CHARGE-TRANSFER ASSOCIATION IN ENZYME INHIBITION

Thesis by

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DEDICATION

To my devoted wife Eileen, whose

love and patience lighted the way.

ACKNOWLEDGMENTS

To the late Dr. Carl Niemann, whose untiring dedication to the science of chemistry provided the initial inspiration for this work and who unknowingly awakened my desire for the broader career of librarianship.

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Abstract

Attempts to determine if charge-transfer bonding forces are involved in enzyme inhibition by gas chromatographic and spectral methods were, in general, unsuccessful.

Direct spectral evidence of formation of the β-naphthoquinolinium cation in the presence of the enzyme, α-chymotrypsin, has been shown.

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INTRODUCTION

For over a century it has been recognized that a variety of aromatic compounds can undergo additive combination with acidic aromatic compounds such as: picric acid (1), polynitroaromatics (2, 3, 4) and quinones (5), to produce stable, isolable, molecular compounds.

The extensive work of Sudborough (2) on the addition compounds of picric acid led to their use in the characterization of polynuclear aromatic hydrocarbons and aromatic ethers (6). Moreover, work with many substituted and unsubstituted aromatic compounds showed that the aromatic nucleus of the donor, or basic addend, was the dominant factor in complex formation (2). The early work of Hildebrand on the colors of iodine solutions led to discovery of non-isolable complexes formed by the interaction of iodine with solvent (7).

Studies of the ultraviolet spectra of solutions of these complexes have been conducted since 1925 (8). In each case a spectrum resulted which was similar enough to that obtained by adding the spectra of the components that it was erroneously concluded that the complex was completely dissociated in solution. Although the color of the complex was invariably different from those of the separate components and corresponded to longer wavelength absorption, no systematic study of the visible spectra was undertaken.

In 1949, the classic paper by Benesi and Hildebrand (9) on the interaction of iodine and aromatic hydrocarbons appeared. Contrary to prior spectrophotometric investigations a strong new ultraviolet

absorption band was found in the spectra of iodine solutions of aromatic compounds which was absent in those of iodine-carbon tetrachloride solutions. Benesi and Hildebrand attributed the anomalous absorption to complex formation and were able to determine the equilibrium constants and extinction coefficients for complexes of iodine with some of the C_6 - C_9 aromatic hydrocarbons.

Assuming that the complex was formed by direct interaction of the acidic and basic components and that it existed in equilibrium with them, Benesi and Hildebrand proposed the following analysis of the observed spectra:

$$A + B \rightleftharpoons AB \tag{1}$$

$$K = \frac{[AB]}{[A_0 - AB][B_0 - AB]} \tag{2}$$

where AB = concentration of complex at equilibrium

A_0 = initial concentration of acidic component

B_0 = initial concentration of basic component

Experimentally, the initial concentration of B was much larger than that of A, so that $[B_0 - AB] \approx B_0$. With the assumption that the absorption followed Beer's Law,

$$D = \Sigma b\,[AB] \tag{3}$$

where D = optical density

Σ = molar extinction coefficient

b = optical path length

equation 4 may be derived,

$$\frac{A_0 b}{D} = \frac{1}{K\Sigma B_0} + \frac{1}{\Sigma} \tag{4}$$

A plot of A_0b/D vs $1/B_0$ would give a linear relationship with a slope of $1/K\Sigma$ and an intercept of $1/\Sigma$. Later, Scott (10) proposed a modification to increase the accuracy of measurements at low concentrations, by rearrangement of equation 4 to give 5.

$$\frac{A_0 B_0 b}{D} = \frac{B_0}{\Sigma} + \frac{1}{K\Sigma} \tag{5}$$

A plot of A_0B_0b/D vs B_0 offers the advantage of extrapolation through regions of low concentration.

Since this time, numerous spectrophotometric investigations have appeared and have been tabulated in an excellent review by Brieglieb (11).

The theoretical basis for understanding the spectra of these complexes was advanced by Mulliken (12). In general agreement with Benesi and Hildebrand, Mulliken suggested that the complexes result from "an acid-base interaction, in the electron-donor sense, in which the iodine functions as the acid or electron acceptor." Mulliken considered the interaction of a no-bond ground state ψ_0 (A, D) and a polar excited state, ψ_1 (D^+, A^-), to produce a stabilized ground state having a wave function

$$\psi_n = a\psi_0 (D, A) + b\psi_1 (D^+, A^-) \tag{6}$$

and an excited charge-transfer state with the wave function

$$\psi_{ct} = a\psi_1 (D^+, A^-) + b\psi_0 (D, A) \quad (7)$$

The anomalous ultraviolet or visible absorption is associated with the electronic transition $\psi_n \rightarrow \psi_{ct}$. The bonding in the ground state is the sum of the van der Waals forces and the resonance energy of interaction between ψ_0 and ψ_1, while the bonding in the charge-transfer state is mainly due to the large electrostatic energy of interaction between D^+ and A^-.

A good approximation (13) to the energy of the charge-transfer transition is given by

$$h\nu_{ct} = I_D - E_A + C \quad (8)$$

where I_D = ionization potential of the donor

E_A = electron affinity of the acceptor

C = the mutual electrostatic energy of D^+, A^- relative to D, A

The approximation involved is that the resonance energy of interaction between ψ_0 and ψ_1, which increases the energy of the charge-transfer transition, is minimal. The validity of this assumption has been demonstrated by McConnell and Dewar (14, 15, 16) for some aromatic systems which are described as weak or π complexes.

As was later pointed out by Dewar (17), since the heats of formation of these complexes are at least an order of magnitude lower than their lowest transition energies, the changes in energy of the

orbitals in forming the complex are small. Thus, the ultraviolet spectra of the components of the complex approximate those of the separate components. The appearance of a new band of lower energy corresponds to the excitation of an electron from a filled orbital of the donor to a anti-bonding orbital of the acceptor. Since the orbital energy is a function of the basicity of a compound, the anti-bonding orbitals of the acceptor will be associated with lower energy values than those of the donor and the charge-transfer transition will occur at longer wavelengths than intramolecular transitions.

An example of strong complexes is given by the interaction of amines and iodine (18). Here, the non-bonding electrons of the amine nitrogen are involved, and the resulting close approach causes a large ground-state interaction between ψ_0 (A, D) and ψ_1 (A^-, D^+), which prevents simplified analysis.

Strong complex formation is also possible with polynuclear aromatic donors which possess an active "K region" which is not accompanied by a strong "L region" (19).

The existence of charge-transfer association in biological systems has been shown by Kosower in work on solutions of N-alkylpyridinium halides (20).

Later work, with DPN and TPN and with the model compound 1-benzyl-3-carboxamidopyridinium chloride, revealed that charge-transfer complex formation occurred with indole derivatives and chymotrypsinogen, the precursor of α-chymotrypsin (21).

Studies on the nature of enzymatic inhibition have shown, in the case of α-chymotrypsin, that aromatic compounds are more effective, by an order of magnitude, than their saturated analogs in binding with the enzyme (22).

The research discussed in this thesis is predicated on the assumption that charge-transfer bonding forces are involved in enzyme inhibition. The strong acceptor, pyromellitic dianhydride (PMDA), was initially selected as a model for the active site of the enzyme. Comparison of the charge-transfer equilibrium constants of the known inhibitors and the model compound with their enzyme inhibition constants could show if charge-transfer bonding forces play a dominant role in the inhibition of α-chymotrypsin. The determination of charge-transfer equilibrium constants was pursued by two distinct methods. The choice of the C_6 - C_8 aromatic compounds for the gas chromatographic method of Muhs and Weiss (23) and the unsubstituted aza-aromatic compounds for the spectroscopic method of Benesi and Hildebrand (9) was necessitated because of the suitability of analyzing sterically similar compounds and the undesirable experimental conditions necessary for gas chromatographic analysis of the latter class of compounds.

GAS CHROMATOGRAPHY

Results and Discussion

As a prelude to the determination of equilibrium constants by gas chromatography, the separation of the isomeric C_8 aromatic compounds was required for the requisite experimental refinements.

This separation was attempted with four systems:

A)	squalane - PMDA	Fig. 1a
B)	diglycerol - PMDA	Fig. 1b
C)	α-chloronaphthalene - PMDA	Fig. 1c
D)	α-chloronaphthalene	Fig. 1d

Table I gives the relative separation factor (33), which is defined as follows, for each of the four systems.

$$S_{12} = \frac{T_2 - T_1}{T_1} \tag{9}$$

T refers to the retention time of a specific compound or mixture and $T_2 > T_1$.

In system A, as the column aged, the absolute retention times and the separation factor decreased, which indicates that the added PMDA has no effect. The lack of effect may be ascribed to the lack of solubility in squalane.

The very low retention times observed in system B are no doubt due to the limited solubility of the aromatic compounds in diglycerol. The general shape of the recorder response curve is that invariably

TABLE I. Gas chromatographic separation factors for equimolar o, m and p-xylene mixtures.

System	T_2	T_1	S_{12}
A	m-xylene	p-xylene	0
A	o-xylene	m, p-xylene	0.176
B	m-xylene	p-xylene	0
B	o-xylene	m, p-xylene	0
C	m-xylene	p-xylene	0.066
D	m-xylene	p-xylene	0.059

found for gas-solid systems, where the separation is due to adsorptive rather than absorptive processes.

The use of α-chloronaphthalene as a liquid support has been previously described by Zlatkis (24) and work in this laboratory confirms his findings. The addition of PMDA to the α-chloronaphthalene support results in an increased value of the separation factor for m- and p-xylene. The limited effect of the added PMDA is due to charge-transfer complex formation between α-chloronaphthalene and PMDA, which necessarily dilutes the effect of the added PMDA. The experiments using α-chloronaphthalene were further complicated by thermal effects. A column temperature of 50°C was required for reasonable retention times and at this temperature the α-chloronaphthalene was sufficiently volatile to prevent reproducible results. The procedure for determination of charge-transfer equilibrium constants (23) requires experimental conditions which remain relatively constant during extensive usage.

Conclusions

The attempt to determine charge-transfer equilibrium constants by gas chromatography is not feasible for high molecular weight compounds. The experimental necessity of using a liquid support in which the π-acid and aromatic mixture are soluble limits the choice to a π-base which necessarily dilutes the acid strength. The use of lower molecular weight π-acids may obviate this problem.

Experimental

Pyromellitic Dianhydride was obtained from du Pont and purified by sublimation at 170° and 70μ Hg. The infrared spectrum of the purified material, which melts at 286°, is shown in Fig. 5. The resublimed PMDA develops a yellow color if the sublimation temperature rises above 175°. Hydrolysis of the purified material followed by titration to the phenolphthalein end point gave a molecular weight of 254.8 compared to the theoretical value of 254.2.

Squalane, Diglycerol and α-Chloronaphthalene were obtained commercially and used without further purification.

o, m and p-Xylene were Eastman reagent grade and were used without further purification.

Chromosorb (80-100 mesh) was obtained from Johns-Mansville and dried at 110° for four hours prior to use.

The squalane and diglycerol columns were prepared by the usual technique of mixing with a easily evaporable solvent. The α-chloronaphthalene was added to the solid support without solvent and mixed by rotation for one week.

An Aerograph A-600A chromatographic system was employed using a hydrogen-flame detector and a Hamilton microliter syringe for sample introduction.

LEGEND TO FIGURES

Fig. 1. Gas Chromatograms of Equimolar Xylene Mixtures

1a. 10% PMDA, 20% Squalane w/w 80-100 Chromosorb

1b. 5% PMDA, 10% Diglycerol w/w 80-100 Chromosorb

1c. 1.8% PMDA, 15% α-Chloronaphthalene w/w 80-100 Chromosorb

1d. 15% α-Chloronaphthalene w/w 80-100 Chromosorb

Fig. 2. Infrared Spectrum of Resublimed PMDA (nujol)

