3620 (OH) and 1700 cm^{-1} (C=O); NMR ($CDCl_3$) δ 0.88 (s, 9), 1.10 (s, 3) (C-4b, C-6a, C-8, and C-10a CH_3), 3.13 (s, 1, epoxy H), and 3.62 (t, 2, J = 6 Hz, CH_2OH).

Anal. Calcd for $C_{25}H_{40}O_3$: C, 77.27; H, 10.38. Found: C, 77.33; H, 10.44.

1β-(3′-Butynyl)-3,4,4aα,4b,5,6,7,8,8a,9,10,10a-dodecahydro-7α-(3′-hydroxypropyl)-4bβ,7β,8aα,10aβ-tetramethyl-2(1*H*)-phenanthrone. To a dry mixture of 112 mg (0.29 mmol) of the epoxy ketone above and 59 mg (0.32 mmol) of *p*-toluenesulfonylhydrazine cooled to −20° under an argon atmosphere was added 4 ml of a −20° solution of 1:2 glacial acetic acid–dichloromethane which had been previously degassed by alternate evacuation and ebullition with argon. After 25 hr at −15 to −25°, followed by 12 hr at room temperature, the reaction mixture was poured into 100 ml of water, and the product was isolated by ether extraction,[33] including a base wash. Purification by chromatography on Florisil (1:1 chloroform–ether) gave 82 mg (77%) of the acetylenic ketone as a slightly yellow oil that was a single-component system by tlc (1:1 chloroform–ether, R_f 0.39): ir ($CHCl_3$) 3610 (OH), 3300 (C≡CH), 2120 (C≡C), and 1700 cm^{-1} (C=O); NMR ($CDCl_3$) δ 0.77 (s, 3), 0.90 (s, 6), 1.18 (s, 3) (C-4b, C-7, C-8a, and C-10a CH_3), and 3.60 (t, 2, J = 6 Hz, CH_2OH). A portion of this oil was crystallized from ethanol–water, mp 99.5–101° (vacuum). However, the resulting solid did not give a satisfactory combustion analysis. Satisfactory results were obtained from a sample prepared by flash distillation of a portion of the original oil at 10^{-4} mm.

Anal. Calcd for $C_{25}H_{40}O_2$: C, 80.59; H, 10.82. Found: C, 80.60; H, 10.85.

1β-(3′-Butynyl)-7α-(3′-hydroxypropyl)-2α,4bβ,7β,8aα,10aβ-pentamethyl-1,2,3,4,4aα,4b,5,6,7,8,8a,9,10,10a-tetradecahydro-2β-phenanthrol (19). To a stirred and ice-cooled mixture of 4.8 ml (8.5 mmol) of 1.7 *M* ethereal methyllithium solution and 7.5 ml of dry tetrahydrofuran under an argon atmosphere was added dropwise a solution of 82 mg (0.22 mmol) of the acetylenic ketone above in 8 ml of tetrahydrofuran. After 10 min of stirring without cooling the excess methyllithium was destroyed with water, and the solution was diluted with 100 ml of brine. Isolation of the product by ether extraction,[33] followed by purification by chromatography on Florisil (10% ether–chloroform), afforded 75 mg (88%) of acetylenic alcohol 19 as a white, crystalline solid. Crystallization of a portion of this material from *n*-hexane–ether afforded analytically pure material that melted at 153–155° (vacuum): ir ($CHCl_3$) 3610 (OH), 3300 (C≡CH), and 2120 cm^{-1} (C≡C); NMR ($CDCl_3$) δ 0.88 (s, 6), 1.0, 1.07, 1.15 (3 s, 3 each) (C-2, C-4b, C-7, C-8a, and C-10a CH_3), and 3.62 (t, 2, J = 6 Hz, CH_2OH).

Anal. Calcd for $C_{26}H_{44}O_2$: C, 80.35; H, 11.41. Found: C, 80.32; H, 11.41.

8α-(3′-Hydroxypropyl)-1α,2α-methano-1,2,3,4,4aα,4b,5,6,-6a,7,8,9,10,10a,10bα,11,12,12a-octadecahydro-4bβ,6aα,8β,10a-β,12aβ-pentamethyl-2β-chrysenol (21). A. Preparation of the Enol Bis Trifluoroacetate. To 85 mg (0.22 mmol) of the acetylenic diol 19 cooled to −25° under an argon atmosphere was added 14.5 ml of a −25° solution of 30% trifluoroacetic anhydride in trifluoroacetic acid. After 45 min of stirring at −25°, the solvents were removed at reduced pressure (~1 mm), and the dark residue was taken up in ether and washed with water and saturated aqueous sodium bicarbonate solution. The resulting oil, which amounted to 136 mg, was used directly in the next experiment: ir ($CHCl_3$) 1780 cm^{-1} (C=O); NMR ($CDCl_3$) δ 0.87 (s, 9), 1.0, 1.03 (2 s, 3 each) (C-4b, C-6a, C-8, C-10a, and C-12a CH_3), 4.33 (t, 2, J = 6 Hz, CH_2–OTFA), and 5.30 (s, 1, C-1 H).

B. Cleavage of the Trifluoroacetate and Methylenation. To a stirred solution of lithium diisopropylamide prepared from 5.1 ml (10.9 mmol) of 2.13 *M* hexane solution of *n*-butyllithium and 1.82 ml (12.0 mmol) of diisopropylamine in 12 ml of dry tetrahydrofuran at 0° under an argon atmosphere was added a solution of 136 mg of the crude bis trifluoroacetate from above in 5 ml of dry tetrahydrofuran. To the cloudy, red-brown solution which resulted after 15 min of stirring were added all at once 43.6 ml (43.6 mmol) of the Simmons–Smith reagent prepared from the zinc–silver couple[36] [4.9 g (75 mmol) of granular zinc, 38 mg of silver acetate, and 38 ml of glacial acetic acid] and 5.78 ml (72 mmol) of diiodomethane in 66 ml of dry ether in the presence of a few strands of silver wool after the procedure of Conia.[36]

After stirring at room temperature for 50 min, the solution was diluted with 200 ml of ice cold, saturated sodium carbonate solution and 30 ml of 40% aqueous ammonium sulfate solution. Isolation of the product by ether extraction[33] including saturated aqueous sodium carbonate and 10% aqueous sodium thiosulfate solution washes afforded a dark red oil which was immediately chromatographed on 130 g of Florisil. Elution with 300 ml of petroleum ether removed diiodomethane, and continued elution with 250 ml of 1:1 ether–petroleum ether and then 350 ml of ether afforded 59 mg (67%) of the cyclopropanol 21 as a yellow, crystalline solid. Crystallization of this material from $CHCl_3$ or hexane–dichloromethane gave colorless crystals, mp ~200° dec (vacuum): ir ($CHCl_3$) 3580 cm^{-1} (OH); NMR (DMSO-d_6) δ 0.449 (m, 2, cyclopropyl CH_2), 0.915 (s, 9), 1.12, 1.17 (2 s, 3 each) (C-4b, C-6a, C-8, C-10a, and C-12a CH_3), and 4.16 (t, 2, J = 6 Hz, CH_2OH); high-resolution, mass measured molecular ion 402.3497 ± 0.0008 (calcd for $C_{27}H_{46}O_2$, 402.34976).

1,4,4aα,4b,5,6,6a,7,8,9,10,10a,10bα,11,12,12a-Hexadecahydro-1β,4bβ,6aα,8β,10aβ,12aβ-hexamethyl-8α-(3′-hydroxypropyl)-2(3*H*)-chrysenone (22). To a solution of 54 mg (0.13 mmol) of the cyclopropanol 21 in 25 ml of ethanol was added 30 drops of concentrated hydrochloric acid solution, and the mixture was refluxed in an argon atmosphere for 40 min. After dilution of the solution with 175 ml of water, the product was isolated by ether extraction,[33] including base wash, and then purified by chromatography on 20 g of Florisil. Elution with 120 ml of ether gave 41 mg (77%) of tetracyclic hydroxy ketone 22. Crystallization of a portion of this material from *n*-hexane afforded analytically pure material: mp 158–160° (vacuum); ir ($CHCl_3$) 3610 (OH) and 1700 cm^{-1} (C=O); NMR ($CDCl_3$) δ 0.72 (s, 3, C-12a CH_3), 0.80, 0.90, 0.93, 1.13 (s, 15, C-1, C-4b, C-6a, C-8, and C-10a CH_3), and 3.62 (t, 2, J = 7 Hz, CH_2OH).

Anal. Calcd for $C_{27}H_{46}O_2$: C, 80.54; H, 11.51. Found: C, 80.43; H, 11.43.

1,4,4aα,4b,5,6,6a,7,8,9,10,10a,10bα,11,12,12a-Hexadecahydro-1β,4bβ,6aα,8β,10aβ,12aβ-hexamethyl-8α-(3′-oxopropyl)-2(3*H*)-chrysenone. To a suspension of 300 mg (3 mmol) of chromic anhydride in 15 ml of dry dichloromethane under an argon atmosphere was added dropwise 0.48 ml (6 mmol) of pyridine. After 20 min of stirring at room temperature, 2.39 ml (0.48 mmol) of this deep burgundy solution was added to 19 mg (0.048 mmol) of the hydroxy ketone 22, and the mixture was stirred for 10 min. The red and black mixture was then filtered with the aid of suction through alumina (III), and the alumina was washed with 150 ml of dichloromethane. Removal of the solvent at reduced pressure afforded 16 mg (84%) of the keto aldehyde as a slightly yellow crystalline solid. Crystallization of a portion of this material from *n*-hexane afforded analytically pure material as colorless crystals: mp 177–179° (vacuum); ir ($CHCl_3$) 2775 (CHO), 1720 (C=O), and 1700 cm^{-1} (C=O); NMR ($CDCl_3$) δ 0.71 (s, 3, C-12a CH_3), 0.88, 0.89, 0.92, 1.11 (4 s, 3 each, C-4b, C-6a, C-8, and C-10a CH_3), 0.88 (d, 3, J = 7 Hz, C-1 CH_3), and 9.8 (s, 1, CHO).

Anal. Calcd for $C_{27}H_{44}O_2$: C, 80.94; H, 11.07. Found: C, 80.93; H, 11.11.

(±)-Shionone. To a solution of 62 mg (1.3 mmol) of triphenylisopropylphosphonium iodide in 4.5 ml of dry tetrahydrofuran under an argon atmosphere was added dropwise 0.50 ml (1 mmol)

of a 2 *M* *n*-hexane–phenyllithium solution. After stirring for 2 hr at room temperature, 3.7 ml (0.74 mmol) of this reagent was added to a solution of 19.6 mg (0.049 mmol) of the above keto aldehyde in 2 ml of dry tetrahydrofuran. After stirring for 20 min at room temperature the product was isolated by ether extraction,[33] including a 10% aqueous hydrogen peroxide wash, and purified by chromatography on 20 g of silica gel. Elution with 100 ml of 10% ether–petroleum ether afforded 17 mg (82%) of (±)-shionone. Analytically pure material was obtained upon crystallization from methanol as small needles: mp 161.5–163° (vacuum); ir ($CHCl_3$) 1700 cm^{-1} (C=O); NMR ($CDCl_3$) δ 0.71 (s, 3, C-12a CH_3), 0.88, 0.90, 0.92, 1.13 (4 s, 3 each, C-4b, C-6a, C-8, and C-10a CH_3), 0.88 (d, 3, J = 7 Hz, C-1 CH_3), 1.61, 1.69 [2 s, 3 each, C=C(CH_3)$_2$], and 5.10 (t, 1, J = 8 Hz, RCH=CR_2).

Anal. Calcd for $C_{30}H_{50}O$: C, 84.44; H, 11.81. Found: C, 84.38; H, 11.90.

The ir, NMR, GLC, and TLC of this material were identical with those found for an authentic sample of natural shionone provided by Professor G. Ourisson.

Registry No.—**1**, 53311-24-3; **2**, 54141-74-1; **3**, 54036-92-9; **4** axial alcohol, 54036-93-0; **4** equatorial alcohol, 54036-94-1; **5**, 54036-95-2; **6**, 54036-96-3; **7**, 54036-97-4; **8**, 54036-98-5; **9**, 54036-99-6; **10**, 54037-00-2; **11**, 54037-01-3; **12**, 54037-02-4; **13**, 54062-79-2; **14**, 53311-25-4; **15**, 54037-03-5; **16**, 54054-05-6; **17**, 53311-26-5; **18**, 54054-06-7; **19**, 54037-04-6; **20**, 54054-07-8; **21**, 54082-41-6; **22**, 54037-05-7; **23**, 53402-15-6; tetramethyldiamidophosphorochloridate, 1605-65-8; 8-methoxy-4aβ,10bβ,12aα-trimethyl-3,4,4a,4bα,5,6,10b,11,12,12a-decahydrochrysene, 54141-75-2; 1β,12aβ-methano-4bβ,6aα,10aβ-trimethyl-1α,2,3,4,4aα,4b,5,6,6a,9,10,10a,10bα,11,12,12a-hexadecahydro-2β-chrysenol, 54037-06-8; 2-acetoxy-1β,12aβ-methano-4bβ,6aα,10aβ-trimethyl-1,4,4aα,4b,5,6,6a,9,10,10a,10bα,11,12,12a-tetradecahydrochrysene, 54062-80-5; 2-trifluoroacetoxy-4bβ,6aα,10aβ,12aβ-tetramethyl-3,4,4aα,4b,5,6,6a,9,10,10a,10bα,11,12,12a-tetradecahydrochrysene, 54037-07-9; 2,9-bisformyl-1-chloro-8-methoxy-4aβ,10bβ,12aα-trimethyl-3,4,4a,4bα,5,6,10b,11,12,12a-decahydrochrysene, 54037-08-0; 8-methoxy-2β,4aβ,10bβ,12aα-tetramethyl-1,2,3,4,4a,4bα,5,6,10b,11,12,12a-dodecahydrochrysen-2α-methanol, 54037-09-1; 8-methoxy-2β,4aβ,10bβ,12aα-tetramethyl-2α-(3′-oxopropyl)-1,2,3,4,4a,4bα,5,6,10b,11,12,12a-dodecahydrochrysene, 54037-10-4; 1,12a-epoxy-4bβ,6aα,8β,10aβ-tetramethyl-8α-(4′-methyl-3′-pentenyl)-1,4,4aα,4b,5,6,6a,7,8,9,10,10a,10bα,11,12,12a-hexadecahydro-2(3*H*)-chrysenone, 54054-08-9; 1β-(3-butynyl)-4bβ,7β,8aα,10aβ-tetramethyl-7α-(4′-methyl-3′-pentenyl)-3,4,4aα,4b,5,6,7,8,8a,9,10,10a-dodecahydro-2(1*H*)-phenanthrone, 54054-09-0; 1,12a-epoxy-1,4,4aα,4b,5,6,6a,7,8,9,10,10a,10bα,11,12,12a-hexadecahydro-8α-(3′-hydroxypropyl)-4bβ,6aα,8β,10aβ-tetramethyl-2(3*H*)-chrysenone, 54037-11-5; 1β-(3′-butynyl)-3,4,4aα,4b,5,6,7,8,8a,9,10,10a-dodecahydro-7α-(3′-hydroxypropyl)-4bβ,7β,8aα,10aβ-tetramethyl-2(1*H*)-phenanthrone, 53311-27-6; 2-trifluoroacetoxy-8α-(3′-trifluoroacetoxypropyl)-4bβ,6aα,8β,10aβ,12aβ-pentamethyl-3,4,4aα,4b,5,6,6a,7,8,9,10,10a,10bα,11,12,12a-hexadecahydrochrysene, 54037-12-6; 4,4aα,4b,5,6,6a,7,8,9,10,10a,10bα,11,12,12a-hexadecahydro-1β,4bβ,6aα,8β,10aβ,12aβ-hexamethyl-8α-(3′-oxopropyl)-2(3*H*)-chrysenone, 54037-13-7.

