PB82-152216

FINAL REPORT

CONTRACT NO. BOSH 099-71-1

Project Name: The Development and Evaluation of an Electronic Emission Spectral Index Which Can Be Related to the Carcinogenic Hazard of the Respirable Portion of Coal-tar Pitch Volatiles

BIBLIOGRAPHIC DATA SHEET	1. Report No.	2. NA	3. Recipient's Accession No. PB82 152216

4. Title and Subtitle

The Development and Evaluation of an Electronic Emission Spectral Index Which can be Related to the Carcinogenic Hazard of the Respirable Portion of Coal-Tar Rich Volatiles

5. Report Date

6. NA 022063

7. Author(s)

8. Performing Organization Rept. No. NA

9. Performing Organization Name and Address

10. Project/Task/Work Unit No. NA

11. Contract/Grant No. 099-71-0001

12. Sponsoring Organization Name and Address

NIOSH
4676 Columbia Parkway
Cincinnati, Ohio 45226

13. Type of Report & Period Covered contract

14. NA

15. Supplementary Notes

16. Abstracts

The possibility of relating an electronic emission spectral index to the carcinogenicity of respirable portions of coal-tar pitch volatiles is investigated. Photophysical properties are determined for 34 polynuclear aromatic hydrocarbons and their heterocyclic analogs. Statistical analysis of the results indicates that there is no significant correlation, with the exception of fluorescence lifetimes, between these properties and the known carcinogenic activity of the compounds studied. An electronic emission spectral index for carcinogenic hazards based on these photophysical properties does not exist for the compounds studied. An index based on fluorescence lifetimes could potentially be used to evaluate the carcinogenic hazard of an untested pure compound, but such an index would not be applicable to mixtures. Testing the photodynamic activities of the compounds using the crustacean Artemia salina shows good statistical correlation between photodynamic activity and carcinogenicity. This method, although untested on mixtures, should be applicable to air samples and other polynuclear aromatic mixtures.

17. Key Words

NIOSH-contract, NIOSH-publication, polynuclear-hydrocarbons, air-monitoring, naphthas, air-sampling, photoelectric-emission, toxic-substances, polynuclear-compounds, carcinogens, monitoring, tars, inhalation, photochemistry, hazards, contract no. 099-71-1, MX8007452

17b. Identifiers/Open-Ended Terms

17c. COSATI Field Group

18. Availability Statement	19. Security Class (This Report) UNCLASSIFIED	21. No. of Pages 152
Available to the public	20. Security Class (This Page) UNCLASSIFIED	22. Price

Final Report BOSH 099-71-1

I. Contract Number, Project Name and Objectives

II. Details of Work Preformed

Table of Contents

		page
I.	Contract Number, Project Name, and Objectives	1
II.	Details of Work Preformed	3
	A. Classification and Source of Compounds	3
	B. Photophysical Properties	26
	C. Photochemical Analysis Data	51
	D. Electronic Emission Spectral Index for Carcinogenic Hazard	125
	E. Photodynamic Bioassay	132
	F. Summary	149
	References	151

Final Report
(Contract period 1 June 71 -- 31 May 72)

I. Contract Number, Project Name and Objectives

Contract No. BOSH 099-71-1

Project Name: The Development and Evaluation of an Electronic Emission Spectral Index Which Can Be Related to the Carcinogenic Hazard of the Respirable Portion of Coal-tar Pitch Volatiles.

Objectives:

1. Evaluation and analysis, including a review of existing information in the literature, of the photophysical properties of a minimum of thirty (30) polynuclear aromatic and heterocyclic compounds found in coal-tar pitch.

2. Develop and evaluate a relatively simple and rapid electronic emission spectral index which can be related to the carcinogenic hazard of a coal-tar pitch volatile mixture.

3. Determine the photochemical analysis data of a minimum of thirty (30) pure polynuclear aromatic and heterocyclic compounds as well as their mixtures found in coal-tar pitch.

4. Develop and evaluate a photodynamic bioassay which can be related to the carcinogenic hazard of coal-tar pitch polynuclear aromatic and heterocyclic compounds using the crustacean *Artemia salina* as a test organism.

II. Details of Work Performed

A. Classification and Source of Compounds used

The carcinogenic and non-carcinogenic compounds used in this work can be divided into four broad categories:

a) *Alternant polynuclear aromatic hydrocarbons.* These compounds contain only the elements carbon and hydrogen and do not contain a ring with an uneven number of centers.

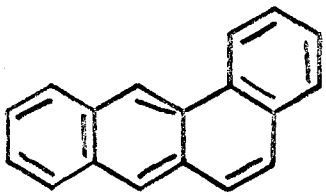
b) *Nonalternant polynuclear aromatic hydrocarbons.* These compounds contain only the elements carbon and hydrogen and have one or more rings with an uneven number of carbon atoms.

c) *Heterocyclic nitrogen analogs of alternant polynuclear aromatics.* These compounds are derived from the alternant polynuclear aromatics by replacing one or more methine groups (=CH–) with a nitrogen atom.

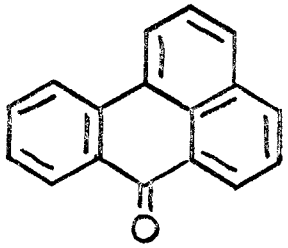
d) *Nonalternant heterocyclic nitrogen polynuclear aromatic compounds.* These compounds may contain a pyrrole nucleus (five-membered ring containing nitrogen) in addition to six-membered rings in their structure.

Each of these broad categories contain both carcinogenic and non-carcinogenic compounds. The compounds chosen for this study are given in Table 1 (see Table 2 for structures.) The nomenclature used throughout this report is that proposed in the 1957 report of the Commission on the Nomenclature of the International Union of Pure and Applied Chemistry [1].

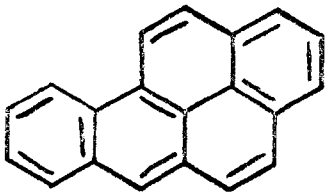
The names, structures and carcinogenic properties of the compounds used are given in Figure 1. The classification of these compounds as carcinogens and non-carcinogens was based on a survey of the literature [2]. The following compounds were obtained from commercial sources: Benzo[a]pyrene, benzo[rst]pentaphene, benz[a]anthracene, benzo[b]triphenylene, benzo[e]pyrene, triphenylene, pyrene, dibenzo[a,e]fluoranthene, fluoranthene, benzo[ghi]fluoranthene, 11H-benzo[a]carbazole, dibenz[a,j]-acridine, 7H-dibenzo[a,g]carbazole, benzanthrone, dibenz[a,h]acridine, dibenz[a,h]anthracene, benz[c]acridine, tetracene, perylene, acridine, phenazine, carbazole, anthanthrene, periflanthene, ovalene, rubicene, anthracene, picene, coronene, benzo[a]fluorene, benzo[b]fluorene, benzo[c]fluorene and fluorene. The remaining compounds given in Figure 1 were prepared by various synthetic methods (see experimental).



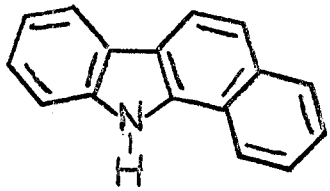
Benz[a]anthracene
Carcinogen



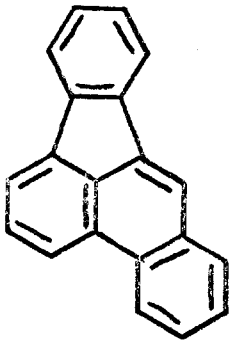
Benzanthrone
Carcinogen



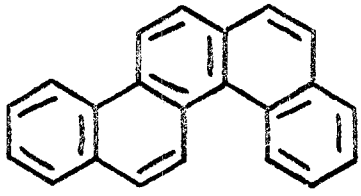
Benzo[a]pyrene
Carcinogen



11H-Benzo[a]carbazole
Carcinogen

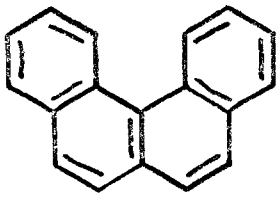


Benzo[b]fluoranthrene
Carcinogen

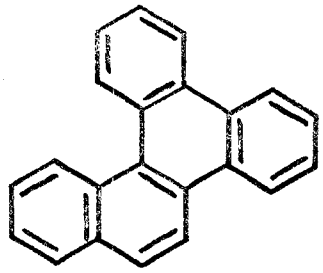


Benzo[c]chrysene
Carcinogen

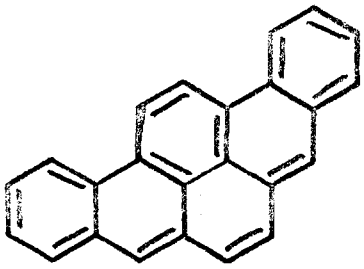
Figure 1. Structures, names, and carcinogenic properties of the compounds used in this work.



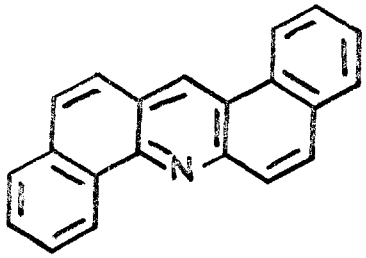
Benzo[c]phenanthrene
Carcinogen



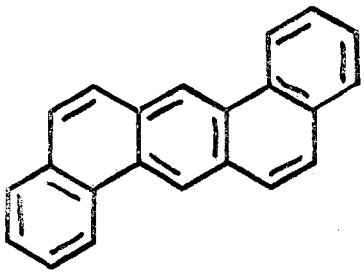
Benzo[g]chrysene
Carcinogen



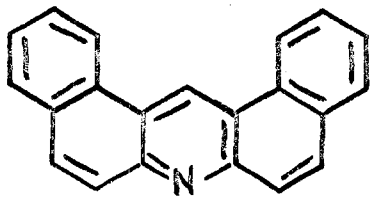
Benzo[rst]pentaphene
Carcinogen



Dibenz[a,h]acridine
Carcinogen

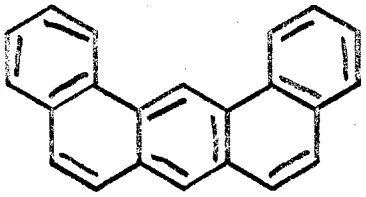


Dib[a,h]anthracene
Carcinogen

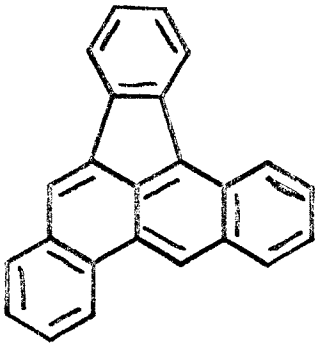


Dibenz[a,j]acridine
Carcinogen

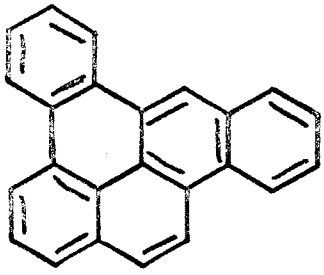
Figure 1. Continued



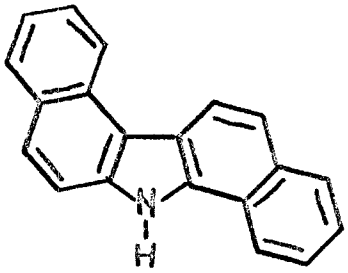
Dibenz[a,j]anthracene
Carcinogen



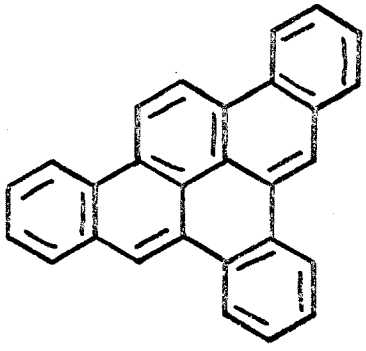
Dibenzo[a,e]fluoranthene
Carcinogen



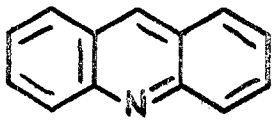
Dibenzo[a,e]pyrene



7H-Dibenzo[a,g]carbazole

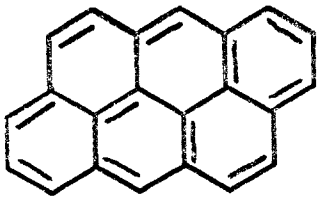


Tribenzo[a,e,i]pyrene
Carcinogen

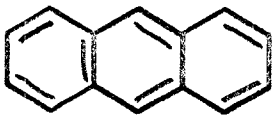


Acridine
Non-carcinogen

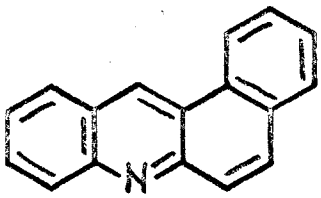
Figure 1. Continued



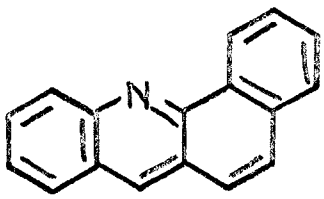
Anthanthrene
Non-carcinogen



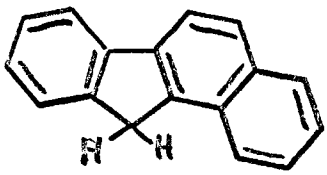
Anthracene
Non-carcinogen



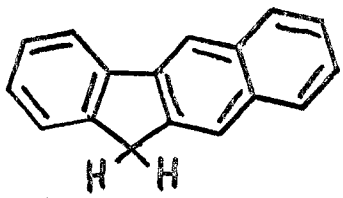
Benz[a]acridine
Non-carcinogen



Benz[c]acridine
Non-carcinogen

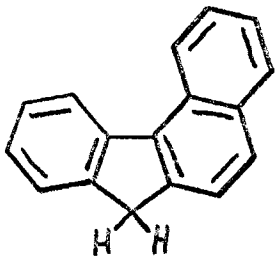


Benzo[a]fluorene
Non-carcinogen

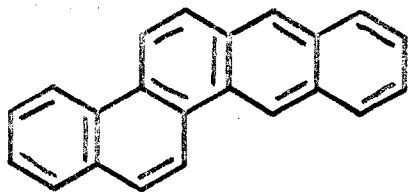


Benzo[b]fluorene
Non-carcinogen

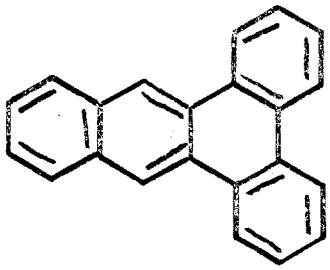
Figure 1. Continued



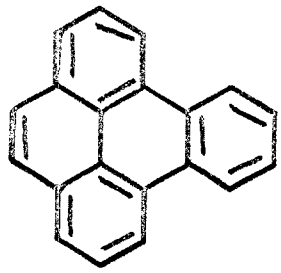
Benzo[c]fluorene
Non-carcinogen



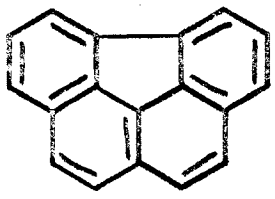
Benzo[b]chrysene
Non-carcinogen



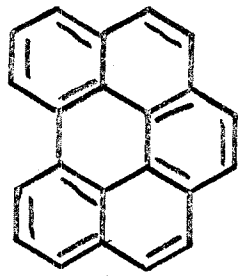
Benzo[b]triphenylene
Non-carcinogen



Benzo[e]pyrene
Non-carcinogen

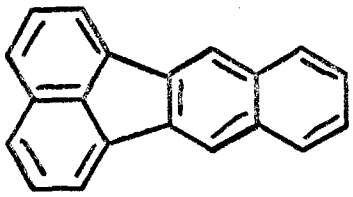


Benzo[ghi]fluoranthene
Non-carcinogen

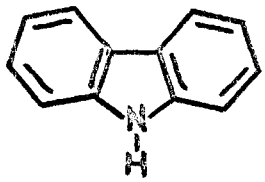


Benzo[ghi]perylene
Non-carcinogen

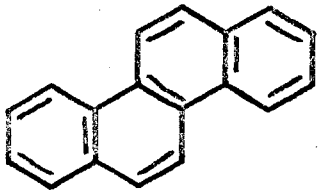
Figure 1. Continued



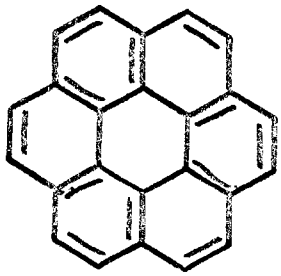
Benzo[k]fluoranthene
Non-carcinogen



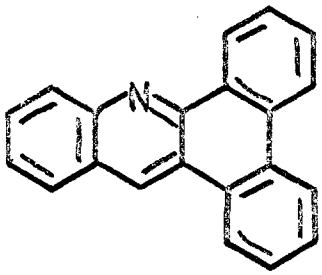
Carbazole
Non-carcinogen



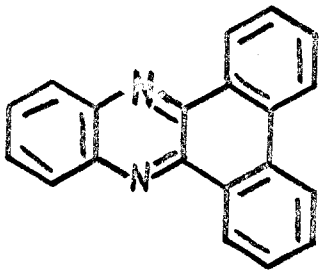
Chrysene
Non-carcinogen



Coronene
Non-carcinogen

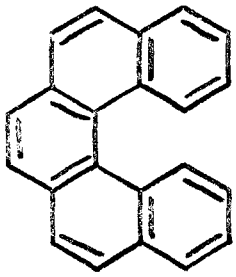


Dibenz[a,c]acridine
Non-carcinogen

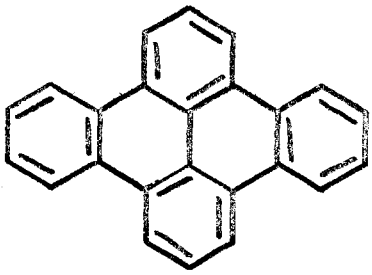


Dibenzo[a,c]phenazine
Non-carcinogen

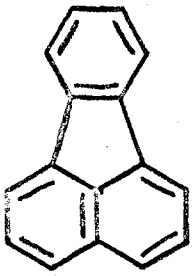
Figure 1. Continued



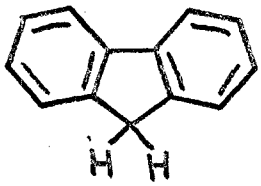
Dibenzo[c,g]phenanthrene
Non-carcinogen



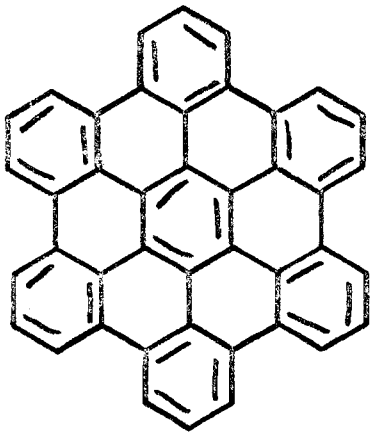
Dibenzo[fg,op]naphthacene
Non-carcinogen



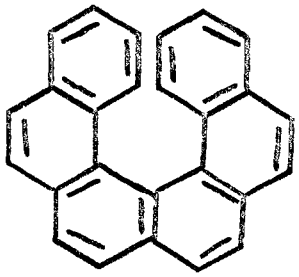
Fluoranthene
Non-carcinogen



Fluorene
Non-carcinogen

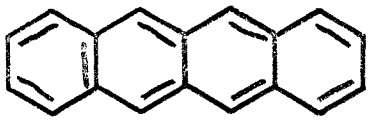


Hexabenzocoronene
Non-carcinogen

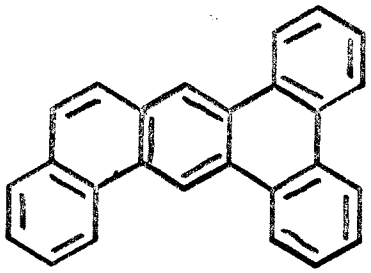


Hexahelicene
Non-carcinogen

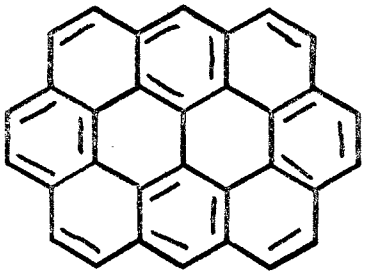
Figure 1. Continued



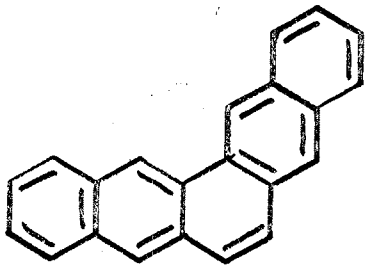
Naphthacene
Non-carcinogen



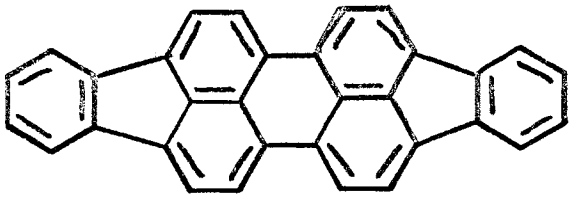
Naphtho[1,2-b]triphenylene
Non-carcinogen



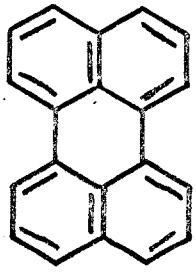
Ovalene
Non-carcinogen



Pentaphene
Non-carcinogen

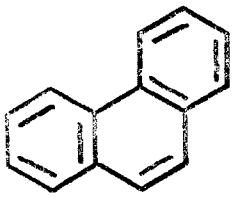


Periflanthene
Non-carcinogen

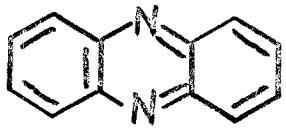


Perylene
Non-carcinogen

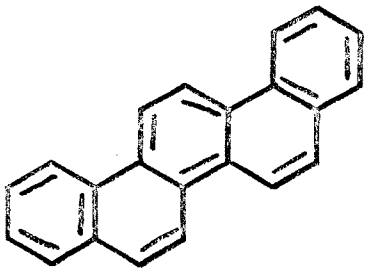
Figure 1. Continued



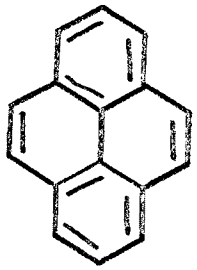
Phenanthrene
Non-carcinogen



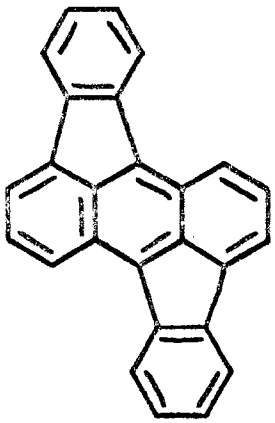
Phenazine
Non-carcinogen



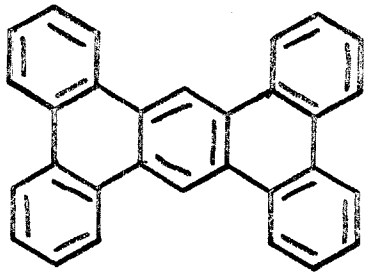
Picene
Non-carcinogen



Pyrene
Non-carcinogen

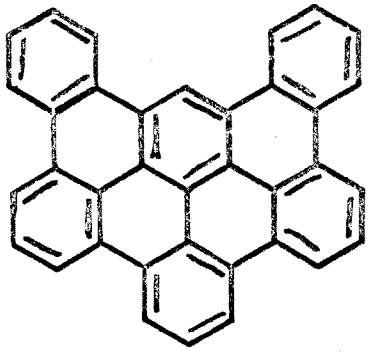


Rubicene
Non-carcinogen



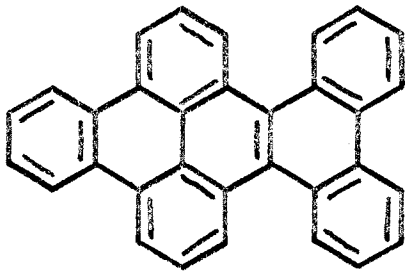
Tetrabenz[a,c,h,j]anthracene
Non-carcinogen

Figure 1. Continued



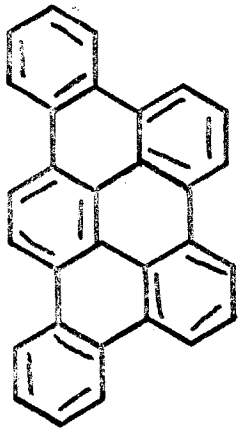
Tetrabenz[a,cd,fg,n]anthanthrene

Non-carcinogen



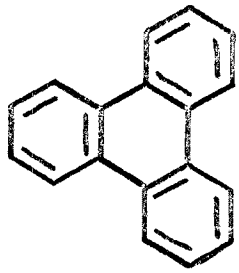
Tetrabenzo[a,c,fg,op]-naphthacene

Non-carcinogen



Tribenzo[e,ghi,k]perylene

Non-carcinogen



Triphenylene

Non-carcinogen

Figure 1. Continued

Experimental

Benzo[c]phenanthrene, dibenz[a,j]anthracene, benzo[c]-chrysene, benzo[g]chrysene, chrysene, benzo[ghi]perylene, phenanthrene, and hexahelicene. These compounds were prepared by the photocyclization of an appropriate diarylethylene under oxidative conditions. One liter of a 10^{-3}-10^{-2} *M* solution of the diarylethylene, containing 0.1 g of iodine, was irradiated with unfiltered light from a Hanovia 550 watt medium pressure mercury arc placed in a water cooled quartz immersion well. The course of the reaction was followed by removing a small aliquot of the reaction mixture at various time intervals and scanning the ultraviolet spectrum. When no further change in the spectrum was observed, the irradiation was stopped. The solution was then concentrated, and passed through a short alumina column. The eluate was then evaporated to dryness and the photoproduct purified either by recrystallization, preparation and recrystallation of the picrate, chromatography, or a combination of these methods. The results obtained were as follows:

Diarylethylene	Product	Yield
2-Styrylnaphthalene	Benzo[c]phenanthrene	70%
1,3-Distyrylbenzene	Dibenz[a,j]anthracene	1%
	Benzo[c]chrysene	70%

9-Styrylphenanthrene	Benzo[g]chrysene	70%
1-Styrylnaphthalene	Chrysene	80%
1,2-Di(2-naphthyl)-ethylene	Benzo[ghi]perylene	60%
Stilbene	Phenanthrene	80%
2-Styrylbenzo[c]-phenanthrene	Hexahelicene	50%

Dibenzo[a,e]pyrene [3]. To a slurry of 2.5 g (0.011 mole) of chrysene in 50 ml of benzene at 63° were added with stirring over a period of 1 hour 5 g (0.0374 mole) of aluminum chloride and 10 g (0.0384 mole) of stannic chloride. After stirring for 4 hours, the reddish complex was broken up by adding 20 ml of 6*N* HCl and 30 ml of ice water. The resulting suspension was extracted with seven 50 ml portions of benzene, the combined extracts washed with water, dried over sodium sulfate, and evaporated to dryness. The resulting orange residue was chromatographed on neutral alumina with benzene and then recrystallized from benzene; yield 113 mg, m.p. 243-244° uncorr.; lit. [13] 240°.