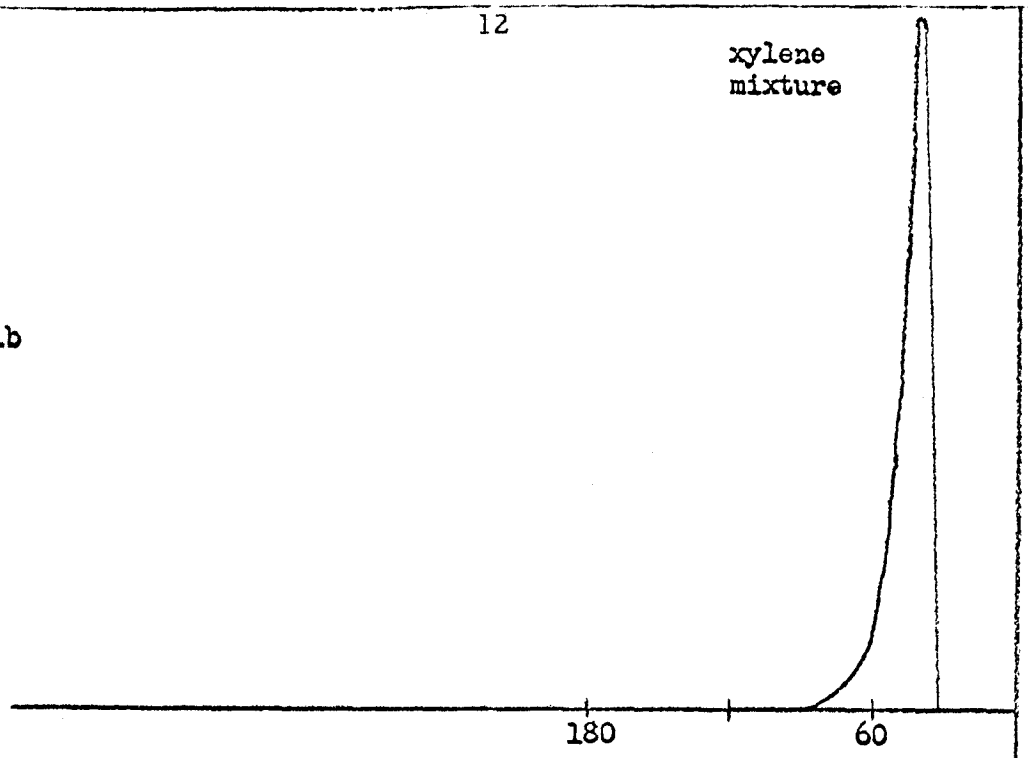

xylene
mixture
180
60
Response
Recorder

Fig. 1b

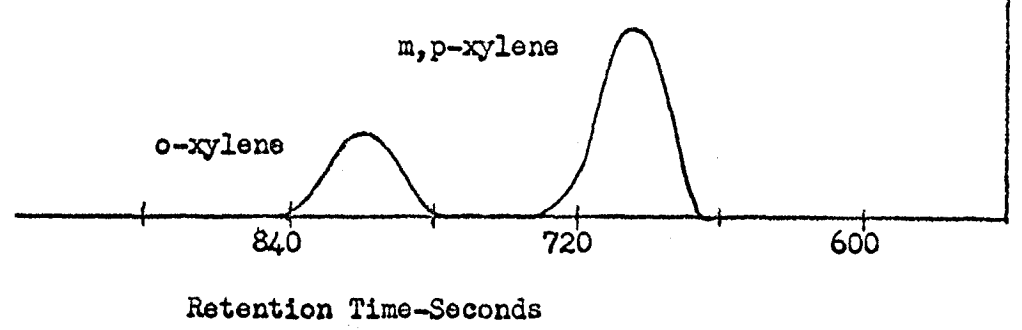

m,p-xylene
o-xylene
840
720
600
Retention Time-Seconds

Fig. 1a

Fig. 1c

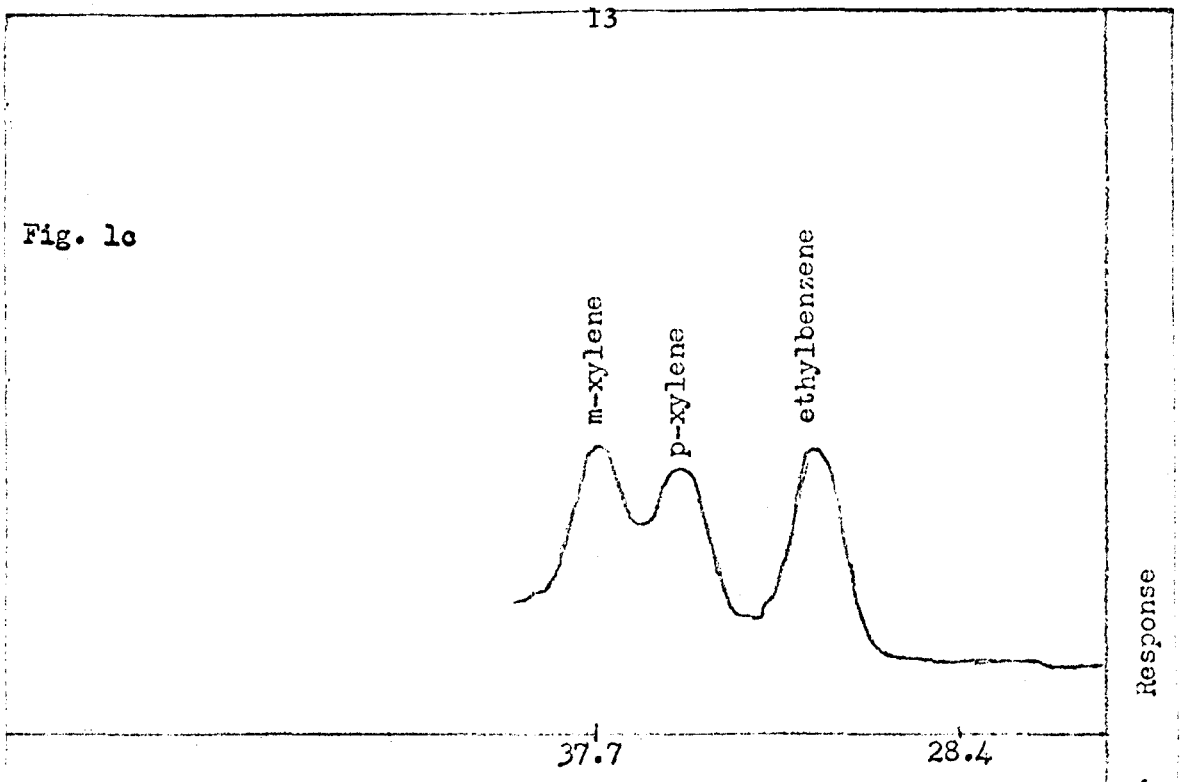

m-xylene
p-xylene
ethylbenzene
37.7
28.4
Recorder Response

Fig. 1d

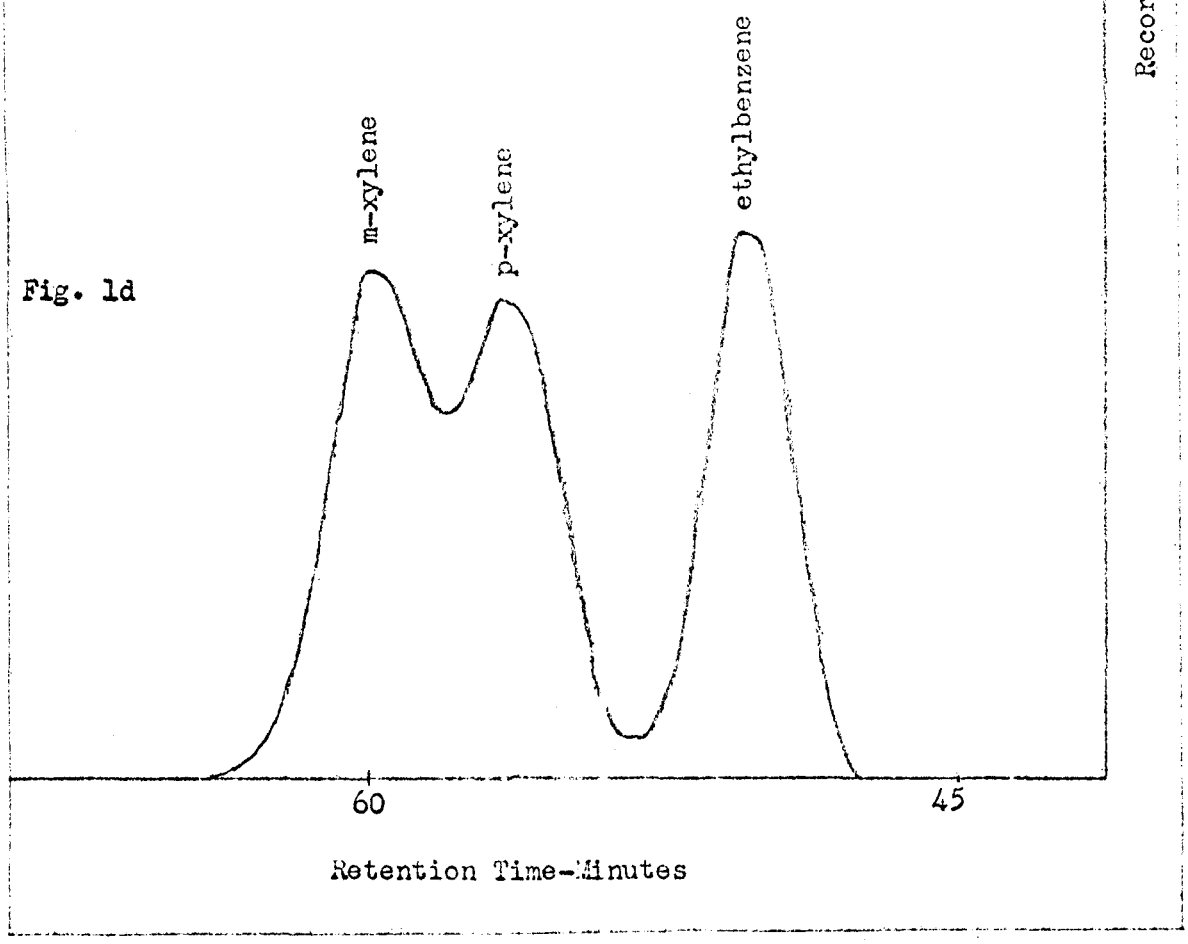
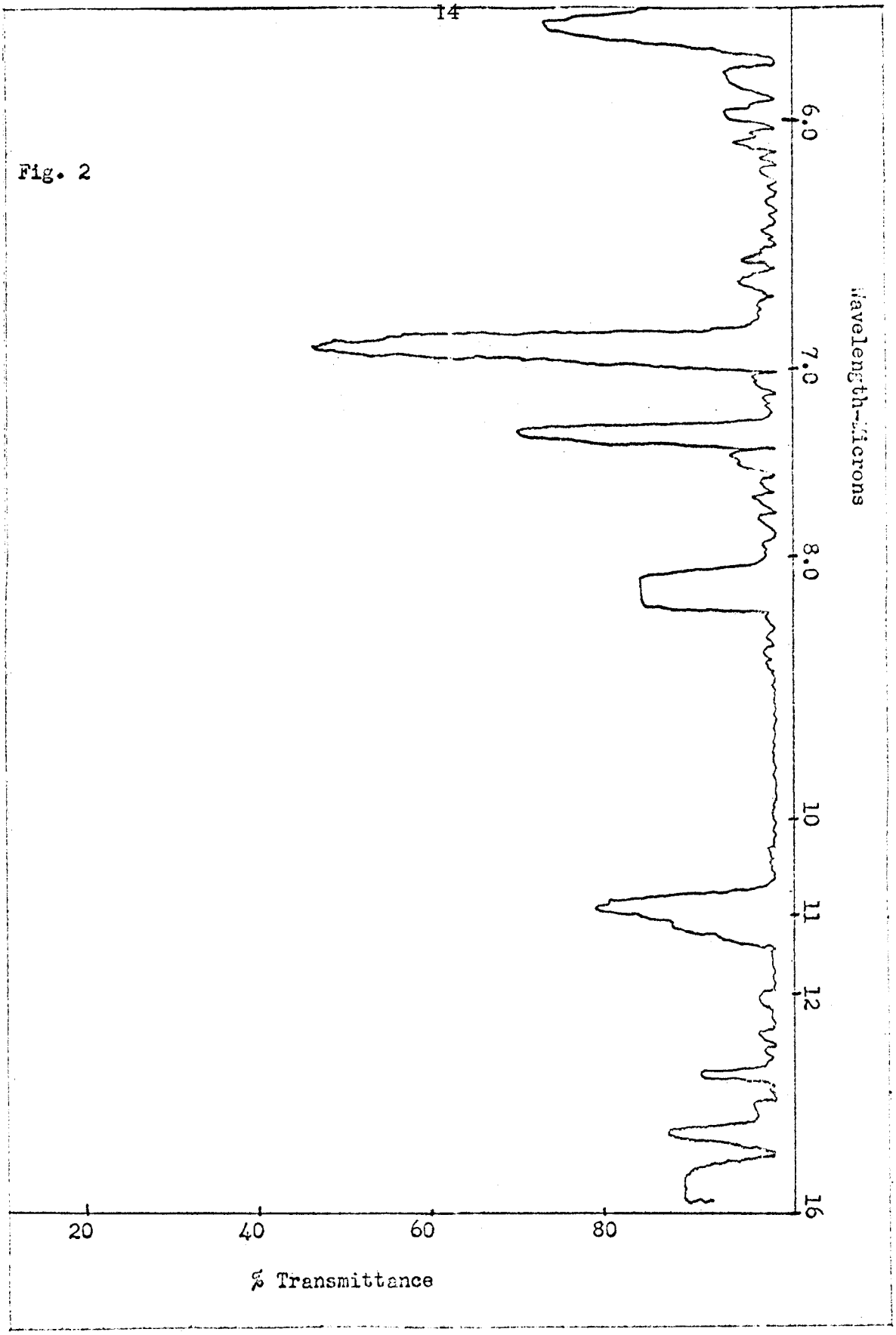

m-xylene
p-xylene
ethylbenzene
60
45
Retention Time-Minutes

Fig. 2


6.0
7.0
8.0
10
11
12
16
Wavelength-Microns
20
40
60
80
% Transmittance

SPECTRAL STUDIES

Results and Discussion

Prior to studies investigating charge-transfer complexes of PMDA, the spectrum of PMDA was examined in a variety of solvents. The long wavelength absorption spectrum appeared as a doublet in chlorinated hydrocarbon solvents (Fig. 3) and as a shoulder in oxygenated solvents (Fig. 4). The effects of solvent on the long wavelength ultraviolet absorption of PMDA are shown in Table II.

TABLE II. Solvent effects on the ultraviolet spectrum of pyromellitic dianhydride.

Solvent	λ_{max}	λ_{max}	Σ_{max}	Figure
1, 2 dichloroethane	316	306		3a
CH_2Cl_2	316	304	3330	3b
CCl_4	312	301		3d
$CHCl_3$	307	299		3c
THF	306		2500	4
DMF	295		2460	4
H_2O	295		2310	4

At shorter wavelengths, PMDA exhibits very strong absorption with a maximum at 257 mμ in CH_2Cl_2. This absorption may be ascribed to a $\pi \rightarrow \pi^*$ transition (25). Analysis of the long wavelength absorption is complicated by the multitude of possible electronic excitations.

The work of Birnbaum (26) on intramolecular charge-transfer and $n \rightarrow \pi^*$ transitions in ketones indicate that absorption due to these effects will occur in the observed range. The spectra in non-chlorinated solvents may additionally be complicated by intermolecular charge-transfer interaction with solvent.