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References and Notes

(1) The structural formulas containing one or more asymmetric carbon atoms depict one enantiomer but refer to racemic compounds unless otherwise designated. In the text the (±) prefix will be omitted, and intermediates are to be assumed to be racemic. The tetracyclic compounds will be described by the chrysene nomenclature and each racemate is arbitrarily represented by that enantiomer that has the C-6a (C-12a) methyl group in the α configuration.
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(3) National Science Foundation Predoctoral Fellow, 1968–1972.
(4) National Defense Education Act Trainee, 1971–1974.
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Gas–liquid phase chromatographic (GLC) analyses were determined on either a Hewlett-Packard 5750 or F & M 810 research chromatograph using helium carrier gas at a flow rate of 60 ml/min. Unless otherwise noted, all analytical GLC was conducted on a 6 ft × 0.125 in. column packed with 4% SE-30 on 60–80 mesh Chromosorb W AW DMCS.

Preparative thin layer chromatography (preparative TLC) was carried out on 20 × 20 × 0.2 cm glass plates coated with silica gel $PF_{254+366}$ (Brinkman Instruments Co). Analytical thin layer chromatography (TLC) was conducted on 1 × 3 in. microscope slides coated with a 0.5-mm layer of silica gel G or $PF_{254+366}$.

Alumina used for column chromatography refers to the grade I, neutral variety manufactured by M. Woelm, Eschwege, Germany, and made up to grade II or III as indicated by the addition of 3% or 6% water prior to use. Silica gel columns used the 0.05–0.2 mm silica gel manufactured "for column chromatography" by E. Merck & Co., Darmstadt, Germany. Preparative medium-pressure column chromatography was performed using ½ × 20 in. or 2 × 20 in. glass columns with fittings supplied by Chromatronix, Inc., Berkeley, Calif., and an instrument minipump supplied by Milton Roy Co., St. Petersburg, Fla. (instrumentation designed by R. H. Mueller, these laboratories, and copies are available on request). The columns were packed with silica gel H "for TLC acc. to Stahl" (10–40 μ) manufactured by E. Merck and Co., Darmstadt, Germany. Solvents were degassed under water aspirator vacuum prior to use.

"Dry" solvents were dried immediately prior to use. Ether, benzene, tetrahydrofuran, and dimethoxyethane were distilled from lithium aluminum hydride; *tert*-butyl alcohol, trimethyl sulfoxide, pyridine, and hexamethylphosphoramide (HMPA) were distilled from calcium hydride; dichloromethane, carbon tetrachloride, diodomethane, and methyl iodide were distilled from phosphorus pentoxide; ammonia was distilled from the tank and then from a blue lithium or sodium solution. "Petroleum ether" refers to the "Analyzed Reagent" grade hydrocarbon fraction, bp 30–60°, which is supplied by J. T. Baker Co., Phillipsburg, N.J., and was not further purified.

Reactions described as run under nitrogen or argon employed a mercury bubbler arranged so that the system could be alternately evacuated and filled with the inert gas and left under a positive pressure.

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was dried over anhydrous sodium or magnesium sulfate, then filtered, and the solvent was evaporated from the filtrate under reduced pressure (water aspirator) using a rotary evaporator. The use of the terms "base wash" or "acid wash" indicate washing the combined organic layers with saturated aqueous sodium bicarbonate solution or with dilute aqueous hydrochloric acid, respectively, prior to the aforementioned washing with water.

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4% SE-30 on 60-80 mesh Chromosorb WAW DMCS.

Preparative layer chromatography (PLC) was carried out on pre-coated PLC plates with a 20x20x2mm layer of silica gel 60F-254 on glass plates manufactured by E. Merck, Darmstadt, Germany.

Alumina used for column chromatography, unless otherwise stated, refers to the grade I, neutral variety manufactured by M. Woelm, Eschwege, Germany, and made up to grade II or III as indicated by addition of 3% or 6% water prior to use. Silica gel columns used the 0.05-0.2 mm silica gel manufactured "for column chromatography" by E. Merck and Co., Darmstadt, Germany. Preparative medium-pressure column chromatography was performed using 1/2 x 20 in or 2 x 20 in glass columns with fittings supplied by Chromatronix, Inc., Berkeley, Calif., and an instrument minipump supplied by Milton Roy Co., St. Petersburg, Fla. The columns were packed with silica gel H "for TLC acc. to Stahl" (10-40μ) manufactured by E. Merck and Co., Darmstadt, Germany. Ether and petroleum ether were degassed under water aspirator vacuum prior to use.

"Dry" solvents were dried immediately prior to use. Ether, benzene, tetrahydrofuran (THF), and dimethoxyethane (DME) were distilled from lithium aluminum hydride, tert.-butyl alcohol, pyridine, diisopropylamine, hexamethylphosphoramide (HMPA), and n-hexane were distilled from calcium hydride; dichloromethane and iodomethane were distilled from phosphorous pentoxide; ammonia and ethylamine were poured from the tank and then distilled from a blue solution

of lithium. "Petroleum ether" refers to the "Analyzed Reagent" grade hydrocarbon fraction, bp 35-60°, which is supplied by J. T. Baker Co., Phillipsburg, N.J., and was not further purified.

Reactions described as run under argon employed a mercury or silicon oil bubbler arranged so that the system could be alternately evacuated and filled with inert gas and left under a positive pressure.

Mass spectral analyses were performed by Ms. Beth Irwin, UCLA, Los Angeles, Calif. Micro-analyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Michigan.

36. In cases where products were isolated"by solvent extraction", the procedure generally followed was to extract the aqueous layer with several portions of the indicated solvent; then the organic layers were combined and washed with water, followed by saturated brine. The organic layer was dried over anhydrous magnesium sulfate, unless otherwise stated, and the solvent was evaporated from the filtrate under reduced pressure (water aspirater) using a rotary evaporator and the resulting residue was dried <u>in vacuo</u> ($\sim$ 1 mm). The use of the terms "base wash" or "acid wash" indicate washing the combined organic layers with saturated sodium bicarbonate solution or with dilute aqueous hydrochloric acid, respectively, prior to the aforementioned washing with water. The term 50% brine refers to the solution resulting when a saturated aqueous solution of sodium chloride is diluted with an equal volume of water.

PROPOSITIONS

Abstracts of Propositions

Proposition 1: It is proposed that synthetic polyethers of the "crown ether" variety be studied as possible analogues of the natural ionophoric antibiotics.

Proposition 2: Possible approaches to the total synthesis of carboxylic acid ionophores in the nigericin group are considered. Specifically, a hemi/acetal approach to the ABCD ring system of monensin, a diels-alder approach to the DE ring system of monensin, and a dihydropyrone approach to the DE ring system of nigericin are proposed.

Proposition 3: A synthesis of the alkaloid loline is proposed.

Proposition 4: A method is proposed for the generation of relatively stable solutions of electrons in solvents other than amines, utilizing crown ethers on a solid phase.

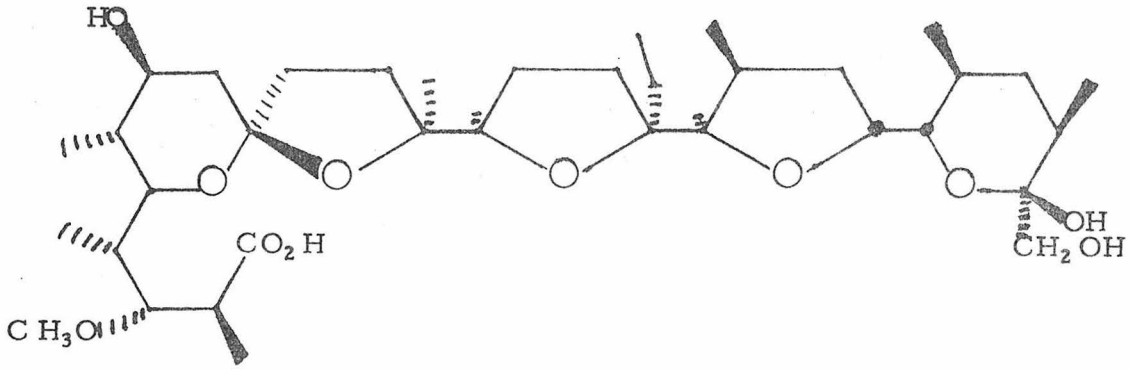
Proposition 5: A method is proposed for the isolation of macromolecules responsible for long term memory using competitive hybridization techniques.

Proposition 1

Synthetic Polyethers as Biologically Active Ionophores: Analogues of X-537A (Lasalocid)

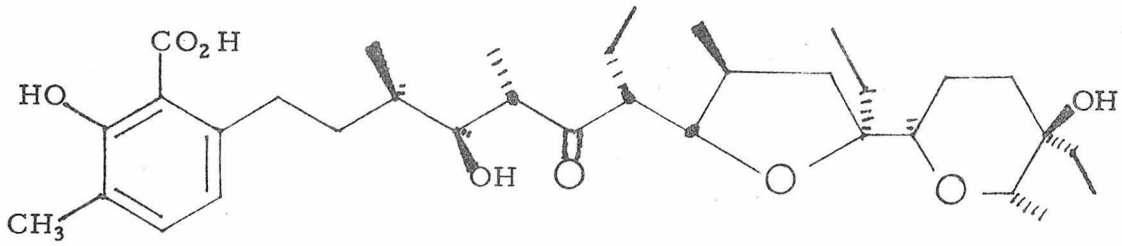
Introduction

The recent discovery that the ionophore monensin (1)[1] possesses useful antibiotic activity stimulated work on isolation



1

monensin



2

X-537A

and characterization of other ionophoric Streptomyces metabolites. This work led to the discovery of X-537A (2)[2] as a new cardiotonic and inotropic agent which in many respects is more ideally suited for therapeutic applications to pathological conditions of the heart than the two inotropic drug classes in current use, the catecholamines and digitalis.[3] It is proposed that relatively easily synthesized polyether ionophores of the "crown ether" variety may be prepared which mimic the activity of X-537A. Furthermore, the synthetic origin of such compounds would allow access to a variety of structural analogues which could be tested and varied within a mechanistic framework allowing preparation of compounds for which desirable properties are maximized.

The Mode of Action of Ionophoric Antibiotics

The naturally occurring ionophoric antibiotics may be divided into two basic classes, differentiated by their charge at physiological pH. The valinomycin class, including valinomycin,[4] the enniatins,[5] and the actins,[6] is composed of neutral macrocyclic polypeptides or lactones which form positively charged lipid soluble complexes with d^0 cations. The nigericin class, including nigericin,[7] monensin (1),[1,7] X-206,[8] lycosellin,[9] X-537A (2),[2] and A-23187,[10] is composed of monocarboxylic acids which are negatively charged at physiological pH and form neutral complexes with d^0 cations. A large variety of studies with compounds of this type involving ion complexation and transport through synthetic and natural membranes have been carried out. The ionophores

have been studied extensively as models for biological ion transport[11] because of their ability to distinguish between cations in a manner reminiscent of biological systems. Members of both classes have also proved useful as tools for the study of various biological processes involving ion transport.[12, 13, 14] In no case has it been shown that any biological activity of the compounds is a result of properties other than the ability to complex cations. The central thesis of this proposal is that the biological activity of the ionophores is indeed a result only of their ability to form lipid soluble complexes capable of transporting cations across membrane barriers and not a result of specific interactions with proteins or other structures in the cell.

This unusual ability of the ionophores to form lipid soluble complexes with d^0 cations[15] may be readily explained by examination of the structures of representative examples. X-ray structural analysis of several complexes have been completed,[15, 16] and many common features may be distinguished:[17] 1) The complexes are cyclic in general, though the shape of the complex may be cylindrical, saddle shaped (as the seam of a baseball), or irregular. 2) The complexes contain between 5 and 8 oxygen atoms (hydroxyl, carbonyl, or ether) within hydrogen bonding distance of the cation and there are few oxygen atoms on the outside surface of the molecule. 3) The oxygens may be regularly arrayed about the cation, or may form an irregular array. 4) The external surface of the complexes are mainly lipophilic, being

composed of hydrocarbon groups or chains. 5) The metal cation is not hydrated. 6) The complexes may be positively charged or neutral.

Properties of the Neutral "Crown" Polyethers

In 1967 the synthesis of some examples of a novel class of compounds termed "crown ethers" was reported.[18] These heteromacrocyclic polyethers possess the ability to complex d^o cations in a manner similar to that exhibited by the natural ionophores. X-ray studies have shown that in a typical 1:1 complex the metal is contained in a cavity surrounded by oxygen atoms in an ordered array with the lipophilic hydrocarbon chain of the "crown" exposed to solvent.

Measurements of a large number of physical properties, including thermodynamics and kinetics of complex formation[19,20,21] and the ability to render natural and synthetic membranes permeable to cations,[22,23,24] indicate that these neutral macrocycles behave in a manner similar to the valinomycin class of antibiotics. There appears to be no reason why the synthetic macrocycles should differ from the natural ionophores in more complex properties such as effects on respiring mitochondria and antibiotic activity. However, in an early study on the effect of some neutral polyethers on mitochondria[23] it was observed that, though the macrocycles (e.g., dibenzo-18-crown-6) rendered mitochondrial membranes permeable to K^+ as evidenced by increased ATPase activity, the important ability to uncouple oxidative

phosphorylation, which is common to all members of the valinomycin class of antibiotics, was not observed. This puzzling result, which seemed to cast doubt upon the utility of synthetic macrocycles as biologically active species, appears now to be explicable in terms of the chemiosmotic theory of oxidative phosphorylation.[25, 26] It seems that energy obtained through electron transport may be stored by the formation of a pH and electrical potential gradient across the mitochondrial membrane. Valinomycin, forming a positively charged complex, moves K^+ down the potential gradient through the membrane, thereby "turning off the power" for oxidative phosphorylation by destroying the gradient. In order to do this the complex must move through the lipid barrier sans counter ion. Though the thermodynamic and kinetic parameters of complexation for valinocycin and some of the synthetic macrocycles are similar as measured for neutral ion pairs, in a single phase or diffusing through lipid barriers, it is not clear that these parameters adequately test for the ability to carry charge across a lipid barrier. Indeed, it has been shown[24] that the "crowns" are much less effective than valinomycin at reducing the electrical resistance of synthetic membranes, though the "crowns" are almost as effective at causing cations to "leak" through blood cell membranes in response to a concentration gradient. This difference in behavior may be rationalized on the basis of the structural differences between the crown complexes, where the M^+ is close to the surface of the complex, and valinomycin complexes, where the M^+ is surrounded more fully by the

lipophilic skin. It should be stressed that the controversy over the mechanism of oxidative phosphorylation is not settled. However, the above arguments do serve to rationalize the apparently anomalous behavior of the neutral synthetic polyethers in the mitochondrial system.

The Properties of X-537A

The compounds X-537A (2) and A-23187 are anomalous monocarboxylic acid ionophores in that A-23187 is specific for dications while X-537A binds mono- and dications efficiently. Examination of the structures of these molecules indicates that no cyclic conformation may be obtained with a cavity of oxygen atoms large enough to accommodate a centrally located cation. X-537A forms dimeric complexes with both monocations[28] and dications in the form of a sandwich. This behavior is alarmingly reminiscent of the behavior of synthetic neutral polyethers with small "holes," e.g., the formation of a 2 : 1 sandwich complex of K^+ and benzo-15-crown-5.[29]

As a result of its unique structure X-537A has many interesting properties not found in other ionophores of its class. Using the aromatic portion of the molecule as a fluorescent probe for complexation[3] a series of disassociation constants (k_{diss}) were obtained for X-537A for various cations in ethanol. It was found that X-537A binds K^+ more effectively than Ca^{++}. Most interesting, however, was the discovery that X-537A also binds ethanolamine hydrochloride, and other ammonium salts, presumably as a 1 : 1

complex. No other natural ionophore in either class has been reported to bind ammonium salts, though most of the synthetic polyethers do.