Tribenzo[a,e,i]pyrene [4]. To a slurry of 0.25 g (0.001 mole) picene in 50 ml of benzene at 70° were added with stirring over a period of 1 hour 0.5 g (0.0037 mole) of aluminum chloride and 1.0 g (0.0038 mole) of stannic chloride. After stirring for 4 hours, the bluish complex

was broken up by adding 30 ml of water and 20 ml of 6*N* HCl. The resulting suspension was extracted with seven 50 ml portions of benzene, the combined extracts washed with water, dried over sodium sulfate, and evaporated to dryness. The yellowish brown product was redissolved in benzene and the solution was chromatographed on a neutral alumina column. The solid obtained on evaporation of the eluate was then recrystallized from benzene; yield 20 mg, m.p. 336-338° uncorr.; lit. [13] 331-332°.

Benzo[b]chrysene [5]. Six grams of 1-vinylnaphthalene and 18.0 g of 1,4-naphthoquinone were dissolved in 150 ml of glacial acetic acid. The solution was heated for 12 hours at 100° and then cooled to room temperature. The precipitated crude benzo[b]chrysene-7,12-quinone was collected and recrystallized once from xylene; yield 4.8 g, m.p. 262-265°. The quinone was reduced by refluxing 4.7 g of it for 5 days with a mixture consisting of 25 g zinc dust, 0.1 g copper sulfate in 100 ml water, 400 ml of 2*N* sodium hydroxide and 100 ml of toluene. The organic layer was then separated and added to 200 ml of benzene. The benzene solution was extracted with two 100 ml portions of water, the benzene solution dried over anhydrous sodium sulfate, and then evaporated to dryness. The solid residue was

recrystallized twice from chloroform; yield 430 mg, m.p. 295-295° uncorr.; lit. [13] 295°.

Benzo[k]fluoranthene [6]. *(1)* 4-(8-Fluoranthenyl)-4-oxobutanoic acid. To 30 g (0.148 mole) of fluoranthene and 16.8 g (0.168 mole) of succinic anhydride in 270 ml of dry nitrobenzene were added in small portions with stirring, 39.9 g (0.3 mole) of anhydrous aluminum chloride. The resulting dark green mixture was kept at room temperature for 4 days and then decomposed with ice. Removal of the nitrobenzene by steam distillation gave a brown solid which after several recrystallizations from acetic acid gave 8.5 g of 4-(8-fluoranthenyl)-4-oxobutanoic acid. *(2)* 4-(8-Fluoranthenyl)-butanoic acid. To 4.6 g (0.015 mole) of 4-(8-fluoranthenyl)-4-oxobutanoic acid in 90 ml of ethylene glycol were added 5.75 g (0.115 mole) of hydrazine hydrate with constant stirring. The mixture was heated to 100° and 5.75 g (0.103 mole) of potassium hydroxide added. The reddish solution was then heated at 160° for 5 hours, cooled to room temperature, and acidified with 6*N* HCl to a pH of approximately 1. The precipitate which formed was collected, dissolved in benzene, and the benzene solution dried over anhydrous magnesium sulfate. The benzene solution was concentrated and cream colored needles of the desired product separated. *(3)* 8,9,10,11-Tetrahydro-

8-oxobenzo[k]fluoranthene. To a stirred solution of 2 g (0.00735 mole) 4-(8-fluoranthenyl)butanoic acid in 35 ml of dry benzene at 10° were added 1.75 g (0.0084 mole) of phosphorous pentachloride over a 5 minute period. The mixture was slowly brought to reflux temperature over a period of 20 minutes during which time evolution of hydrogen chloride occurred. The mixture was then refluxed 10 minutes. The solution was cooled to 10° and 3.64 g (0.014 mole) of stannic chloride in 40 ml of dry benzene was added over a 1 hour period. The mixture was then stirred for an additional hour at 10°, decomposed with 60 g ice and 50 ml concentrated hydrochloric acid, and the mixture heated gently until the evolution of hydrogen chloride ceased. The cooled reaction mixture was transferred to a separatory funnel and the water layer after separation was washed with three 25 ml portions of benzene. The benzene extracts were combined with the original benzene layer and the combined solution was washed successively with two 50 ml portions of water, two 25 ml portions of 10% sodium carbonate, two 50 ml portions of water, and two 25 ml portions of saturated sodium chloride solution. The benzene solution was dried over magnesium sulfate, evaporated to dryness, and the yellow residue recrystallized from benzene, giving 1 g of 8,9,10,11-tetrahydro-8-oxobenzo[k]fluoranthene.

(4) 8,9,10,11-Tetrahydrobenzo[k]fluoranthene. To 1 g (0.0037 mole) of 8,9,10,11-tetrahydro-8-oxobenzo[k]-fluoranthene in 40 ml of ethylene glycol was added 1.25 g (0.025 mole) of hydrazine hydrate with constant stirring. The solution was heated to 100° and 1.25 g (0.0223 mole) of potassium hydroxide added. The temperature of the reaction was then raised to 160° and kept at this temperature for 5 hours. The reddish solution was cooled and diluted with water. The yellow precipitate was collected, washed with water, air-dried and recrystallized from ethanol. The yield of 8,9,10,11-tetrahydrobenzo[k]fluoranthene was 0.4 g.

(5) Benzo[k]fluoranthene. To 0.4 g (0.00156 mole) of 8,9,10,11-tetrahydrobenzo[k]fluoranthene in 5 ml of benzene was added 0.78 g (0.0034 mole) of 2,3-dichloro-5,-6-dicyano-1,4-benzoquinone (DDQ). The solution was refluxed for 45 minutes and the initially blue colored solution became colorless. Another 0.78 g portion of DDQ was added and the solution refluxed an additional 75 minutes. After dilution with petroleum ether (30-60°) and ethyl ether, the solution was filtered and the filtrate evaporated to dryness. The solid residue was dissolved in benzene and chromatographed on neutral alumina. The eluate was evaporated and the residue was recrystallized from hexane-benzene; yield 0.13 g, m.p. 216-217° uncorr.; lit. [13] 217°.

Dibenzo[a,c]phenazine [7]. Phenanthra-9,10-quinone, 1.04 g (0.0002 mole) and *o*-phenylenediamine, 0.54 g (0.0002 mole) were placed in a 25 ml flask and the mixture heated at 100° for 30 minutes with occasional stirring. The resulting solid was dissolved in benzene and chromatographed on neutral alumina with benzene. The fractions containing dibenzo[a,c]phenazine were combined and evaporated to dryness. The solid residue was recrystallized from ethanol; yield 300 mg, m.p. 222.5-223.5° uncorr.; lit. [7] 218-220°.

Dibenzo[g,p]chrysene [8]. To 10 g (0.0555 mole) of fluorenone ground together with 10 g (0.153 mole) of zinc dust were added 10 g (0.171 mole) sodium chloride and 10 g (0.366 mole) of zinc chloride. The mixture was stirred and the temperature raised to 320-340° and kept there for 5 minutes. The initial green color turned to dark red. On cooling, a dark red layer separated from the zinc chloride. The cold mixture was decomposed with acetic acid, extracted with benzene, the benzene solution dried over magnesium sulfate and concentrated. The yellowish precipitate (1.8 g) of 9-diphenylene-10-oxo-9, 10-dihydrophenanthrene was collected by filtration. To 1.2 g (0.00348 mole) of this diphenylene-phenanthrene was added 1.2 g (0.0184 mole) of zinc dust, 1.2 g

(0.0205 mole) of sodium chloride, and 6 g (0.044 mole) of zinc chloride. The mixture was stirred and heated to 320-330° for 15 minutes. After cooling, the mixture was decomposed with 30 ml of acetic acid, extracted with 75 ml of benzene and the benzene extract was dried over magnesium sulfate. The solvent was evaporated and the residue was taken up in hexane and chromatographed on a neutral alumina column. Evaporation of the eluate and recrystallization of the residue from acetic acid gave 230 mg of product; m.p. 217-218° uncorr.; lit. [13] 218-220°.

Dibenz[a,c]acridine [9]. To 4 g (0.019 mole) of phenanthrene-9,10-quinone stirred in 30 ml of refluxing methanol was added 36.5 g (0.102 mole) of stannous chloride over a period of one-half hour. Concentrated hydrochloric acid (20 ml) was gradually added, and the mixture refluxed until solution occurred. To the resulting clear red solution was added 3 g (0.0175 mole) of *o*-nitrobenzylchloride whereupon the solution turned black. On heating, a brownish-yellow precipitate formed, and heating was continued for an additional 30 minutes. The reaction mixture was cooled and filtered. The yellow product was treated with boiling 5*N* potassium hydroxide for 1 hour, the solution filtered, and the residue dissolved in benzene. The benzene solution was dried over sodium sulfate, concentrated, and the desired

product allowed to crystallize; yield 2.4 g, m.p. 201° uncorr.; lit. [15] 201°.

Benzo[b]fluoranthene [10]. To 1.0 g (0.006 mole) of fluorene in 20 g of quinoline, 5 g (0.085 mole) of potassium hydroxide was added and then 0.84 g (0.006 mole) of 2-chlorobenzaldehyde was added at 100°. The mixture was heated, under nitrogen, at 210° for 3 hours [10b,c]. The black reaction mixture was cooled, 100 ml of benzene was added and after separation the organic layer was washed with two 50 ml portions of water, two 50 ml portions of 6*N* hydrochloric acid, two 50 ml portions of water and the organic solution dried over sodium sulfate. After evaporation of the solvent, the residual brownish material was dissolved in hexane and chromatographed on a neutral alumina column. The eluate was evaporated and the residue was recrystallized from hexane giving 1.1 g of product, m.p. 168-169° uncorr.; lit. [13] 168°.

Benz[a]acridine [11]. *(1)* *o*-Toluidine hydrochloride. To a stirred solution of 20 g (0.185 mole) *o*-toluidine, at 0°, was added excess concentrated hydrochloric acid. The resulting white crystalline hydrochloride was recrystallized from water and dried under vacuum.

(2) *o*-Tolyl-2-naphthylamine. *o*-Toluidine hydrochloride, 10 g, (0.07 mole) and 11 g (0.076 mole) of recrystallized

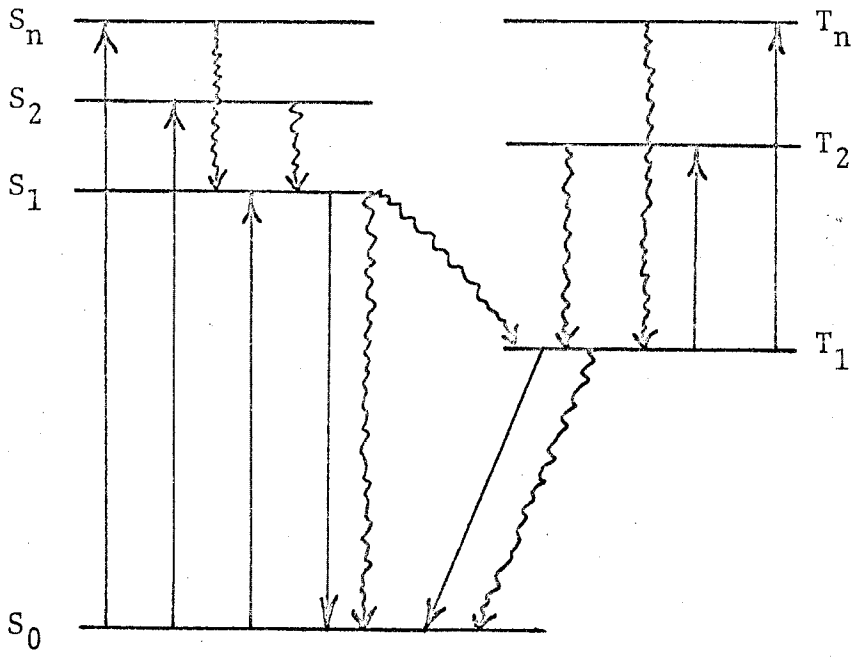
2-naphthol were heated together in a flask at 230-240° for 5 hours. The reaction mixture was cooled and made alkaline with 6*N* sodium hydroxide. The solution was extracted with ether-ethanol and the extracts washed with water, dried over sodium sulfate and evaporated to dryness. The resulting brownish black material was chromatographed on a neutral alumina column using 700 ml of hexane and benzene (1:1), then 1100 ml of carbon tetrachloride. After removal of the solvent the residue was vacuum distilled at 2 mm giving 8 g of a reddish brown amine. *(3)* Benz[a]acridine. To 8 g (0.0342 mole) of *o*-tolyl-2-naphthylamine was added 80 g (0.0358 mole) of lead oxide (PbO) and the mixture heated at 280° for 1 hour. After cooling, the reaction mixture was extracted with ethanol and ether, the extracts evaporated to dryness, and the residue dissolved in benzene and chromatographed on a basic alumina column. After evaporating the benzene, the resulting yellowish solid was recrystallized from hexane; yield 0.64 g, m.p. 131° uncorr.; lit. [11] 131°.

11H-Benzo[a]carbazole [12]. *(1)* 5,6-Dihydro-11H-benzo[a]-carbazole. To 10 g (0.0685 mole) of 1-tetralone was added 7.3 g (0.0675 mole) of phenyl hydrazine and the mixture heated for 1 hour at 100°. Fifty milliliters of 10% sulfuric acid were added and the mixture heated an

additional 4 hours at 100°. The mixture was then extracted with ether, the ether dried and evaporated to dryness. The resulting residue was vacuum distilled and the liquid fraction boiling at 90° (2mm) removed. The residue left behind in the pot was recrystallized from petroleum ether (30-60°) and benzene, yielding 6.8 g of 5,6-dihydro-11H-benzo[a]carbazole. *(2)* 11H-Benzo[a]-carbazole. To 5 g (0.024 mole) of lead oxide (PbO) was added 0.5 g (0.0023 mole) of 5,6-dihydro-11H-benzo[a]-carbazole and the mixture was heated at 290° for 1 hour. The cooled reaction mixture was extracted with ether and ethanol, filtered, and evaporated to dryness. The residue was dissolved in benzene and chromatographed on a basic alumina column. Evaporation of the eluate gave a white solid which was recrystallized from hexane-benzene (1:1); yield 0.22 g, m.p. 232-233° uncorr.; lit. [12] 288°.

B. Photophysical Properties of Polynuclear Aromatic Compounds

The interaction [14, 15, 16, 17, 18] of a polynuclear aromatic hydrocarbon with ultraviolet and visible light can be described by the following diagram:



Those processes represented by solid lines involve either absorption or emission of a photon and the radiationless processes are represented by wavy lines. The electronic absorption spectrum results from the promotion of a molecule from its ground state (S_0) to one of its excited states ($S_1, S_2, \ldots\ldots S_n$) through the interaction of the molecule with electromagnetic radiation of the appropriate frequency. This transition takes place in 10^{-15} sec. The energy of each excited singlet state,

relative to the ground state, can then be determined from the 0–0 band of each electronic transition. Molecules promoted to singlet states other than S_1 are rapidly converted back to the zero vibrational level of S_1. This process is called internal conversion.

Once a molecule is in its first excited singlet state, it can react a number of ways as shown below.

$$S_1 \longrightarrow S_0 + h\nu \qquad (1)$$

$$S_1 \longrightarrow S_0 + \text{heat} \qquad (2)$$

$$S_1 \longrightarrow T_1 \qquad (3)$$

$$S_1 + Q \longrightarrow S + Q^* \qquad (4)$$

$$S_1 \longrightarrow \text{chemical change} \qquad (5)$$

It can emit a quantum of radiation as indicated in reaction (1). This process, usually over in 10^{-6}-10^{-9} sec, is called fluorescence and gives rise to a fluorescence emission spectrum. The quantum yield for fluorescence or the quantum efficiency for this step is defined as the number of molecules undergoing this process per number of quanta absorbed by the system. A second way in which the molecule can return to the ground state is by loss of electronic excitation energy to the surroundings in the form of heat (reaction (2)). This

process is also called internal conversion. The excited singlet state can also undergo intersystem crossing (reaction (3)) with formation of the molecule in its triplet state.

In reaction (4) the excited singlet state of the molecule interacts with the ground state of another molecule and the electronic energy associated with S_1 is transferred to Q, the acceptor molecule, promoting it to an excited state. Usually this reaction is described as quenching of the S_1 state or energy transfer. Finally, reaction (5) involves some chemical change, such as fragmentation or rearrangement, resulting from absorption of light. This last process is relatively unimportant for aromatics.

The intersystem crossing process allows the molecule to enter the triplet manifold where it rapidly relaxes to the lowest vibrational level of T_1. Then the molecule in its lowest triplet state can react according to the following scheme:

$$T_1 \longrightarrow S_0 + h\nu_p \qquad (6)$$

$$T_1 \longrightarrow S_0 + \text{heat} \qquad (7)$$

$$T_1 + Q \longrightarrow S_0 + Q^* \qquad (8)$$

$$T_1 \longrightarrow \text{chemical change} \qquad (9)$$

$$T_1 + h\nu \longrightarrow T_n \qquad (10)$$

$$T_1 + T_1 \longrightarrow S_1 + S_0 \qquad (11)$$

$$S_1 \longrightarrow S_0 + h\nu_f \qquad (12)$$

The molecule can emit a quantum of radiation from the triplet state (reaction (6)). This process is called phosphorescence and gives rise to the phosphorescence emission spectrum. The rate of phosphorescence is slow compared to the other processes since it is a spin forbidden transition, and therefore it is observed only when the other rates are slowed. Phosphorescence spectra are therefore usually run at liquid nitrogen temperatures (77°K) in a solvent mixture which sets as a transparent glass. The energy of the lowest triplet state is then determined from the position of the highest frequency band (the 0–0 band) in the phosphorescence spectrum.

Reaction (7) represents an intersystem crossing, followed by internal conversion, and results in the conversion of the electronic energy in the triplet state into heat lost to the surrounding environment. Reaction (8) is an energy transfer from the triplet to some acceptor molecule. It is similar to reaction (4) except here the acceptor molecule is promoted to its triplet state. Reaction (9) involves a chemical transformation

from the triplet state of the molecule and as in reaction (5) is relatively unimportant.

Triplet-triplet absorption is shown in reaction (10). The triplet state may persist long enough, in solution, to undergo an absorption of another photon of appropriate frequency and promote the molecule to a higher triplet state.

Finally, if the triplet excited state is sufficiently populated, reaction (11) will be observed. This process will last about 10^{-4} sec for aromatics and gives rise to delayed fluorescence through reaction (12).

The singlet (E_S), triplet (E_T) and singlet-triplet splittings ($\Delta E_{S,T}$) energies for 59 compounds were determined and given in Table 1. The E_S and E_T values were calculated from the frequency of the 0–0 band in the fluorescence and phosphorescence spectra respectively or were taken from the literature.

The mean fluorescence lifetimes, τ_f, of 18 compounds were determined by the method of Berlman [16] and are given in Table 1. Fluorescence spectra were determined in the presence of oxygen. Nitrogen was then bubbled through the samples for two minutes to remove the oxygen, and the fluorescence spectra were then retaken.

Oxygen acts as a quencher of fluorescence and the fluorescence quantum yield in the absence of oxygen (ϕ_f^o) is therefore larger than the quantum yield in the presence of oxygen (ϕ_f). The quantity ϕ_f^o/ϕ_f can then be related to the mean fluorescence lifetime by the Stern-Volmer equation:

$$\phi_f^o/\phi_f = 0.9372 + \tau_f(5.828 \times 10^7)$$

This equation was derived by doing a least squares analysis on ϕ_f^o/ϕ_f versus τ_f values for 50 compounds described by Berlman. The ratio of the quantum yields were determined by cutting and weighing the individual fluorescence spectra and then calculating the ratio of their weights, i.e.

$$\frac{\phi_f^o}{\phi_f} = \frac{\text{weight of fluorescence spectrum without oxygen}}{\text{weight of fluorescence spectrum with oxygen}}$$

The mean phosphorescence lifetimes, τ_p, for 40 compounds were determined and are given in Table 1. The phosphorescence decay of a pure compound follows the equation:

$$I_p = I_p^o \exp(-t/\tau_p)$$

where I_p^o = phosphorescence intensity at t=0

I_p = phosphorescence intensity at time t

t = time

τ_p = mean phosphorescence lifetime

A plot of $\ln I_p$ vs time is a straight line with a slope of $1/\tau_p$. The decay times were obtained with the assistance of a minicomputer. Details are given in the experimental section.

Triplet lifetimes in fluid solution (methanol in this work) can be determined by monitoring the decay of delayed fluorescence. Delayed fluorescence in polynuclear aromatics is the result of triplet-triplet annihilation. Parker [18] has shown that the mean decay time of delayed fluorescence is equal to one-half the mean lifetime of the triplet state. In this work the mean delayed fluorescence lifetimes for 12 compounds were determined with the aid of a minicomputer and from these values the triplet lifetimes were calculated. The results are given in Table 1.

The determination of relative fluorescence efficiencies, ϕ_f, of two substances with the aid of a spectrofluorometer is relatively simple. The solutions are usually dilute (10^{-6}M in ethanol or methanol) so that errors due to excessive absorption of the exciting light or self-absorption of fluorescence are negligible. Under these conditions the following equation holds:

$$I_f = (I_o 2.303 A_\lambda)\phi_f$$

where I_f = the total fluorescence intensity of a compound

I_o = the light intensity incident on the sample

A_λ = the absorbance of the compound at the exciting wavelength, λ

ϕ_f = the fluorescence efficiency

The integrated area under the fluorescence emission spectrum is proportional to the total intensity of fluorescence emitted by the solution and in turn is proportional to $I_o 2.303 A_\lambda$. Thus if the emission spectra of two solutions are measured with the same apparatus and with the same intensity of exciting light, then

$$\frac{\text{area}(1)}{\text{area}(2)} = \frac{A_\lambda(1)\phi_f(1)}{A_\lambda(2)\phi_f(2)}$$

If one of the substances has a known fluorescence efficiency, the value of the other is obtained. It is usually desirable to have a standard having an emission band situated in the same spectral region as that of the unknown compound. In this work anthracene with an efficiency of 0.27 was used as a standard, if the compound being tested had a spectral distribution in the region of 370-450 nm. If the compound being tested had a spectral distribution in the region of 450-550 nm, then perylene with an efficiency of 0.93 was used as a standard. Before running the spectra, the solutions were oxygenated by passing a current of nitrogen through the solution for two minutes. The results for 20 compounds are given in Table 1. These results are not corrected for the phototube-grating response.

Triplet formation efficiencies, ϕ_{ST}, were determined by using the sensitized delayed fluorescence technique described by Parker [18]. In this method the integrated intensity and lifetime of delayed fluorescence emitted by two solutions containing the same fluorescence acceptor (perylene in this work) but different donors are compared. One of the donors has a known ϕ_{ST} (anthracene in this work). The intersystem crossing efficiency of

the unknown is then given by

$$\phi_{ST}(1) = \frac{I_a(2)\phi_{ST}(2)\tau_{DF}(2)}{I_a(1)\tau_{DF}(1)} \left(\frac{I_{DF}(1)}{I_{DF}(2)}\right)^{1/2}$$

The results for 6 compounds are given in Table 1.

Literature values for mean fluorescence lifetimes, fluorescence efficiencies and triplet formation efficiencies are given in Table 2. Only a limited number of polynuclear aromatics have been studied and when these have been studied in different laboratories the results are sometimes drastically different. The experimental techniques for determining these quantities are, at present, not sufficiently developed to give good, reproducible values. Consequently τ_f, ϕ_f, and ϕ_{ST} values are probably not reliable enough to be used as an index for carcinogenic hazard.

The excited state energy values, E_S, E_T, and $\Delta E_{S,T}$, are precise and easily reproducible. Values for the phosphorescence lifetimes of selected compounds can be found in the literature. However the technique used in this work for obtaining phosphorescence lifetimes is far superior to those previously used. Consequently no literature values are quoted.