In general, the long wavelength, low intensity, absorption of carbonyl groups is due to $n \rightarrow \pi^*$ transitions, while in anhydride groups the $n \rightarrow \sigma^*$ transitions are possible for singly bonded oxygen atoms. The solvent effects on the transition are directly related to the hydrogen bonding ability of the solvent (25).

As shown in Table II, the energy required for electronic excitation in PMDA solutions increases with a corresponding increase in hydrogen bonding ability. The anomalous position of the spectral bands in CCl_4 may be ascribed to the fact that this compound is devoid of hydrogen atoms and thus is not a member of this quasi-homologous series of hydrocarbons.

Solutions of PMDA-isoquinoline gave anomalous absorption bands at 331 mμ in CH_2Cl_2 and at 326 mμ in DMF (Fig. 5). Attempts to determine if this absorption was due to a charge-transfer transition were prevented by the long wavelength absorption of isoquinoline which has a maximum at 319 mμ in CH_2Cl_2 (Fig. 6). These solutions had no visible coloration.

Owing to the unsuitability of PMDA as an acceptor in the study of the aza-aromatic inhibitors which may in part be ascribed to its absorption above 315 mμ and the additional possibility that charge-

transfer in these basic compounds involves the non-bonding nitrogen electrons, a new model acceptor was chosen. Tetracyanoethylene (TCNE), which has two electron-deficient carbon atoms, is susceptible to charge-transfer complex formation with donors having loosely held non-bonding electrons.

The ultraviolet spectrum of TCNE in CH_2Cl_2 shows a very strong doublet with maxima at 277 mμ and 267 mμ and extinction coefficients of 13,400 and 14,900 respectively (Fig. 7).

Solutions of TCNE-isoquinoline, TCNE-quinoline and TCNE-β-naphthoquinoline in CH_2Cl_2 solvent were prepared and analyzed by the method of Benesi and Hildebrand (9) for charge-transfer complex formation. The results are shown in Table III.

TABLE III. Absorption maxima of various aza-aromatic TCNE charge-transfer complexes.

Donor	Figure	λ_{max}	λ_{max}	λ_{max}	Color
isoquinoline	8a		418	398	yellow
quinoline	8b	510	417	398	orange
β-naphthoquinoline	8c	508	417		orange
pyridine (27)			422	400	yellow

The 422 mμ absorption of the TCNE-pyridine solution follows a Benesi-Hildebrand relationship (27), as does the 418 mμ absorption of the TCNE-isoquinoline solution. The solutions of TCNE and quinoline and β-naphthoquinoline do not reveal a Benesi-Hildebrand relationship.

The equilibrium constants and extinction coefficients are given in Table IV.

TABLE IV. Equilibrium constants and extinction coefficients of TCNE-pyridine and TCNE-isoquinoline charge-transfer complexes.

Donor	λ_{max}	K_{eq}	max	T, °C
pyridine (27)	422	12.0	10,500	22°
isoquinoline	418	512.0	6,360	25°

The Benesi-Hildebrand plot for the TCNE-isoquinoline solution is shown in Fig. 13 and the data is given in Table V.

TABLE V. Concentration and spectral data for the TCNE-isoquinoline charge-transfer complex.

[TCNE]	$X_{isoquinoline}$	Optical Density
8.96×10^{-5}	1.43×10^{-2}	0.165
1.45×10^{-4}	12.62×10^{-2}	1.035
9.37×10^{-5}	24.84×10^{-2}	0.580
8.74×10^{-5}	50.96×10^{-2}	0.565

The anomaly of decreasing extinction coefficients with increasing equilibrium constants has been reported frequently and is discussed by Murrell and McConnell (14-28).

The appearance of a new band in the spectrum of TCNE-quinoline that was not present in the TCNE-isoquinoline spectrum is difficult to explain. Pullman and Pullman (29) report that the difference in the energies of the highest occupied molecular orbitals of the aromatic compounds is only 0.045 β. Moreover, Krishna and Chowdhury (30) in studies on the charge-transfer spectra of iodine and the unsubstituted aza-benzenes, found that the λ_{max} varied by only 11 mμ, which would indicate a narrow range of ionization energies for the non-bonding nitrogen electrons.

An estimate for the maximum absorption of the n-π complexes of TCNE-pyridine can be obtained by the method of Batley and Lyons (31); it occurs at 291 mμ. Moreoever, the absorption maximum of the TCNE radical anion in tetrahydrofuran has been reported at 430 mμ suggesting that this species may be responsible for the 510 mμ absorption reported above.

Conclusions

The determination of the charge-transfer equilibrium constants for a series of aza-aromatic inhibitors by the method of Benesi-Hildebrand using the very strong inhibitors, PMDA and TCNE, is not feasible due to abnormal side reactions. The further study of the complexes which absorb at 510 mμ might be possible by investigation of their EPR absorption.

The significance of charge-transfer bonding in enzyme inhibition is evidenced by the similarity of the ratios of the free energies of dissociation of the pyridine- and isoquinoline-TCNE charge-transfer complexes to the ratio of the free energies of the corresponding enzyme-inhibitor complexes (22).

TABLE VI. Enzyme-inhibitor and charge-transfer complex dissociation constants for pyridine and isoquinoline and the ratios of the corresponding free energies.

Compound	K_{ct}	K_I	$(\Delta F/\Delta F)_{ct}$	$(\Delta F/\Delta F)_I$
pyridine	0.083	27.0	1.63	1.94
isoquinoline	0.0019	0.32		

Enzymatic Studies

Following the work of P. R. Hammond (32) on the acridine-acridinium cation charge-transfer complex, in which it was shown that the acridinium cation had an acceptor strength comparable to TCNE, the possibility that, in the special case of interaction with aza-aromatic inhibitors, α-chymotrypsin was in fact acting as the donor in forming a charge-transfer complex was considered.

Solutions of α-chymotrypsin with and without added β-naphthoquinoline, a very effective inhibitor (22), were prepared using water 0.1 N in NaCl as the solvent.

The spectrum of α-chmyotrypsin showed a shoulder at 290 mμ and a peak at 282 mμ (Fig. 16).

The solvent dependence of the ultraviolet absorption of β-naphthoquinoline is given in Table VII.

TABLE VII. Solvent dependence of the ultraviolet absorption of β-naphthoquinoline.

Solvent	λ_{max}	λ_{max}	Figure
H_2O	345	330	11a
CCl_4	346	328	11b
5% H_2SO_4	360		11c

The difference spectrum of β-naphthoquinoline and α-chymotrypsin at pH 7 shows absorption from both β-naphthoquinoline and the β-naphthoquinolinium cation (Fig. 20). Since β-naphthoquinoline does

not exist in the protonated form at pH 7 the results here indicate that the enzyme induces formation of significant quantities of the cation.

Experimental

Pyromellitic Dianhydride was obtained commercially and purified as previously described.

Tetracyanoethylene was obtained from Eastman Kodak Company and purified by sublimation at 100° (70 μ Hg) or by preparation of the charge-transfer complex with chlorobenzene and subsequent removal of the chlorobenzene at low pressure.

Quinoline was obtained from Eastman and redistilled. The material collected boiled at 34-35° (70 μ Hg). The ultraviolet spectrum is shown in Fig. 13.

Isoquinoline was obtained from Eastman and redistilled. The material collected boiled at 45° (80 μ Hg). The ultraviolet spectrum is shown in Fig. 6.

β-Naphthoquinoline was obtained from K & K Laboratories and was recrystallized from 0.1 N aqueous acetic acid.

Methylene Chloride was Eastman spectroscopic grade, as were the other solvents.

The spectra were recorded either on a Cary model 11 or model 14 spectrophotometer. Matched 10-mm silica cells were used. The solutions were prepared in volumetric flasks. The liquids were weighted into the flasks on a Mettler balance and the solids were weighed on an analytical balance.

LEGEND TO FIGURES

Fig. 3. Ultraviolet Spectra of Pyromellitic Dianhydride in Chlorinated Hydrocarbon Solvents.

3a. PMDA (saturated) - 1, 2 Dichloromethane

3b. PMDA (3.45×10^{-4} M) - Methylene chloride

3c. PMDA (saturated) - Chloroform

3d. PMDA (saturated) - Carbon tetrachloride

Fig. 4. Ultraviolet Spectra of Pyromellitic Dianhydride in Oxygenated Solvents.

PMDA (6.28×10^{-4} M) - H_2O

PMDA (5.9×10^{-4} M) - DMF

PMDA (6.8×10^{-4} M) - THF

Fig. 5. Ultraviolet Spectra of PMDA-Isoquinoline Solutions in Methylene Chloride and Dimethylformamide.

PMDA (2.57×10^{-4} M) Isoquinoline (1.06×10^{-1} M) - CH_2Cl_2

PMDA (5.1×10^{-4} M) Isoquinoline (1.13×10^{-2} M) - DMF

Fig. 6. Ultraviolet Spectrum of Isoquinoline (10^{-4} M) in Methylene Chloride Solvent.

Fig. 7. Ultraviolet Spectrum of Tetracyanoethylene (7.46×10^{-5} M) in Methylene Chloride Solvent.

Fig. 8. Ultraviolet Spectra of TCNE-Aza Aromatic Hydrocarbon Solutions in Methylene Chloride Solvent.

8a. TCNE (9.4×10^{-5} M) Isoquinoline (6.4×10^{-2} M) - CH_2Cl_2

8b. TCNE (9.0×10^{-5} M) Quinoline (3.1×10^{-2} M) - CH_2Cl_2

8c. TCNE (1.2×10^{-4} M) β-Naphthoquinoline (10^{-2} M) - CH_2Cl_2

Fig. 9. Benesi-Hildebrand Plot for the TCNE-Isoquinoline Complex.

Fig. 10. Ultraviolet Spectrum of α-Chymotrypsin in 0.1 N NaCl (H_2O) Solvent.

Fig. 11. Ultraviolet Spectra of β-Naphthoquinoline in Various Solvents.

11a. β-Naphthoquinoline (1.16×10^{-4} M) - 0.1 N NaCl (H_2O)

11b. β-Naphthoquinoline (2.65×10^{-4} M) - CCl_4

11c. β-Naphthoquinoline (7.95×10^{-5} M) - 5% H_2SO_4 (H_2O)

Fig. 12. Ultraviolet Difference Spectrum of β-Naphthoquinoline (10^{-4} M) and α-Chymotrypsin (10^{-4} M) - 0.1 N NaCl (H_2O)

Fig. 13. Ultraviolet Spectrum of Quinoline (3.2×10^{-4} M) in Methylene Chloride.