Since k_{diss} in a single phase is not expected to be a reliable indicator for ability to transport cations across a lipid barrier, a simple vertically stacked bulk phase transport system (Fig. 1) was used to investigate rates of transport for various ions.[3] It

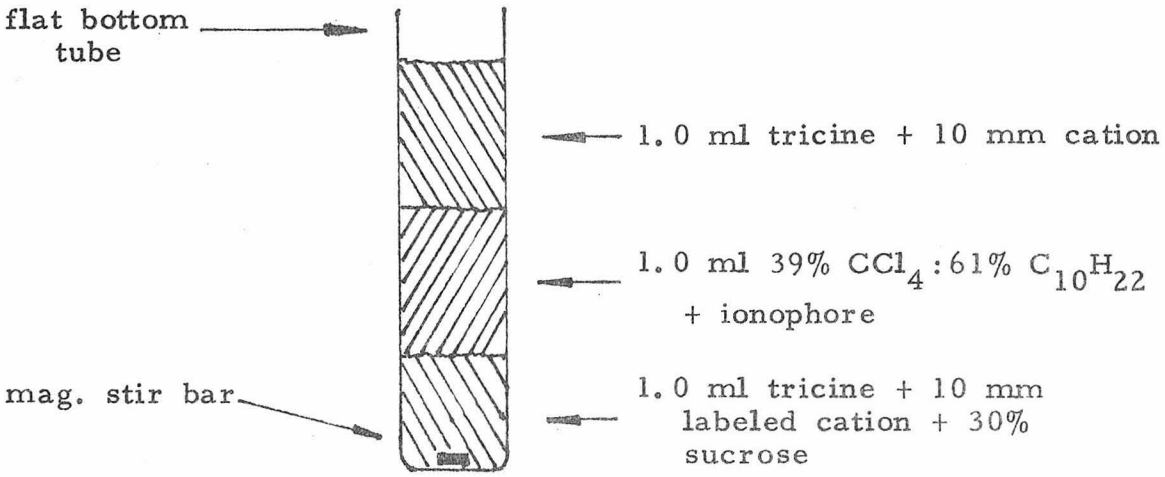


Fig. 1

was shown that Ca^{++} and Cs^{+} are transported fastest by X-537A. Also, X-537A shows a selectivity pattern for catecholamines predictable on the basis of steric factors, with mediated transport rates in the order norepinephrin > epinephrin > isopropylepinephrin.

As a result of the observed affinity of X-537A for Ca^{++} its effect upon isolated sarcoplasmic reticulum (SR) vesicles was studied.[30, 31, 32] It was found that SR which had absorbed Ca^{++}

by an ATP linked transport mechanism lost this Ca^{++} upon addition of X-537A to the medium. It appears that this effect is due solely to the ability of X-537A to render SR membranes permeable to Ca^{++} so that simple passive transport down a concentration gradient occurs. This effect coupled with the observed ability of X-537A to complex catecholamines led investigators to test X-537A on muscle tissue.[3] The results of these studies are perhaps the most exciting of all natural ionophore studies to date. It was found that X-537A initiates contraction of the smooth muscle of the rabbit aorta and increases the strength and rate of contraction of perfused rabbit heart. In in vivo studies on live dogs a dramatic increase in contractile tension (IVST) of 81% was observed, while the heart rate and aortic pressure were increased by much smaller amounts (3% and 10%/16%, respectively). The peripheral resistance actually decreased by 23%. No conductive anomalies appeared on EKG or HBE traces and no other toxic manifestations were observed. Also, preliminary studies indicate that the hemodynamic responses of dogs in surgically induced cardiac shock are even more striking. The results indicate that X-537A may become a useful inotropic or cardiotonic agent in humans.

Synthetic Analogues of X-537A

In order to prepare a series of synthetic analogues of X-537A designed to mimic its cardiotonic activity in vivo, it is necessary to determine the structural requirements for such activity. It would be possible to simply begin testing of analogues in vivo and

in this way develop empirical correlations between cardiotonic activity and structure. However, it seems likely that better analogues would result if as many parameters as possible, including equilibrium association constants, kinetics of complexation-decomplexation, ability to transport ions across bulk phases, and ability to transport ions across natural membrane barriers, were determined and correlated to in vivo cardiotonic activity. In this way the contribution to the in vivo activity of various detailed processes may be determined, and eventually it is possible that a mechanistic model for the in vivo activity could be developed. This would allow intelligent design of new ionophoric molecules possessing desired activity. This process would be analogous to that used in determining the parameters necessary for activity in uncoupling oxidative phosphorylation.

Unfortunately, very few correlations may be made with existing data. One is hindered by the diversity of methods used in the literature to characterize various ionophores and by the large number of different parameters actually measured. The first step in an investigation of this sort is the choice of a parameter to use as a screen for activity. It appears in the present case that measurement of the rate of ion transport through a bulk phase in response to a concentration gradient, using a system similar to that of Cram, et al.[27] (Fig. 2), provides such a parameter. The experiments are simple in execution and provide a crude model for the activity in vivo. After a number of synthetic analogues are tested and related to the behavior of X-537A by this criterion,

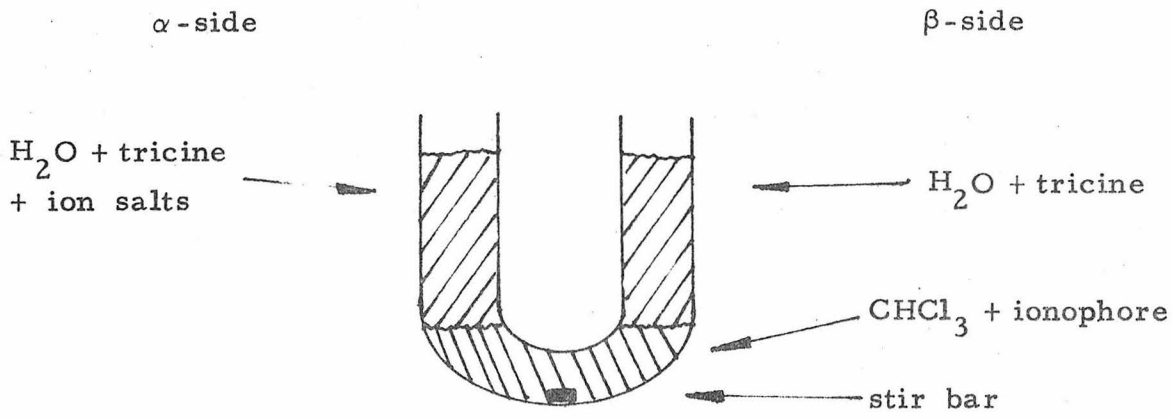


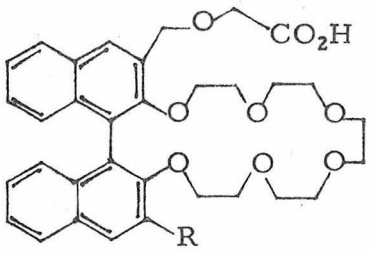
Fig. 2

testing of likely compounds by other methods (e.g., Ca^{++} transport in SR vesicles and rate data in single phases) should lead to analogues active in vivo, and point the way to design of new compounds.

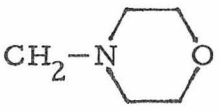
The limited number of structure-function correlations already obtained for X-537A derive from the previously mentioned structural data for X-537A complexes and measurements made on some analogues of X-537A already prepared.[33] It has been shown[3] that Br-X-537A, a derivative brominated at C-6, has a partition coefficient in the 1-octanol/water system which is a factor of 2 larger (more soluble in the organic phase) than X-537A itself. Also, the transport rates through a bulk phase (Fig. 1) for Ca^{++} and Cs^{+} are greater for Br-X537A than for X-537A, though not by a factor of 2. The transport rates for Rb^{+}, Sr^{++}, Ba^{++} and ethanol ammonium^{+} are comparable for Br-X-537A and X-537A. Thus there appears at least some correlation between the presence of lipophilizing

groups on the molecule and transport rates through a bulk phase. However, the cardiotonic activity of Br-X-537A was not reported.

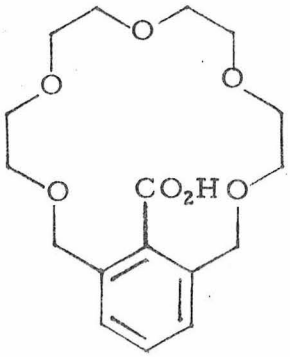
Examination of all pertinent data indicates that a synthetic analogue of X-537A should conform to the following structural requirements. The synthetic analogues should be mono-carboxylic acids which possess the ability to form neutral lipid soluble complexes with dications in the form of a sandwich. The compounds should also form lipid soluble complexes with monocations and ammonium salts, and the rates of complexation-decomplexation should not be so slow as to render the ionophore incapable of transporting cations across lipid barriers at a reasonable rate. With these criteria in mind a large number of candidates for testing which have already been prepared spring to mind. These are the acidic ionophores of Cram, et al.,[27] which have been designed as hosts for the binding of metal cations and ammonium salts in a very specific manner simulating the binding of substrates by enzyme systems. These compounds appear to offer an ideal starting point in the search for X-537A analogues. The macrocycles 5-8 represent only a small fraction of the total number of mono-acid ionophores prepared to date but serve to exemplify structures available through synthetic procedures already worked out. The number and kind of heteroatoms in the macrocycle, the total number of atoms in the cycle, and the type of connecting and shaping units and appended functional groups have been varied to give numerous analogous compounds with widely differing properties.[27, 34, 35]



5, R = H

6, R = 

7, R = 



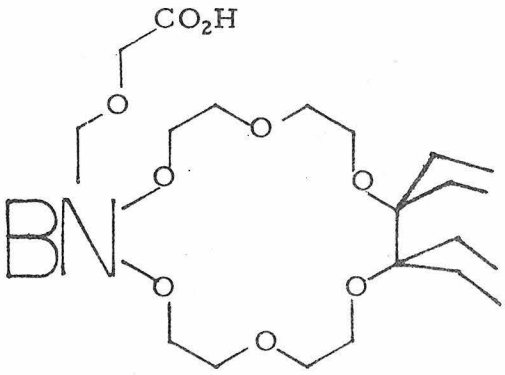
8

Some of the properties of 5-8 with respect to binding of metal and ammonium ions have been reported and will be discussed briefly.

Compound 5 has been shown to complex valine, and though no report of the properties of (5^--M^+) complexes are available, this molecule would appear to fulfill the above structural requirements. More data is available for 6, and this cycle serves to point up a possible problem with compounds of this type. Compound 6 has been shown to form $CHCl_3$ soluble complexes with alkali and alkalin earth cations. However, the sandwich complex (6_2^- - Ba^{++}) has such a slow rate of decomplexation that a methanolic solution of (6_2^- - Ba^{++}) is stable for short periods to treatment with H_2SO_4. If Ca^{++} also exhibits this slow rate behavior in polar solvents, 6 would not be expected to serve as a good model for X-537A. It is possible, however, that making the cycle of 6 larger or smaller would increase the rate of decomplexation and provide a better

analogue. The cycle 7 and the hydroxy-acid resulting from selective reduction of the ester function are two more members of the class of ionophores using the binaphthyl connecting and shaping unit. The unique class of ionophores with the carboxyl group placed in the center of a macrocycle is represented by 8. When complexed, the phenyl ring is out of the plane of the oxygen atoms in the macrocycle with the carboxylate sitting above the metal atom. This compound has been shown to complex tert.-butyl ammonium thiocyanate, Ca^{++}, K^{+}, and Na^{+}. Presumably the Ca^{++} complex is a sandwich structure.

With information gained by testing compounds of the general type 5-8 it should be possible to design new ionophores with desirable properties. It is hard to predict what sort of structures would be most interesting to try, but some possibilities are: 1) macrocyclic ionophores with more lipophilic hydrocarbon units appended to the chain, e.g., 9; 2) macrocycles with different connecting and shaping units, e.g., 10, 11, 12, and 13; open chain derivatives using connecting and shaping units more closely resembling those in the natural ionophores, e.g., 14; 4) and dl X-537A itself. Though preparation of compounds of the type 14 and total synthesis of X-537A will require the development of new synthetic methodology and understanding of acyclic conformational analysis, it is clear that molecules of this type will be available in the near future.



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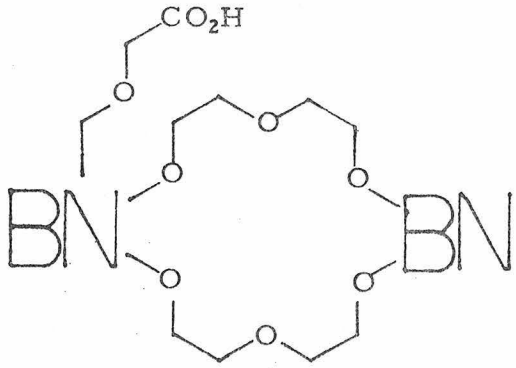

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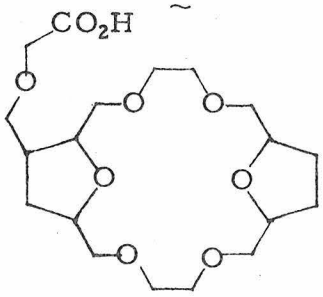

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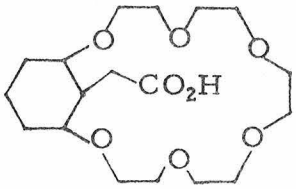

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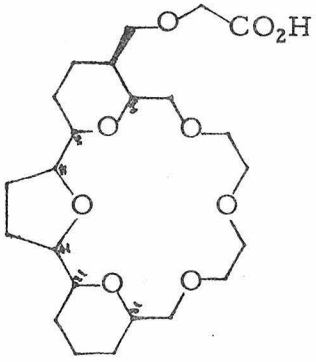

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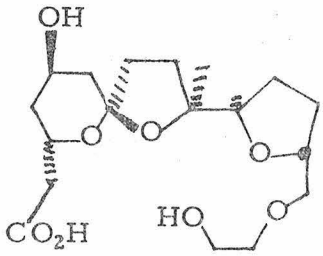

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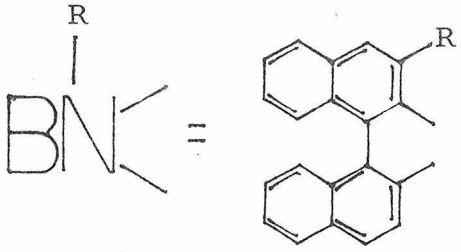

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Proposition 2

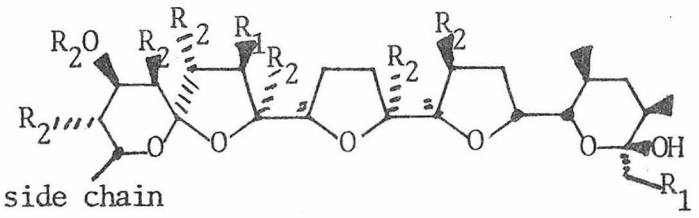
Total Synthesis of the Carboxylic Acid Ionophores: The Nigericin Group

INTRODUCTION

Since 1967 the structures of a number of novel mold metabolites possessing interesting biological properties have been determined. Among these are the structurally unique ionophoric antibiotics. This class includes monensin,[1] nigericin,[2] grisorixin,[3] septamycin,[4] A-204A,[5] dianemycin,[6] X-206,[7] alborixin,[8] salinomycin,[9] lysocellin,[10] X-537A,[11] and A-23187[12] (see chart). Inspection of the structures of these examples indicates that the monocarboxylic acid ionophores may be divided into four structural groups: 1) those molecules containing the nigericin ABCDE pentacyclic ring system; 2) those containing the X-206 pentacyclic skeleton; 3) those containing the ketonic 5-carbon chain present in X-537A; and 4) the group represented by A-23187, which so far contains but one member. This proposal consists of the outline of a possible strategy for the synthesis of members of group 1, the nigericin type ionophores.