Table 1. Photophysical Properties

Carcinogens:

Compound	E_s	E_T	$\Delta E_{s,t}$	τ_f(nsec)	τ_p(sec)	τ_t(msec)	ϕ_f	ϕ_{st}
Benz[a]anthracene	74.2	47.9	26.3	30.9	0.359	0.718	0.48	
Benzanthrone	72.3	46.0	26.3	1.4	0.024		0.43	
Benzo[a]pyrene	71.0	42.2	28.8	37.2	0.105			
11H-Benzo[a]carbazole	81.4	61.1	20.3		4.74			
Benzo[b]fluoranthene	78.3	54.7	23.6	10.4	1.80		0.09	
Benzo[c]chrysene	74.0	55.6	18.4		1.53			
Benzo[c]phenanthrene	76.8	57.1	19.7	52.2	3.34		0.02	
Benzo[g]chrysene	77.0	53.9	23.1	19.2	1.46	0.528	0.04	
Benzo[rst]pentaphene	66.0	40.3	25.7		0.052	0.482		
Dibenz[a,h]acridine	73.1	54.8	18.3		2.31			
Dibenz[a,h]anthracene	72.5	52.2	20.3		1.60	0.272		
Dibenz[a,j]acridine	72.5	53.4	19.1		1.04			
Dibenz[a,j]anthracene	72.4	52.9	19.5		2.51			
Dibenzo[a,e]fluoranthene	60.3	46.8	13.5		0.546			
Dibenzo[a,e]pyrene	72.2	47.0	25.2		0.570	0.380		
7H-Dibenzo[a,g]carbazole	72.7	55.4	17.3	8.6	2.25		0.86	
Tribenzo[a,e,i]pyrene	68.4	46.2	22.2	47.7	0.490		0.06	

Table 1. Continued

Non-Carcinogens:

Compound	E_s	E_T	$\Delta E_{s,t}$	τ_f(nsec)	τ_p(sec)	τ_t(msec)	ϕ_f	ϕ_{st}
Acridine	75.2	45.5	29.7	2.2			0.07	0.18
Anthanthrene	66.0	34.2	31.8					
Anthracene	76.3	42.6	33.7	4.3			0.27	0.70
Benz[a]acridine	74.6	50.8	23.8	6.9	0.406		0.39	0.24
Benz[c]acridine	74.6	50.8	23.8		0.281		0.28	0.60
Benzo[a]fluorene	78.0	57.7	20.3		4.5			
Benzo[b]fluorene	74.4	55.2	24.2		1.2			
Benzo[c]fluorene	79.4	55.2	24.2		1.2			
Benzo[b]chrysene	73.0	45.3	27.7	8.4	0.185		0.34	
Benzo[b]triphenylene	76.4	50.8	25.6		0.794	0.552		
Benzo[e]pyrene	78.1	52.8	25.3		2.12	0.704		
Benzo[ghi]fluoranthene	67.4	54.1	13.3		0.378	0.886	0.30	
Benzo[ghi]perylene	70.3	46.2	24.1		0.438	0.592		
Benzo[k]fluoranthene	71.4	50.5	20.9		0.828	1.06		
Carbazole	84.9	70.1	14.8	9.4	8.04		0.01	
Chrysene	79.3	57.2	22.1		2.54	0.436		
Coronene	66.8	55.4	11.4		9.5			
Dibenz[a,c]acridine	77.0	54.2	22.8	3.9	0.741		0.24	

Table 1. Continued

Compound	E_s	E_T	$\Delta E_{s,t}$	τ_f(nsec)	τ_p(sec)	τ_t(msec)	ϕ_f	ϕ_{st}
Dibenzo[a,c]phenazine	70.9	53.4	17.5		0.286			
Dibenzo[c,g]phenanthrene	72.3	56.6	15.7					
Dibenzo[g,p]chrysene	74.6	49.4	25.2	9.0	0.821		0.48	
Dibenzo[fg,op]naphthacene	76.8	58.2	18.6					
Fluoranthene	70.6	52.8	17.8		0.990		0.22	0.37
Fluorene	94.9	67.8	27.1					
Hexabenzocoronene	64.4	50.1	14.3					
Hexahelicene	69.2	54.6	14.6		2.07			
Naphthacene	60.6	29.3	31.3	4.9			0.20	
Naphthol[1,2-b]triphenylene	73.6	55.3	18.3					
Ovalene	61.2	44.0	17.2					
Pentaphene	67.5	48.4	29.1					
Periflanthene	52.8	34.8	18.0					
Perylene	65.8	36.2	29.6					
Phenanthrene	82.8	61.8	21.0		3.85			
Phenazine	71.4	44.4	27.0	40.3			0.04	
Picene	75.9	57.3	18.6		2.7			
Pyrene	76.9	48.5	28.4	107.8	0.63	0.730	0.47	0.23
Rubicene	53.9	49.6	4.3					

Table 1. Continued

Compound	E_s	E_T	$\Delta E_{s,t}$	τ_f(nsec)	τ_p(sec)	τ_t(msec)	ϕ_f	ϕ_{st}
Tetrabenz[a,c,h,j]-anthracene	74.7	58.8	15.9					
Tetrabenz[a,cd,fg,n]-anthanthrene	66.5	52.8	13.7					
Tetrabenzo[a,c,gf,op]-naphthacene	63.0	56.0	7.0					
Tribenzo[e,ghi,k]perylene	71.2	53.2	18.0					
Triphenylene	84.1	67.4	16.7		15.2			

Table 2. Literature values for τ_f, ϕ_f, and ϕ_{ST}.[a]

Compound	τ_f(nsec)	ϕ_f	ϕ_{ST}
Benz[a]anthracene	44.1	0.19-0.23	0.55-0.82
Benzo[a]pyrene	49.0	0.42	0.58
Dibenz[a,h]anthracene			0.98
Acridine	0.9		
Anthanthrene		0.73	0.23
Anthracene	4.2-5.8	0.27-0.36	0.58-0.72
Carbazole	16.1	0.38	
Chrysene	44.7	0.14-0.23	0.67-0.85
Coronene		0.23	0.56
Fluoranthene	53.0	0.21-0.25	0.79
Fluorene	10.0	0.54-0.80	0.31-0.32
Hexahelicene	14.5	0.041	
Naphthacene	6.4	0.16-0.21	0.63
Perylene	4.68-6.9	0.87-0.98	0.01-0.06
Picene			0.36
Pyrene	290-530	0.32-0.72	0.27-0.38
Triphenylene	36.6	0.066-0.15	0.85-0.95

(a) Taken from references 14, 16, and 18. Consult these works for the original articles.

Values of the triplet lifetimes in solution should approach the values of the phosphorescence lifetimes. Self quenching and impurity quenching lower the lifetime values in fluid solution several orders-of-magnitude. Triplet lifetimes for selected compounds can be found in the literature but these are highly sensitive to the experimental conditions under which they are determined. In this work lifetimes were determined with the aid of a computer and are far superior to previous values.

EXPERIMENTAL

The absorption spectra of the polycyclic aromatics were determined in 95% ethanol on a Cary 14 absorption spectrophotometer. The concentrations of the samples were in the range of 10^{-4}-10^{-6}M. The rest of the photophysical measurements were obtained on an Aminco Bowman Spectrophotofluorometer.

Instrumentation shown in Figure 2 was used in measuring the fluorescence spectra, fluorescence lifetimes, and quantum yields. The light source was a Coherent Radiation Laboratories Model 53 Argon Laser, with excitation wavelengths of 350 and 366 nm. After passing through a 1.0 cm quartz cell and the emission monochromater the monochromatic light entered the phototube. The photomultiplier tube, an RCA 1P28 with an S4 response or an RCA 1P21 with an S5 response, with the voltage set at 800 converted the light into an electrical signal which was then amplified and recorded on an x-y recorder. The 10^{-5}M sample solutions were made in MCB spectrograde methanol or U.S.I. 95% ethanol.

The phosphorescence spectra were obtained by means of the instrumentation shown in Figure 2. Using the argon laser as a light source, the radiation passed through the lower part of a quartz vacuum Dewar which contained

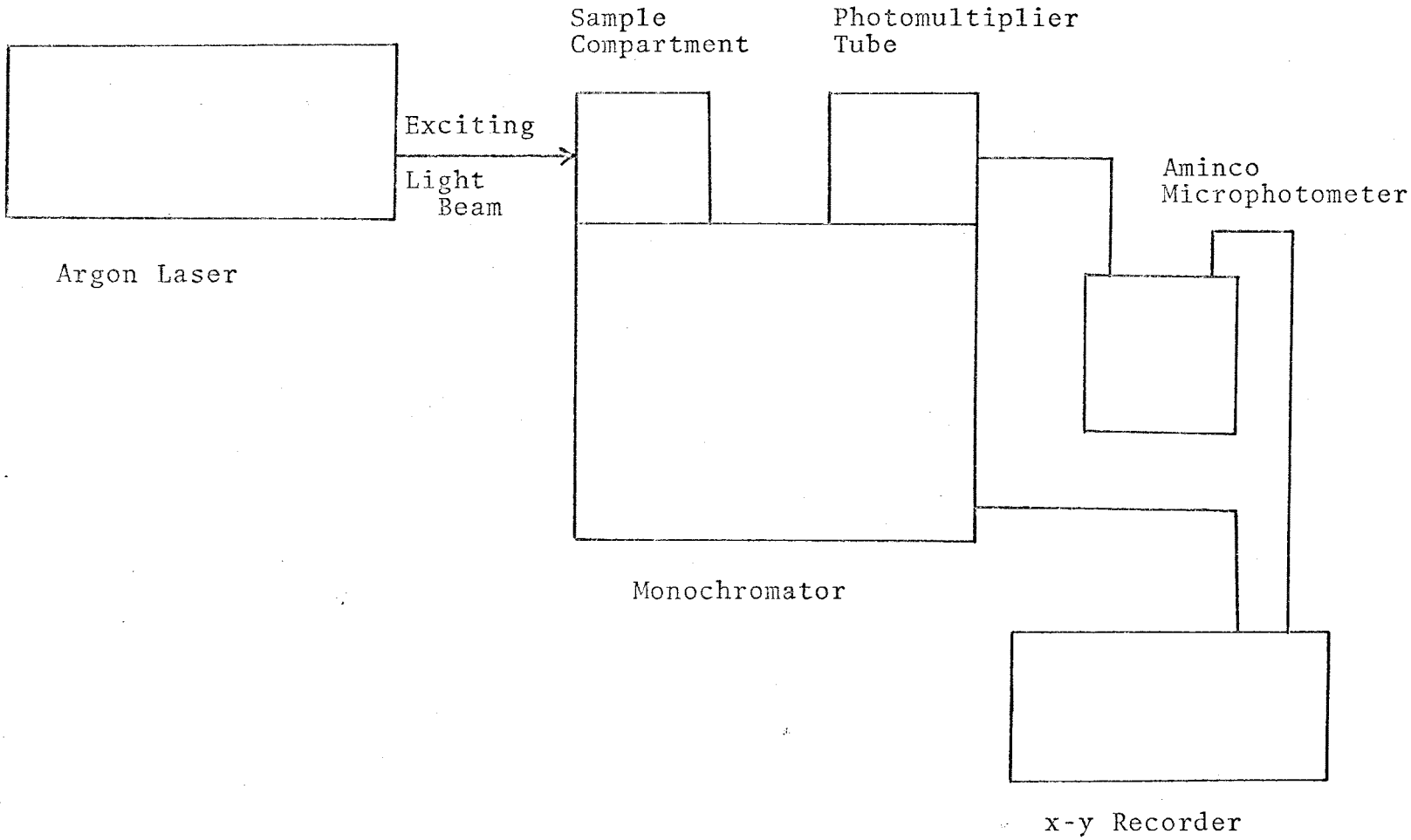
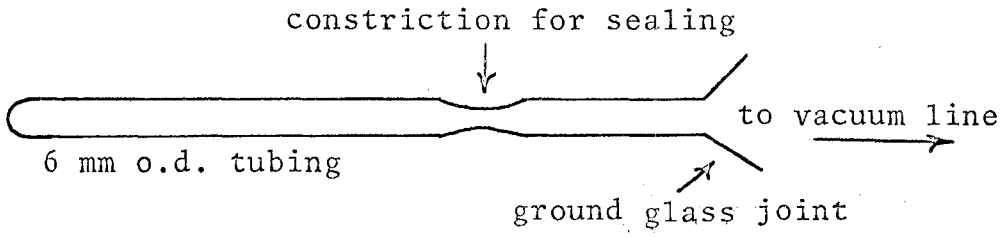


Figure 2. Block diagram of instrumentation used in obtaining fluorescence and phosphorescence spectra, fluorescence lifetimes and quantum yields.

a 6 mm outside diameter Pyrex degassed sample tube at liquid nitrogen temperatures. A rotating shutter with varying speeds and two 60° angle apertures 180° opposite each other surrounded the lower part of the Dewar. Once through the emission monochromater, the light was converted into an electrical signal by either an RCA 1P28 photomultiplier tube or an RCA 7102 with S-1 response, amplified, and recorded on an x-y recorder. The voltage from the RCA 7102 tube was usually set at 1300 and the tube was cooled to dry ice temperatures to keep the dark current noise to a minimum.

The samples were dissolved in U.S.I. 95% ethanol or distilled 95% ethanol as described by Parker [18] and anhydrous ether in a ratio of 1:2. For the more insoluble aromatics, the samples were made in ethanol, ether and toluene in a ratio of 1:2:2. Four-tenths of a ml of 10^{-3}M sample solutions were then placed in sample cells of the type shown below.



The sample solutions were then degassed on a high vacuum line using five freeze-pump-thaw cycles at 10^{-6} torr and the sample was sealed during the fifth cycle.

The phosphorescence lifetimes were obtained by means of instrumentation shown in Figure 3. The modified Aminco Bowman spectrophotometer was the same as for the phosphorescence spectra except that the rotating shutter was removed from the cell compartment. Also, an Avco Everett Research Laboratory Pulsed Nitrogen Laser Model C950 was used as a light source which generated coherent light at 337.1 nm. The electrical signals which were generated, passed from an Aminco microphotometer into a voltage amplifier and were then displayed on a Tektronix 7704 oscilloscope and sent over phone lines to a Ratheon 704 minicomputer. All of the data acquisition and data reduction was handled by the computer (described below) and the results were printed out on the teletype. The samples used and the method in making them were the same as for phosphorescence spectra.

The delayed fluorescence lifetimes were obtained by means of instrumentation shown in Figure 3, except that the Dewar was removed and the light source used was the argon laser. The 10^{-3}M sample solutions were made in MCB spectrograde methanol or spectrograde cyclohexane

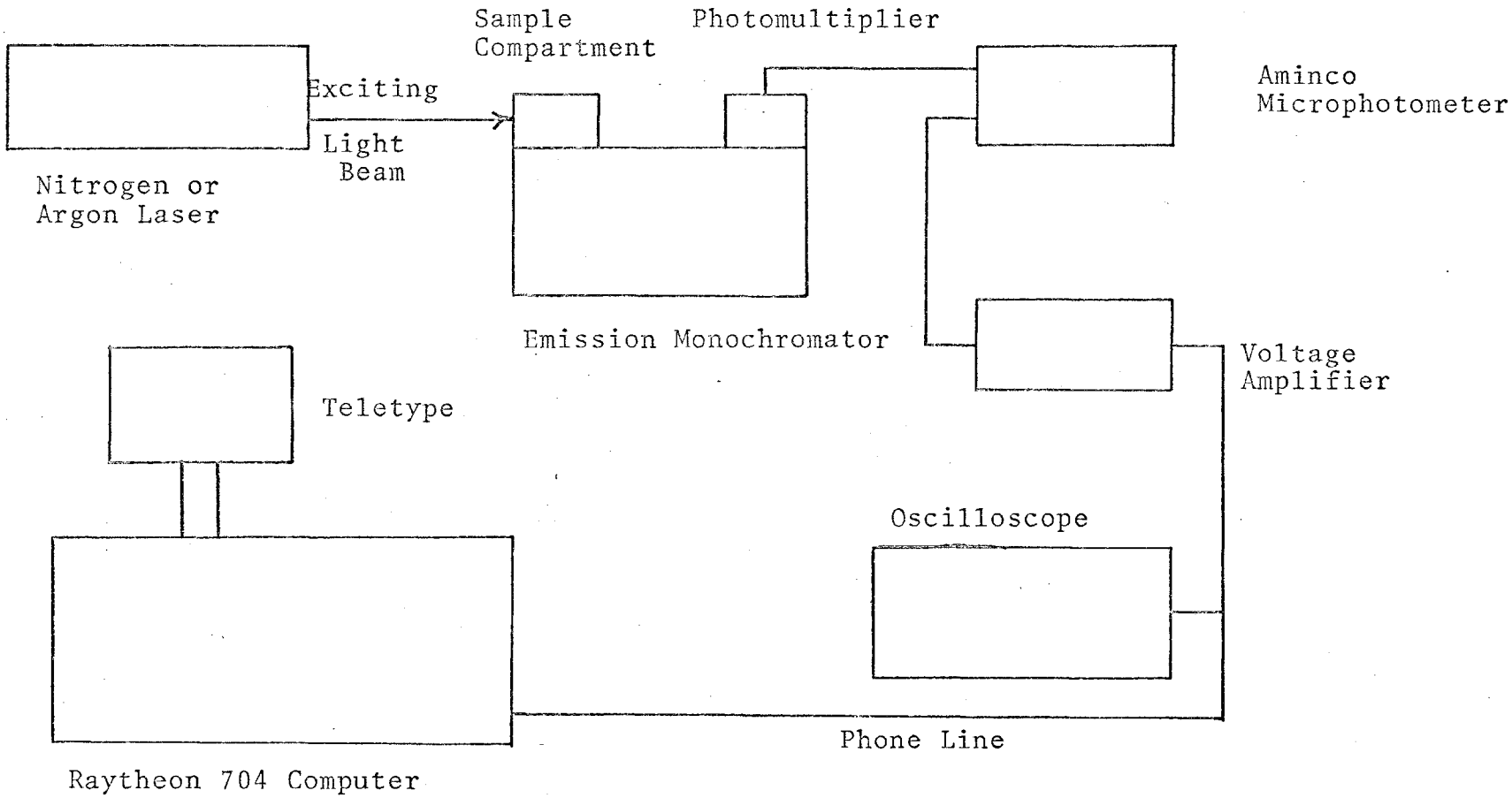


Figure 3. Block diagram of instrumentation used in obtaining Phosphorescence, delayed fluorescence and sensitized delayed fluorescence lifetimes.

and degassed as described for the phosphorescence spectra.

The sensitized delayed fluorescence lifetimes were obtained on the same instrumentation as delayed fluorescence lifetimes except that the 363 nm line was filtered out by the use of a Baush & Lomb monochrometer. The samples were dissolved in spectrograde methanol and degassed according to the procedure above. The donor molecule concentrations were 5 X 10^{-5} M, and the concentration of the acceptor molecule, perylene, was 4 X 10^{-5}M.

Once the electrical signal passed through the voltage amplifier it was carried over the phone lines to a 12 bit A to D converter on the Raytheon 704 minicomputer which had a storage capacity of 16K, 16 bit words (see Figure 4). The 10 volt analog signal was then converted into an 11 bit binary number and a one bit sign indicator with an accuracy of 4.5 millivolts. Since information was collected a number of times for each sample tested, the data was stored on the magnetic tapes for later reduction. The 704 CPU then processed all the data, and the results were printed out on the ASR 33 teletype or plotted out on the 10 bit storage scope.

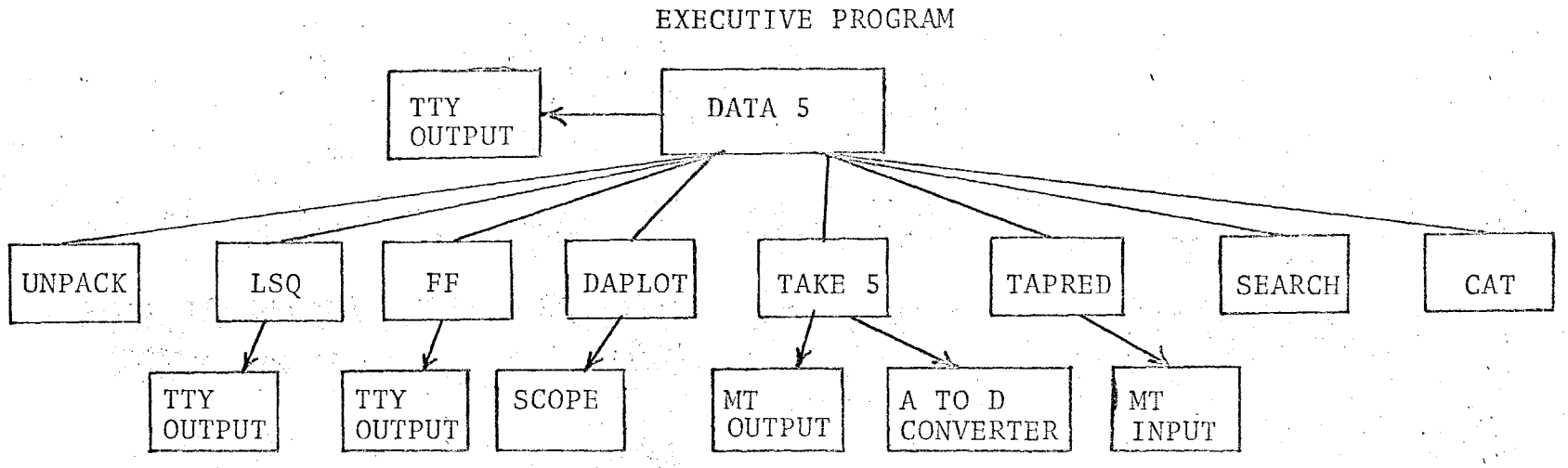
All the programs used were subroutines of the executive program, DATA 5 (figure 4)[19]. After loading DATA 5 the subroutine, TAKE 5, then collected the data and

stored the information on magnetic tapes. TAKE 5 had the capacity of acquiring 1400 points per buffer (NOP) using CAT or 2100 points per buffer if CAT was not needed and could acquire the data at a rate of 8 to 32000 microseconds between points (TBP). The acquisition usually was initiated either with the phosphorescence or delayed fluorescence signal or with a pulse from the laser.

TAPRED then read the data from the magnetic tapes into core, DUMP contained in TAKE 5 indicated what was in the work area and FF read data in free format into core from TTY. SEARCH then averaged the background, CAT averaged N number of runs for each sample and the results were plotted on the storage scope by DAPLOT. By using DAPLOT the number of points in the exponential part of the curve was determined. LSQ then determined the logarithm of the intensity, least squared the log of the intensity versus time, after subtracting out the background, and printed the results on the teletype. The results were given in both first order rate constants and lifetimes where

$$\text{slope} = k = \frac{1}{\tau_p} \quad \text{or} \quad \tau_p = \frac{1}{k}$$

If, however, the results gave a correlation coefficient of less than 0.99, LSQ was repeated with fewer points, i.e.



SOFTWARE

DATA 5 - EXECUTIVE PROGRAM
UNPACK - UNPACK BUFFER
LSQ - LEAST SQUARE PROGRAM
FF - FREE FORMAT TTY INPUT
DAPLOT - PLOT DATA ON SCOPE
TAKE 5 - AQUISITION PROGRAM
TAPRED - READ MAGNETIC TAPES
SEARCH - AVERAGES DATA IN WORK AREA
CAT - AVERAGES N NUMBER OF RUNS

HARDWARE

RAYTHEON 704 CPU - CENTRAL PROCESSING UNIT
HSTR - HIGH SPEED TAPE READER
MADC 12 F/G - A TO D CONVERTER - 12 BIT
ANALOG TO DIGITAL CONVERTER
ASR 33 TTY - TELETYPE
9 TRACK DMA - MAGNETIC TAPE DRIVES
TEKTRONIX 601 WITH RAYTHEON INTERFACE -
10 BIT STORAGE SCOPE
RAYTHEON 704 CORE - STORAGE CAPACITY 16K,
10 BIT WORDS

Figure 4. Diagram of computer program on Raytheon 704.

one data per word, into the Fortran format i.e. one data point per two words so that DUMP and LSQ both of which were in Fortran could execute.

C. Photochemical Analysis Data

The photochemical analysis data consists of the absorption spectrum, the fluorescence, and the phosphorescence emission spectra of the compounds of interest in this study. The absorption spectra of 36 compounds are given in Figure 5 and were obtained on a Cary 14 spectrophotometer using 95% ethanol as solvent in all cases. The emission spectra of the same 36 compounds were obtained on a modified Aminco emission spectrophotometer using an argon laser as the exciting source. The spectra are given in Figure 6 and are uncorrected for grating-photomultiplier response. Two of the compounds (perylene and tetracene) do not phosphoresce. The fluorescence emission spectra were obtained in highly purified 95% ethanol or spectrograde methanol at room temperature and the concentration of polynuclear aromatics were between 10^{-6} to 10^{-3} M. The phosphorescence emission spectra were obtained at 77°K in an EA glass (2 parts ethyl ether and 1 part 95% ethanol). All solutions were approximately 10^{-3} M in polynuclear aromatic. The a absorption, fluorescence, or phosphorescence spectrum of a pure compound serves as a "finger print" for that compound. It is therefore relatively easy to identify a pure but unknown polynuclear aromatic from either

absorption or emission spectral data or a combination of these. The identification of the polynuclear aromatics in a complex mixture such as coal tar is at best difficult and in some cases impossible. In fact mixtures containing six or more major compounds would, in general, have to be subjected to further separation before an accurate spectral analysis could be performed.

Figure 5 . Absorption spectra.