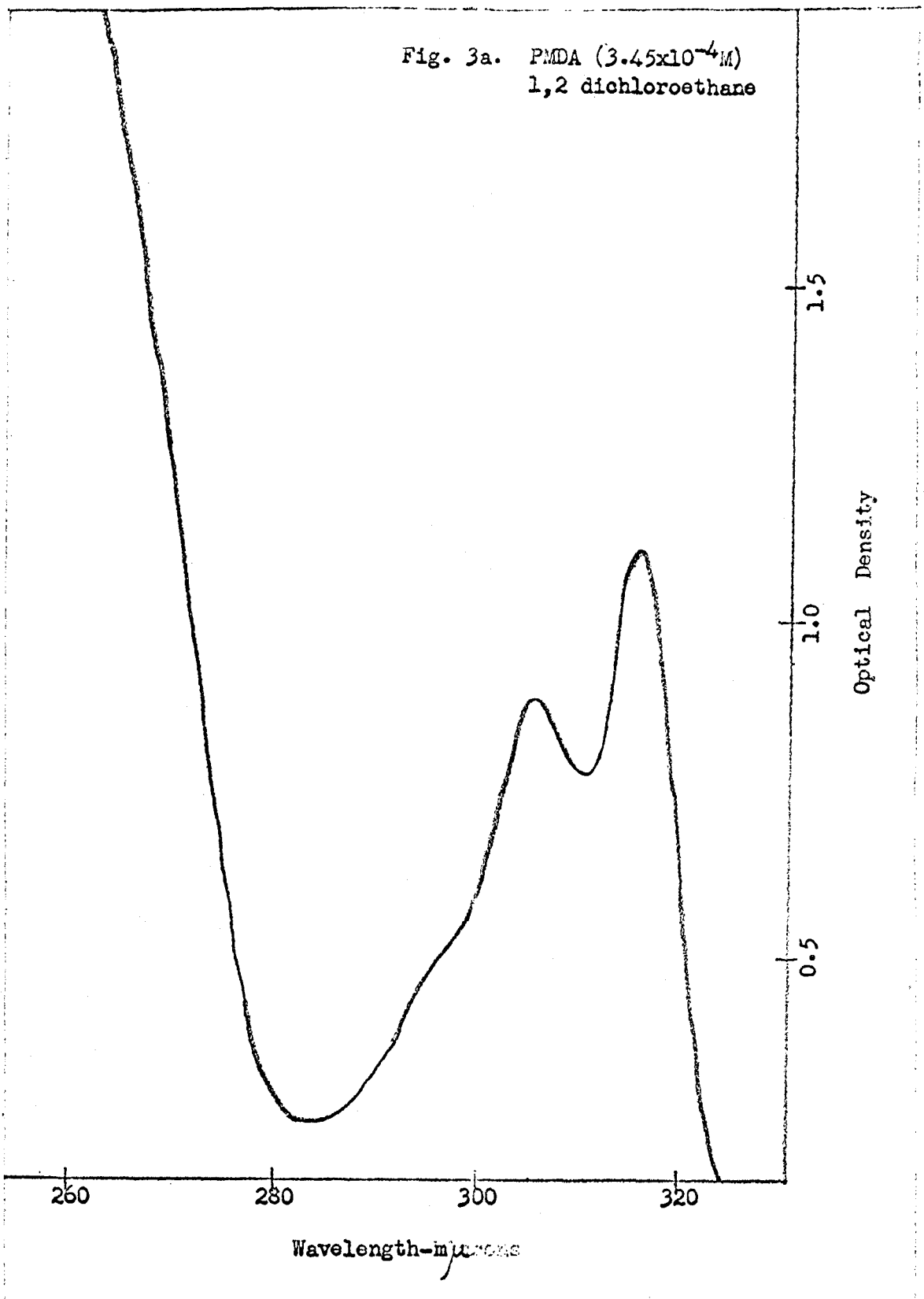
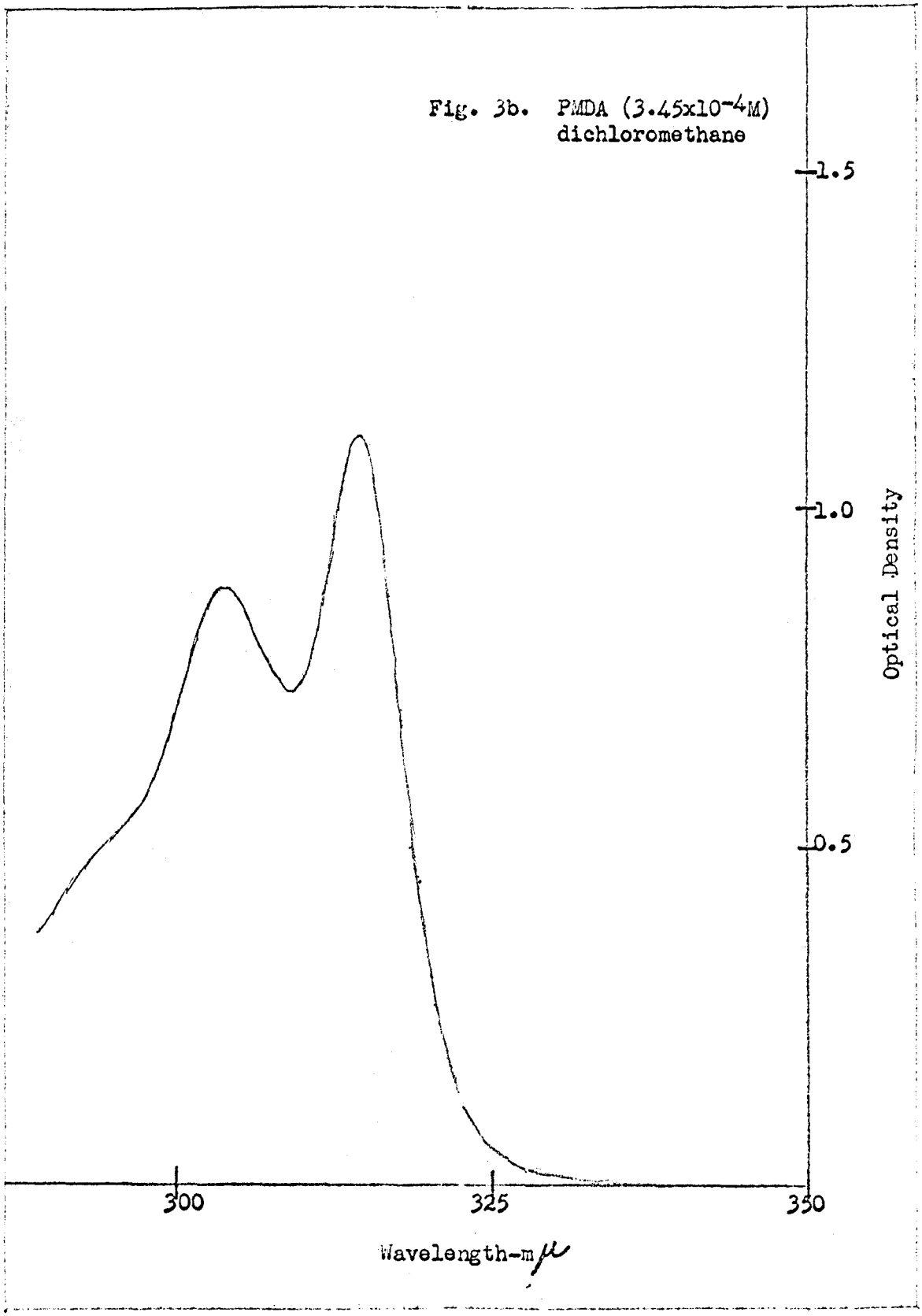


Fig. 3a. PMDA (3.45×10^{-4} M) 1,2 dichloroethane

Fig. 3b. PMDA (3.45×10^{-4}M) dichloromethane



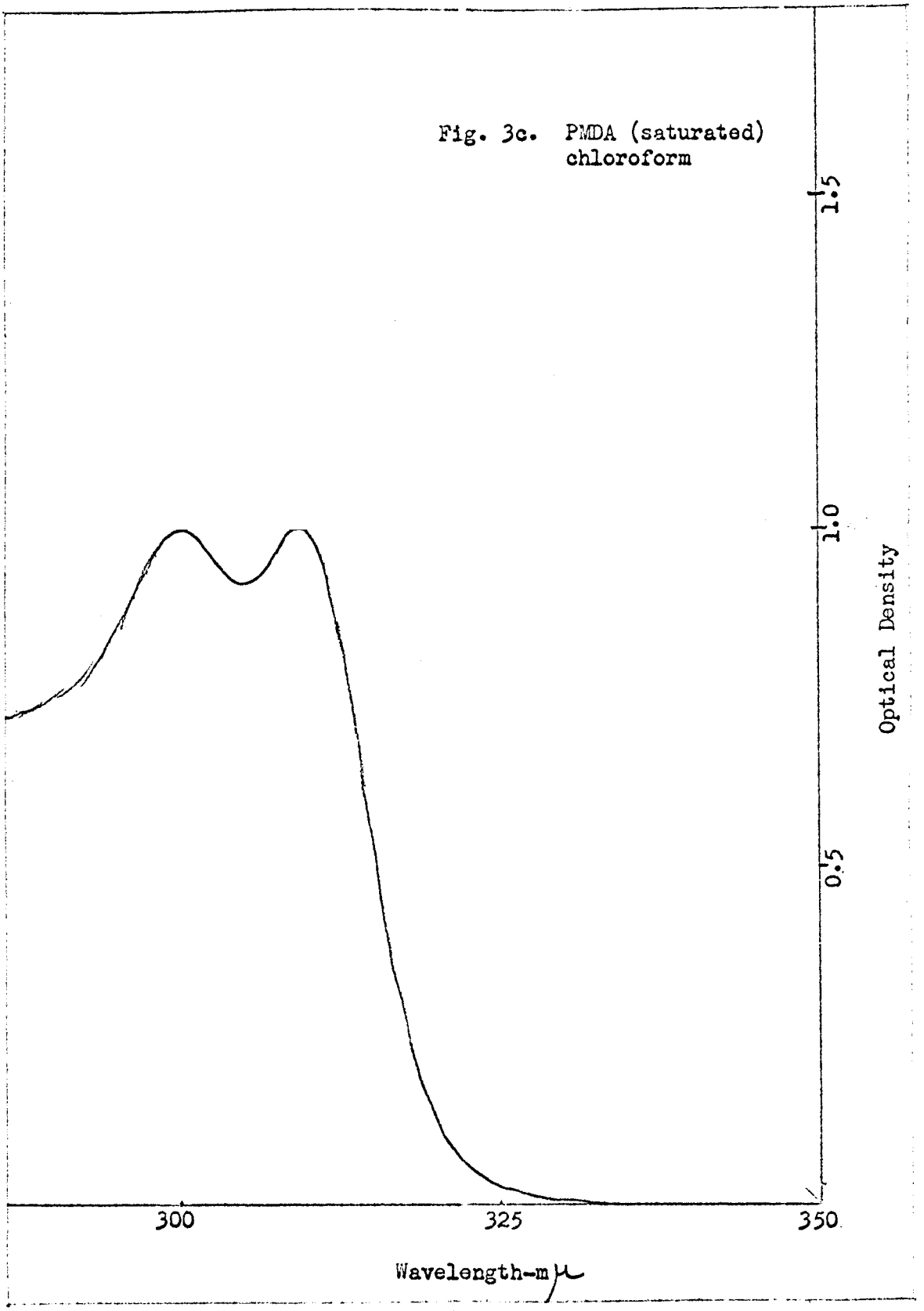
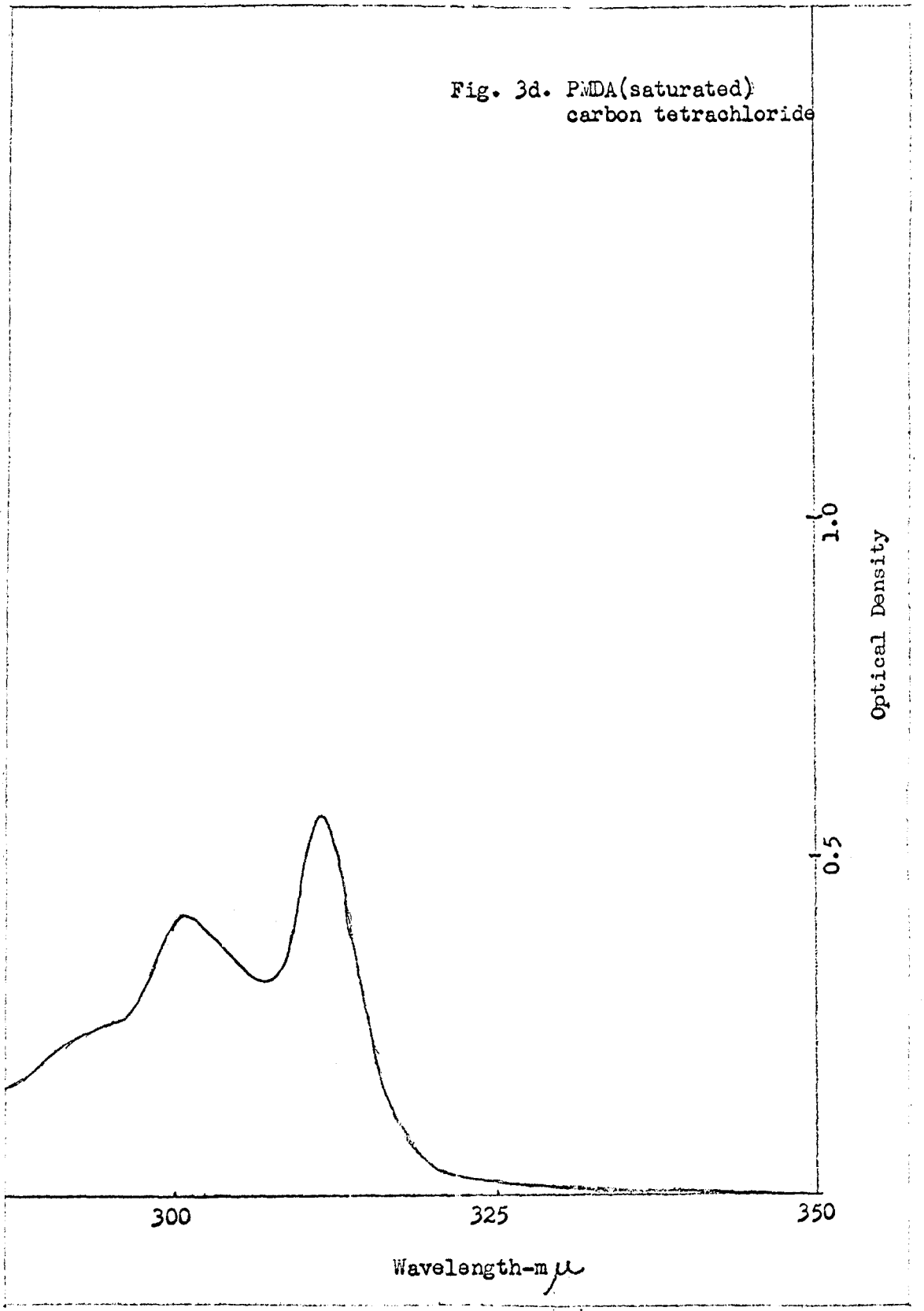