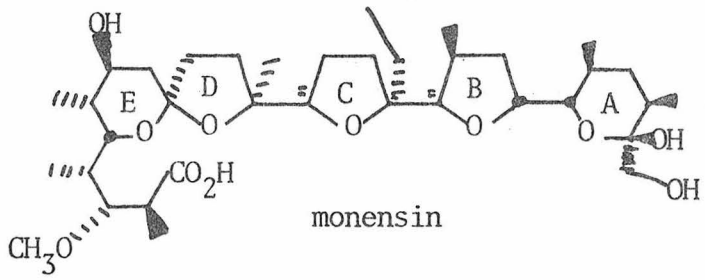
THE PENTACYCLIC BACKBONE

Inspection of the structures of members of the nigericin group shows that the pentacyclic skeleton may be generalized as in structure 1.

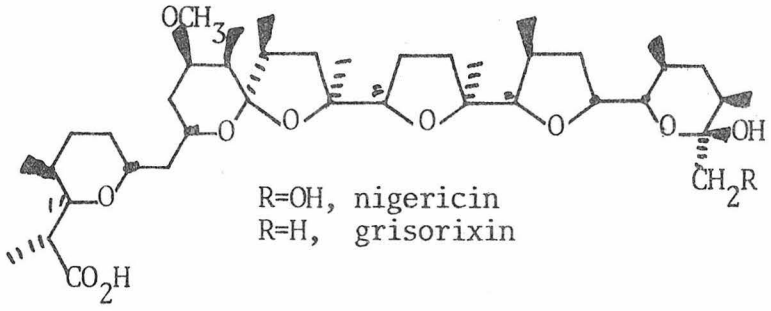


R_1 = H, OH, or OCH_3 R_2 = H, CH_3, or CH_2CH_3 1

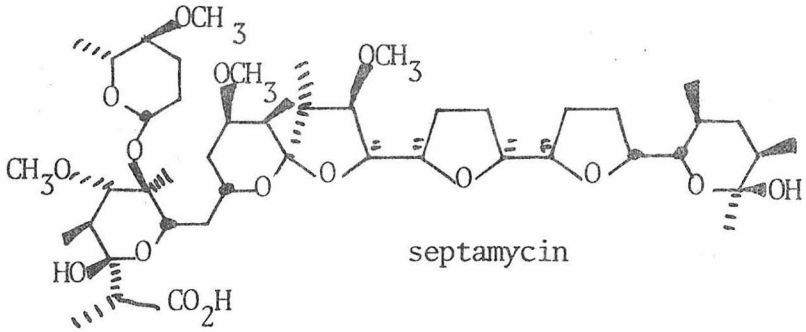
Ionophoric Antibiotics



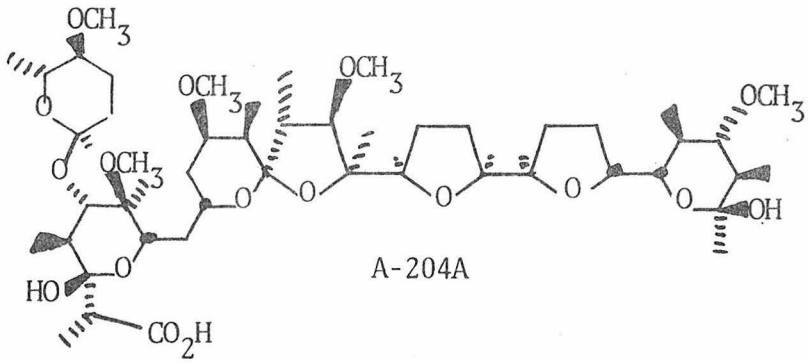
monensin



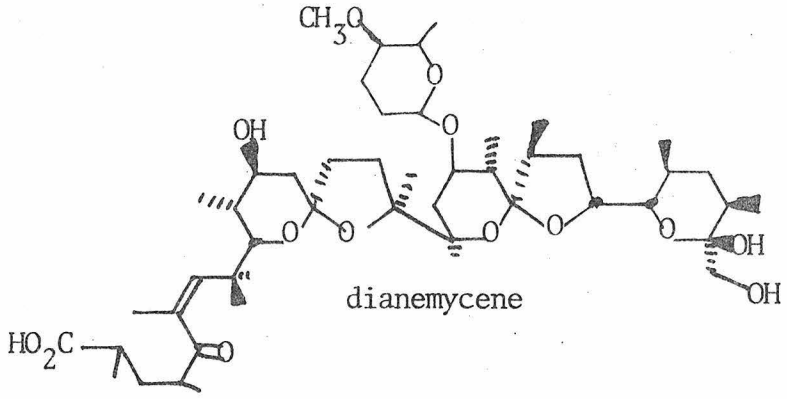
R=OH, nigericin
R=H, grisorixin



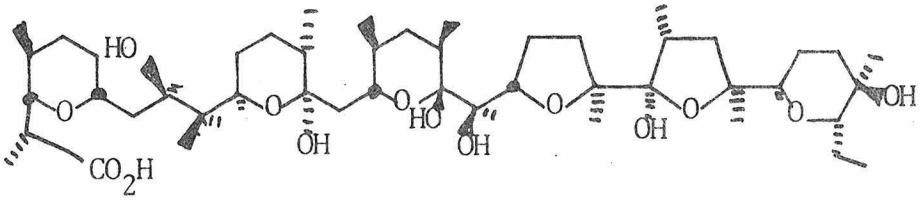
septamycin



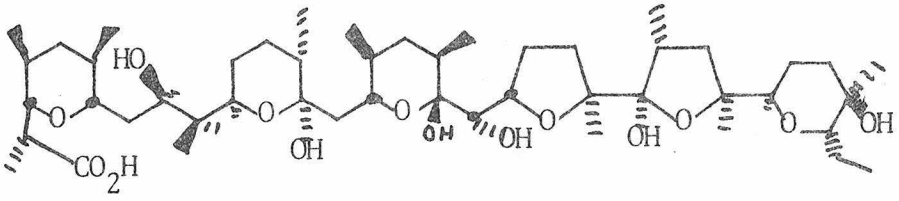
A-204A



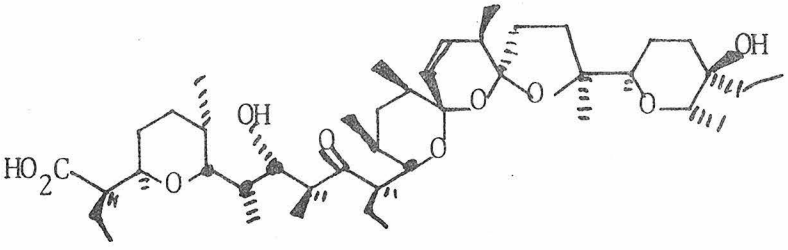
dianemycene



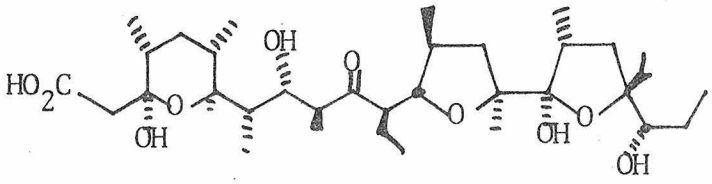
X-206



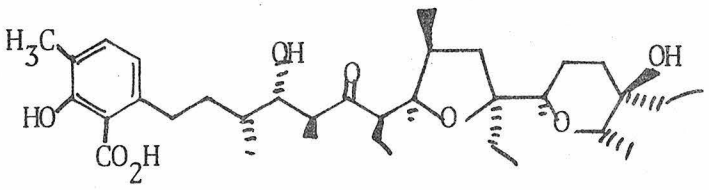
alborixin



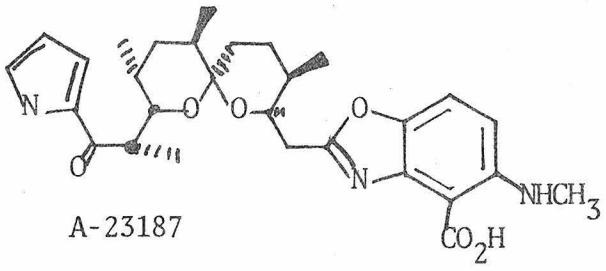
salinomycin



lysocellin



X-537A

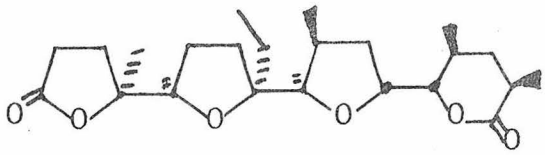


A-23187

Although a variety of structures are represented, a number of features common to all members of the group are evident: 1) the stereochemistry along the chain is identical for each member of the group; 2) the A-ring is very similar, differing only in R_1 = H or OH; 3) the oxygen substituent at C-3 and the ketal oxygen atom of ring D are in a 1,3 diaxial orientation. These similarities suggest that a basic unified approach applicable to the synthesis of all these compounds may be devised. However, the structural variety apparent within the group also demands that each molecule be considered as an individual with routes designed for each structural type requiring their own specialized methodology.

MONENSIN: THE ABCD RING SYSTEM

During the course of the structural determination of monensin some chemical degradative work was carried out.[1a] It was found that monensin was stable in basic solution but upon treatment with acid followed by oxidation with KIO_4-$KMnO_4$ a crystalline dilactone with the formula $C_{23}H_{36}O_6$ was formed as part of a mixture. The properties reported for this dilactone are consistent with structure 2.



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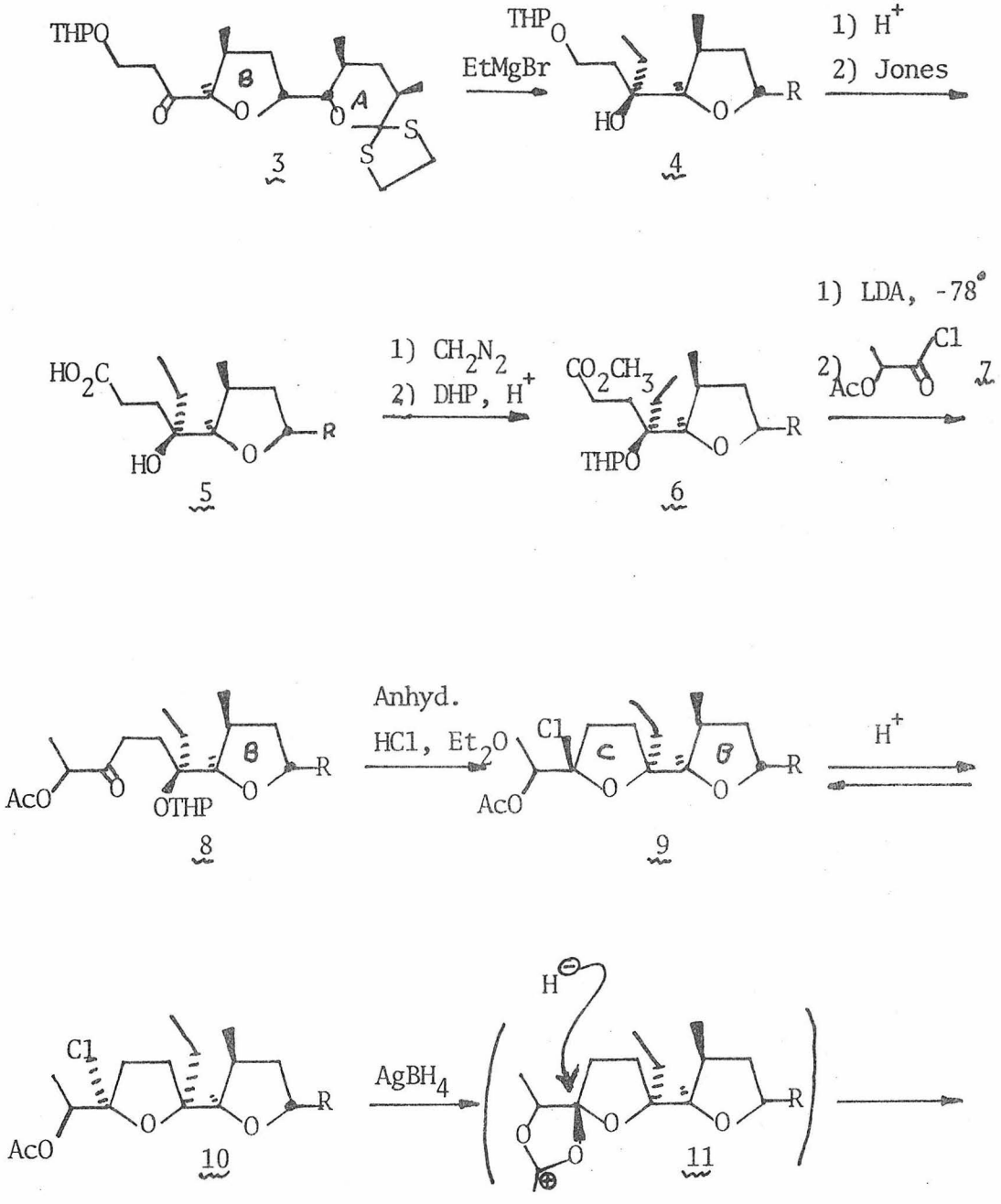
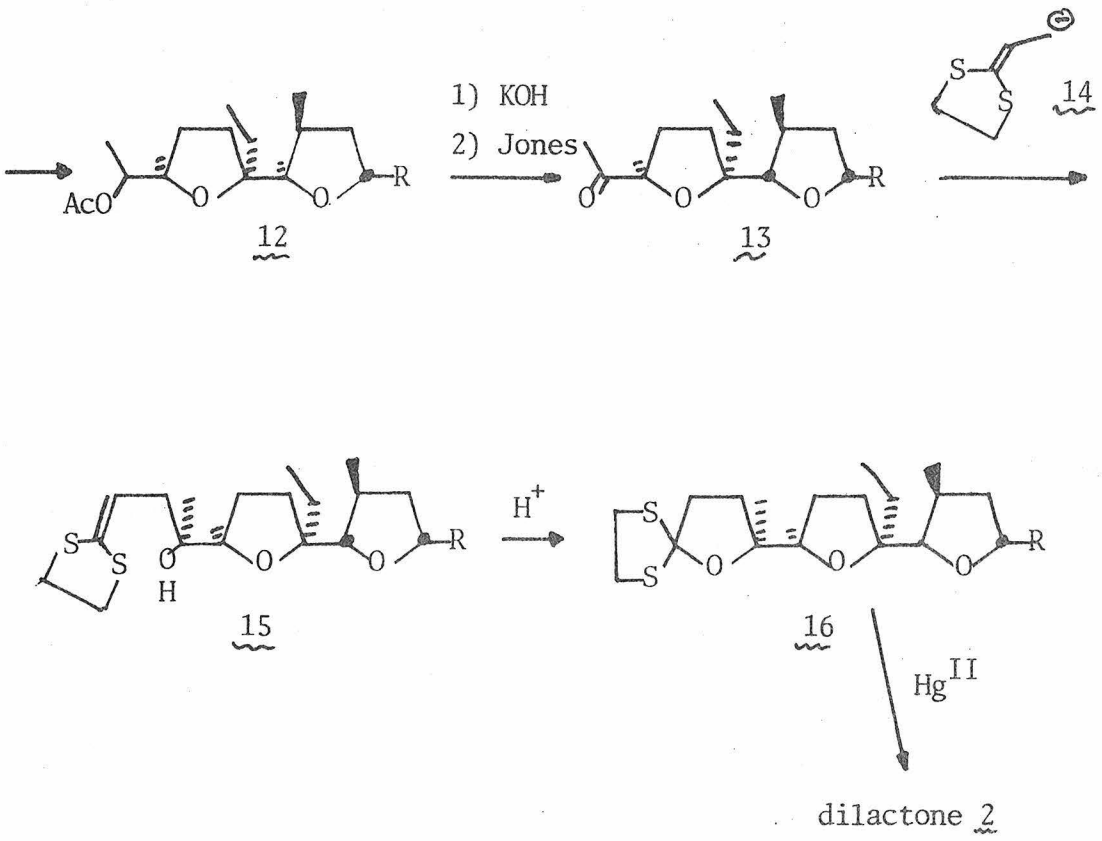
This molecule appears to offer an ideal initial goal for total synthesis since it contains many of the most difficult synthetic problems present in the molecule while still much less difficult than monensin itself. Also, at least in principle, routes may be designed

Scheme 1 Route to the Dilactone 2 from an AB Fragment



Scheme 1, Continued



which elaborate 2 back to the parent structure. Scheme 1 outlines a tentative route from an AB fragment 3 to the dilactone 2 by consecutive formation of the C and D rings. Although the proposed route is long and non-convergent, it serves to point out some of the problems associated with synthesis of polyethers of this type via a hemiketal approach.