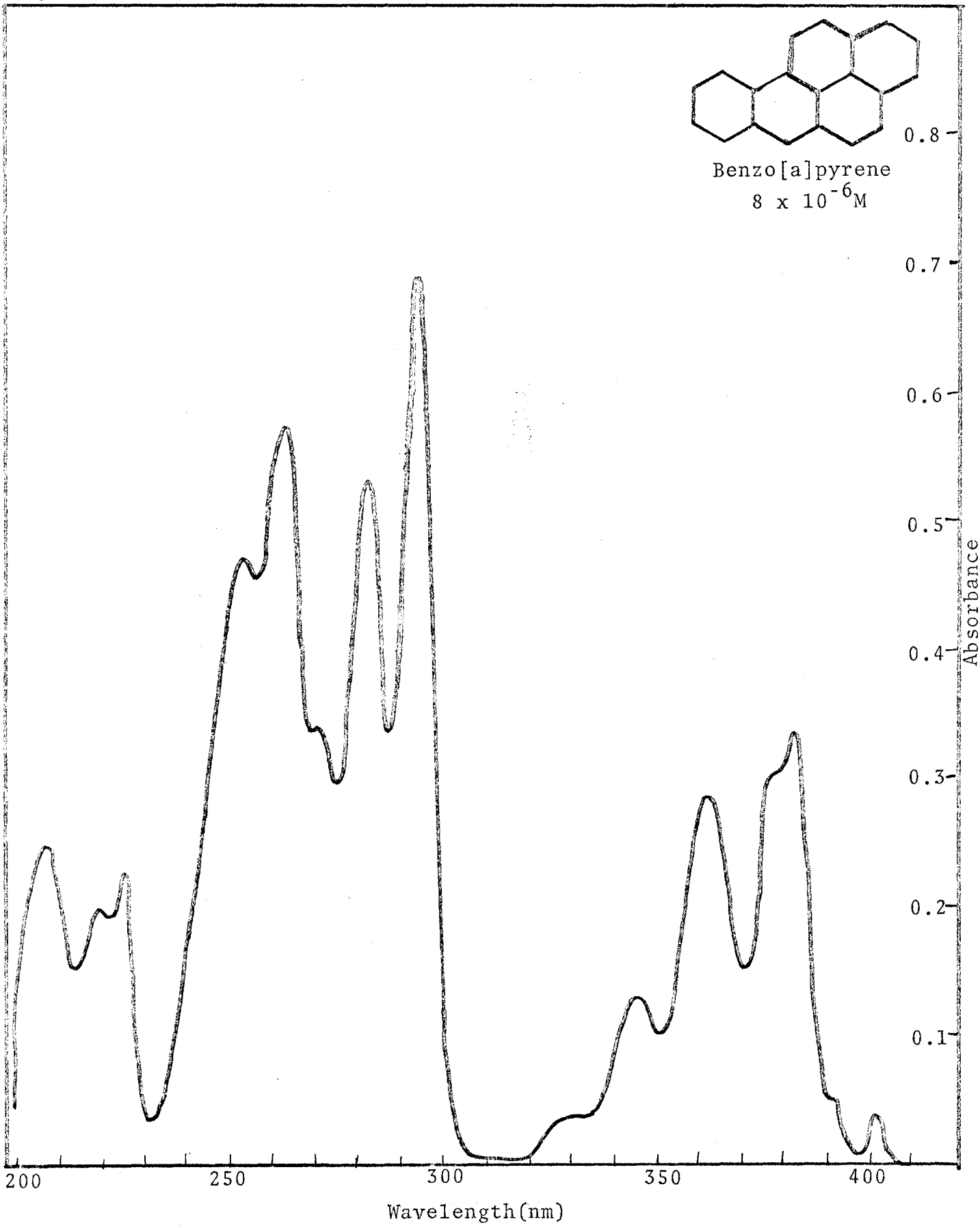
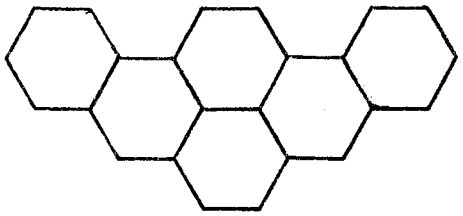


Figure 5. Cont.



54

Benzqrst]pentaphene

6.35×10^{-7}M

Figure 5. Cont.

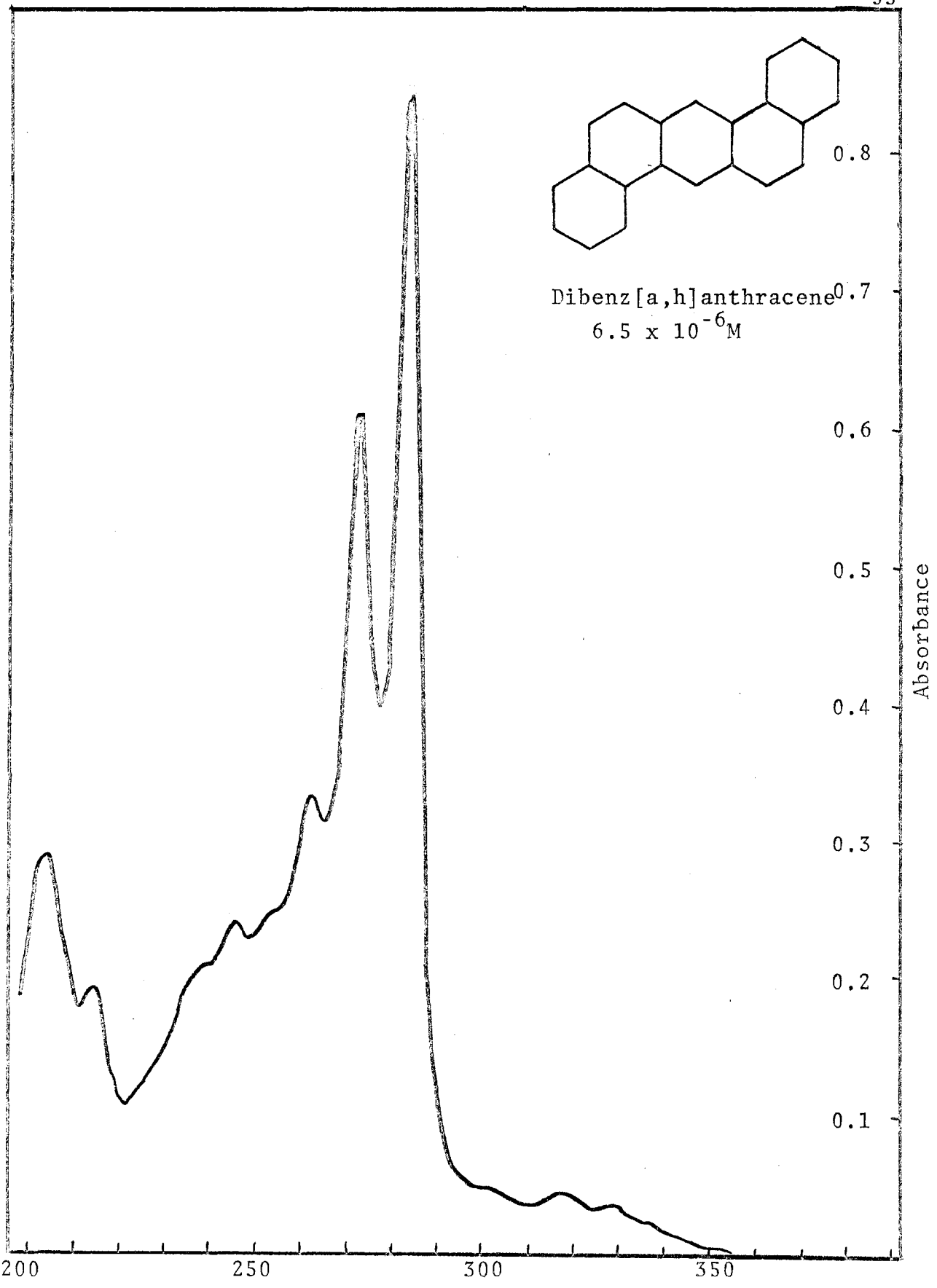


Figure 5. Cont.

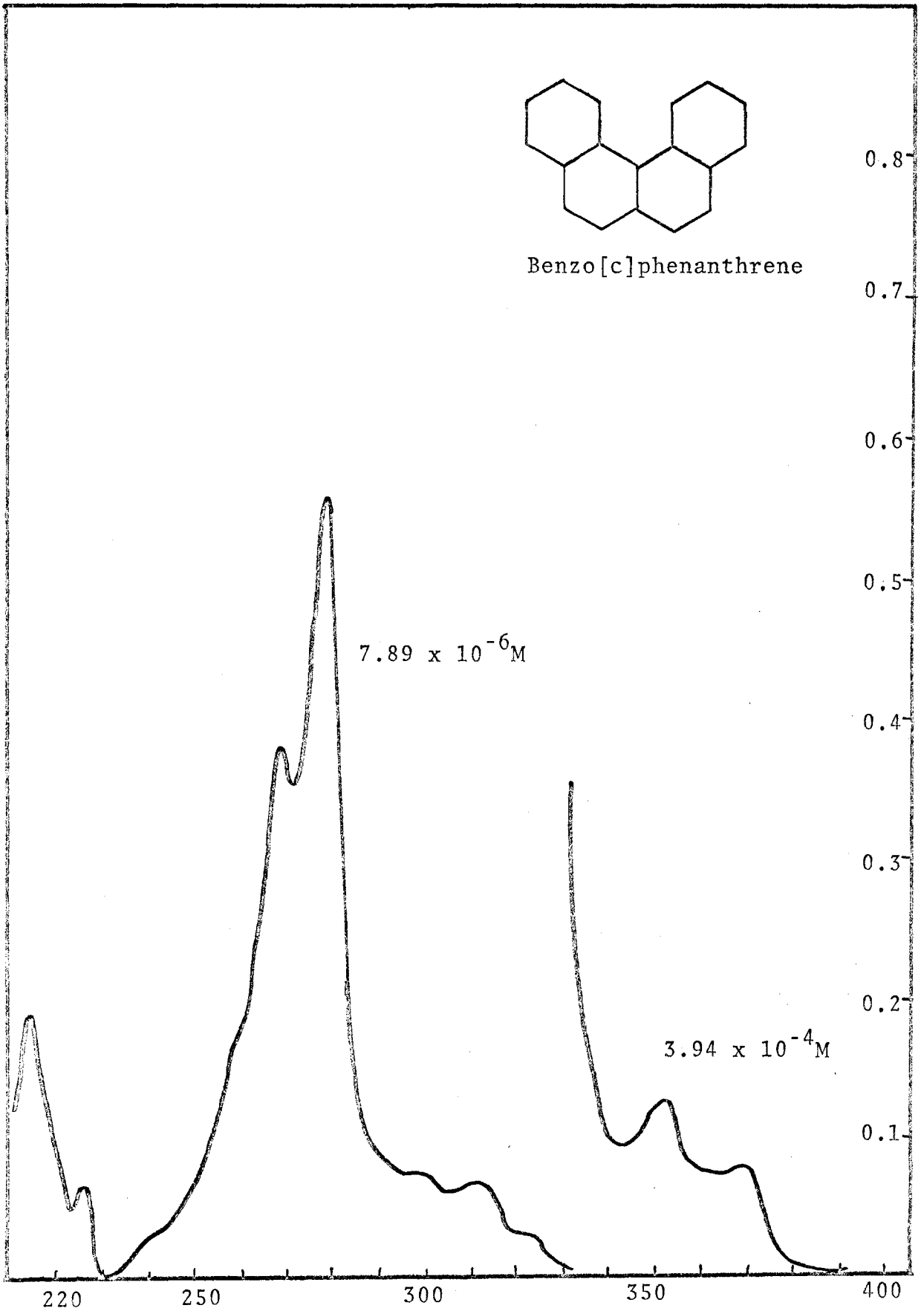


Figure 5. Cont.

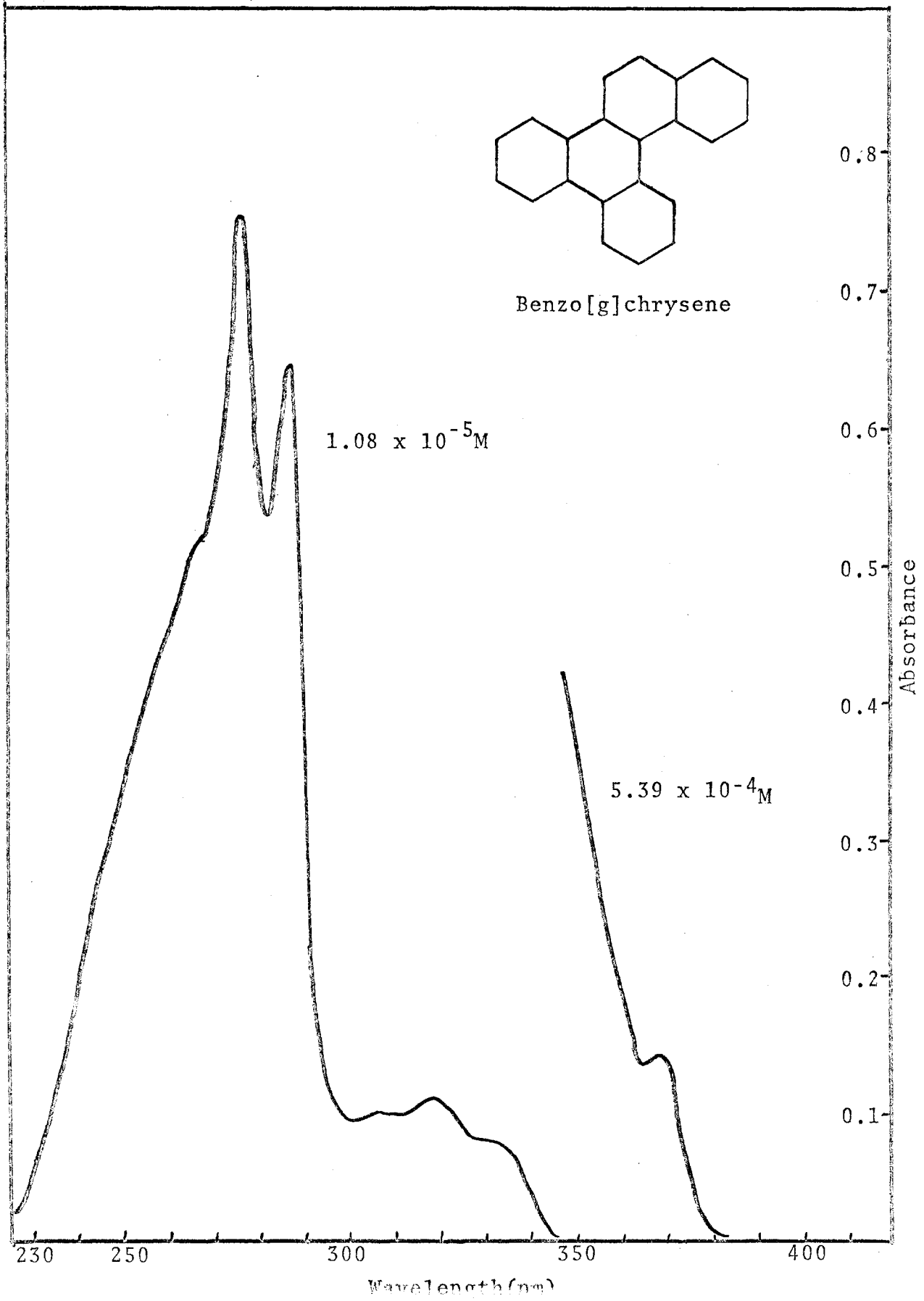


Figure 5. Cont.

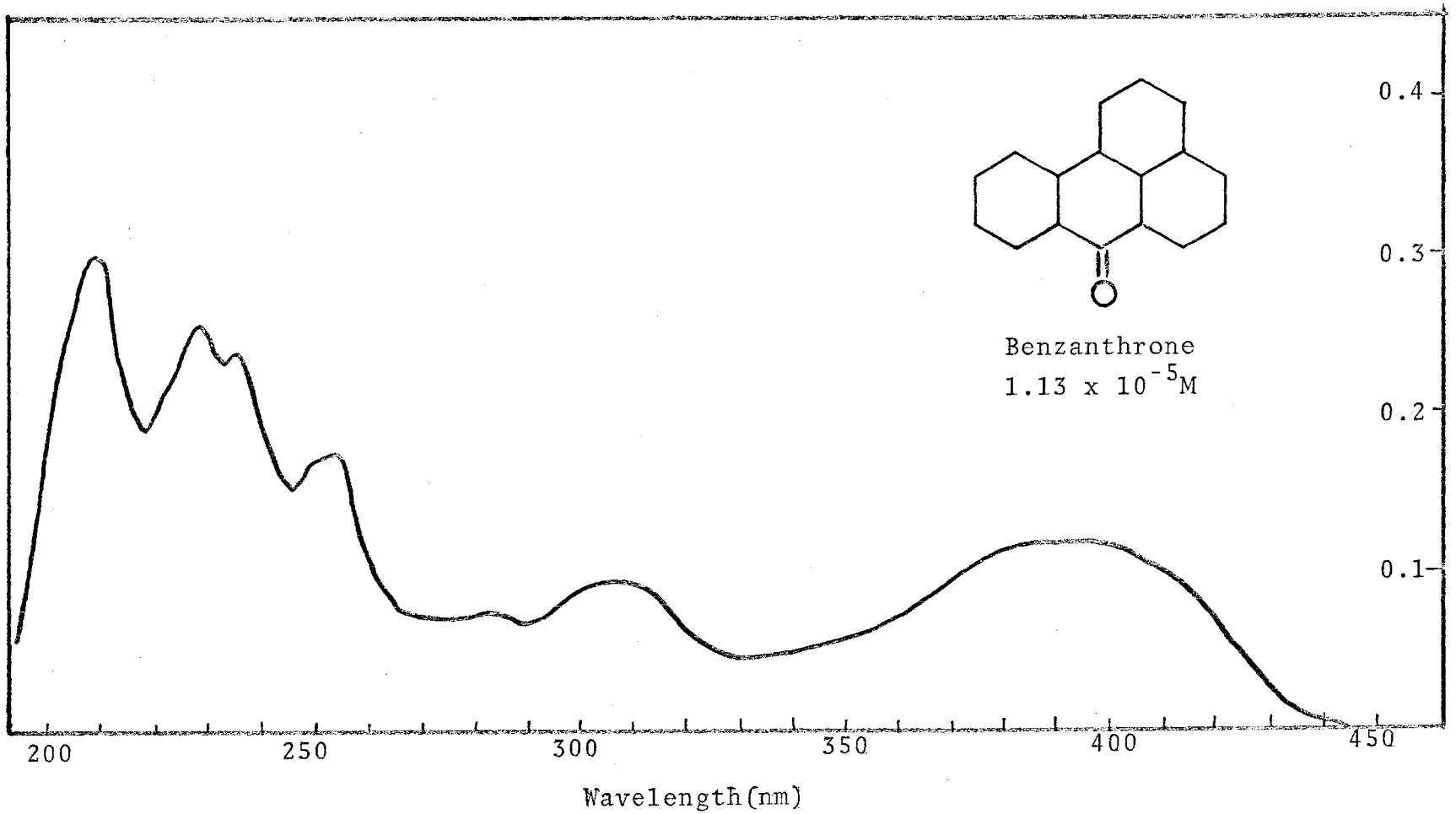


Figure 5. Cont.

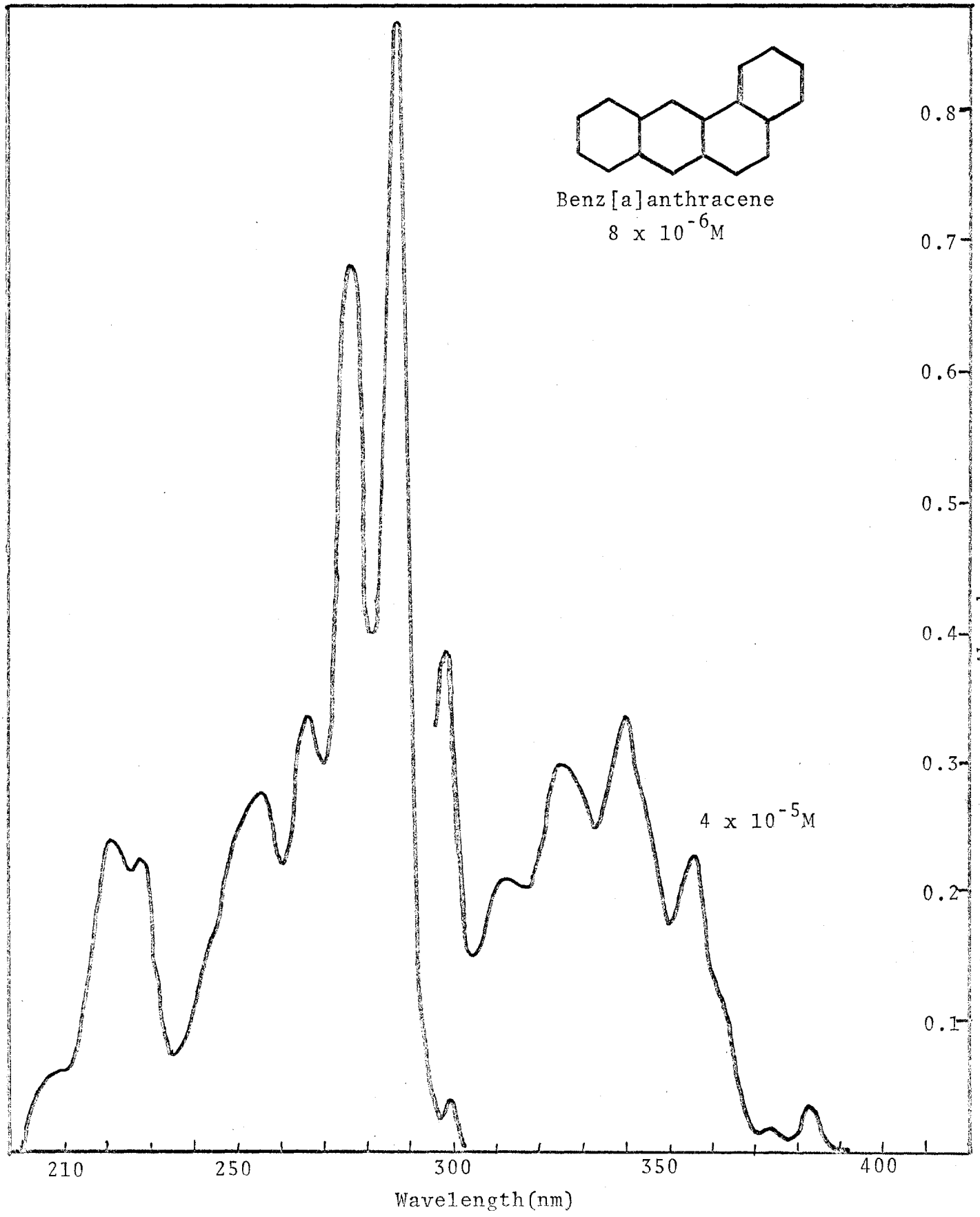


Figure 5. Cont.

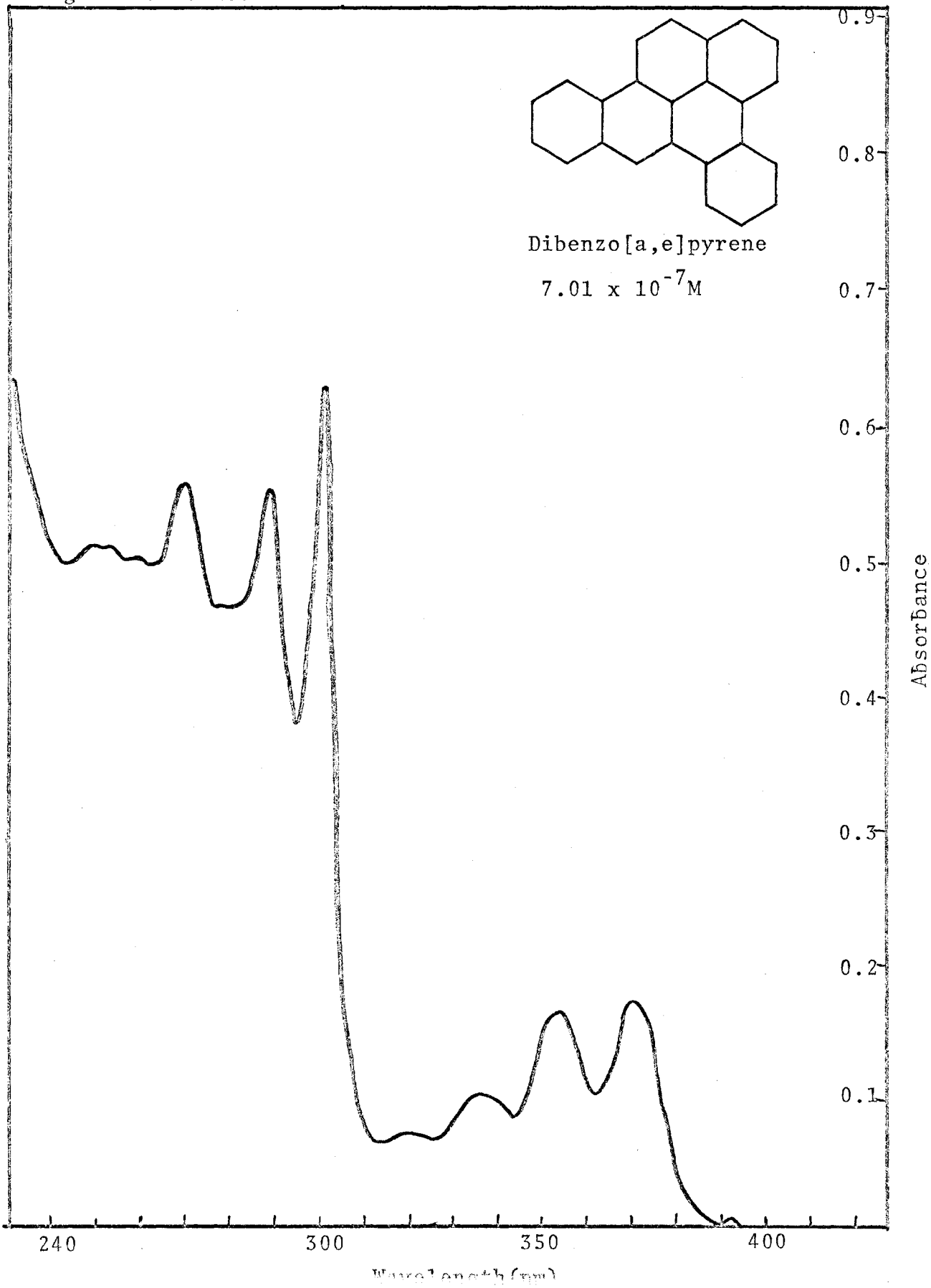


Figure 5 . Cont.