Fig. 3c. PMDA (saturated) chloroform

Fig. 3d. PMDA(saturated) carbon tetrachloride



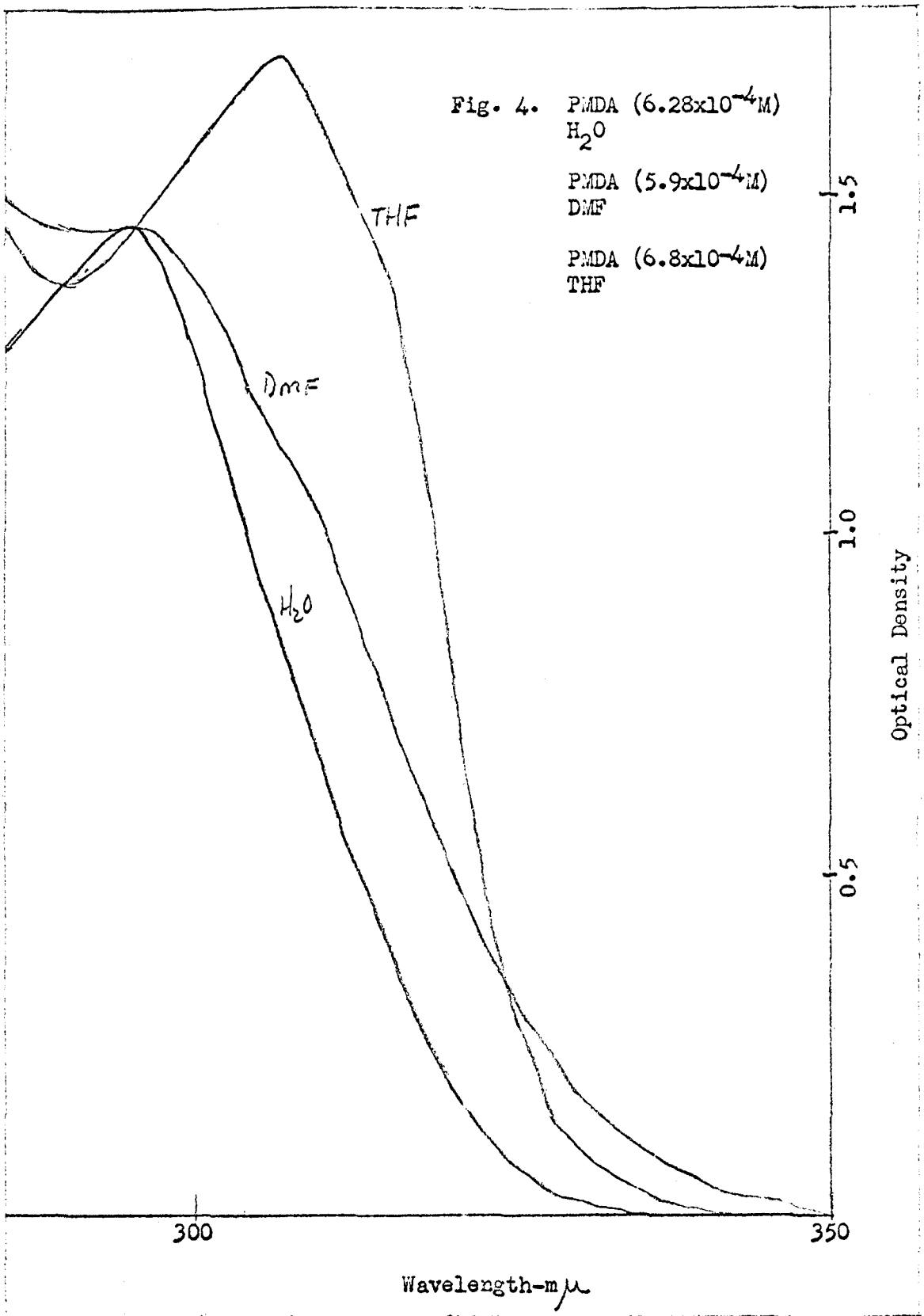


Fig. 4. PMDA (6.28×10^{-4}M) H_2O

PMDA (5.9×10^{-4}M) DMF

PMDA (6.8×10^{-4}M) THF

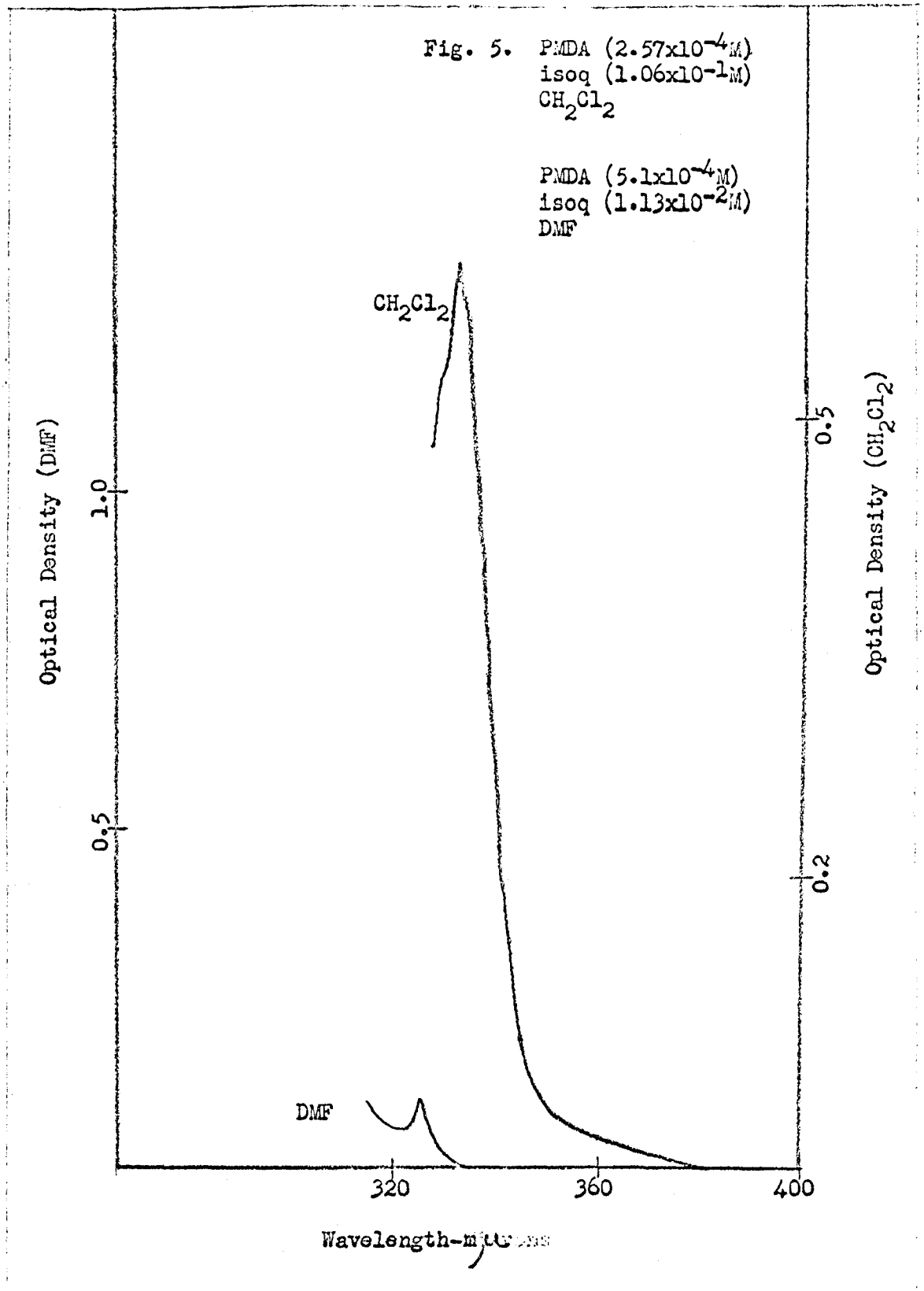


Fig. 5. PMDA (2.57×10^{-4}M) isoq (1.06×10^{-1}M) CH_2Cl_2

PMDA (5.1×10^{-4}M) isoq (1.13×10^{-2}M) DMF

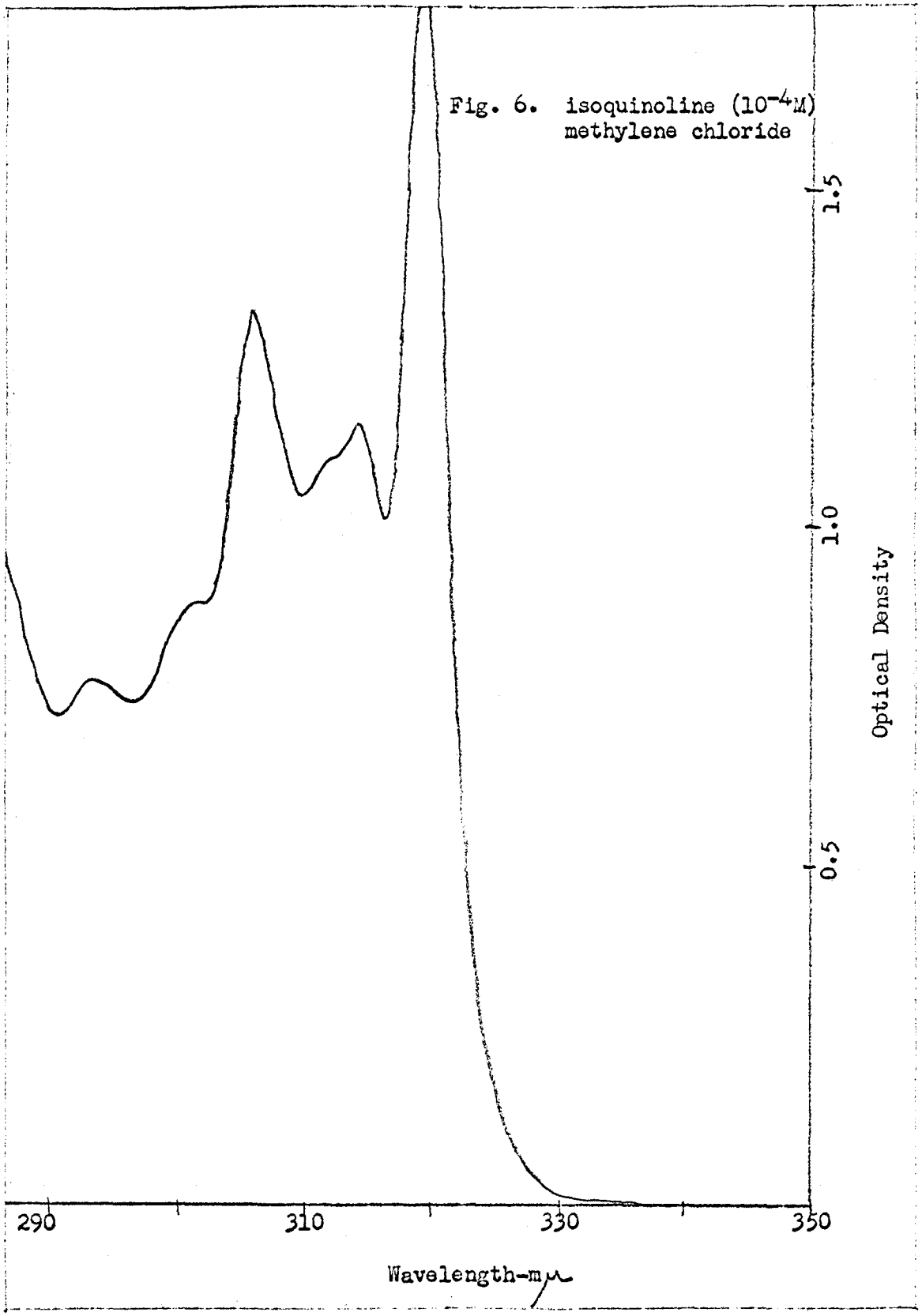


Fig. 6. isoquinoline (10^{-4}M) methylene chloride