Inspection of models of the AB fragment 3 indicates that because of the methyl substituent at C-14 the ketone carbonyl should adopt a configuration which by application of Cram's rule should lead to predominate formation of the correct stereochemistry at C-12 upon nucleophilic attach by ethyl grignard giving the alcohol 4. If conditions can be found which afford a large excess of the correct diastereomer, this technique could prove of general utility in these systems. Conversion of alcohol 4 to the ketoacetate 8 is straightforward. Formation of a substituted tetrahydrofuran derivative such as 12 from a hydroxy ketone derivative such as 8 appears to offer some advantages. The central feature of this route involves reduction of a hemiketal derivative such as the chloroether 10 to the saturated ether 12 in a stereoselective manner. Since the anomeric effect is operative in 5-membered rings[13] the chlorine atom will prefer a pseudo-axial orientation. Thus, in order to minimize an undesirable 1,3 interaction the chlorine should prefer to be cis to the smaller substituent at C-12. In the present case it is likely that a nearly 50:50 mixture will result, though separation and reequilibration could lead to isolation of a good yield of the desired epimaric chloroether 10. The crucial reduction of 10 must then proceed with retention. It is reported in the literature[14] that Ag^+ catalyzed nucleophilic displacement on an α-acetoxy chloroether by an amine proceeds presumably

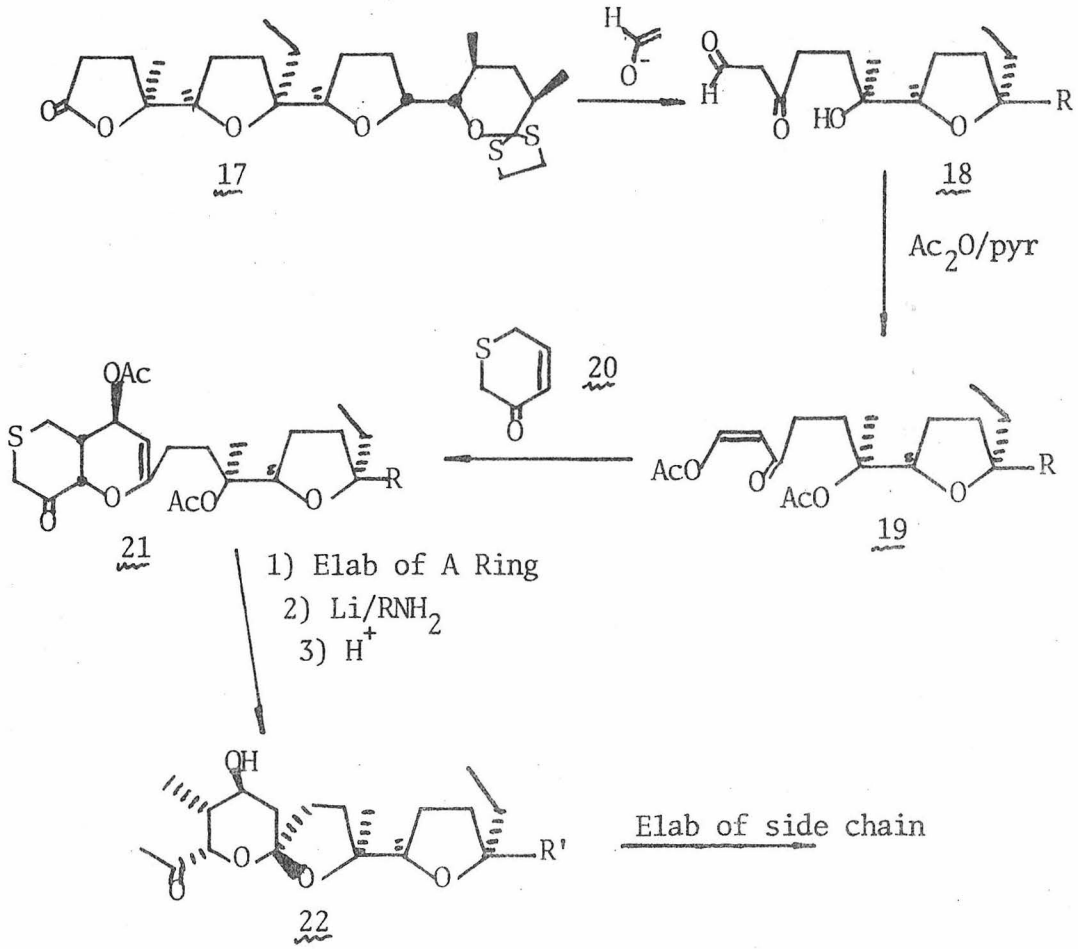
with participation via a cation such as 11 to the product corresponding to displacement with retention. In the present case, displacement by hydride with retention would give the desired acetate 12. Many variations of this general reaction type may be envisaged. For example, reduction of a hemiketal corresponding to chloroether 10 with the mixed hydride reagent,[15] or displacement of a hemiketal hydroxyl by CN^- in the presence of Et_3Al-HCN. A variety of mechanisms, and stereochemical consequences, are possible for these transformations. From the acetate 12, conversion to the ketone 13 is straightforward and adds the possibility of epimerization to other methods of control of the C-14 stereochemistry. The transformation of the ketone 13 to the alcohol 15 points out another general problem in construction of 5-membered rings by this route. An anion of the type 17 is required to place the necessary alcohol and carbonyl functions appropriately. A possible solution to this problem is the

17 14

ketene thioacetal anion 14.[16] Conversion of the alcohol 15 to the desired dilactone 2 via the protected lactone 16 [17] is straightforward. Thus, while a possible route to the CD system of monensin is outlined, several problems and many unanswered questions have become apparent.

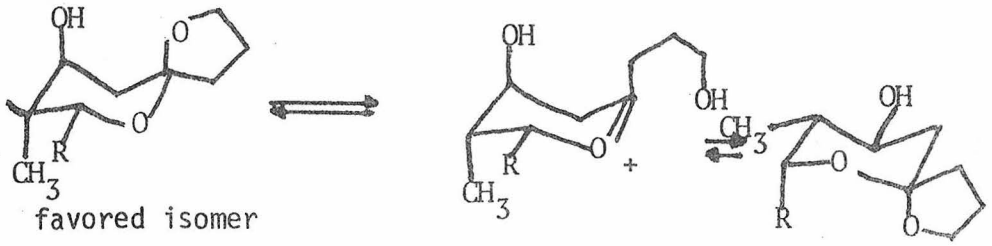
Although a more convergent approach is desirable, the elaboration of the dilactone 2 to monensin is a reasonable goal. This entails the

Scheme 2 Conversion of 2 Into Monensin



addition at C-5 of the elements of the E-ring and side chain, followed by formation of the spiroketal DE ring system and elaboration of the side chain. A route designed to accomplish this task is outlined in scheme 2. Although no stereochemical control in the attachment of the two pieces is attained, the route serves to point out some interesting features of the DE system.

Inspection of models of the monesin DE ring system indicates that once the stereochemical relationship between the side chain at C-1, the methyl group at C-2, and the hydroxyl at C-3 are established, formation of the thermodynamically more stable spiro ketal should produce the correct orientation as indicated below. This observation suggests the use

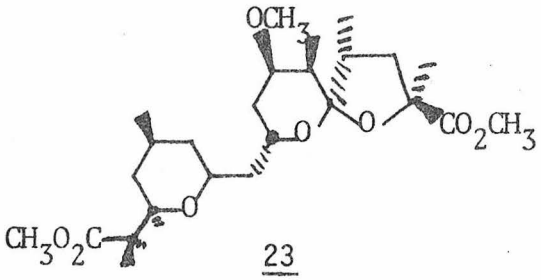


of a dihydropyran derivative as precursor to the spiro-ketal 22. Also, the cis relationship of the alkyl groups at C-1 and C-2 suggests the use of a Diels-Alder reaction for formation of such a dihydropyran. Thus, addition of acetaldehyde enalate to a non-protected lactone 17, followed by treatment with acetic anhydride under appropriate conditions should allow formation of the diene 19. Condensation with a large excess of the cyclic dienophile 20 would hopefully lead to formation of two diastereomers of the dihydropyran 21 (starting with resolved lactone 17). The carbonyl group of the dienophile 20 serves to direct the sense of addition in the Diels-Alder.[18] Cleavage of the C-S bonds and acetate

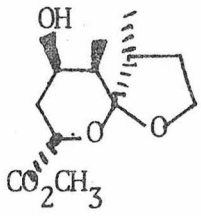
grouping of the adduct 21, followed by acid catalyzed ring closure could afford spiro ketal 22, which offers the possibility of elaboration to monensin.

THE NIGERICIN DE RING SYSTEM

During the course of structural work on grisorixin[3] the DE fragment 23 was isolated. Much as the dilactone 2 represents an ideal



23



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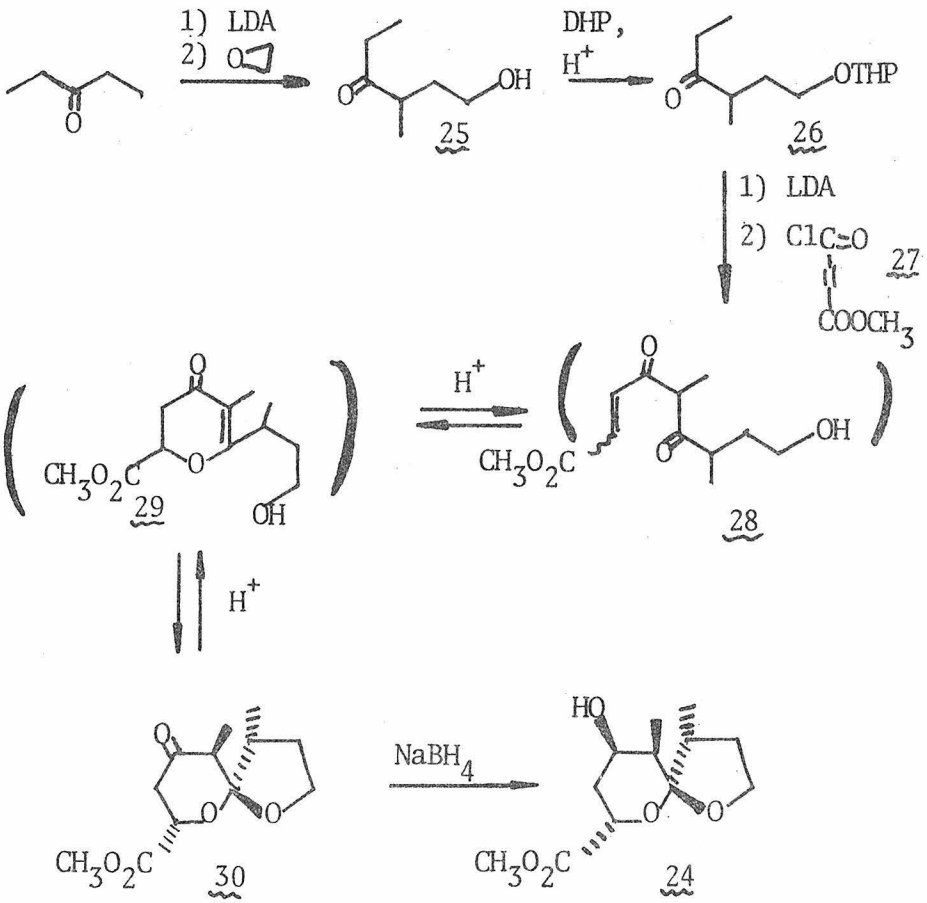
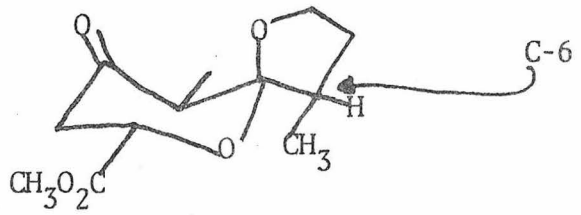
initial goal for development of methods for synthesis of the ABCD rings of monensin, the spiro ketal 23 represents such a goal for the DE rings and side chain of nigericin and grisorixin. Inspection of models indicates that, with the exception of the axial methoxyl at C-3, this system represents the most stable conformation at C-3, C-4, C-5, and C-6. Also, since the oxygen at C-5 is axial, one would expect that reduction of a C-3 ketone grouping with a hydride reagent should result in formation of the proper orientation of the oxygen atom at C-3. In order to test these ideas, a synthesis of the spiro ketal 24 is proposed as outlined in scheme 3.

Preparation of the ketone 26 is straightforward. Trapping of the derived enclate with acid chloride 27 followed by hydrolysis should afford the unsaturated dione 28. The cyclization of intermediates of

Scheme 3 The Nigericin DE Rings



this type to dihydropyrones such as 29 is well precedented, and proceeds in high yield with simple alkyl side chains.[19] Cyclization to the spiro ketal 30 should also represent a facile process. Since positions C-1 and C-4 are epimerizable and the cyclization is reversible, one would expect the most stable pyrone to result. As indicated in the perspective drawing, the carbomethoxy group at C-1 is equatorial, as is



the methyl group at C-4. The methyl group at C-6 is not epimerizable. However, depending upon the mode of cyclization of the side chain either the α or β methyl configuration may result. Although a mixture is expected, it is likely that the correct orientation, in which the methyl group eclipses a C-O bond instead of a C-C bond, will predominate at equilibrium. Hydride reduction by equatorial attack would then afford the desired alcohol 24.