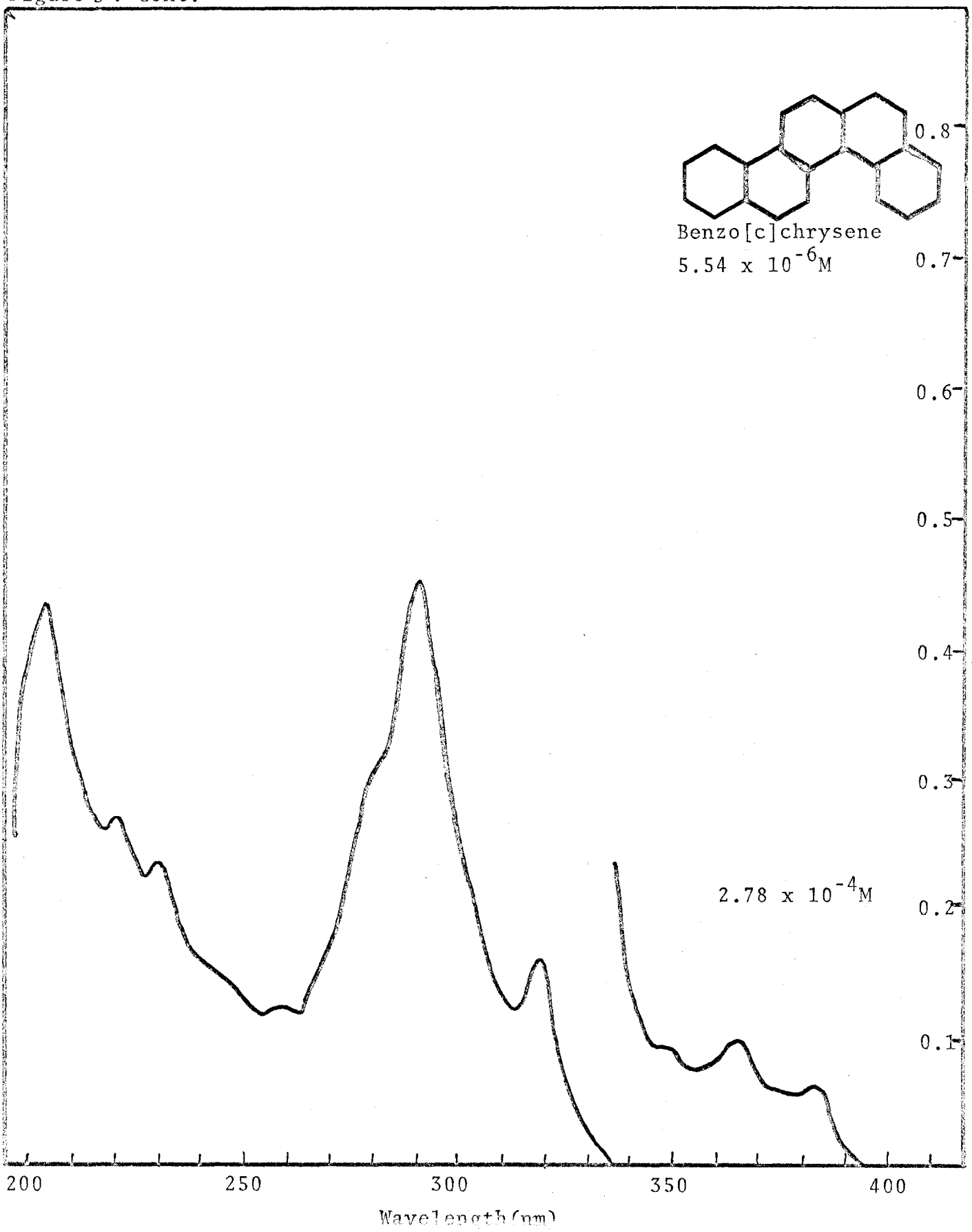


Figure 5. Cont.

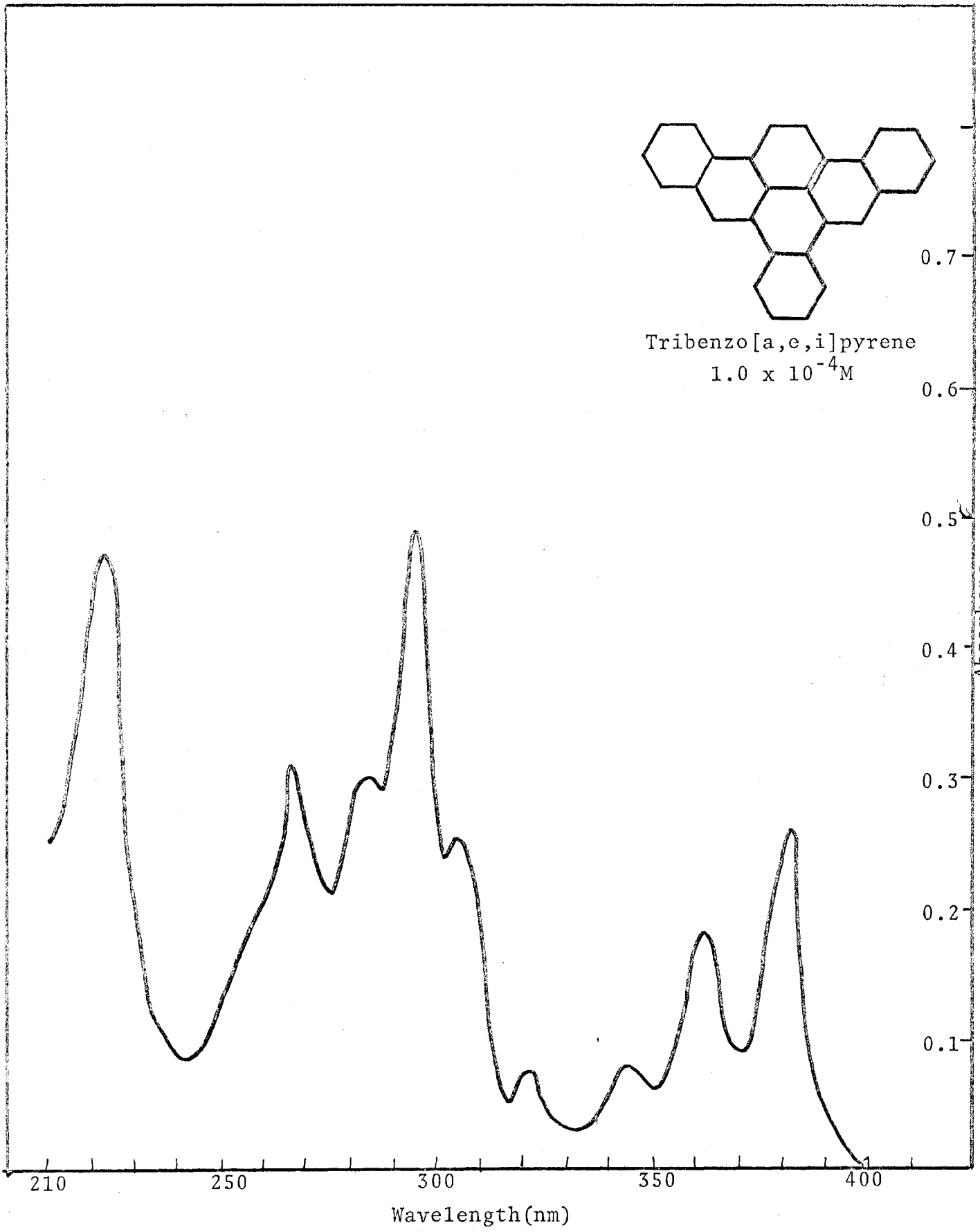


Figure 5 . Cont.

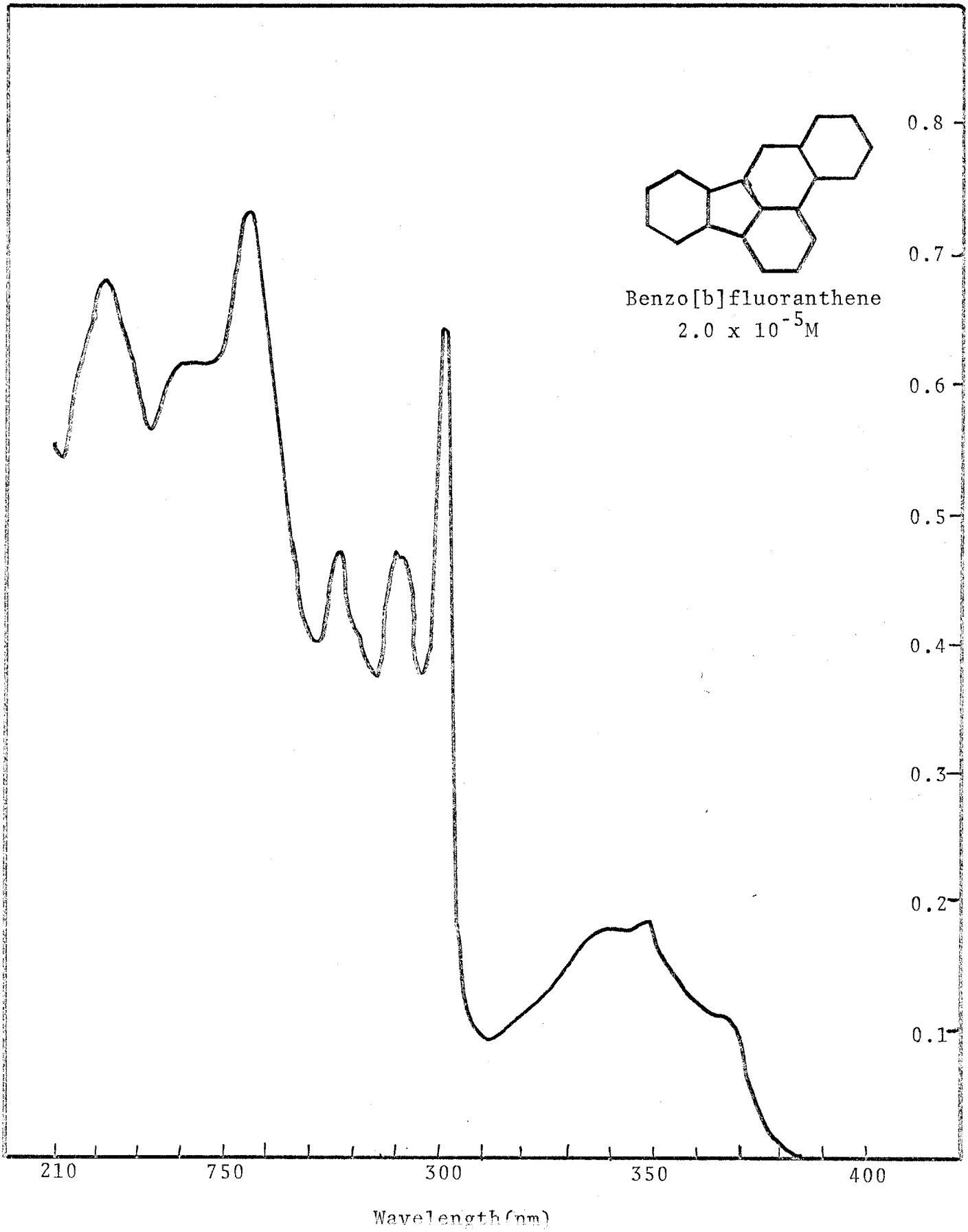


Figure [illegible]

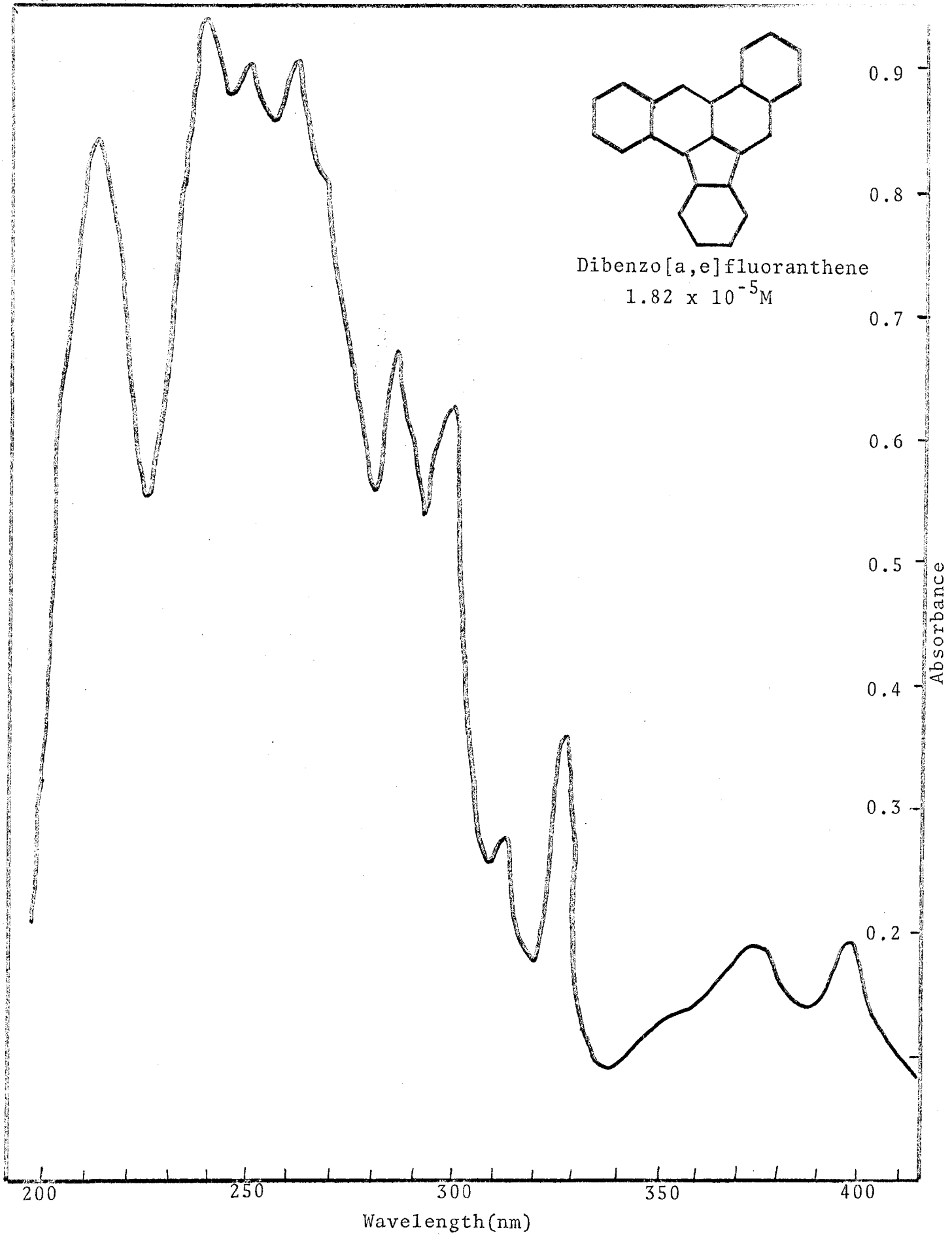

Dibenzo[a,e]fluoranthene
1.82 x 10^-5 M
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
Absorbance
200
250
300
350
400
Wavelength(nm)

Figure 5 . Cont.

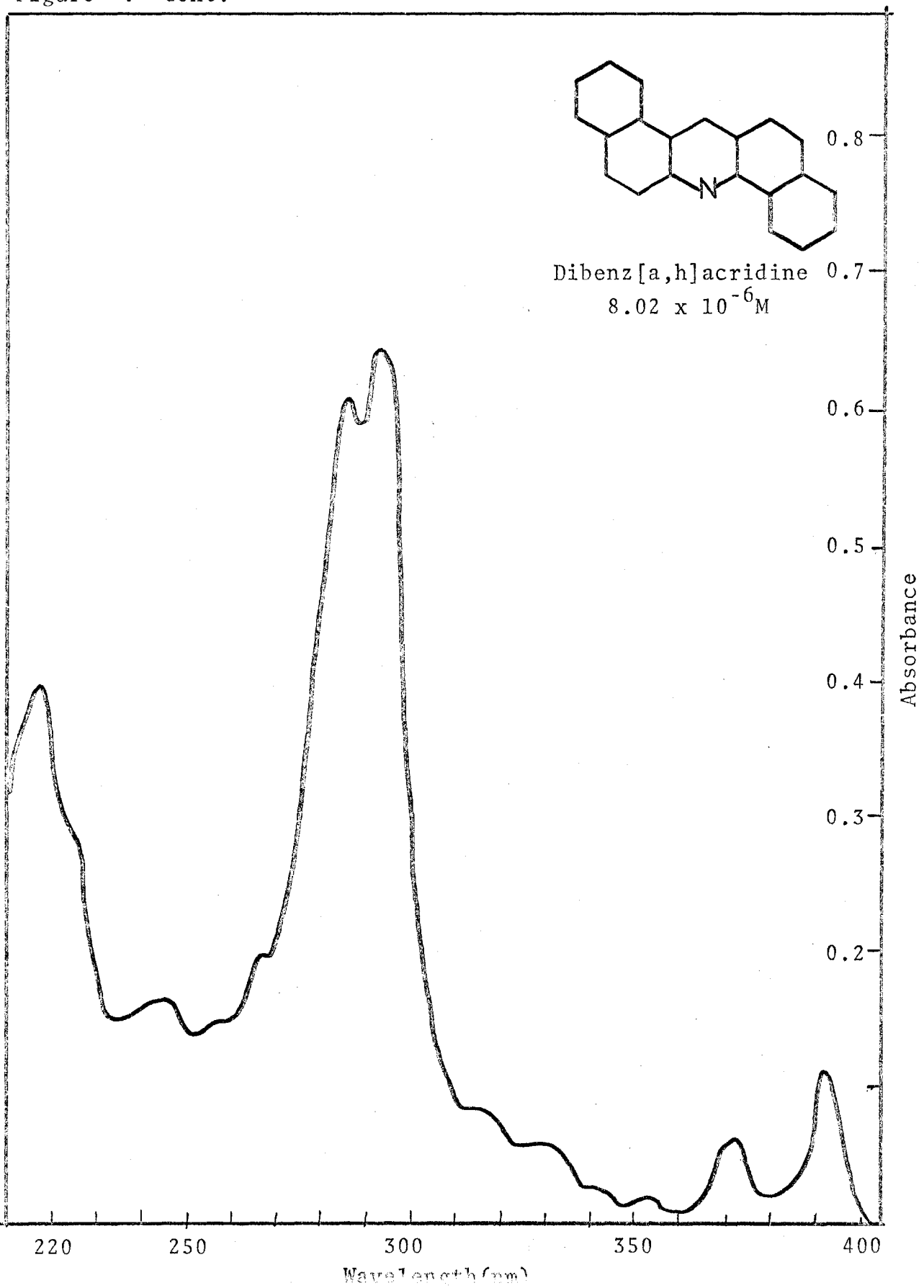
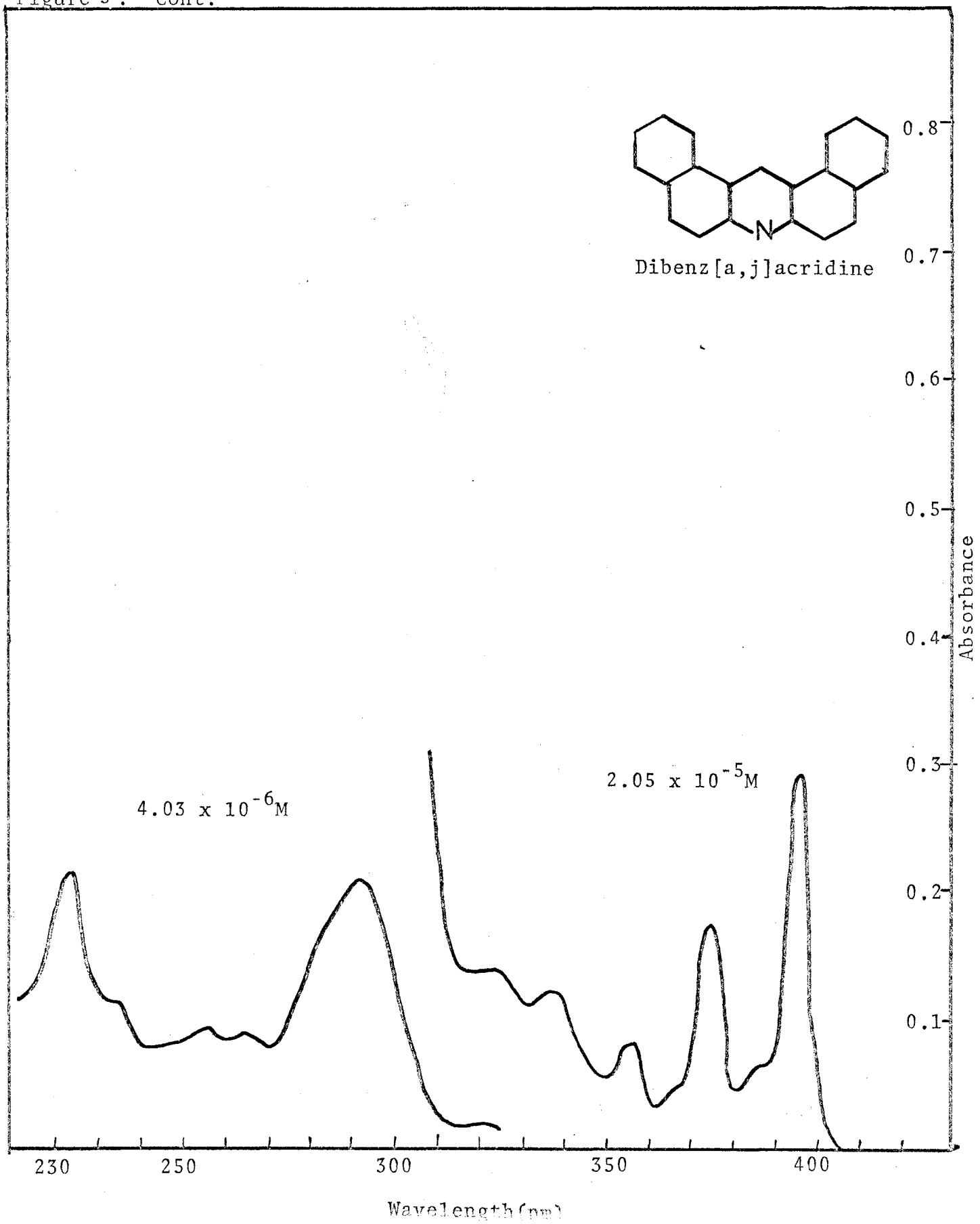


Figure 5 . Cont.



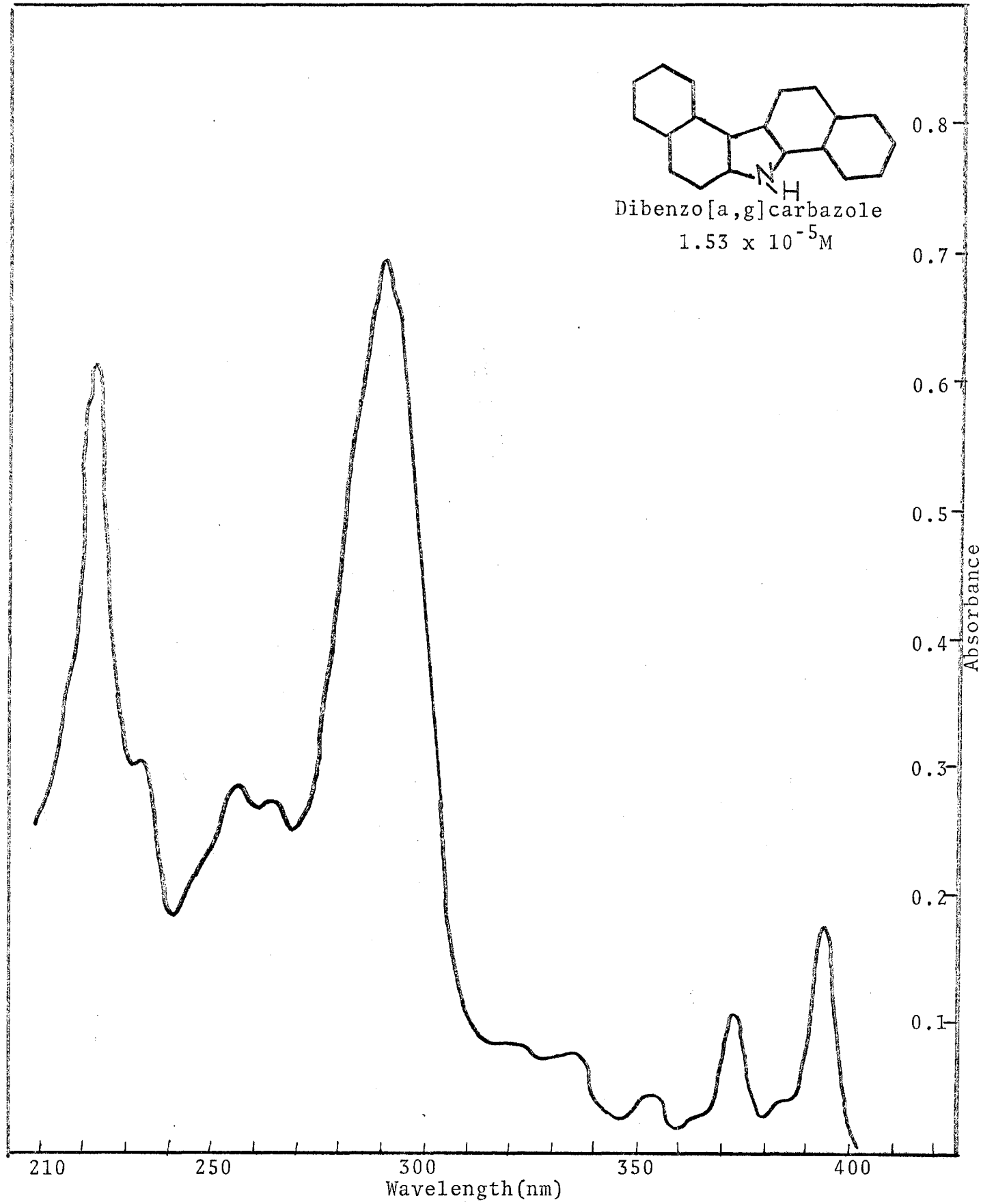

Dibenzo[a,g]carbazole
1.53 x 10⁻⁵M
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
Absorbance
210
250
300
350
400
Wavelength(nm)

Figure 5. Cont.