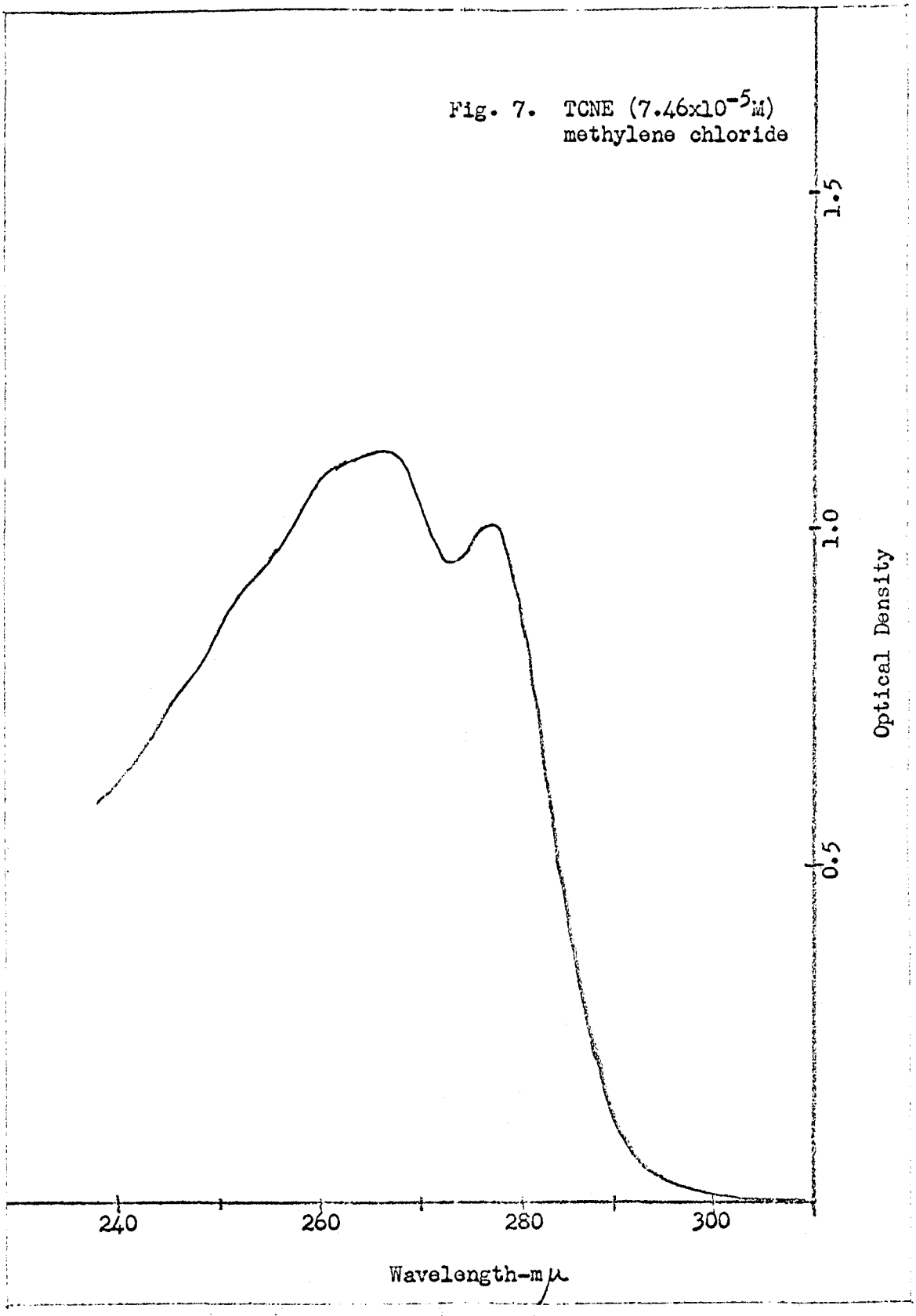


Fig. 7. TCNE (7.46×10^{-5}M) methylene chloride

Fig. 8a. TCNE (9.4×10^{-5}M)
isoquinoline (6.4×10^{-2}M)
methylene chloride

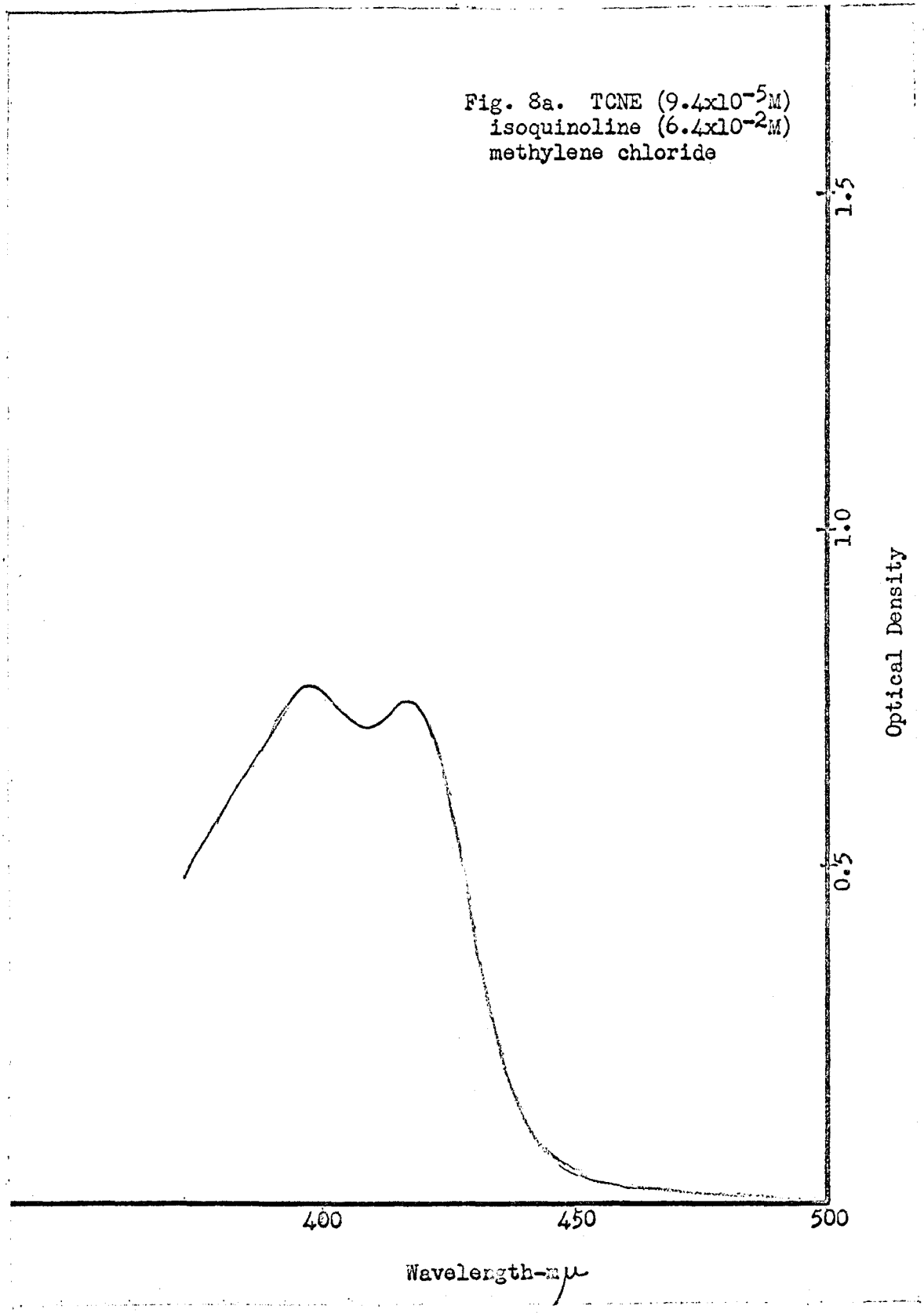
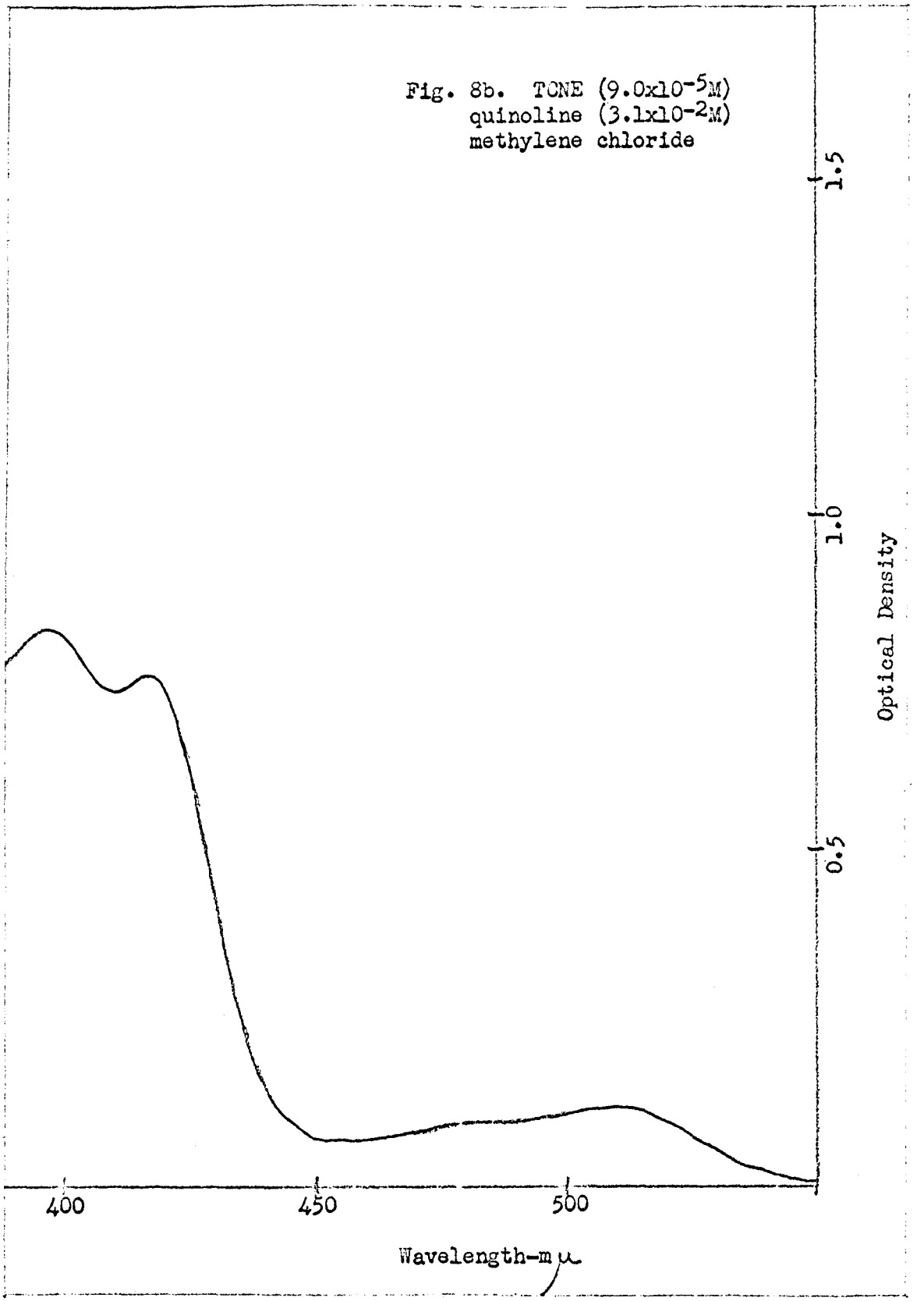


Fig. 8b. TCNE (9.0×10^{-5}M)
quinoline (3.1×10^{-2}M)
methylene chloride



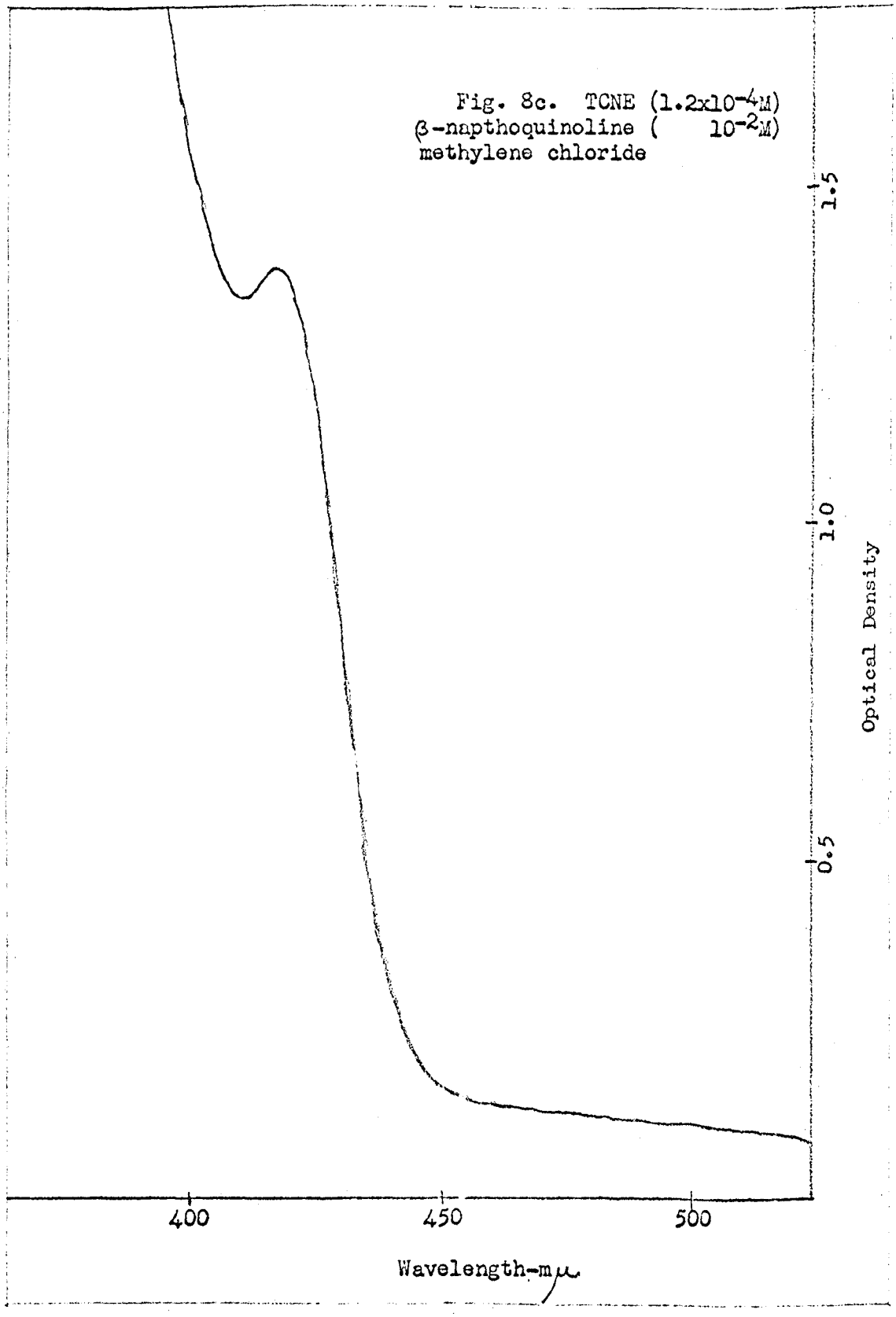


Fig. 8c. TCNE (1.2×10^{-4}M)
β-napthoquinoline (10^{-2}M)
methylene chloride

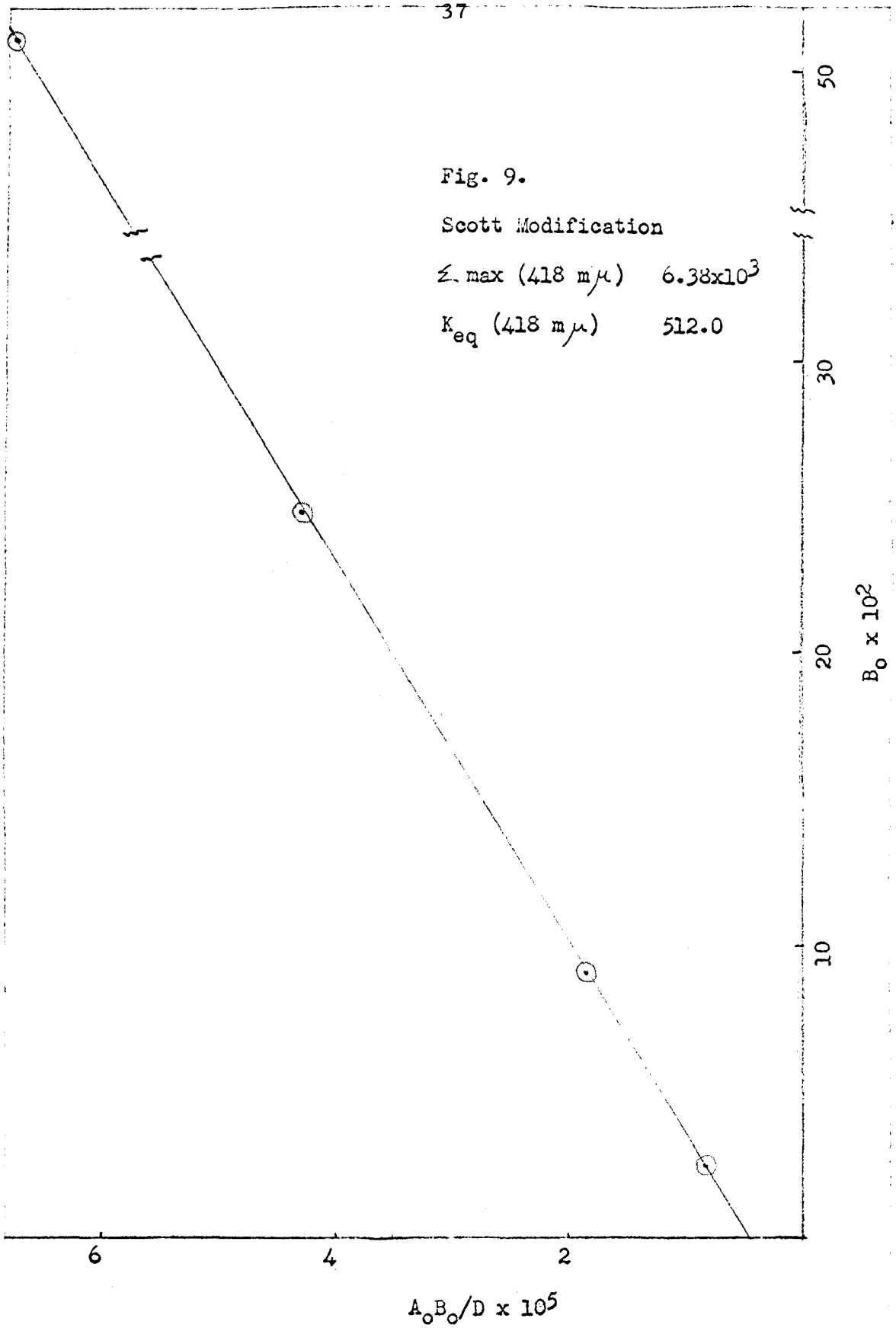


Fig. 9.