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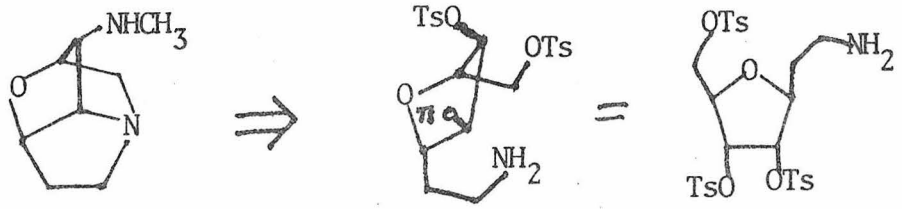
Proposition 3

The Total Synthesis of Loline

A total synthesis of the tricyclic alkaloid loline (1)[1] is proposed as outlined in scheme 1. The key step in this route is cyclization of



1 8

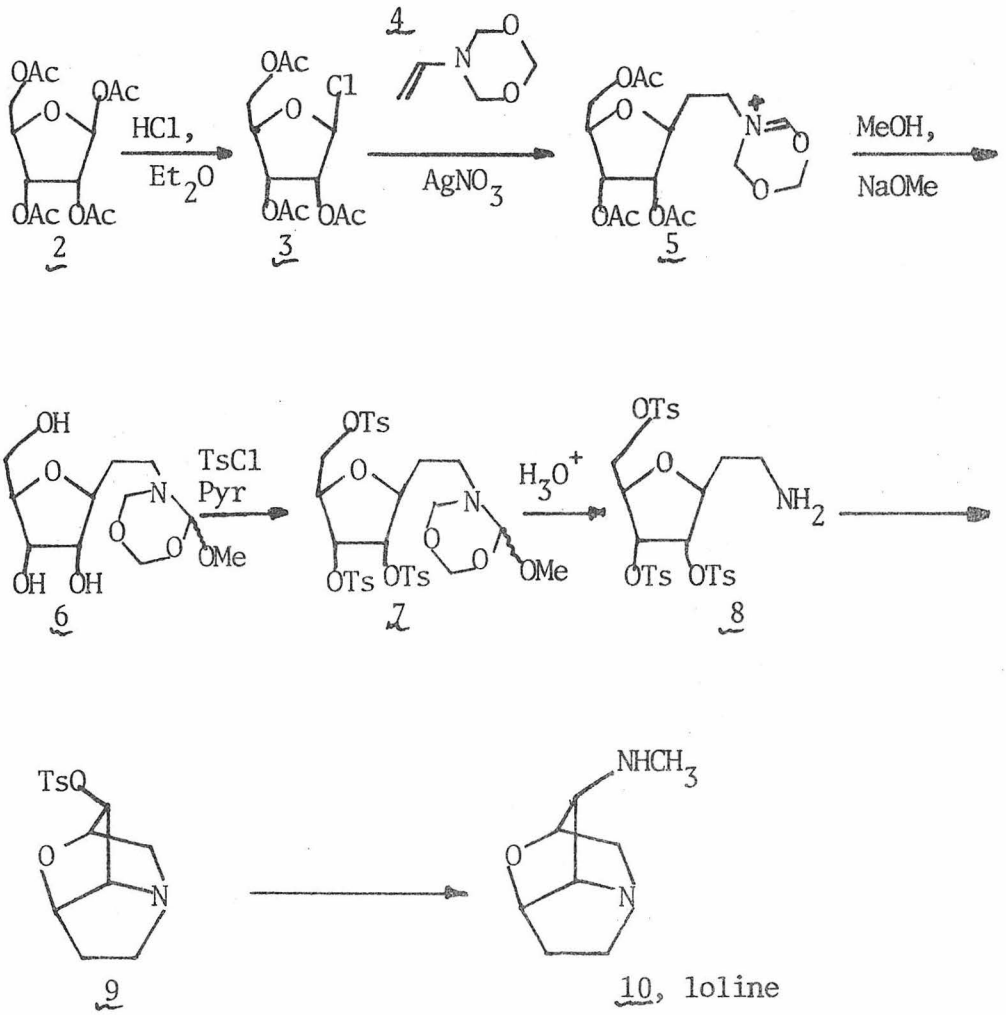
the tritosytate 8 to give tosylate 9. Condensation with methylamine would then afford loline stereoselectively.

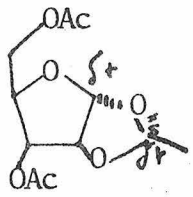
Examination of the proposed intermediate 8 suggests that it may be derived from ribose. The previously prepared chloroacetate 3[2] has been shown to undergo nucleophilic displacement with retention with amine nucleophiles in the presence of Ag^+.[2] Thus, for synthesis of 8 one requires a carbon nucleophile of the type 4a. Enamine 4 appears a

4a 4

possible candidate. Generation of an electrophilic species such as cation 39 in the presence of 4 may afford the immonium salt 5 after tautomeriza-

Scheme 1


4
OAc
OAc
OAc OAc
2
HCl,
Et2O
OAc
Cl
OAc OAc
3
AgNO3
OAc
OAc OAc
5
MeOH,
NaOMe
OH
OH OH
6
TsCl
Pyr
OMe
OTs
OTs OTs
7
H3O+
OTs
OTs OTs
8
NH2
TsO
9
NHCH3
10, loline

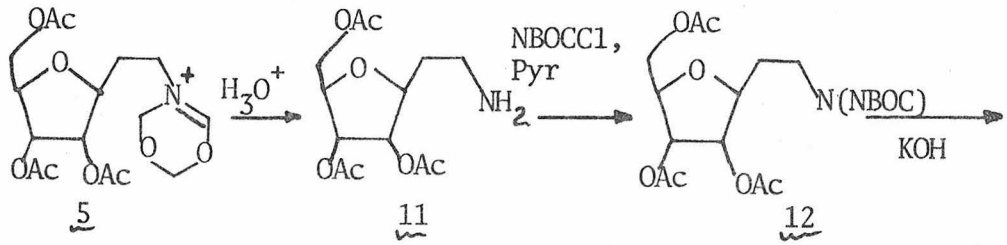


3a

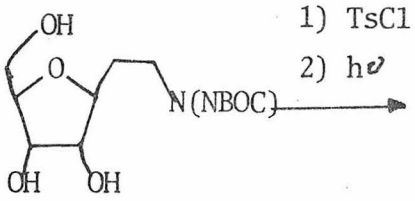
tion. Trapping of the immonium salt 5 with sodium methoxide with concomitant cleavage of the acetate functions gives the protected amine 6. Tosylation and careful removal of the ketal protecting group could afford the desired tritosylate 8, which may cyclize to the tricyclic 9.

An alternative route from the immonium salt 5 is indicated in scheme 2. This route does not require an acid catalyzed hydrolysis in the presence of the sensitive tosyl esters, but utilizes the photolabile m-nitrobenzyloxycarbonyl protecting group for the amine function.[3]

Scheme 2



5 11 12



13 8

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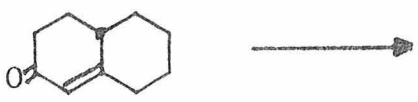
Proposition 4

A method is proposed for the generation of relatively stable solutions of electrons in solvents other than amines, utilizing crown ethers on a solid phase.

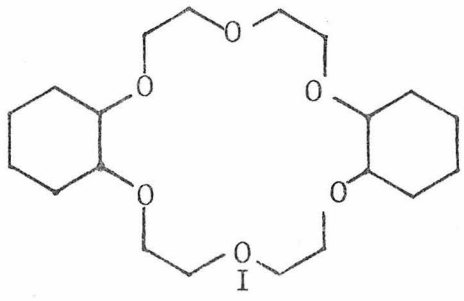
One of the principal limitations of the dissolving metal reduction is the relatively narrow spectrum of solvents available. There are several advantages to running solvated electron reactions in solvents other than amines. Aside from the advantages derived from use of solvents with different solvent properties, there is evidence that some reductions, specifically that of an enone, give different stereochemical results depending upon whether they are run in trimesityl borane-ether, or ammonia.[1] Also, in some cases it is desirable to be able to control



			starting material
Li, liq.NH_3, tBuOH	13%	87%	7%
Et_2O, Na, tBuOH, trimesitylborane	42%	58%	34%

the proton source in a reaction to avoid side reactions resulting from the strongly basic amide anions resulting from reductions in ammonia. Also, since primary amines and ammonia are quite nucleophilic compared to THF or Et_2O the advantages of these solvents with molecules sensitive to nucleophiles are apparent. Several investigators have been working

to devise ways of producing relatively concentrated solutions of solvated electrons in solvents other than amines. Recently several advances have been made, for example, use of HMPA [2] or trimesityl borane (c.f. above), both of which involve a radical anion as electron transfer agent. One of the most exciting is the discovery of Dye et al.[3], that blue solutions of potassium or cesium in THF or diethyl ether may be prepared by addition of a crown ether, specifically dicyclohexyl-18-crown-6 (I) to the reaction mixture. These solutions were prepared by addition of DCC to

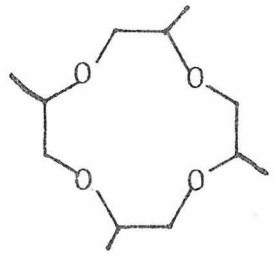


I

dicyclohexyl-18-crown-6 (DCC)

to THF or Et_2O in the presence of a mirror of potassium or cesium, and were stable for several hours at room temperature. The concentrations of metal obtained (1×10^{-4}M in the presence of 5×10^{-3}M (I)) were not close to that obtainable in, for example, a saturated solution of sodium in liquid ammonia at -33.8°C (4.8 M)[4]. However, the result seems to be worthy of further investigation as a possible source of solvated electrons in an aprotic medium. In fact, the paper of Dye is not the first indication in the literature that crown ether type compounds may be valuable as sources of stable solvated electron solutions. In 1959, G. Wilkinson, et al.[5] reported a study of stabilities of the blue solutions formed when potassium and sodium-potassium alloy were added to a number

of acyclic and cyclic ethers. Results were rather qualitative, the extent of dissolution for most species being estimated by the intensity of the color produced. In a few cases, including DME and THF, a more quantitative approach was tried, that of measuring the amount of hydrogen formed upon addition of water. The general conclusion was that the metal solutions in ethers were metastable, decolorizing at room temperature at varying rates depending upon the ether. Wilkinson set the upper limit of solubility at 10^{-4}M. He did, however, report one anomalous case. He observed that "the cyclic tetramer of propylene oxide (II) gives a deep blue solution of appreciable stability". However, though



(II)

realizing that the chelating abilities of II are obviously important, he felt that some of the stability of this solution was due to the viscosity of the material, noting that the stability of the filtered blue solutions of other ethers seemed to be higher for more viscous ethers. Today we recognize II as a 12-crown-4 derivative, and the stability of alkali metal solutions in this compound are not surprising. It would indeed be interesting to study a solution of II in THF with lithium added, since the lithium complex would be predicted to be the most stable by correlating ring size to ionic diameter.[6] (The most stable lithium-crown ether complex is obtained with DCC. However, di(t-butylperhydrobenzo)-14-crown-4

is the most selective for lithium.[7])

In a more recent paper relating to the work of Wilkinson, Catterall et al.[8], they studied the potassium-THF solution using optical spectra and esr methods. The solutions appear similar to metal-primary amine solutions. The concentration of metal, estimated from optical spectra, was 6×10^{-6}M. The concentration of unpaired spin was 2-5% in concentrated solutions and up to 25% in dilute solutions. The solutions are closely related to ethylamine-potassium solutions, except that the total concentration of unpaired spin is lower, and the electrons are closer to the potassium nucleus in the monomer species present in solution (larger coupling constant in the esr.)

It is clear that the simplest procedure for running a reduction using crown ethers in THF or Et_2O would be to dissolve the metal in a solution of crown ether and THF, then add the substrate. After the reaction is judged complete, a standard workup could be performed. However, further steps would be necessary to separate crown ether from product, since crown ethers are soluble in most organic solvents. In fact, DCC (one of the most likely candidates for the reaction because of its affinity for cations) is soluble even in petroleum ether, and yet displays appreciable water solubility.[7] Of course a liquid phase column chromatography could be used to get pure product, but the procedure also has the disadvantage that at least molar quantities of crown ether (with respect to electrons transferred) must be used, and reductions involve the transfer of at least two electrons. Thus two moles of crown ether would be necessary to run the reaction for every mole of substrate in the favorable case of reduction of an enone to a ketone. This involves

quite large quantities of crown ether. It is possible that the crown ether used in the reaction could be recovered and regenerated by conventional methods. However, this would be a tedious process. A solution to this problem, and one which has many other applications as well, is to attach the crown ether molecules to a polymeric support. In fact, Christensen, et al.[9] in a review of crown ether chemistry, suggested the use of crown ethers in ion chelating resins, and Pedersen and Frensdorf,[7] in their review, mention that dibenzo-18-crown-6 has in fact been condensed with formaldehyde to give resins containing polyether rings, though no mention is made of the properties of these resins.

If crown ether rings could be attached to a suitable polymeric support, a simple filtration of a quenched reduction reaction mixture would be all that is necessary to separate product from crown ether. Also, the polymer bound crown ethers could be regenerated by simply washing the polymer with water. One more important advantage gained by use of crown ethers on a solid support could be enhanced solubility of the metal. As previously stated, Dye only obtained 10^{-4}M solutions of potassium in THF. It is not clear that this is the maximum concentration of metal obtainable by using free crown ethers, but it is probably true that solutions comparable to those obtainable with ammonia and amines would be very difficult to produce using free crown ethers. However, with the crown ether attached to a polymer, the solubility of the metal may be increased. If only a small percentage of possible polymeric reactive sites were crown ether (i.e., the molecules were isolated from one another in space), the properties of a very dilute solution could be obtained in a relatively "concentrated" one. This is perhaps the most

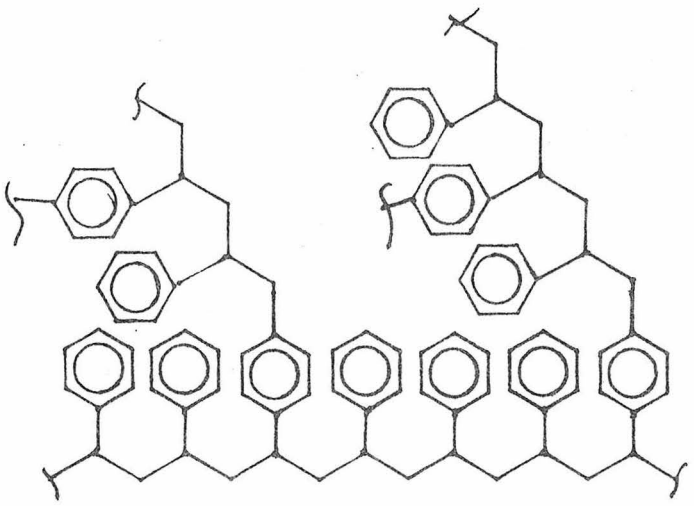
fascinating consequence of polymer bound crown ethers. The general principle has already been demonstrated by Potchornick and Kraus.[10] In order to obtain the monoacylation of an ester enolate with an acyl halide without formation of products derived from self-condensation of the ester, or diacylation, the ester was attached to a polymeric support during the reaction. Thus, the properties of a very dilute solution were achieved, in that only monoacylation product resulted after cleavage of the ester from the support.

SYNTHESIS OF THE POLYMER

There are numerous cases in which polymers containing functionality attached covalently to the polymer backbone have been used to carry out reactions on small substrate molecules, atoms or ions. Three of most well known cases are the vulcanization of natural and synthetic rubbers, the many ion exchange resins currently available (both the synthesis of these resins, and their function), and the solid phase peptide synthesis developed initially by R. B. Merrifield.[11] Recently, much work has been done to expand the method, including the monoacylation previously described, a polymer bound Wittig reagent developed at Dow Chemical Co.[12], and a unidirectional Dieckmann cyclization achieved by H. Rapoport.[13]

By far the most common polymeric support used for these reactions is a suspension polymerized copolymer of styrene and divinyl benzene (DVB). Suspension polymerization involves free radical polymerization of the monomers in small globules in a vigorously agitated aqueous solution containing various stabilizers to keep the partially polymerized

beads from sticking together. Using this technique beads as large as ~1 cm and as small as 1 μ have been obtained.[14] The most common beads for synthetic work have been about 30μ in diameter, containing about 2% DVB. The resulting bead is insoluble in all solvents, but appreciably swelled by most. Its structure is indicated below.