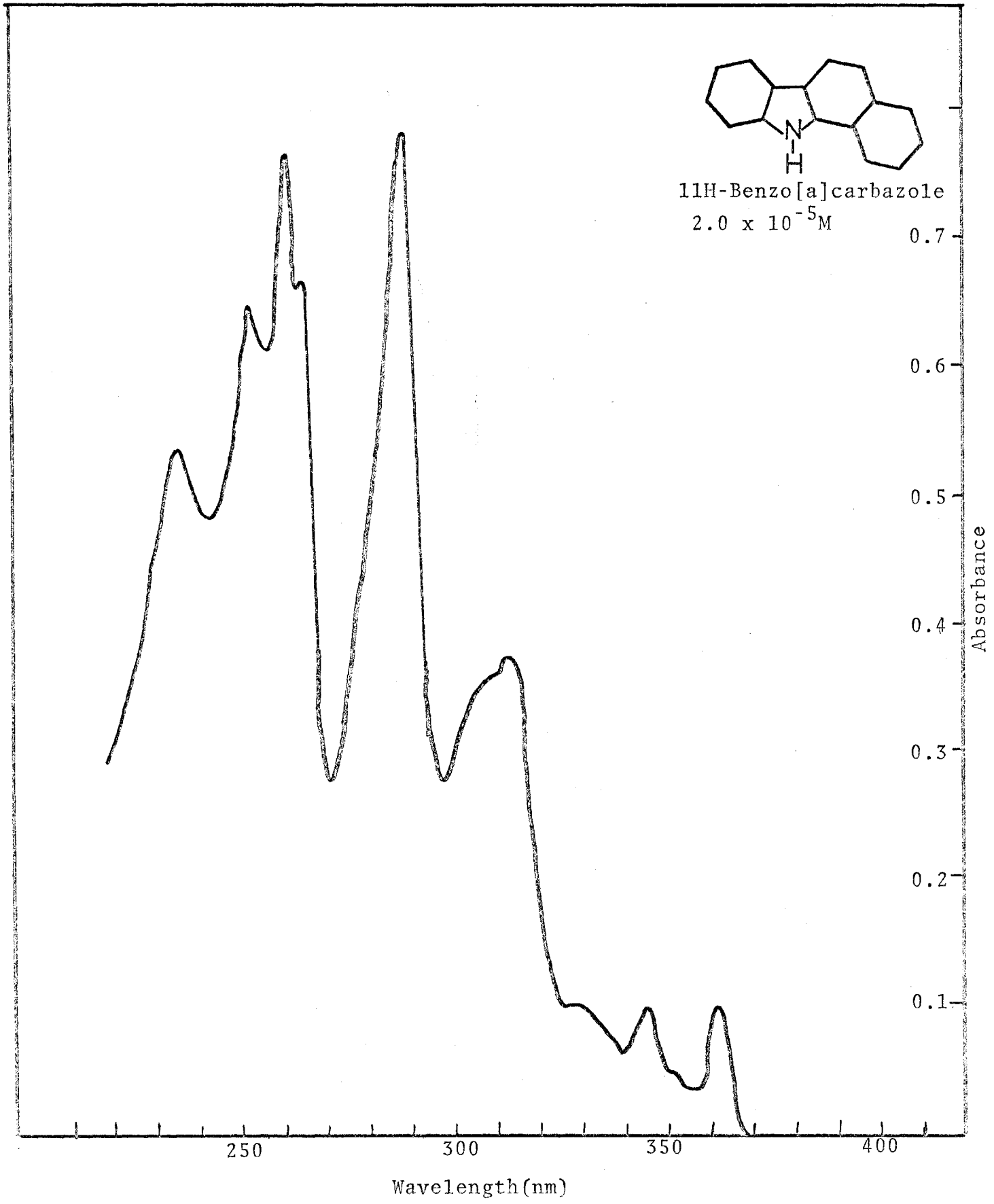


Figure 5. Cont.

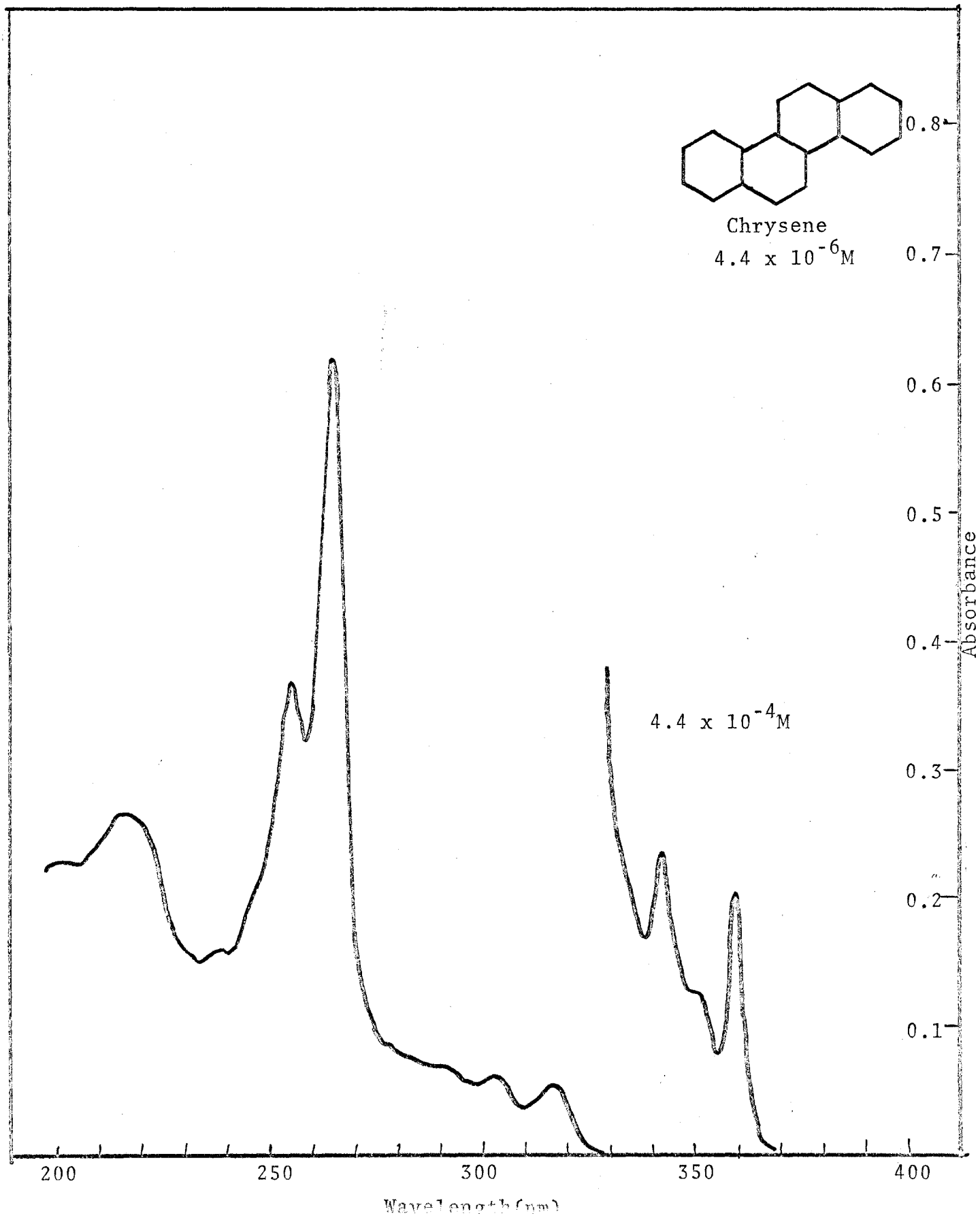


Figure 5 . Cont.

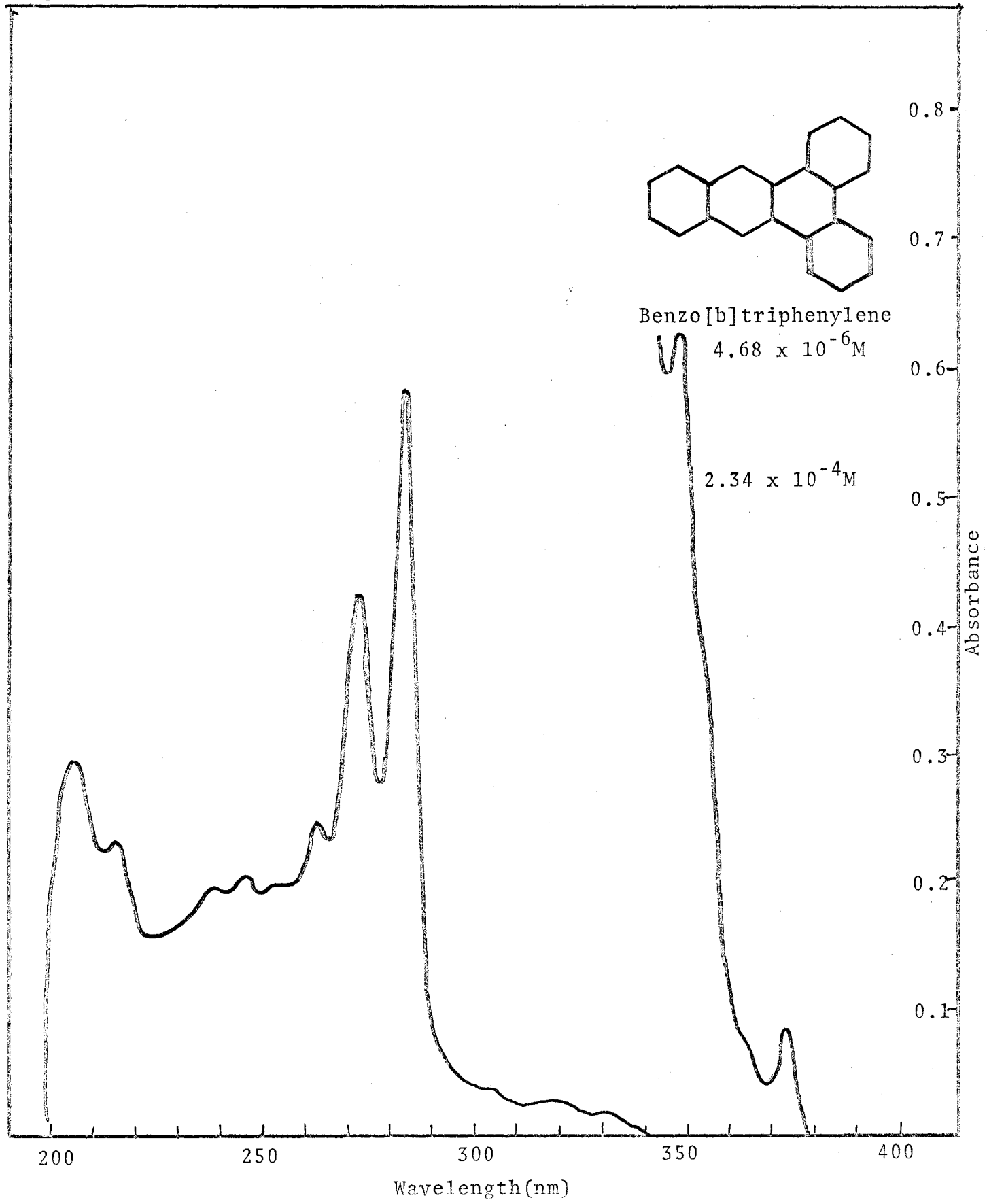


Figure 5. Cont.

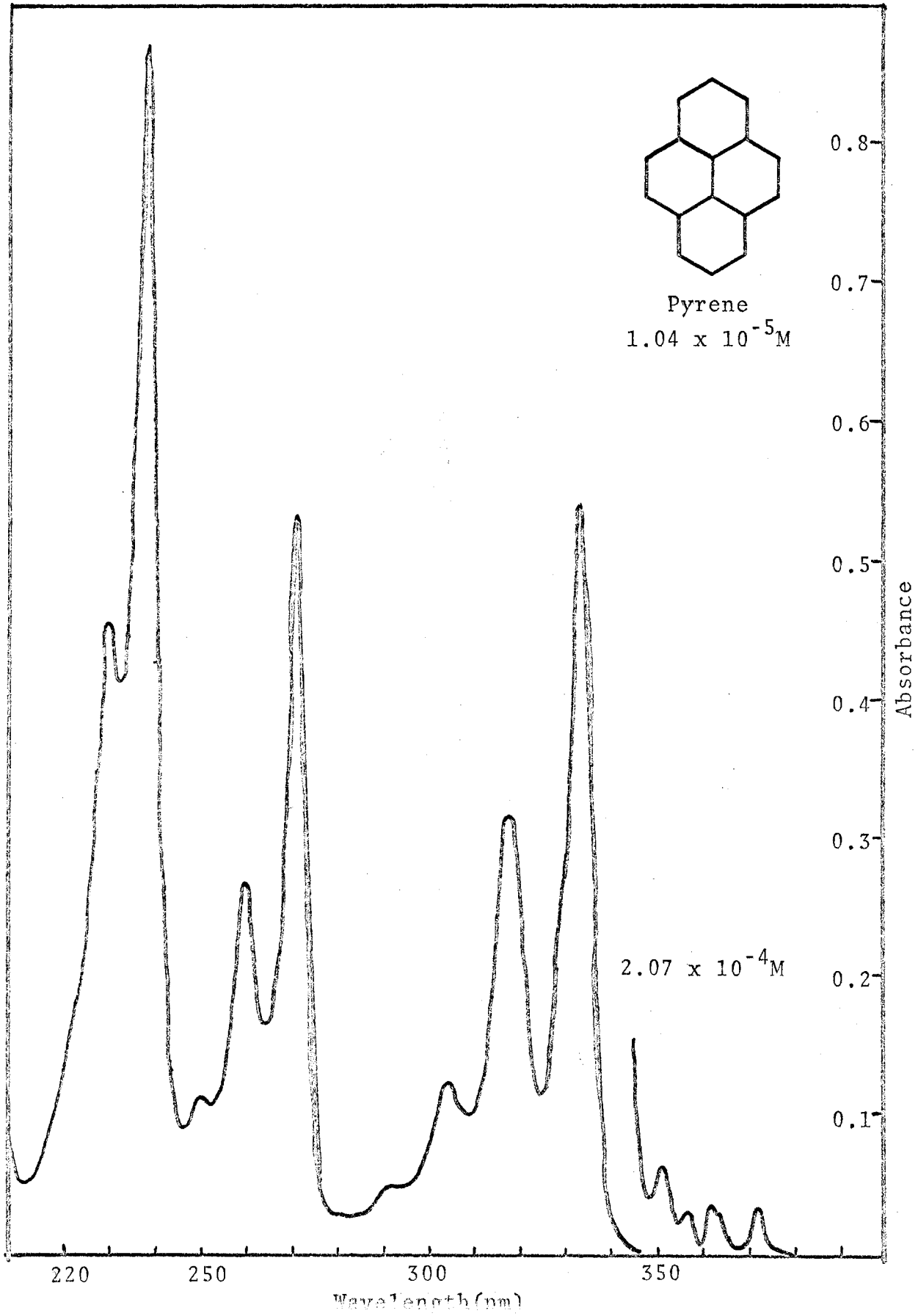


Figure 5. Cont.

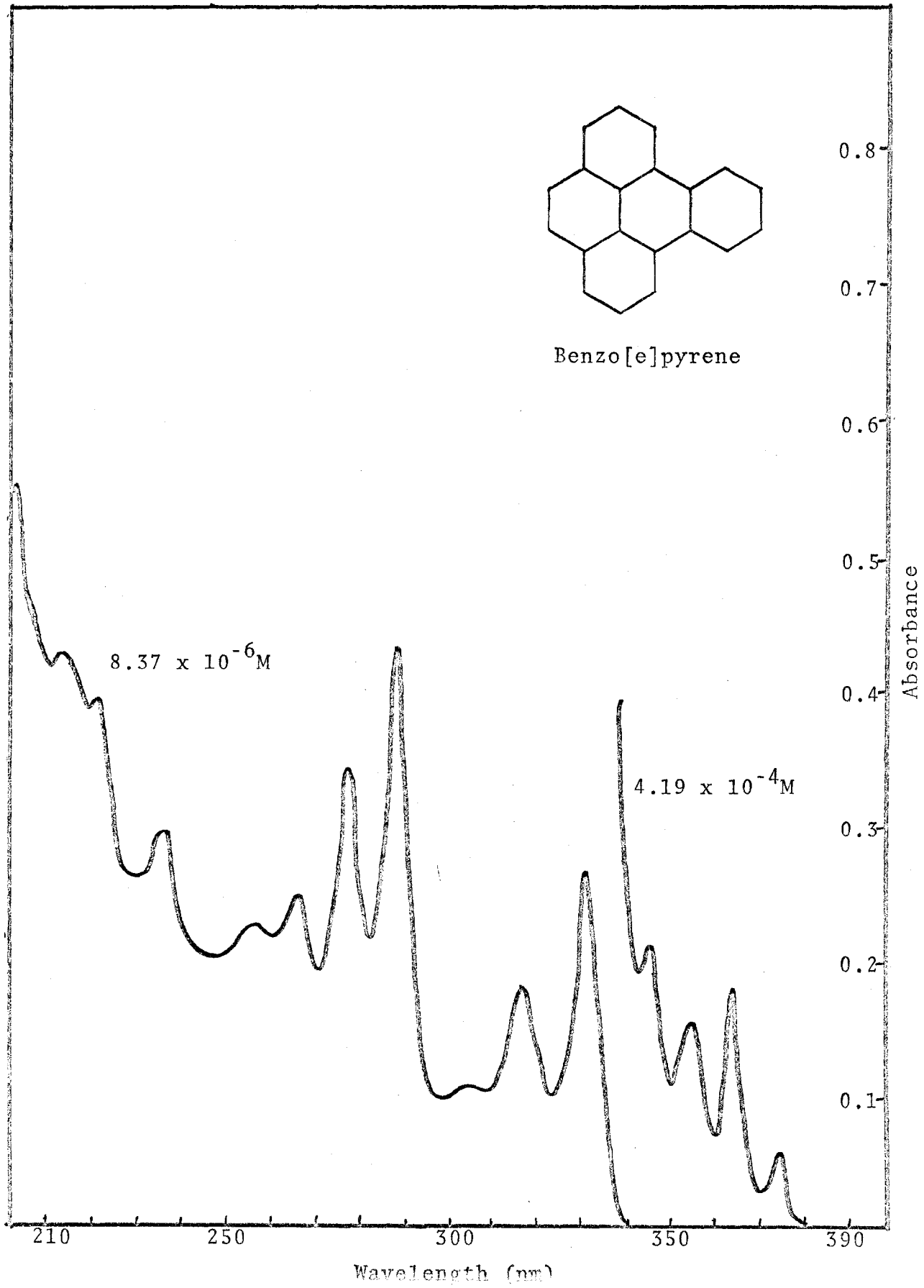


Figure 5. Cont.

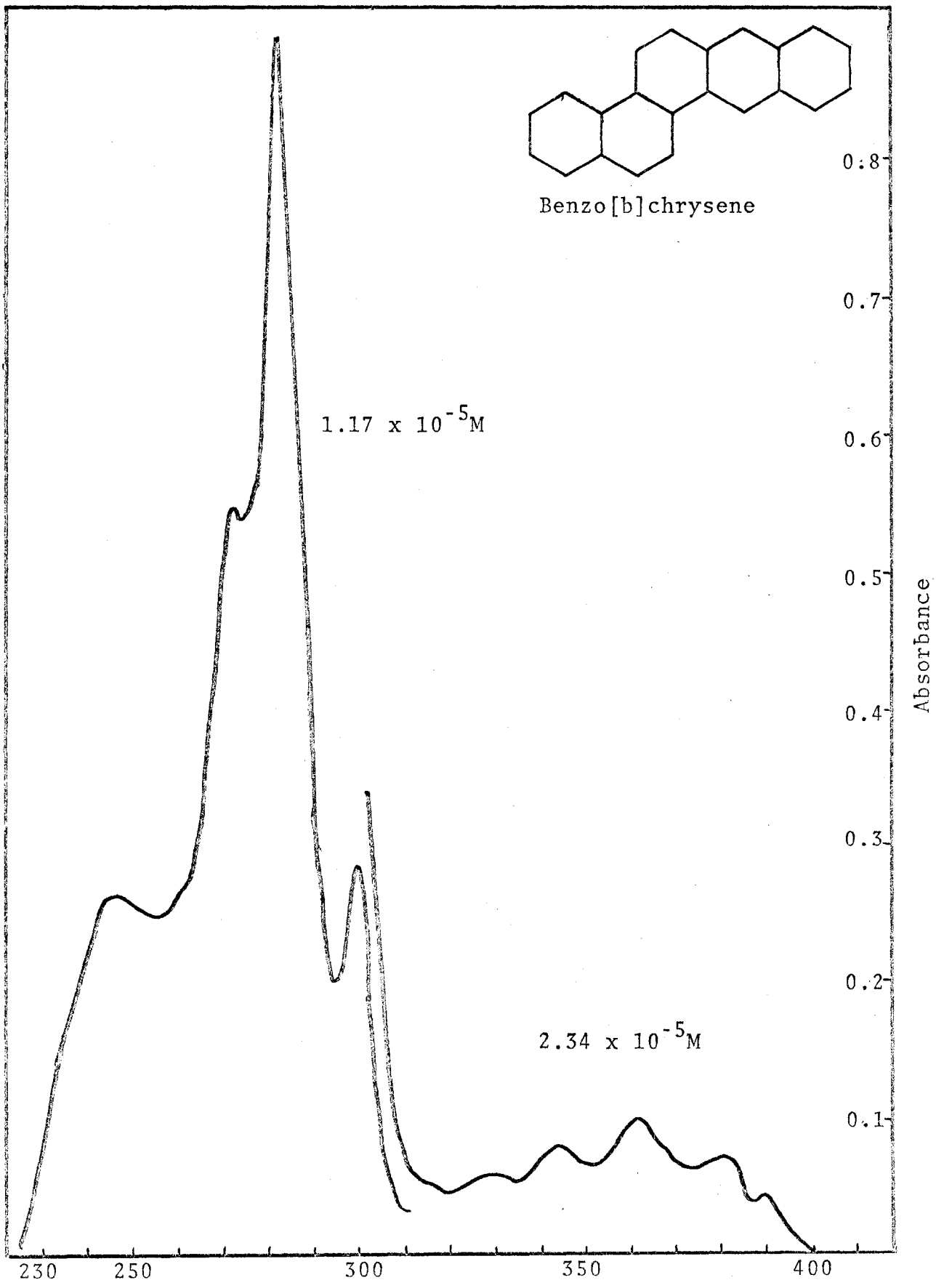


Figure 5 . Cont.

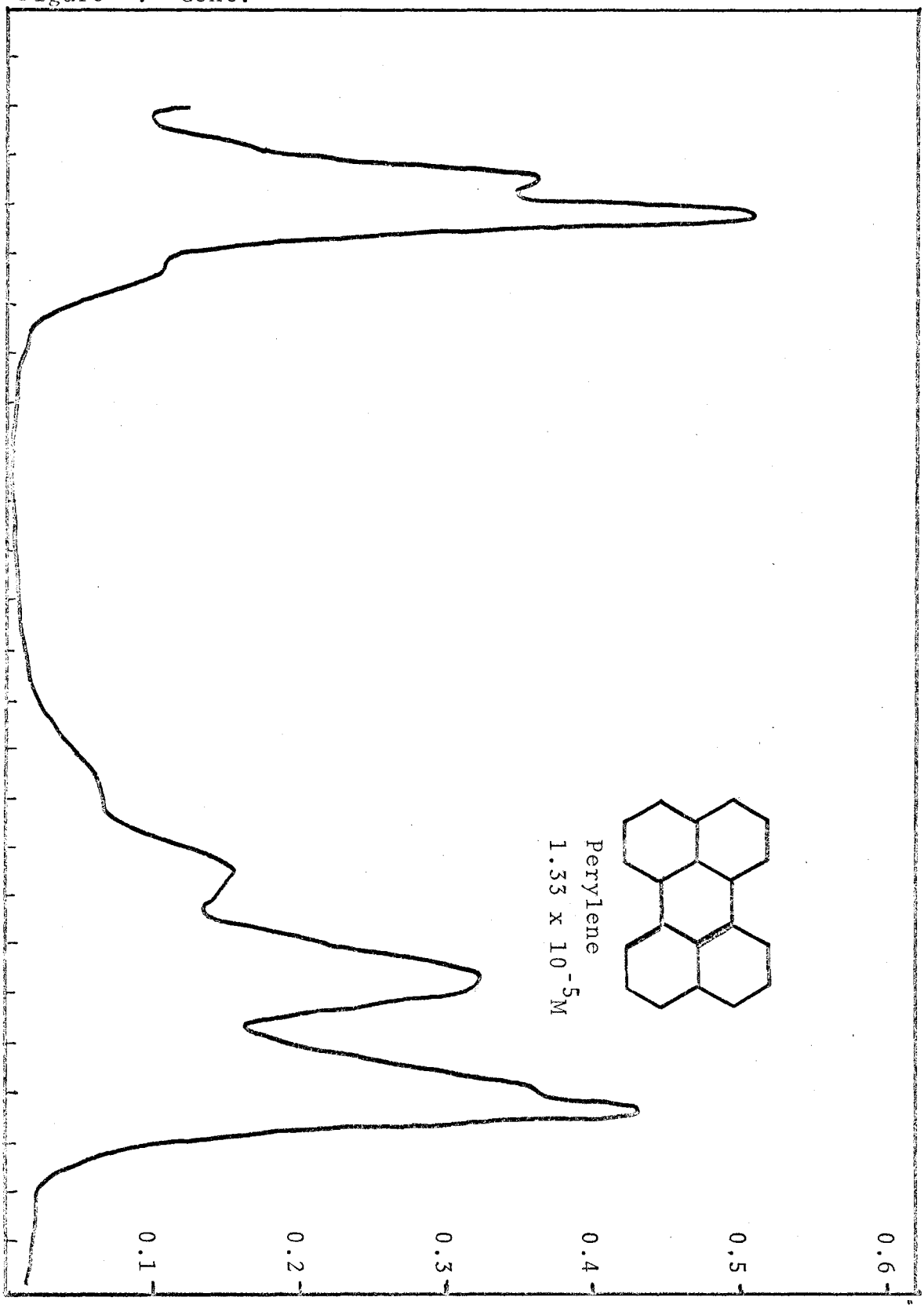


Figure 5 . Cont.