Scott Modification

ε max (418 mμ) 6.38×10^3

K_{eq} (418 mμ) 512.0

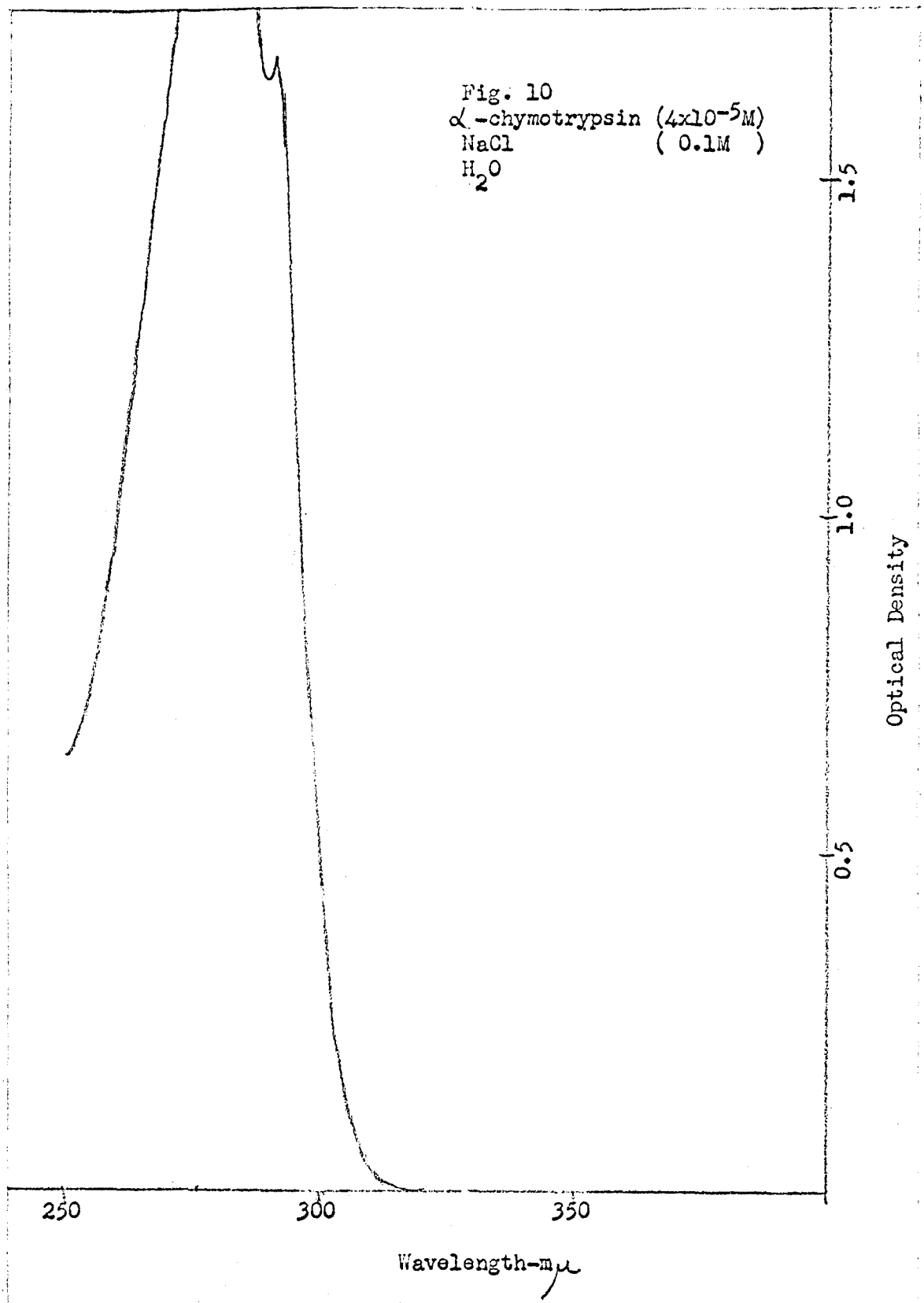


Fig. 10
α-chymotrypsin (4x10-5M)
NaCl (0.1M)
H2O
1.5
1.0
0.5
Optical Density
250
300
350
Wavelength-mμ

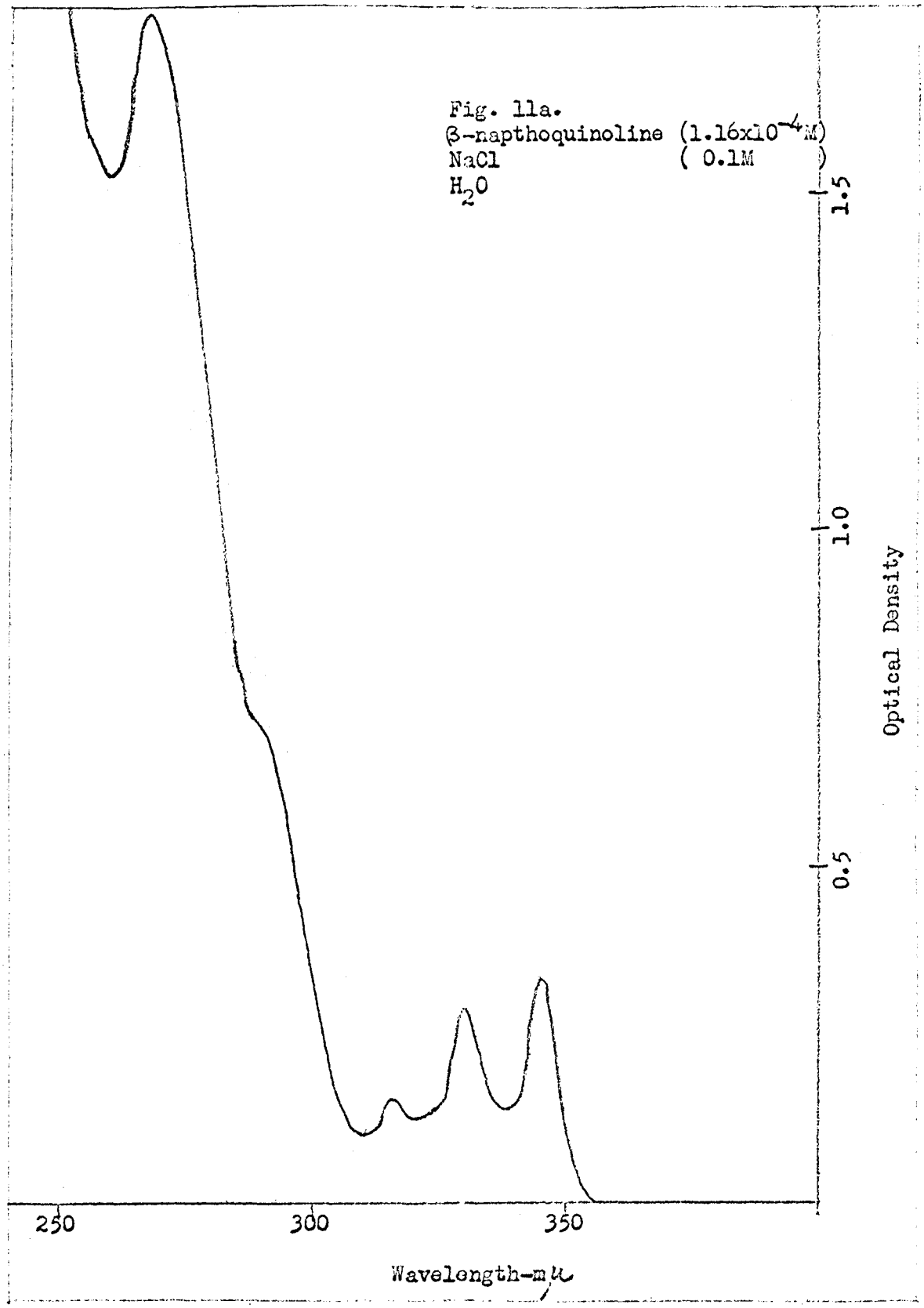


Fig. 11a.
β-napthoquinoline (1.16x10⁻⁴M)
NaCl (0.1M)
H₂O
1.5
1.0
0.5
Optical Density
250
300
350
Wavelength-mμ

Fig. 11b.
β-napthoquinoline (2.65×10^{-4}M)
carbon tetrachloride

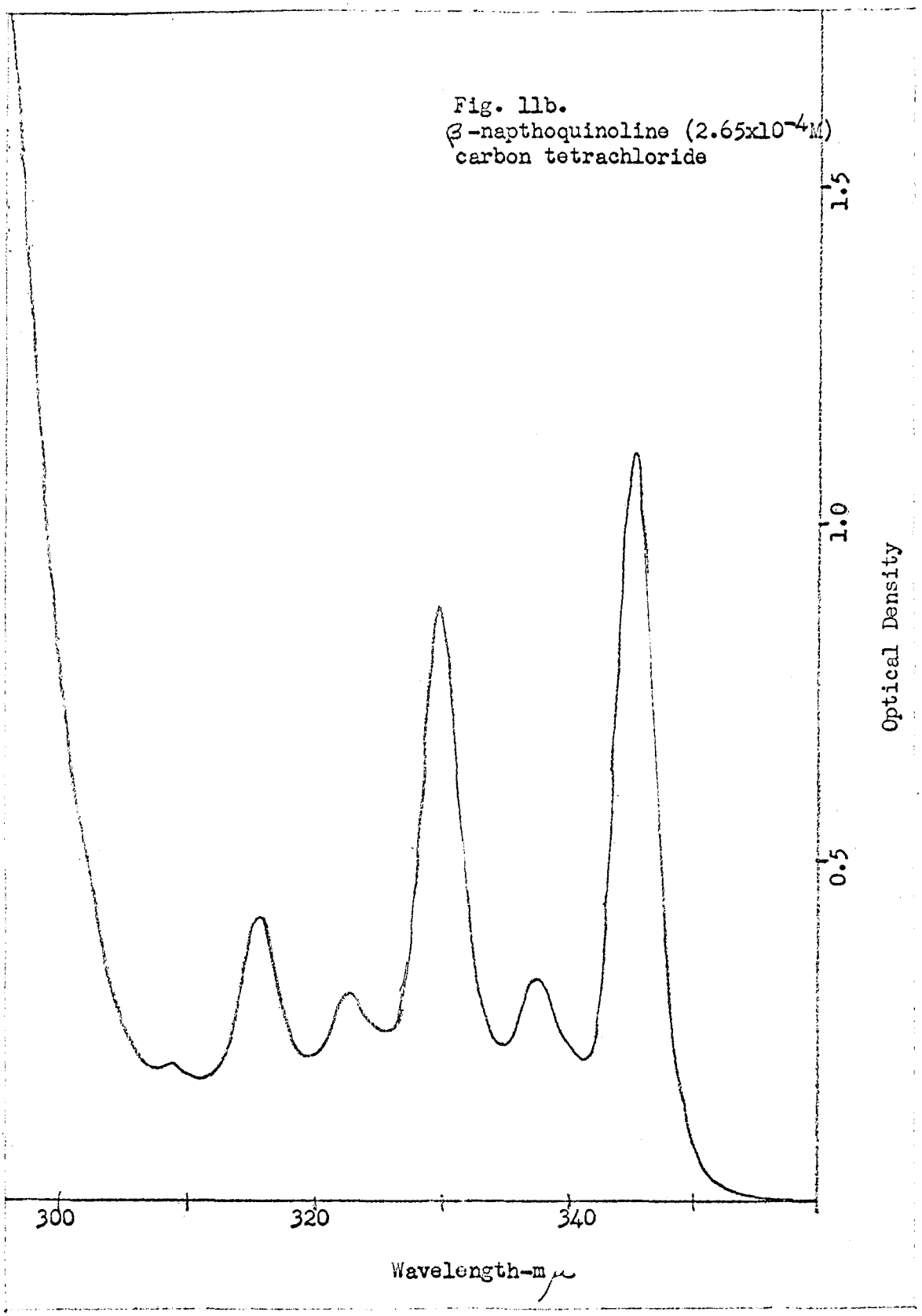
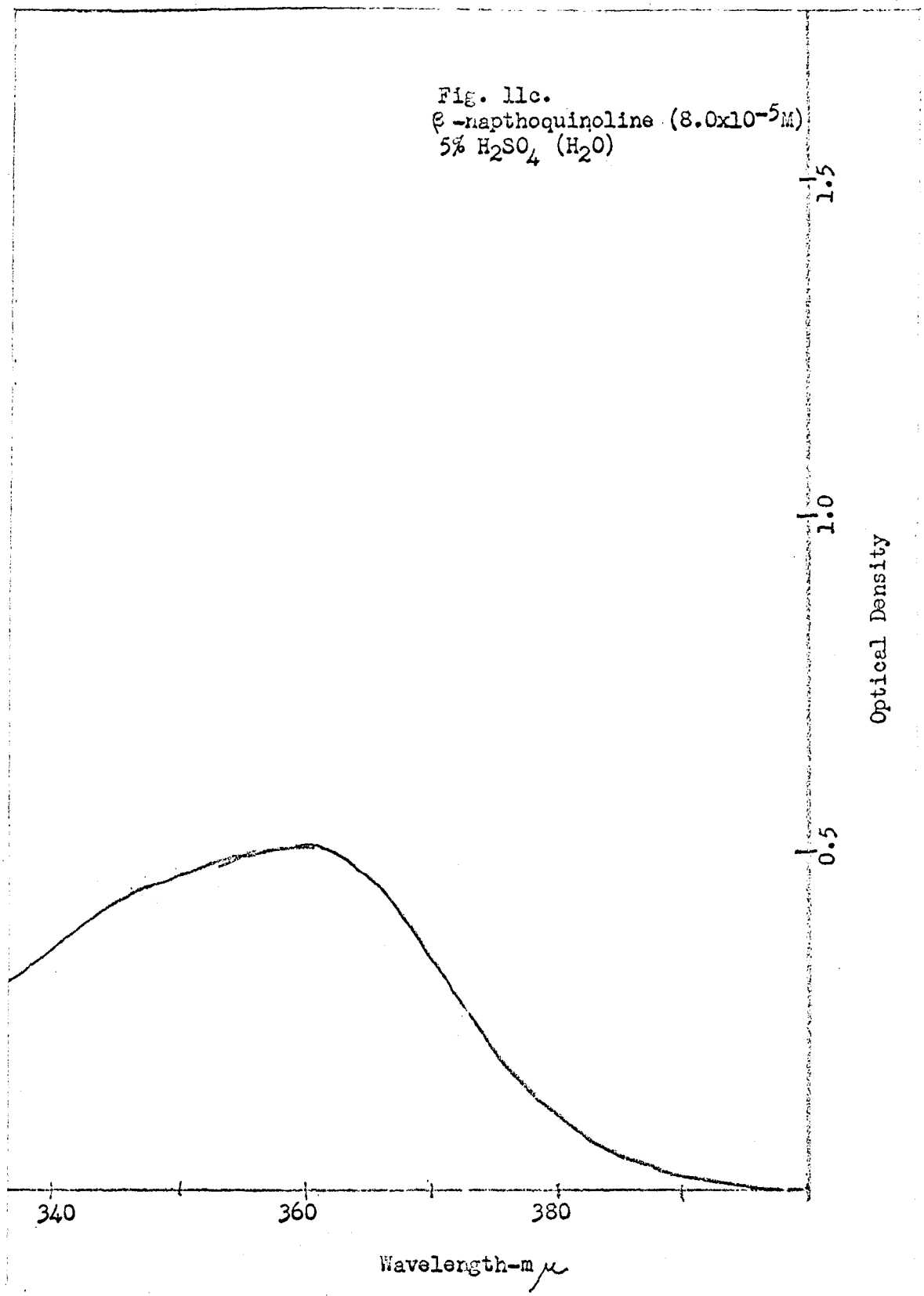