Essentially every benzene ring in the entire polymer may be functionalized by reaction of the beads with chloromethyl methyl ether in the presence of stannic chloride. This chloromethylation may be controlled to yield polymers with a very wide range of functionalization by varying the temperature and time of reaction. For example, the polymer used by Rapoport[13] contained 1.5% chlorine, and was prepared by reaction of the beads in $ClCH_2OCH_3$ in the presence of $SnCl_4$ at 0°C for 8 min. A great variety of functional groups may be attached to the polymer via the chloromethyl groups. For example, reaction of the chloromethyl polymer

with the sodium salt of a half ester at 150° for 10 hours gives the ester linkage.[13] Alternatively, the resin may be converted to the acetoxymethyl derivative with KOAc in hot benzyl alcohol, then saponified to give the hydroxymethyl derivative, which may be esterified under mild conditions with dicyclohexylcarbodiimide. Anion exchange resins may be prepared by reaction of the chloromethyl polymer with a tertiary amine, to give the quaternary ammonium salt.[15] Another method used to functionalize the polymer is reaction of substituted styrenes which yield substituted polymer upon which various reactions may be carried out. For the preparation of the Wittig resin,[12] p-bromostyrene was suspension copolymerized with styrene and 2% DVB. The resulting p-bromostyrene polymer (about 25% brominated) was treated with n-butyllithium in benzene, then with chlorodiphenylphosphine to give the poly-p-styryldiphenylphosphine.

A few other types of polymers have been used in solid phase peptide synthesis. Inukai, et al.[16] used a phenol resin prepared by reaction of phenol with s-trioxane in bis-(2-ethoxyethyl)ether containing a catalytic amount of PTSA at 150° in a sealed tube. The resin obtained was amorphous, but was swelled by the usual solvents used in peptide synthesis. The first N-protected amino acyl group was attached to the resin via an ester bond with the phenolic hydroxyl group. Several workers have used styrene-DVB popcorn polymers in peptide synthesis. The popcorn polymer is formed in a neat mixture of the monomers in which the polymer is somewhat insoluble and forms as hard nodules which are not swelled by the solvent, but are evidently quite freely permeable. A study of the rates of aminolysis of p-nitro phenyl ester groups on a

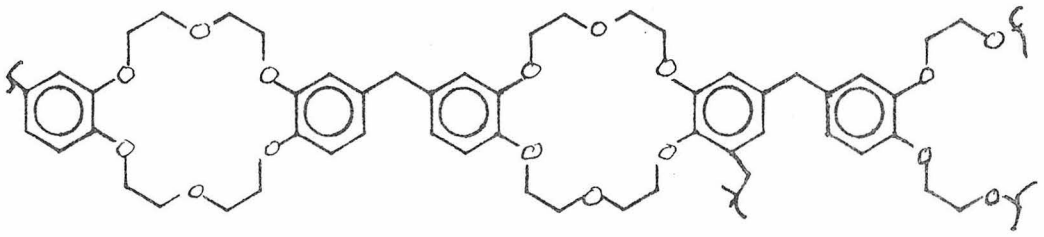
swellable s-DVB bead polymer, a popcorn polymer, and in homogeneous solution of p-nitrophenyl benzoate[17] showed that both polymers reacted at the same rate, which was about 1/5 the homogeneous reaction rate.

Perhaps the largest departure from the norm is the work of Shemyakin, et al.,[18] in which peptide synthesis was carried out on a styrene polymer chain which was soluble in the reaction solvents (no cross linking). After reaction, the polymer-peptide could be precipitated with water and washed.

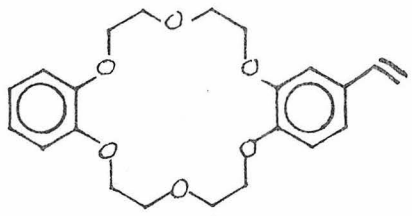
There are numerous possible routes to a suitable polymer with attached crown ether molecules. The requirements are that the polymer be insoluble (therefore probably crosslinked), fusable, and stable to the reaction conditions. The latter requirement, that the polymer be stable to the vigorous conditions of the Birch reduction, puts severe limitations on the possible polymeric supports. Obviously, an ester linkage of crown ether molecule to polymeric support is unsuitable, but the most limiting factor of all is the requirement that no aromatic rings be present. The reason styrene polymers are so simple to prepare in useful forms is that styrene monomer may be polymerized by free radical processes initiated by simple organic (benzoyl peroxide) or inorganic (potassium persulfate) radical sources. Unfortunately, unactivated olefins do not undergo this reaction, as the necessary radical intermediate is not stabilized by the aromatic ring. Olefins such as ethylene may be polymerized at high pressures and temperatures or by stepwise mechanisms, but these polymers are long, straight or branched chain molecules, which do not have properties ideal for the present purpose. Thus, for this application, the property of the monomer which

makes it convenient for polymerization and functionalization is also not acceptable in the final product.

There are a number of ways around this problem. The most obvious is the attempted reduction of an unsaturated, crosslinked polymer. Synthesis of such a polymer, containing crown ethers, would be quite simple and straightforward. In fact, it is possible that under the right conditions a polymer obtained by reaction of H_2CO with dibenzo-18-crown-6 [7] could be useful. This type of polymer would be analogous to Inukai's[15] phenol, s-trioxane polymer, and would probably have a crosslinked structure as shown. If crosslinking could be controlled such that the final

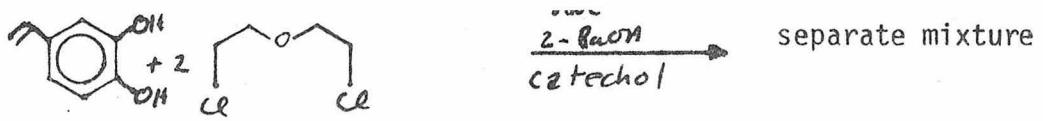


polymer were insoluble but fusable, a potentially useful material would result. It would also be relatively straightforward to make a bead type polymer by simple suspension copolymerization of styrene, DVB, and a crown ether monomer such as III. Such a monomer could be prepared by

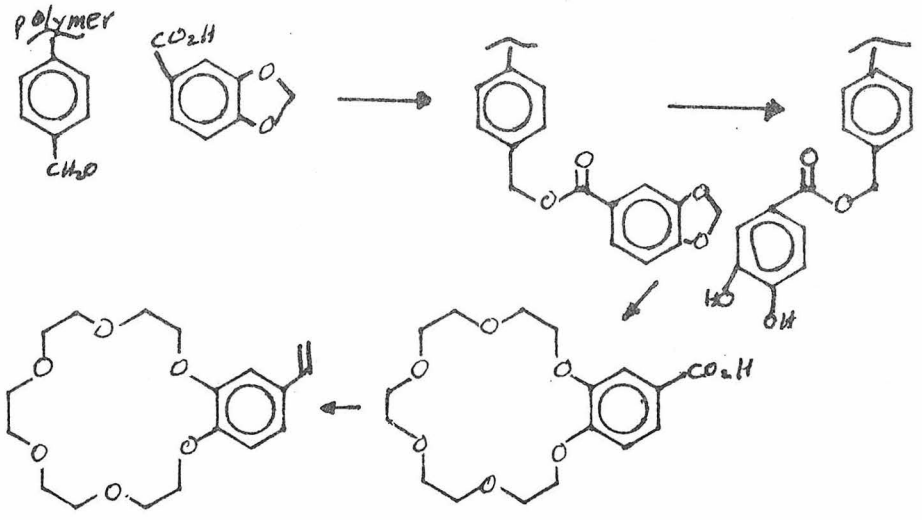


(III)

reaction of 3-4 dihydroxy styrene, with catechol and bis(2-chloromethyl) ether as shown in the diagram.[7] The desired product should make up half the total product. The yield of this reaction to make dibenzo-18-crown-6 is 45%. Thus the total yield of desired product should be about 22%.



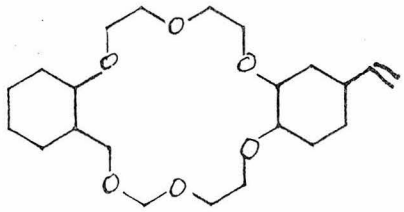
A perhaps more elegant and very fascinating way of preparing a suitable monomer would be to prepare the crown ether from a catechol derivative attached to a solid phase, taking advantage of the immobility of the reactant to increase the yield of desired product. For example, a protected 3,4 dihydroxy benzoic acid could be esterified to a hydroxymethylated styrene-DVB bead using dicyclohexylcarbodiimide, followed by deprotection. Reaction with triethylene glycol ditosylate in the presence of base, followed by cleavage using the standard procedures developed in peptide synthesis (e.g., TFA[10]) would give benzo-18-crown-6 with a carboxyl group at the 4 position. Esterification, reduction with DIBAH, and Wittig would give the required vinyl monomer.



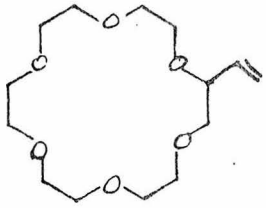
The next problem, reduction of the polymer, is not so amenable to simple solution. There are few ways of reducing an aromatic ring. The most common is catalytic hydrogenation. Unfortunately, an inhomogeneous hydrogenation of an insoluble polymer would not be likely to succeed. However, there is a report of a Ziegler type nickel catalyst which converts benzene to cyclohexane under 70 atm of H_2 at 150-210° in essentially quantitative yield.[19a,b] If this catalyst could be made to convert an aromatic polymeric material into the saturated analogue in high yield, the problem would be solved. If not, other routes are available. Although Birch reduction normally does not lead to saturated compounds, the 1-4 dihydro product out of a Birch could possibly be reduced using tris (triphenyl phosphine) rhodium chloride to yield the saturated polymer. There are drawbacks to this procedure, however, since a possible side reaction in the Birch is the reductive cleavage of the ether[20] which would result in some loss of activity of the polymer.

Although a polystyrene type backbone would be very convenient and could possibly lead to useful product, a method of obtaining saturated polymer from mono-olefinic monomers would be very useful. There are many methods of polymerizing non-activated olefins such as ethylene. The two most common are high pressure free radical polymerization and use of the Ziegler-Natta catalysts ($R_3Al + TiCl_4$). The convenience and applicability to lab scale operations make the use of complex catalysts the preferred method for the purposes of this discussion. As an example of the efficiency of the complex catalysts, linear ethylene may be polymerized at 190-210°, with 0.03% O_2 at 1500 atm to give highly branched polyethylene (low density). Using Ziegler-Natta catalysts

(e.g., $TiCl_4$ and Et_2AlCl in xylene) an all linear, high molecular weight polyethylene may be prepared in about 30 min at room temperature.[14] Poly (vinycyclohexane) has been prepared by reaction of vinylcyclohexane with triisobutyl aluminum-$TiCl_4$.[21] It should be possible to prepare an insoluble, loosely crosslinked, fusable, saturated polymer by reaction of a mixture of vinylcyclohexane-divinylcyclohexane with mono-olefinic monomers containing crown ethers, using Ziegler catalysts. Monomers could be of type IV or V. Evidence indicates that a polyether of type

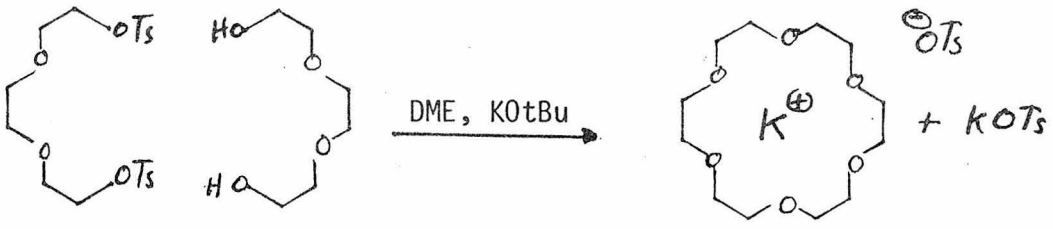


(IV)



(V)

V would be at least as good as a complexing agent for cations as IV.[22] The parent compound of V has been prepared in 93% yield as shown. The reason for the high yield of V is postulated to be the action of the K^+ ion as a template for ring closure. Stability constants indicate that 18-crown-6 complexes Na^+ and K^+ in methanol even better than DCC. The synthesis of monomers of this type should be possible using reactions already well worked out and straightforward.



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Proposition 5

A method is proposed for the isolation of macromolecules responsible for long term memory using competitive hybridization techniques.

In the last two decades a considerable amount of effort has been expended by many workers trying to understand the mechanisms of memory and learning on a molecular scale. Advances have been made, but very little is known about the details of the biochemistry of memory storage and even less is known about retrieval. The literature has been full of reports of results which later proved irreproducible and workers in the field are in agreement on only a very few points. Some scientists feel that the problem is either not amenable to solution at all, or that technology is in much too primitive a state to even begin background work. These arguments, however, sound very much like arguments against the study of the molecular mechanisms of heredity before the double helix was suggested, and though higher nervous function is perhaps the most complex biological phenomenon of all, it seems clear that work in the area today can yield useful and interesting data.

Before continuing, it will be useful to define "memory" and "learning". It has been proposed[1] that the term "learning" not be used in scientific language since different researchers have differing opinions concerning a definition. However, for our purpose the following definitions are of value. Memory is the storage of some type of information which may, under certain conditions, be observed as a behavioral change. Evidence (see below) strongly indicates that there are two kinds of memory:

short term and long term. Short term memory may be detectable only on the order of hours, whereas long term memory is detectable under certain circumstances after very long periods of time (the lifetime of the subject?). Learning will be defined as the aquisition of long term memory.

There have been three relatively successful approaches toward understanding the mechanisms of memory. First is the study of inhibition of memory by application of various chemical or physical agents. It has been shown[2] that generalized convulsions induced either by electric shock or chemical means shortly after training produce a memory deficit in animals. Perhaps the most interesting studies in this general area involve the administration of various antimetabolites which block either the synthesis of RNA (actinomycin D) or protein (cycloheximid) in the brain. This work has been reviewed by Barondes.[3]

The general results of the inhibitor work are as follows. An animal (e.g., goldfish or rat) may learn a specific response by methods of operant conditioning. The learned response is retained for many days. If an inhibitor (protein or RNA) is injected into the brain of a subject during or just after training, the response is retained for up to 6 hours (short term memory), but is then lost. If inhibitors are injected about 1/2 hour after training, there is no memory inhibition. This evidence indicates that there are two memory mechanisms and that long term memory is dependent upon protein synthesis. It must be remembered that these metabolic inhibitors exhibit very complicated activity in organisms besides their primary functions. Even so, it looks as though the conclusions based on these experiments are correct.

A second general approach towards elucidation of the mechanisms of memory is the transfer effect. This is certainly the most controversial of the techniques, and probably one of the most controversial subjects in the life sciences today. The supposition that macromolecule synthesis is necessary for memory is consistent with two basically different mechanisms. One possibility is that the synthetic activity is merely a quantitative increase in the synthesis of molecules and tissues already present in the brain (e.g., new synaptic connections). The other possibility is that the new molecules are qualitatively different from substances already present in the brain and that somehow the structures of the new molecules are related to the learned response. The latter mechanism is necessary to explain transfer of memory. The philosophy behind the transfer experiments is that if the macromolecules responsible for long-term memory are unique, then introduction of these molecules into the brain of a naive subject could cause the subject to "remember" training which he never received. The experiments in general are very similar, differing only in detail. At least two groups of donors and two groups of recipients are used. One donor group is given some sort of training (a conditioned response) and the other donor group is handled in very close to the same manner, but not trained (the control group). Soon after the last training period, the brains of the trained and control donors are withdrawn, and either a whole brain homogenate, or some fraction of the brain matter is introduced into the recipient's brain. This is accomplished in one of four ways--either by feeding, or injection intracranially (IC), intravenously (IV), or intraparitoneally (P). The two recipient groups (trained and control) are then tested for altered

behavior. Statistical analysis is used to show whether the trained and control animals behave differently to a certain probability. (In any test of behavior, differences between two groups of animals may be caused by random chance. Psychologists have worked out tests to determine, with any two groups, what the odds are that differences in behavior are random fluctuations. Usually two groups are deemed statistically different if the odds are about 1 in 100 or less that differences are due to random chance.)