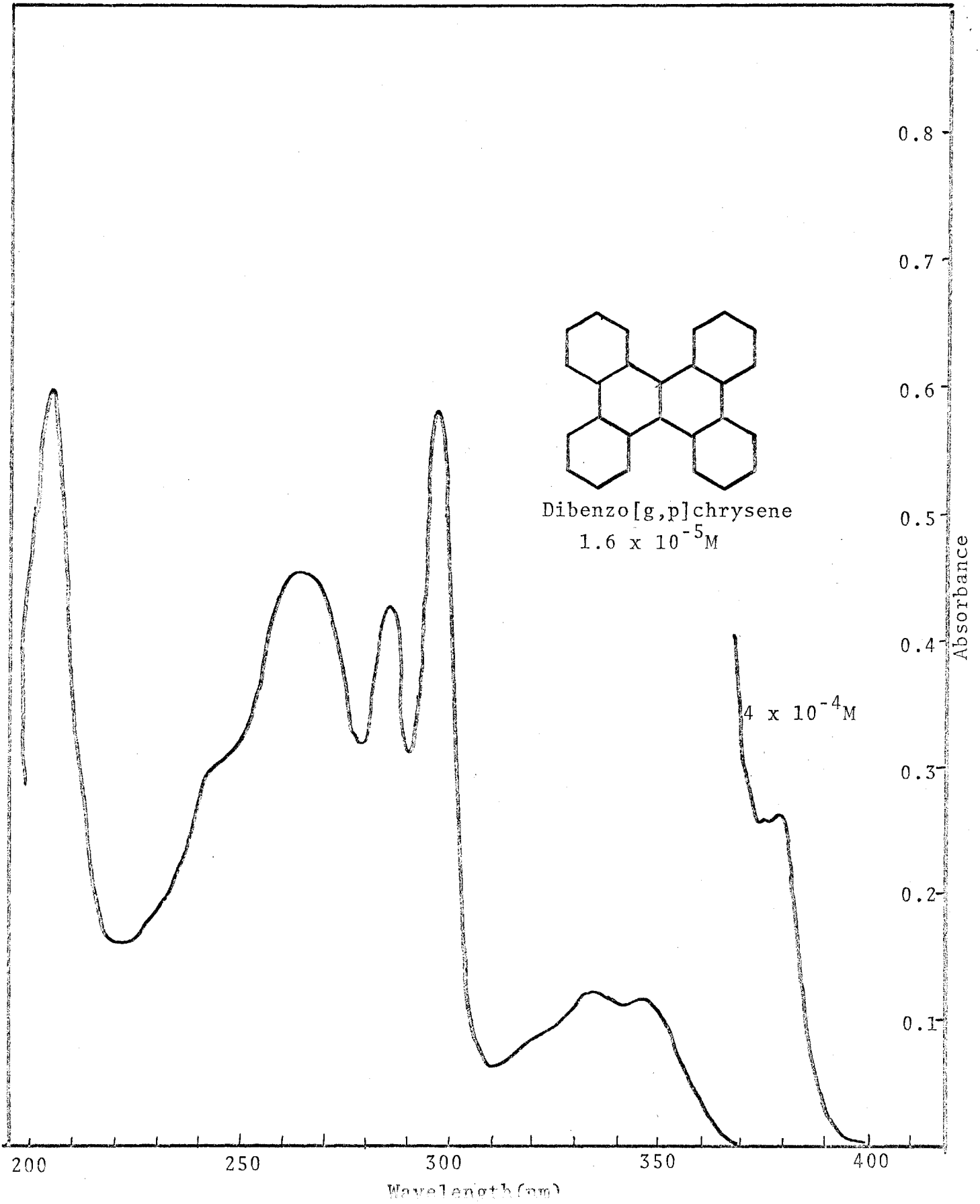


Figure 5. Cont.

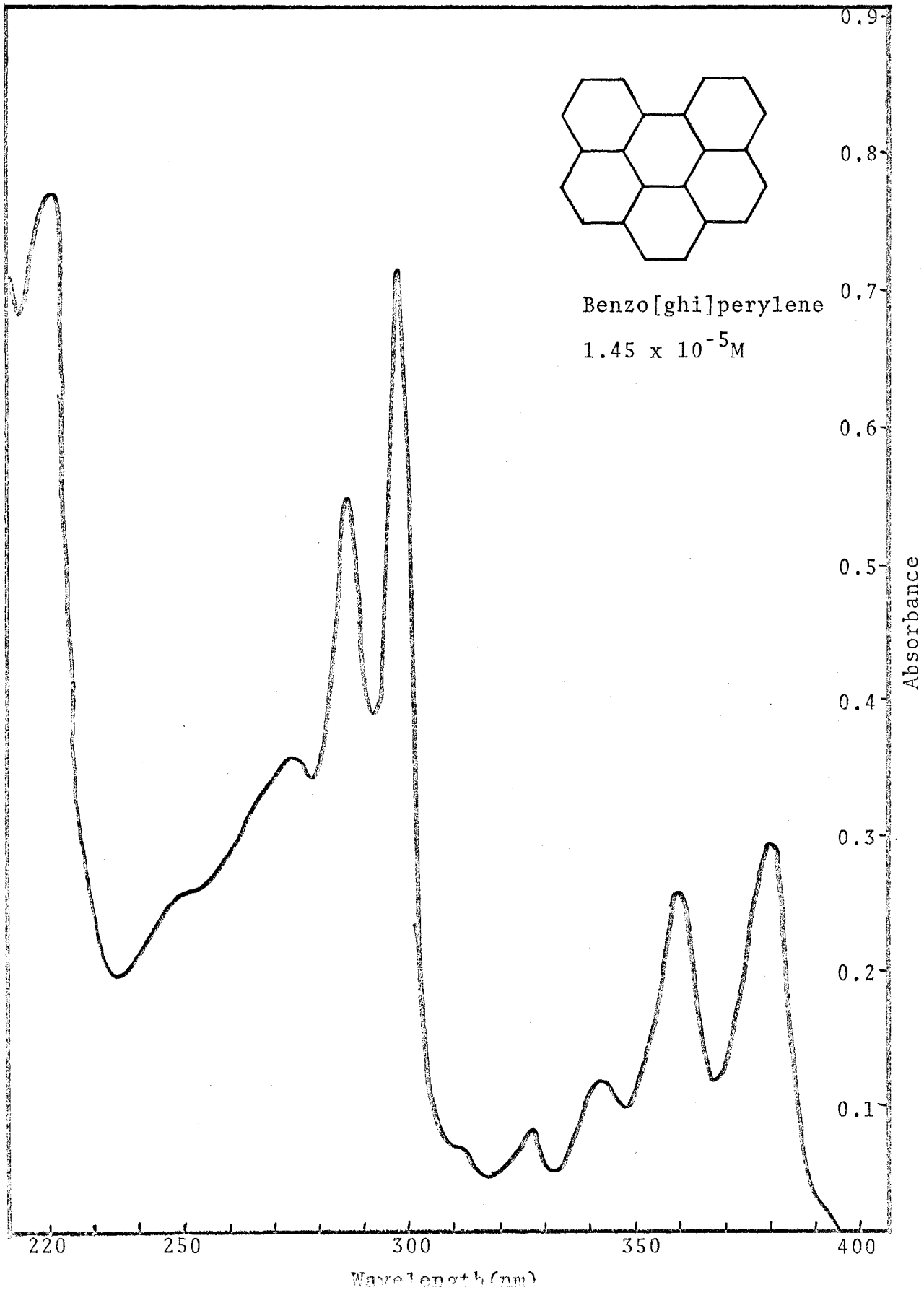


Figure 5. Cont.

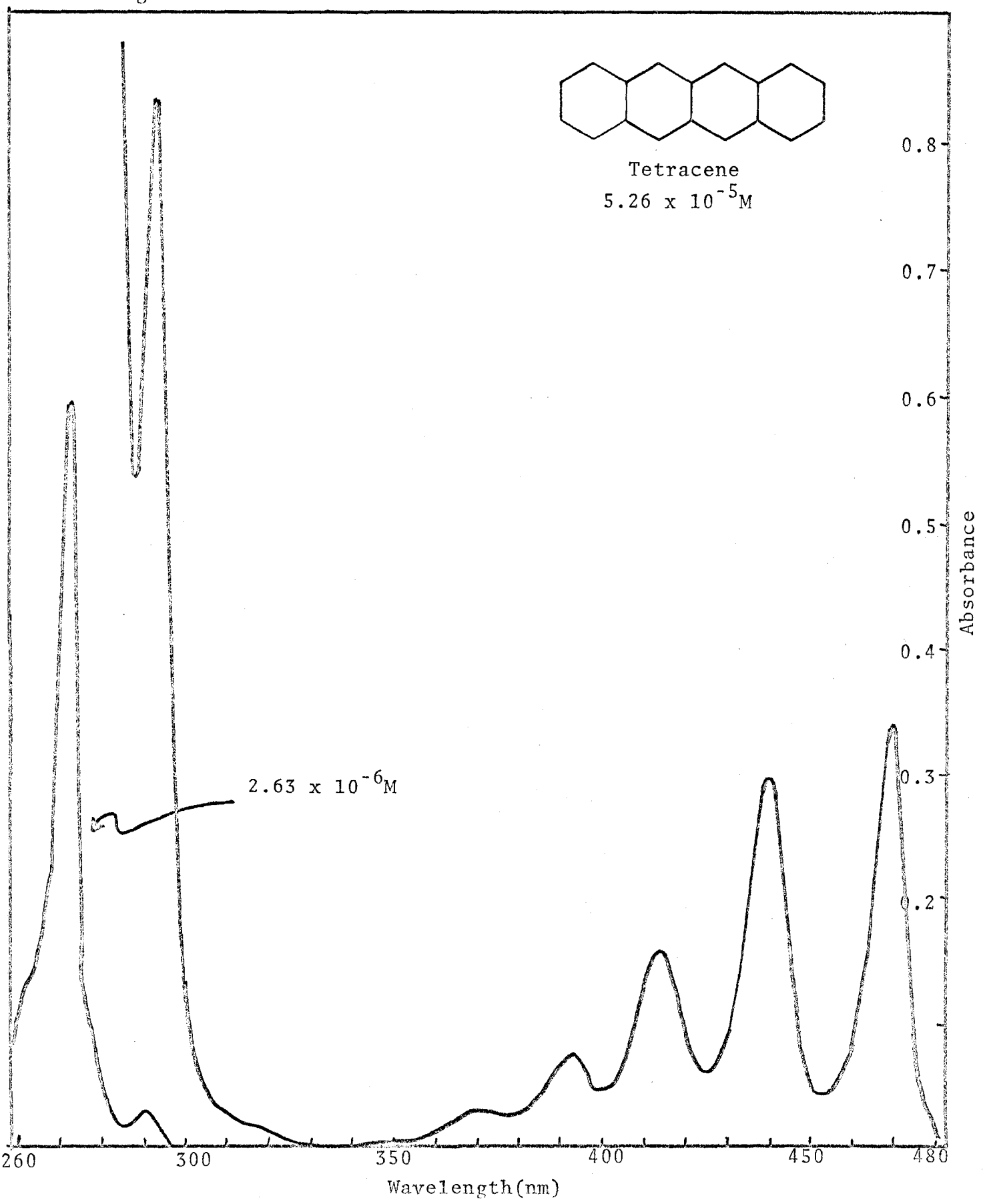


Figure 5 . Cont.

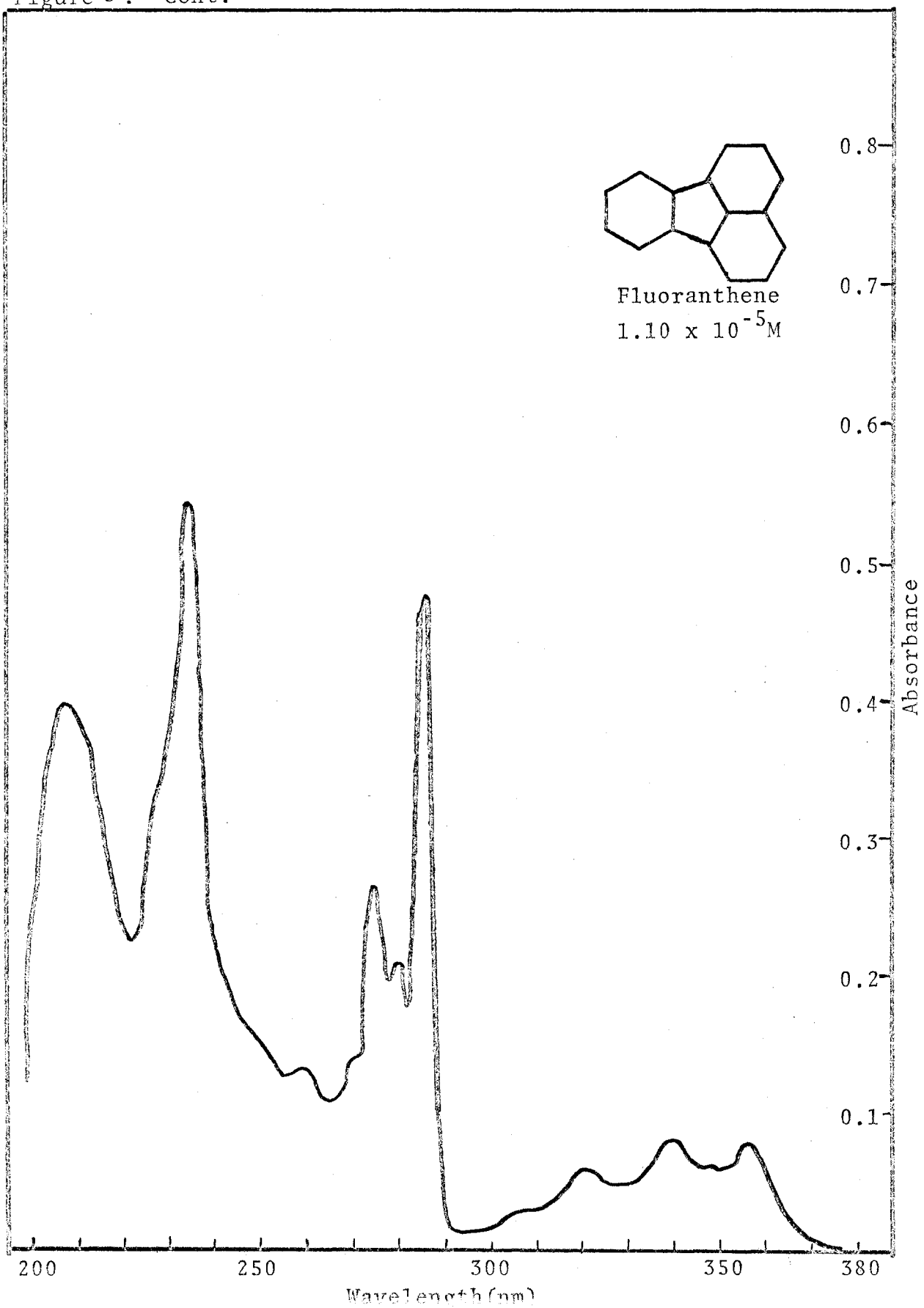


Figure 5 . Cont.

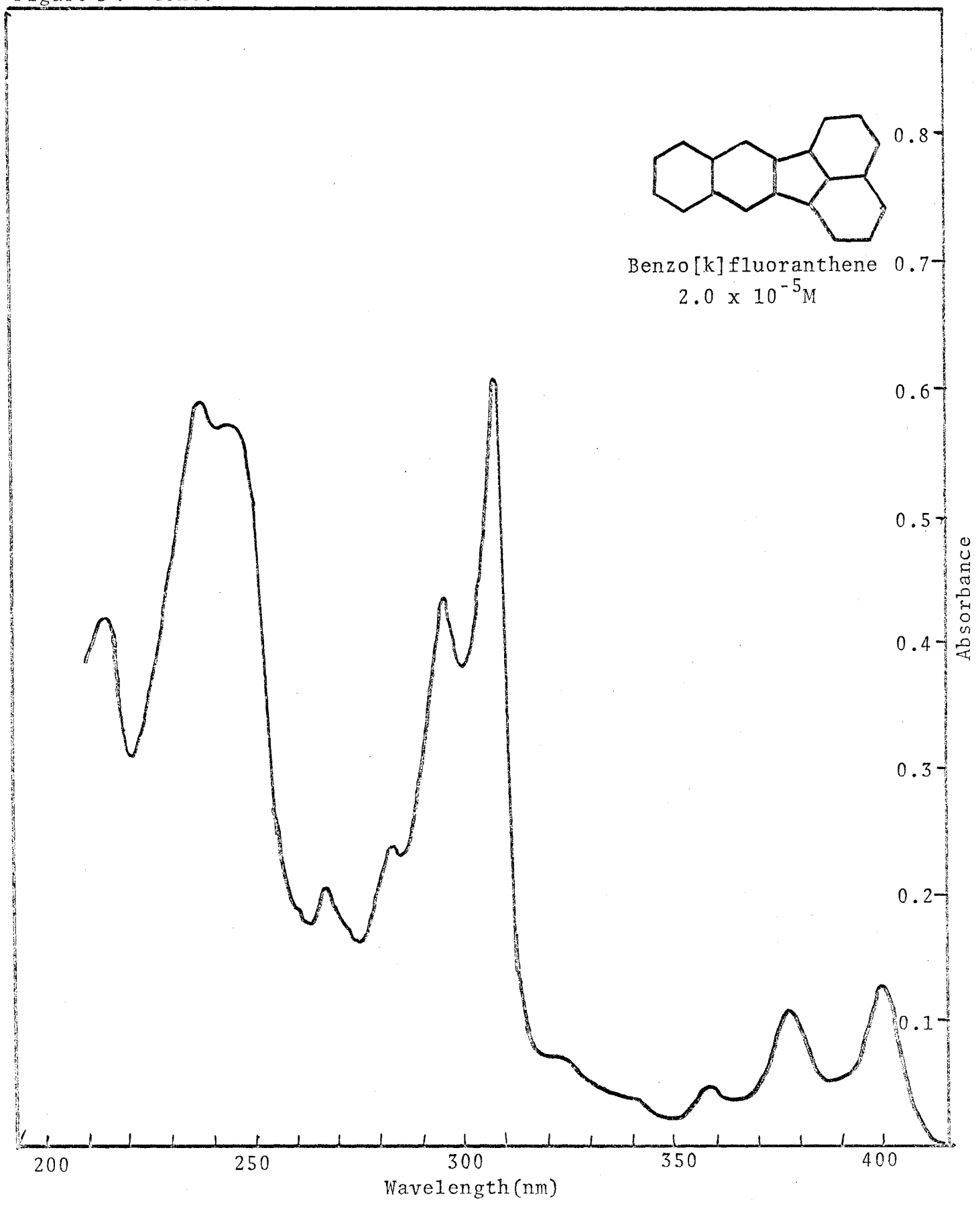


Figure 5 . Cont.

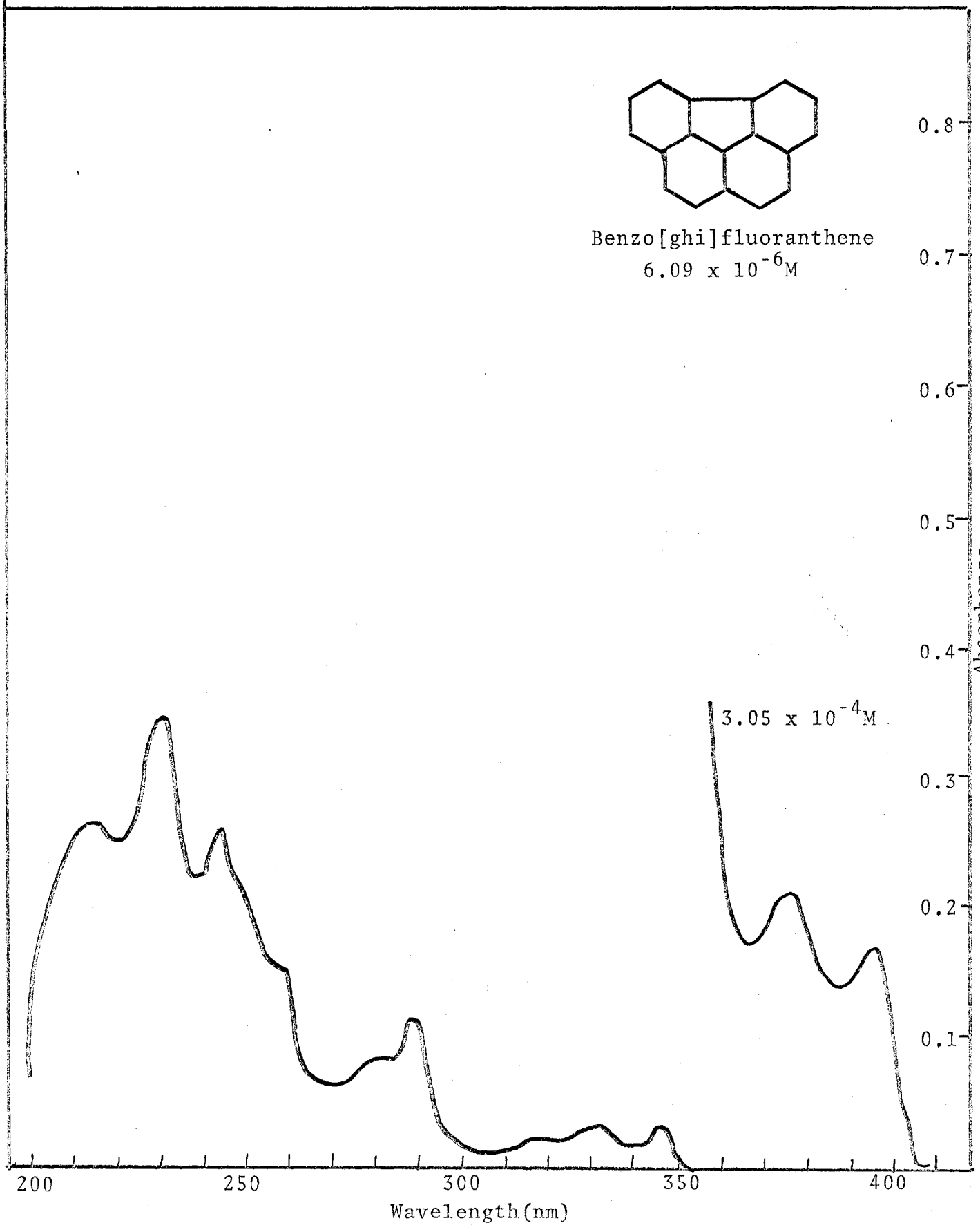


Figure 5 . Cont.

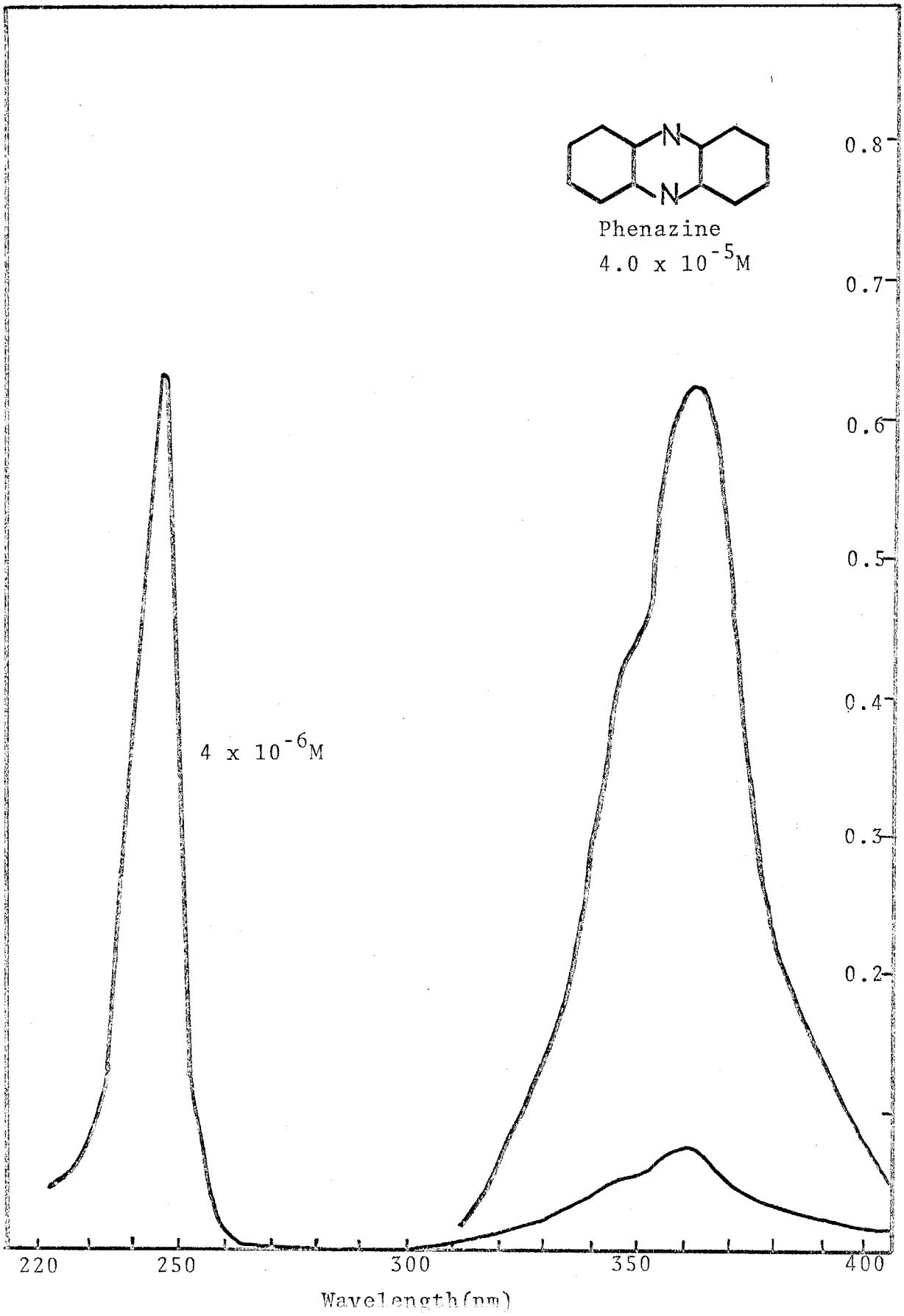


Figure 5. Cont.