Fig. 11c.
β-napthoquinoline (8.0×10^{-5}M)
5% H_2SO_4 (H_2O)



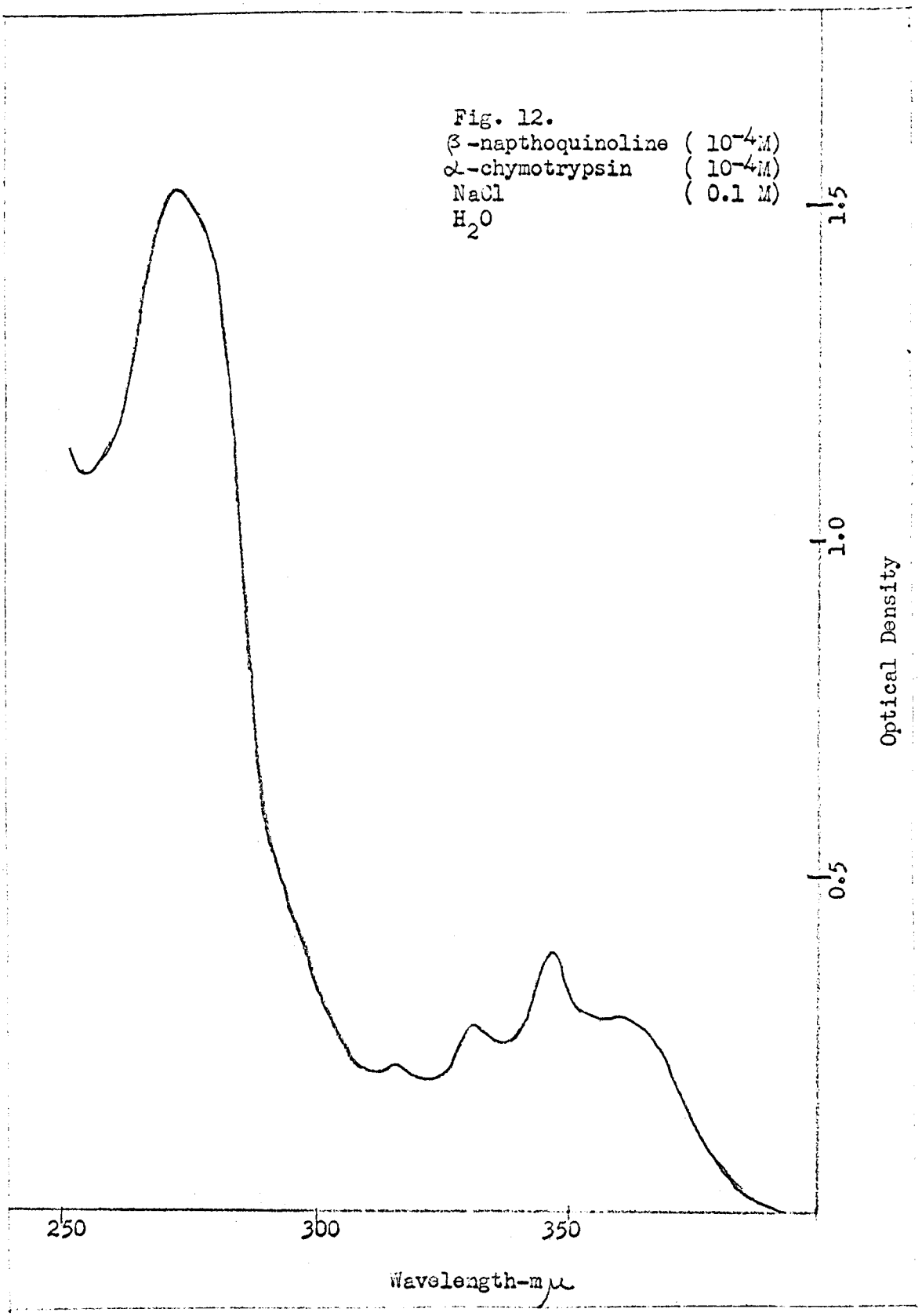


Fig. 12.
β-napthoquinoline (10⁻⁴M)
α-chymotrypsin (10⁻⁴M)
NaCl (0.1 M)
H2O
Optical Density
0.5
1.0
1.5
250
300
350
Wavelength-mμ

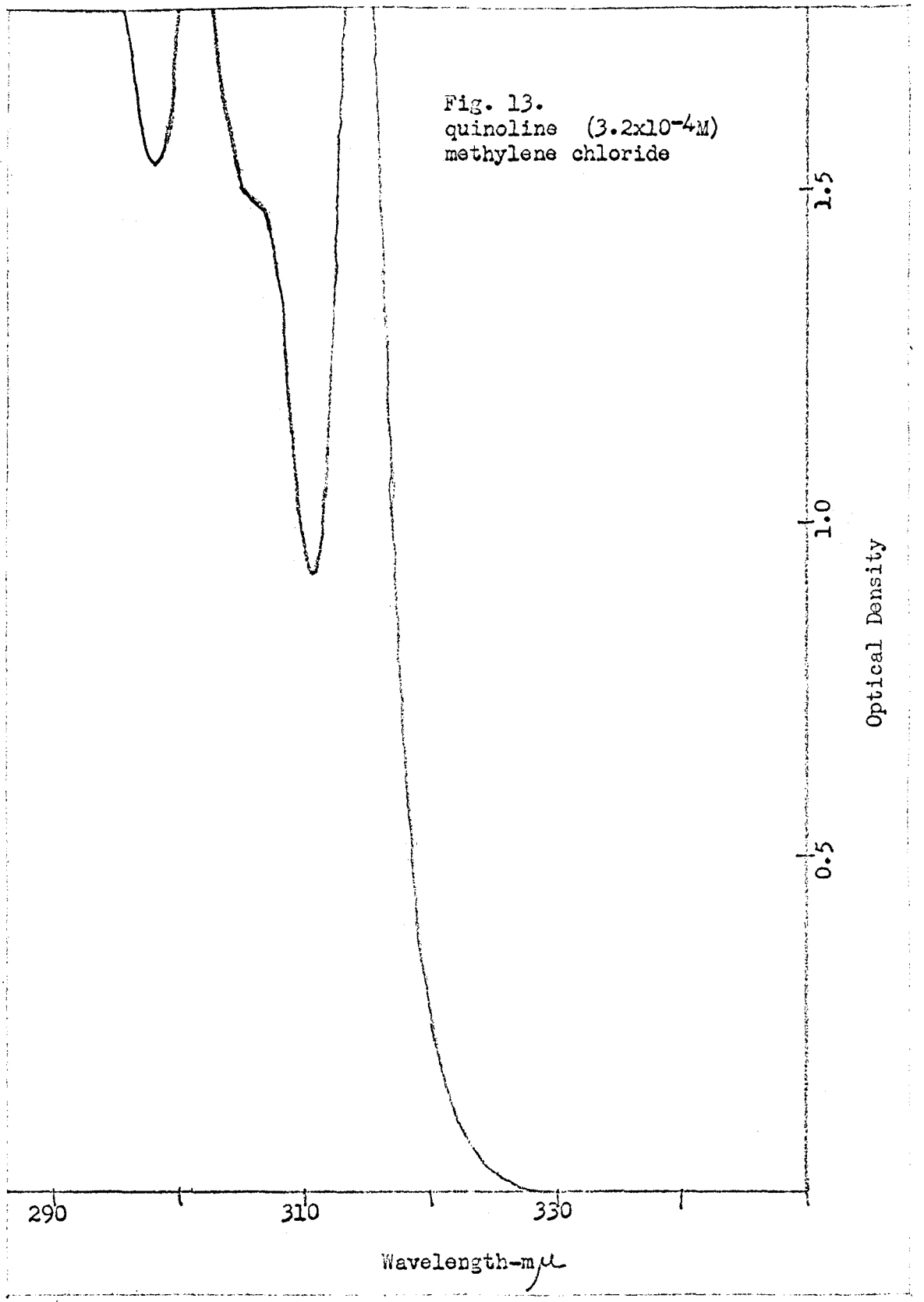


Fig. 13.
quinoline (3.2×10^{-4}M)
methylene chloride

Annual Report - 1963

A Model for a Portion of the Active Site of α-Chymotrypsin

Investigators: D. L. Roth and C. Niemann
Support: National Institutes of Health

It is known that many aromatic molecules combine with a locus at the active site of α-chymotrypsin. The nature of this interaction is unknown. In order to determine whether or not a charge-transfer type of interaction is involved a model based upon vapor-phase chromatography has been developed. This model entails use of a solution of a set of π-acids in squalene and determination of the retention coefficients of a series of π-bases. The retention coefficients should be dependent not only upon electronic but also upon steric factors. Comparison of retention coefficients so determined with the enzyme-inhibition constants should determine whether or not a charge-transfer type of interaction is of any significance in the inhibitor--active-site interaction.

Annual Report - 1964

Spectrophotometric Determination of Charge-Transfer Equilibrium Constants

Investigators: D. L. Roth and C. Niemann

Support: Public Health Service, Division of General Medical Sciences

Comparison of charge-transfer equilibrium constants with enzyme inhibition constants should give some information concerning the role of charge-transfer binding in the inhibitor-enzyme complex. The equilibrium constant for the isoquinoline-tetracyanoethylene complex has been determined by the method of Benesi and Hildebrand [J. Am. Chem. Soc., 71, 2703 (1949)]. Studies on solutions of the quinoline-tetracyanoethylene and α-naphthoquinoline-tetracyanoethylene complexes are complicated by significant concentrations of a species believed to be the tetracyanoethylene radical ion. The difference spectrum of a solution of α-chymotrypsin and β-naphthoquinoline exhibits a shoulder at 360 mμ which can be ascribed to charge-transfer absorption.

A Model for a Portion of the Active Site of α-Chymotrypsin

Investigators: D. L. Roth and C. Niemann

Support: Public Health Service, Division of General Medical Sciences

Experimental studies on the relationship between retention coefficients for various π-bases in gas chromatography and enzyme inhibition constants, described in the 1963 Annual Report, have been completed. The low temperatures required for formation of charge-transfer complexes limited the study to C_6-C_8 aromatic hydrocarbons. Addition of pyromellitic dianhydride to the liquid phase of the column produced an increased separation between m- and p-xylene, but the order of elution corresponded to the boiling points of these π-bases, and no conclusions were reached.

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