Since the first report of this type of transfer experiment in 1962[4] at least 53 laboratories have performed transfer experiments. James A. Dyal[5] has made what he claims to be an impartial survey of these experimenters, and finds that out of 263 experiments reported (in this field negative results are often published; however, Dyal communicated privately with many of the workers in the field to find out if significant negative results were not published, and found this not to be the case) 115 showed no transfer, 133 showed statistically significant transfer, and 15 were equivocal. Considering the tremendous difficulty of the experiment it is not surprising that reproduction of results is very difficult. The surprising fact is that so many laboratories have reported positive results. It would be very hard indeed to rationalize a theory of memory without taking the transfer phenomenon into account. One is almost forced, against his will, to believe the effect is real. Still, the argument for transfer is of the "overwhelming evidence" type, and no single paradigm has proved to give a robust reproduceable effect in many different laboratories.

Research in the transfer field, once the effect is considered real, is directed towards two goals. Is the transfer specific and what is the chemical agent of the transfer? Much work has gone into elucidation of these points and still no general agreement has been reached. (Indeed, many reputable scientists still doubt the reality of the effect.) The most ambitious work has come from the lab of Georges Ungar, at Baylor College of Medicine, in Houston. In fact, Ungar, using the transfer effect as a biological assay, claims to have succeeded in isolating the substance, a pentadeca peptide, responsible for transfer of the dark avoidance response in rats.[6] In fact, he has deduced the amino acid sequence of the material, called scotophobin, and a total synthesis using the Merrifield technique has been performed.

The experiments are run as follows. Donor rats are trained to fear the dark by conditioning using electrical shock in a dark cage. Rats, when given a choice, will spend more time in a dark part of their enclosure than in a lighted part. However, rats conditioned as above spend much more time in the light. Brains of conditioned and control rats are excized and various fractions injected IP into recipient mice. The recipients are then put in a cage with a dark part and a light part. The amount of time spent in each is recorded automatically. The recipients of trained brain extract spent significantly more time ($p < 0.001$) in the light part of the cage than the recipients of control brain extract. Synthetic scotophobin is as active as the natural scotophobin in causing fear of the dark in rats.

Ungar's experiments seem quite straightforward and conclusive. However, replication of the dark avoidance transfer has been difficult

(though some labs have done it) and it is interesting to note that, while Ungar's article in Nature is 4-1/2 pages long, the critical comments of the referee, which were published as a paper directly following Ungar's paper, are 7-1/2 pages long.

It is easy to see that even assuming the transfer phenomenon is real, use of the effect as a tool to elucidate the mechanism of memory is very difficult. This brings us to the third general approach which has been of considerable value in studying memory. This involves direct studies of chemical changes which occur in the brain during learning. Most of the work in this area has been of the following type. Either an increase in the uptake of radioactively labeled RNA precursors is shown to take place during learning, or differences in base-pair ratios of brain RNA are observed between control animals and recently trained animals. While these experiments elegantly show that indeed synthesis of some RNA species is increased during learning, they do not give a clue as to whether the RNA is unique. Clearly, if transfer is real, and whether RNA, as some claim, or protein as Ungar claims, is the transfer agent, RNA species unique to certain training conditions must be formed in the brain during training. At first thought, direct proof of the existence of such unique RNA's would seem almost impossible, especially when it is remembered that, assuming the scotophobin work is correct, the unique m-RNA species coding for scotophobin would be a mere 45 nucleotides long. However, recent advances in biochemical genetics make proof of the existence of these unique RNA's possible, and I will propose a method of showing their existence and isolating the RNA's in question.

The technique that makes this type of detection and fractionation possible is based upon the following simple argument. Any RNA (and therefore any protein) produced in a cell must be coded for in the genome of the cell, at least according to one prevalent theory. If an RNA molecule with a certain nucleotide sequence is produced in the cell, a complementary sequence must be present in the DNA of the cell. Therefore, if one isolates the DNA of a cell and separates it into single strands, then incubates this DNA with purified RNA from that cell, every RNA species will base-pair with its complementary DNA sequences. This process is called hybridization, and the DNA-RNA double strand is referred to as a hybrid. Incubation of this mixture with radioactively labeled RNA from a similar cell should show no incorporation of radioactivity into the hybrid molecules, since all the complementary DNA sequences corresponding to the labeled RNA are complexed with cold RNA. However, if labeled RNA from a cell containing any different RNA species is incubated with the DNA mixture, the new RNA species will bind to their (unoccupied) complementary sites on the DNA, causing radioactive label to be incorporated into the hybrid. This is, in principle, a very sensitive and specific test for unique RNA molecules (see Fig. 1). In 1965 a method for the quantitative assay of DNA-RNA hybrids was described[7] based on the fact that single stranded DNA will bind and be immobilized by a nitrocellulose filter, whereas RNA will not. One merely passes a solution of single stranded DNA through the filter. The DNA sticks and is, in effect, held rigid on a solid phase. The entire filter is incubated in a solution of labeled RNA which is to be tested. The filter is then washed and counted in a scintillation counter.

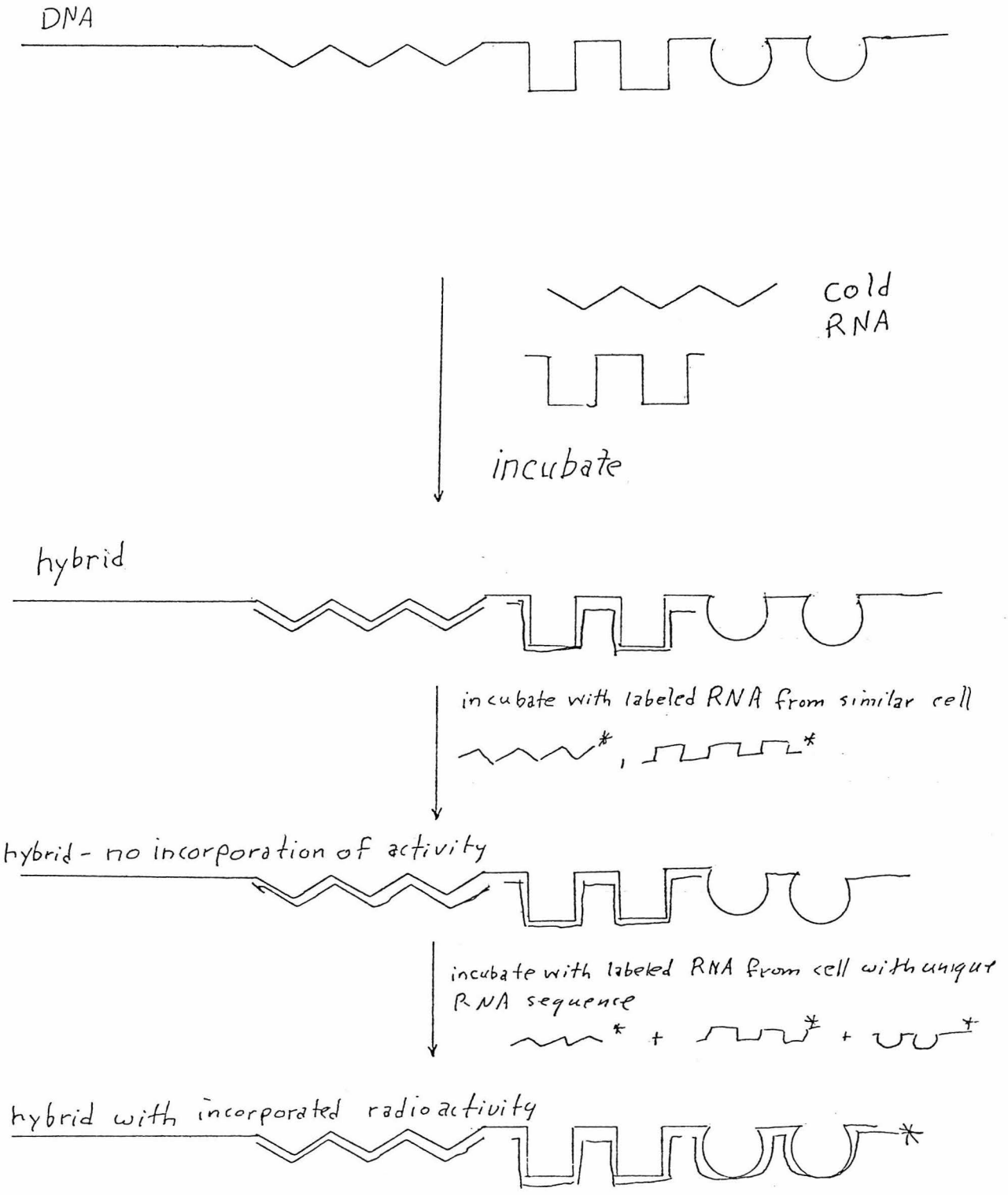
DNA
cold RNA
incubate
hybrid
incubate with labeled RNA from similar cell
hybrid - no incorporation of activity
incubate with labeled RNA from cell with unique RNA sequence
hybrid with incorporated radioactivity

Fig. 1

The hybridization reaction obeys second order kinetics and some of the RNA species may be very dilute. For this reason the time of reaction is critical. It is important to incubate the mixture until saturation of the DNA occurs, that is, until every sequence of DNA which is represented by an RNA molecule in the solution has been bound.

In 1968 Machlus and Gaito published a series of three papers[8a,b,c] describing the use of this technique to prove the existence of unique RNA species formed during learning. They followed the procedure of Fig. 1, called successive competition hybridization. Rats were divided into three groups. One group was given shock avoidance training; the other two were given shocks but could not escape from the shock chamber, and therefore no avoidance training resulted. Unlabeled orotic acid was injected IC into one control group, and labeled orotic acid was injected IC into the other control group and the trained group. After the training the rats were killed, their brains were removed, and DNA extracted and purified. This DNA was put on nitrocellulose (50 µg). RNA was extracted and purified and the competition performed (50 µg of RNA in solution was used and incubation was performed on the order of hours). Results indicated that a sizeable proportion (about 37%) of the brain RNA in shock avoidance trained rats was unique.

The above described results were received with enthusiasm by workers in the field. However, in 1971 Kern von Hungen, from Berkeley, reported that his own data and other reports in the literature indicated that Machlus' values were "unrealistic and incorrect".[9] Von Hungen repeated Machlus' work and showed that under those conditions the DNA was far from saturated. (In fact, the latest evidence indicates that long

DNA, bound to a solid phase and hybridized with equal amounts of RNA would probably not reach saturation in a year.) Later in 1971 Gaito et al. published a retraction of their previous work[10] admitting that their findings were possibly due to artifacts. Recently it has been shown[11] that with IC injections of labeled precursors into rat brain an unequal distribution of label is obtained. This unequal distribution is undesirable because in the cells of higher organisms there is a type of RNA synthesized in the cell which turns over very rapidly and never leaves the nucleus.[9] Slight changes in precursor pools in the cell could cause different amounts of labeling to occur giving rise to quantitatively false values in a Machlus type experiment. This is probably the reason for the false conclusions reached by Machlus and Gaito. Another general fault of the competitive hybridization experiment pointed out by von Jungen[9] is the fact that since the genome in the cells of higher organisms contains a large fraction of repetitive sequences[12] (10% of mouse DNA is present as a segment of about 300 nucleotides repeated about one million times in a single cell), saturation may be difficult if not impossible.

Techniques are now available which should lead to extremely sensitive tests for the presence of unique RNA's using competitive hybridization. First, it was shown by Kottler[11] that IV injection of labeled precursors leads to equal distribution in the brain. Second, and most important, it has become possible to separate the repetitive sequences of the genome from the single copy DNA.[12] This is accomplished by taking advantage of two very powerful tools. First, a special type of calcium phosphate (hydroxyapatite, or HAP) when used as column packing, binds double stranded DNA but not single strands. Second, the rate of

renaturation of repetitive sequences is much faster than the rate of renaturation of single copy DNA. In fact, the number $C_o t_{1/2}$, where $t_{1/2}$ is the time to reach 1/2 renaturation and C_o is the initial concentration of DNA, is a constant characteristic of different types of DNA, dependent upon the length of non-repetitive segments. Thus in order to separate repetitive and non-repetitive DNA's one first shears the DNA to about 400 nucleotides in length. The DNA is then heated to above T_m and allowed to cool to T_m-25°. At a certain time essentially all of the repetitive sequences will be double stranded and all the unique sequences will still be single stranded. HAP chromatography then separates the double stranded, repetitive DNA from the single stranded, unique DNA.

This unique DNA could be used in a competitive hybridization experiment similar to Machlus', using pulse-labeled RNA. However, Grouse[13] has reported a much improved procedure for detection of the amount of hybridization which takes place in experiments of this nature. DNA is highly labeled, sheared to a M.W. of about 1.7×10^5 (about 500 nucleotides long) and the unique sequences isolated. RNA is then added in great excess (about 1000-fold) and after saturation (about 4 days) HAP chromatography separates any DNA which hybridized (i.e., which contained sequences corresponding to the RNA present) from that which remained single stranded. Both fractions are counted, and the percent DNA hybridized is calculated.

Grouse used pulse labelling techniques to label his DNA with H^3. A procedure which is an improvement over that of Grouse is to label the DNA with I^{125}. Iodine reacts with the double bonds of cytosine. Reaction of I^{125} ($T_{1/2}$= 57 days) with only 1% of the cytosine in a sample can result in DNA with a specific activity of 2×10^7CPM/µg. Also, when the

I^{25} does decay it leaves the cytosine intact.[14]

Combining these procedures we have an extremely sensitive method for determining whether a unique RNA is produced during learning. The experiment would be as follows. Rat DNA from the strain chosen for the experiment is purified, sheared, and the unique sequences isolated. This DNA is treated with I^{125} to give DNA of very high specific activity. The test rats are divided into two groups, learning (L) and nonlearning (NL). The rats are raised in as close to identical conditions as possible, then the L group is subjected to dark avoidance training. The NL group is shocked, but not trained. Immediately after training both groups are killed, the brains excised, and RNA removed. Note that no injections of labeled precursors are necessary.

The RNA_{NL} is then hybridized with the hot DNA using a 100-1000-fold excess of RNA. Aliquots are removed and HAP chromatography is used to determine the percent of double strands. After saturation is reached (about 4 days) the entire reaction mixture is chromatographed on HAP. As a test, the hybridization procedure may be repeated using RNA_{NL}. No activity should remain on the column after HAP chromatography. If this is true, then the single stranded DNA coming off the column contains no sequences complementary to any RNA_{NL}. This DNA is then hybridized to the RNA_L. If any activity remains on the column after HAP chromatography this will be due to unique RNA's which were present in the trained rat brain. Note also that we have effectively separated this unique RNA from all other RNA's that were present.

This procedure could be performed with many different training tasks, levels of training, etc. in a relatively rapid, efficient manner.

If it was found that unique RNA's were indeed formed during various learning experiences, it would be very interesting to be able to compare these RNA's. Since nucleotide sequencing is extremely tedious I feel that the following alternative might be tried first.

The double stranded DNA-RNA hybrids containing the unique RNA may be treated with a DNase specific for single stranded DNA.[15] This would leave a small, double stranded DNA-RNA hybrid which could be melted, and the DNA separated by filtration on nitrocellulose. This DNA would contain the complementary sequences of the specific learning RNA to which it was originally bound. Other learning RNA's could then be tested for ability to hybridize with this DNA. If the other RNA's are close to the first, considerable hybridization should take place. A semi-quantitative measure of the extent of hybridization would be obtained by study of the melting curves of the various hybrids.

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