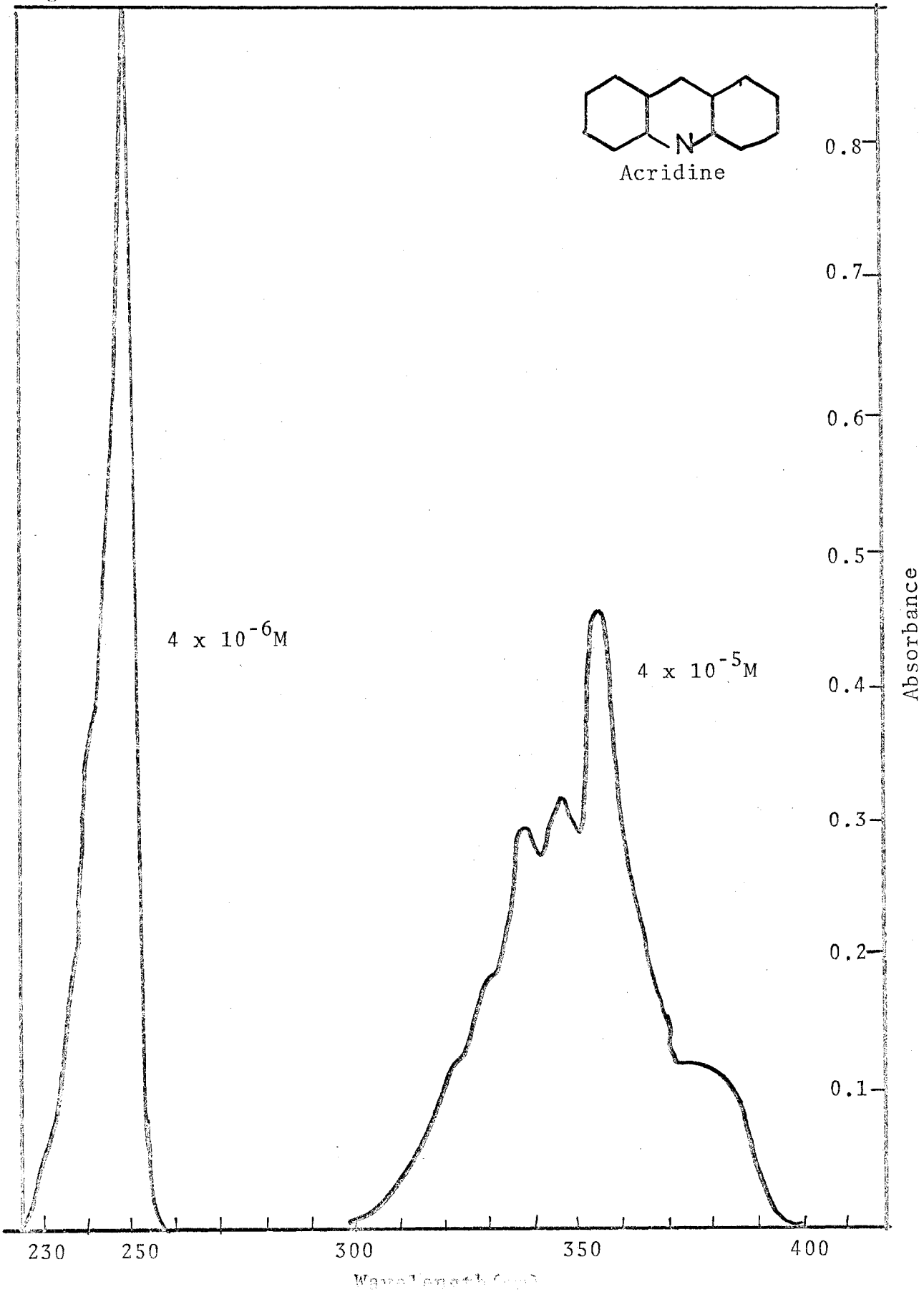


Figure 5. Cont.

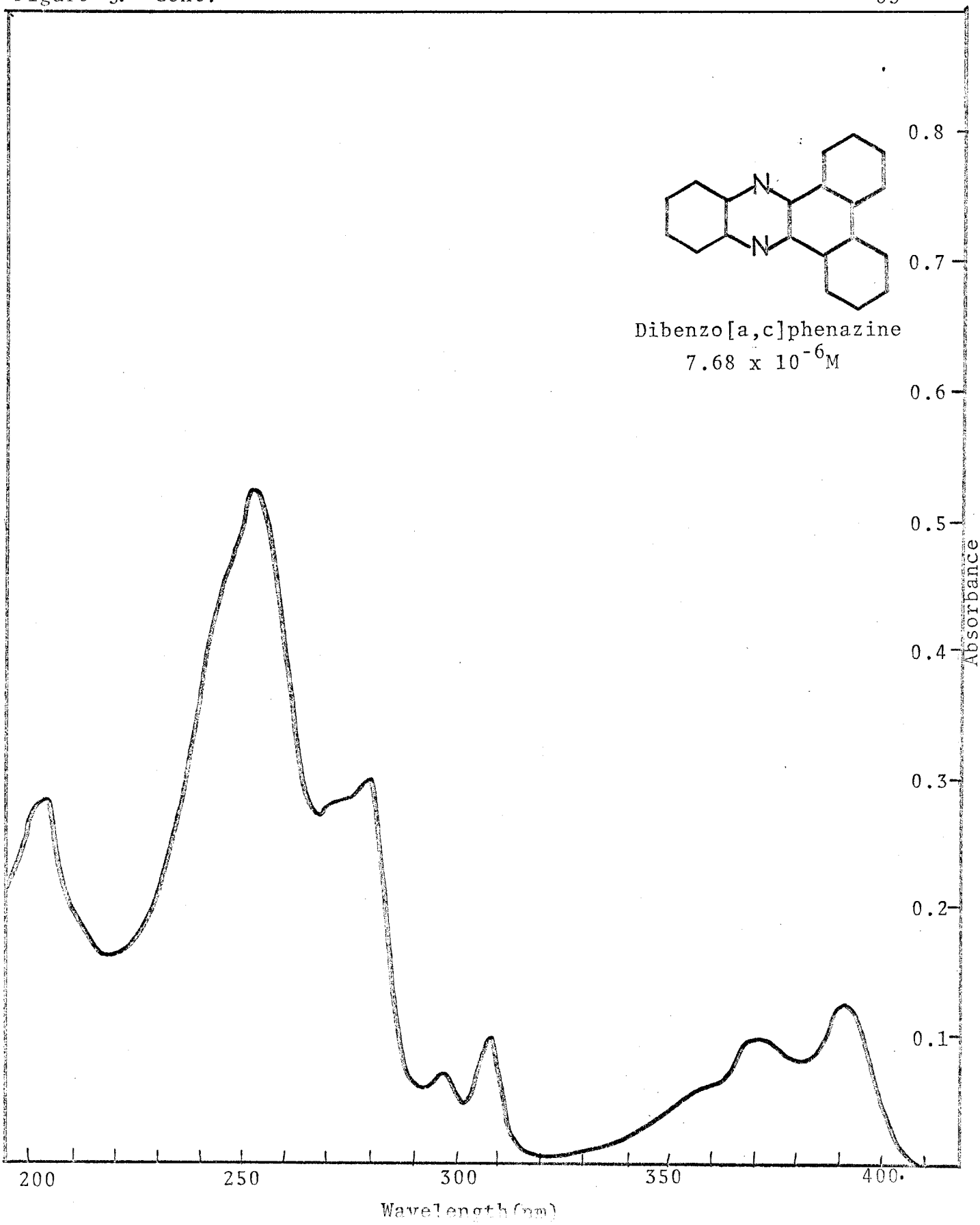


Figure 5 . Cont.

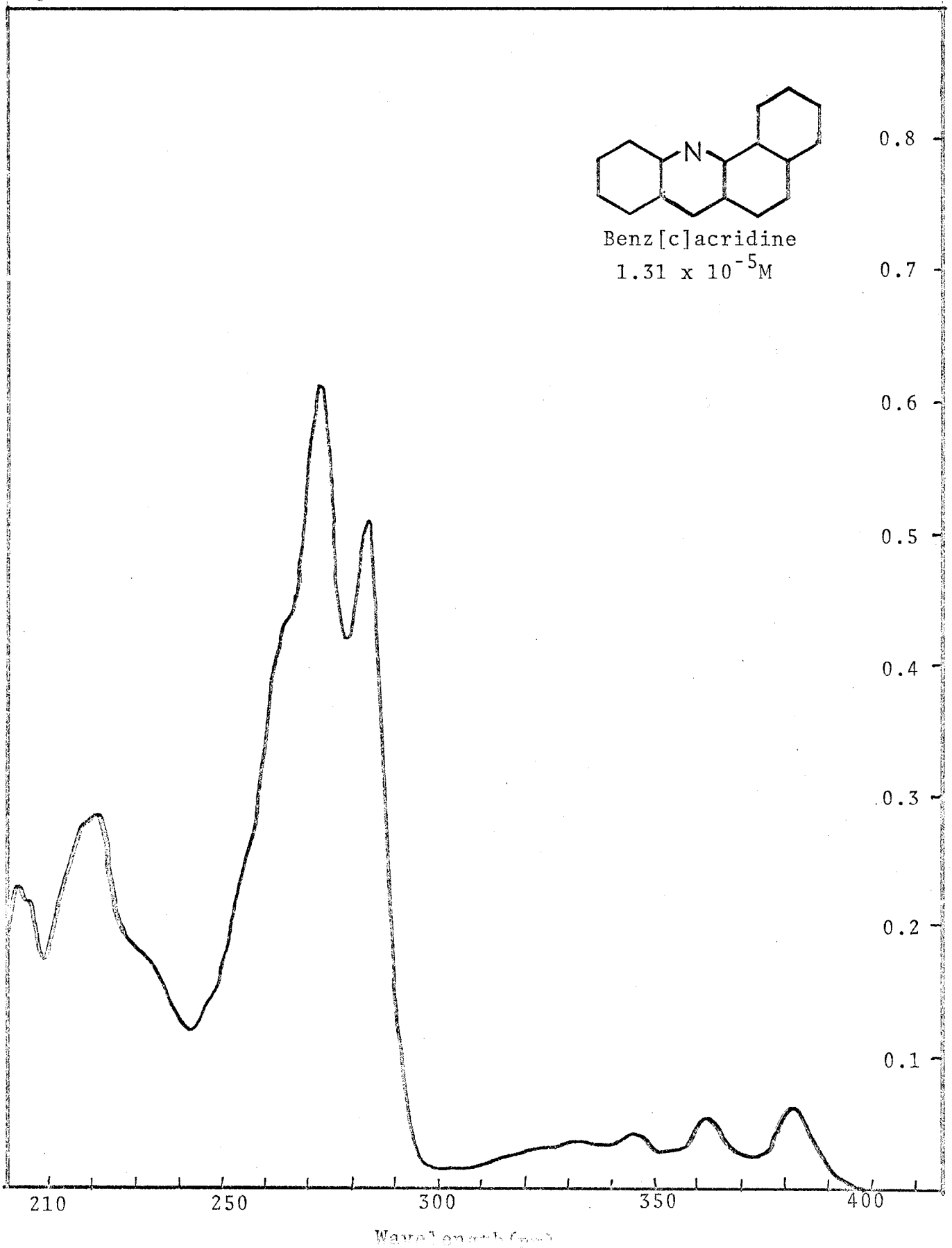


Figure 5 . Cont.

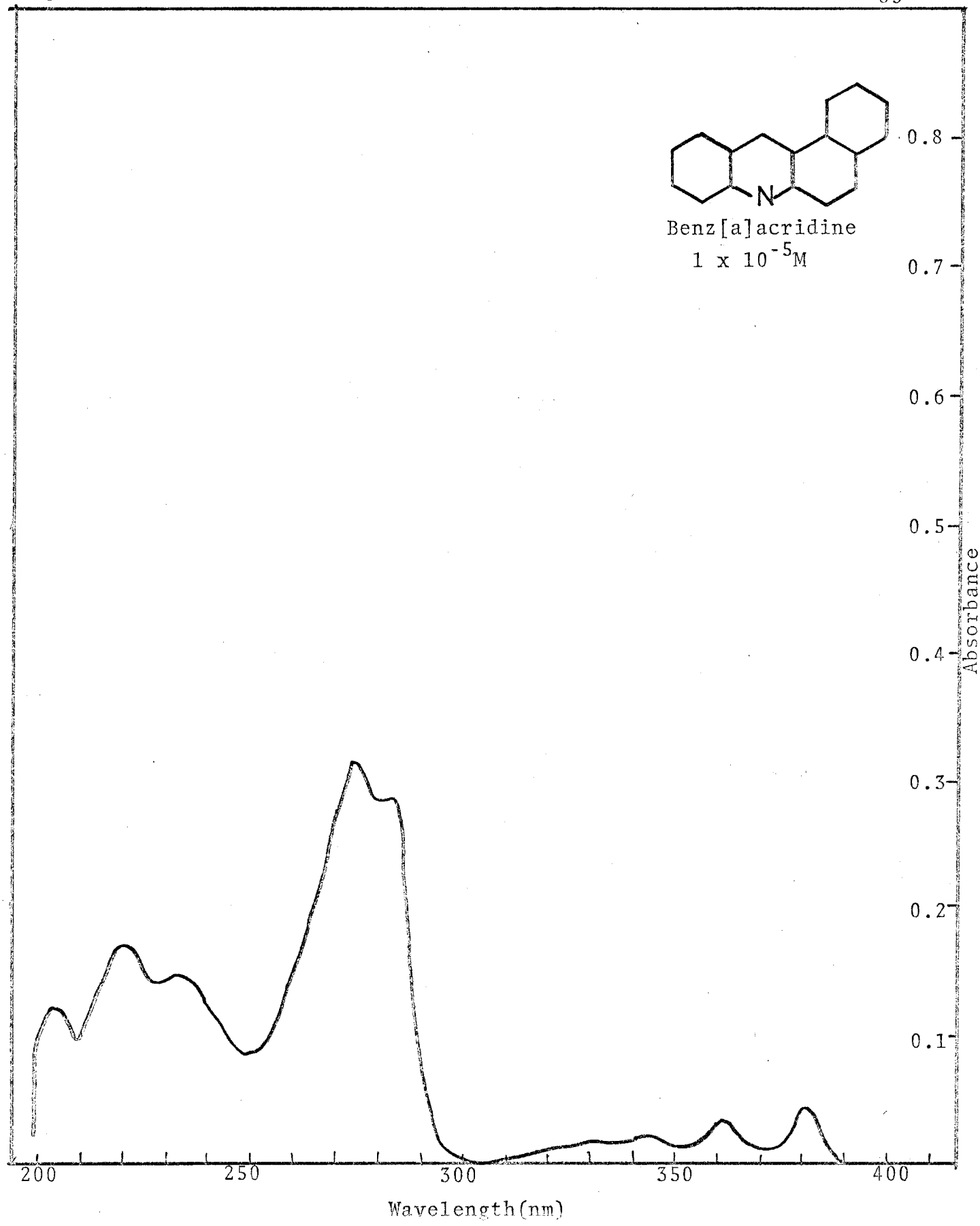


Figure 5. Cont.

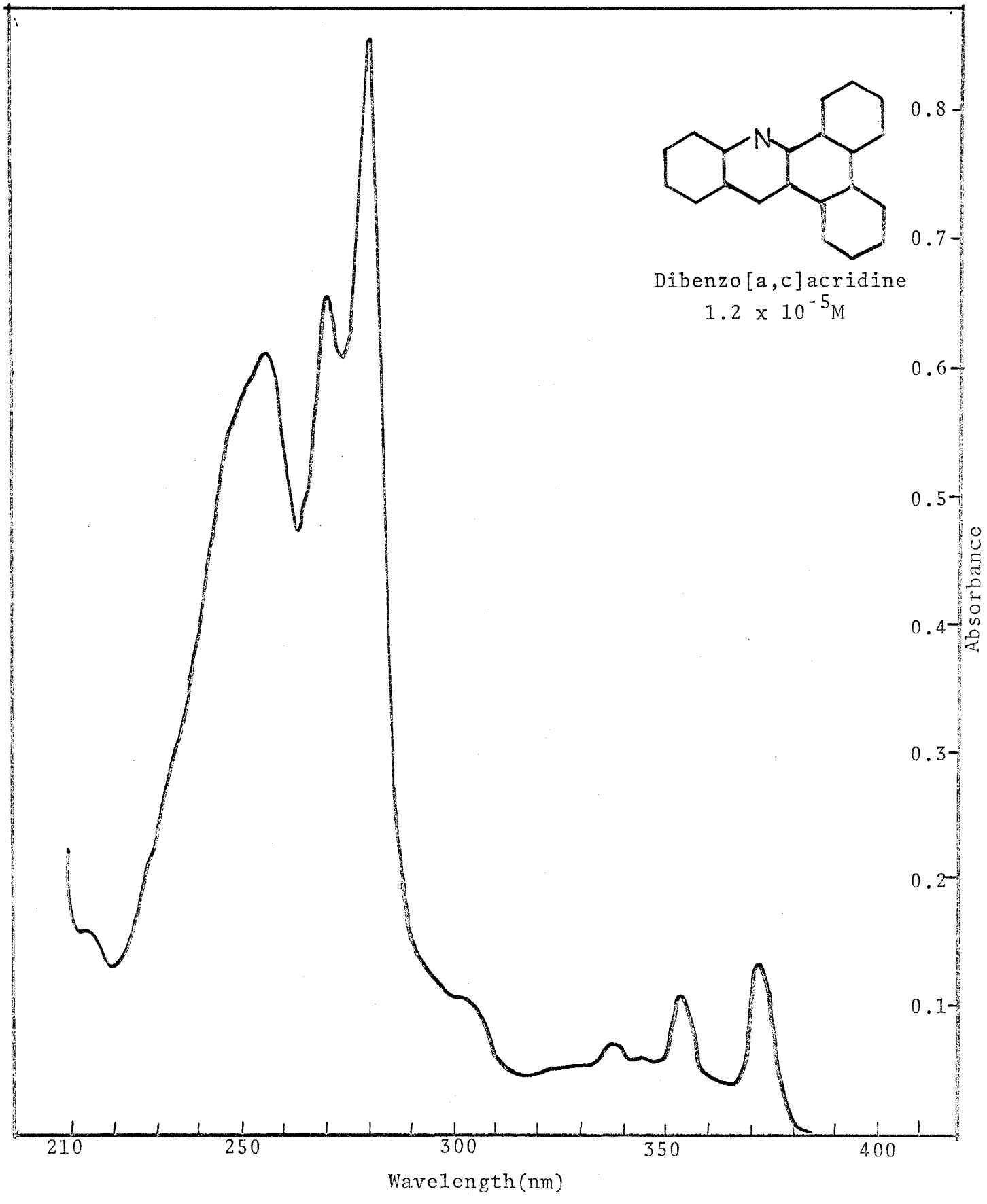


Figure 5 . Cont.

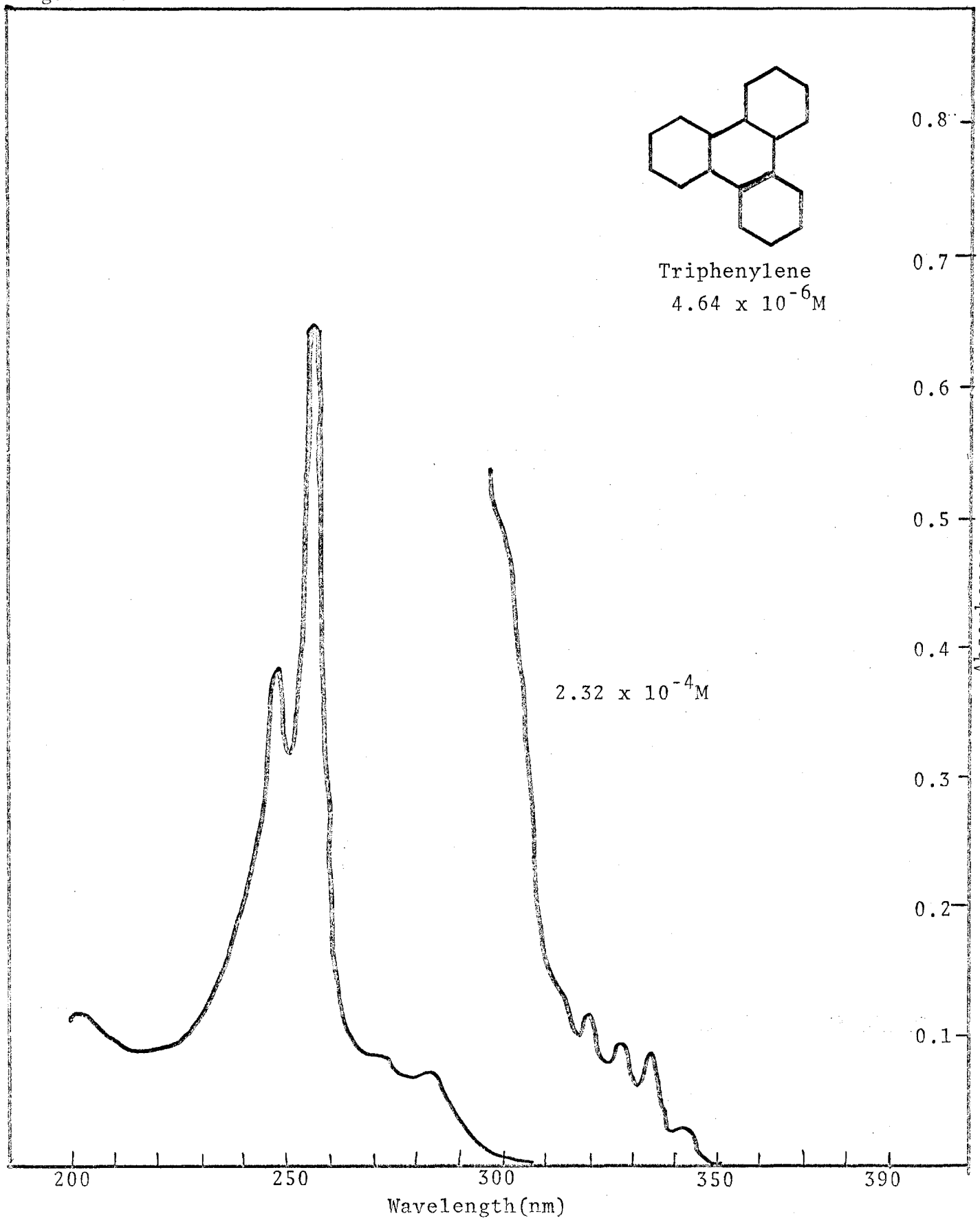


Figure 5. Cont.

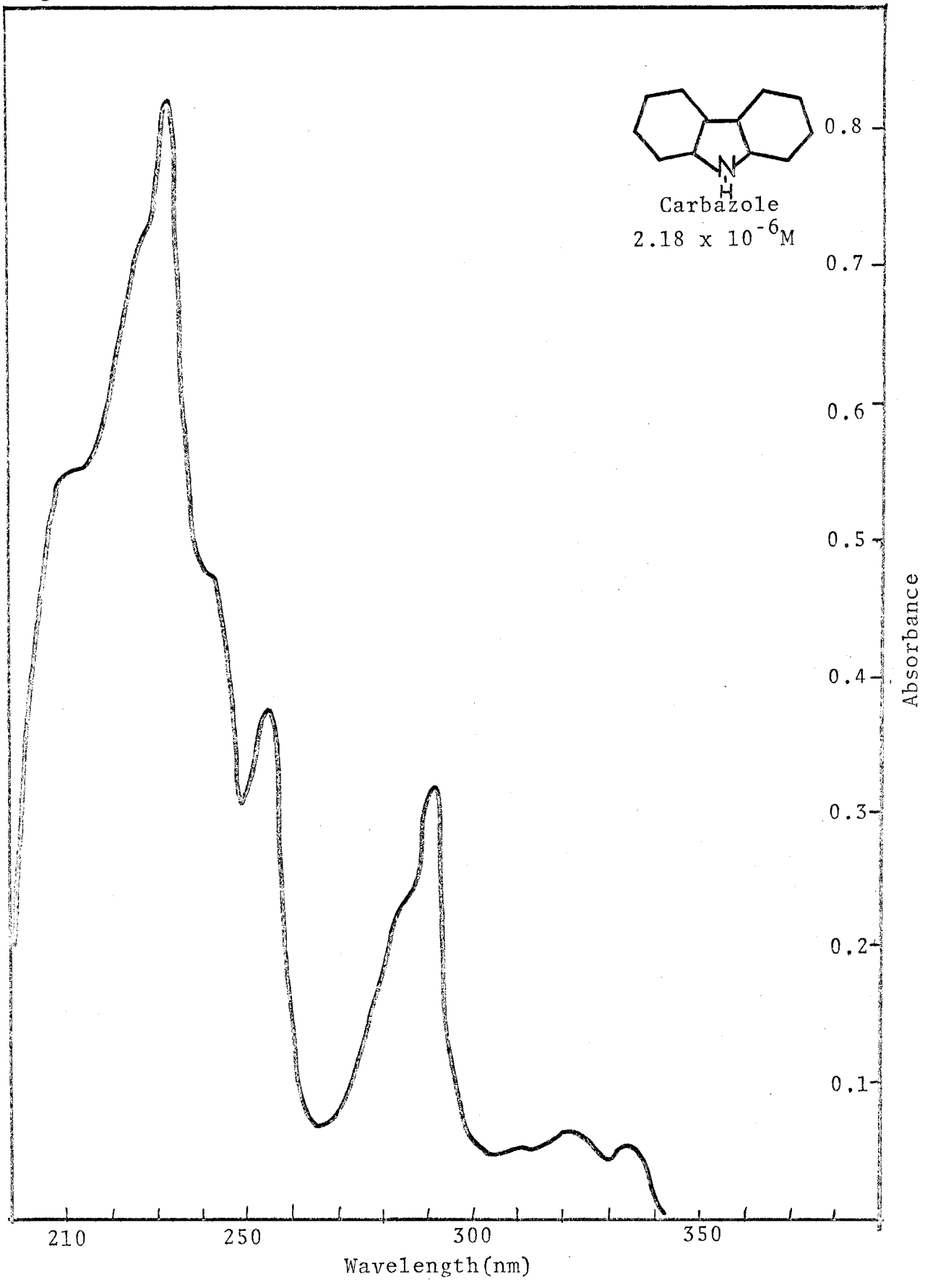


Figure 6. Fluorescence and phosphorescence emission spectra

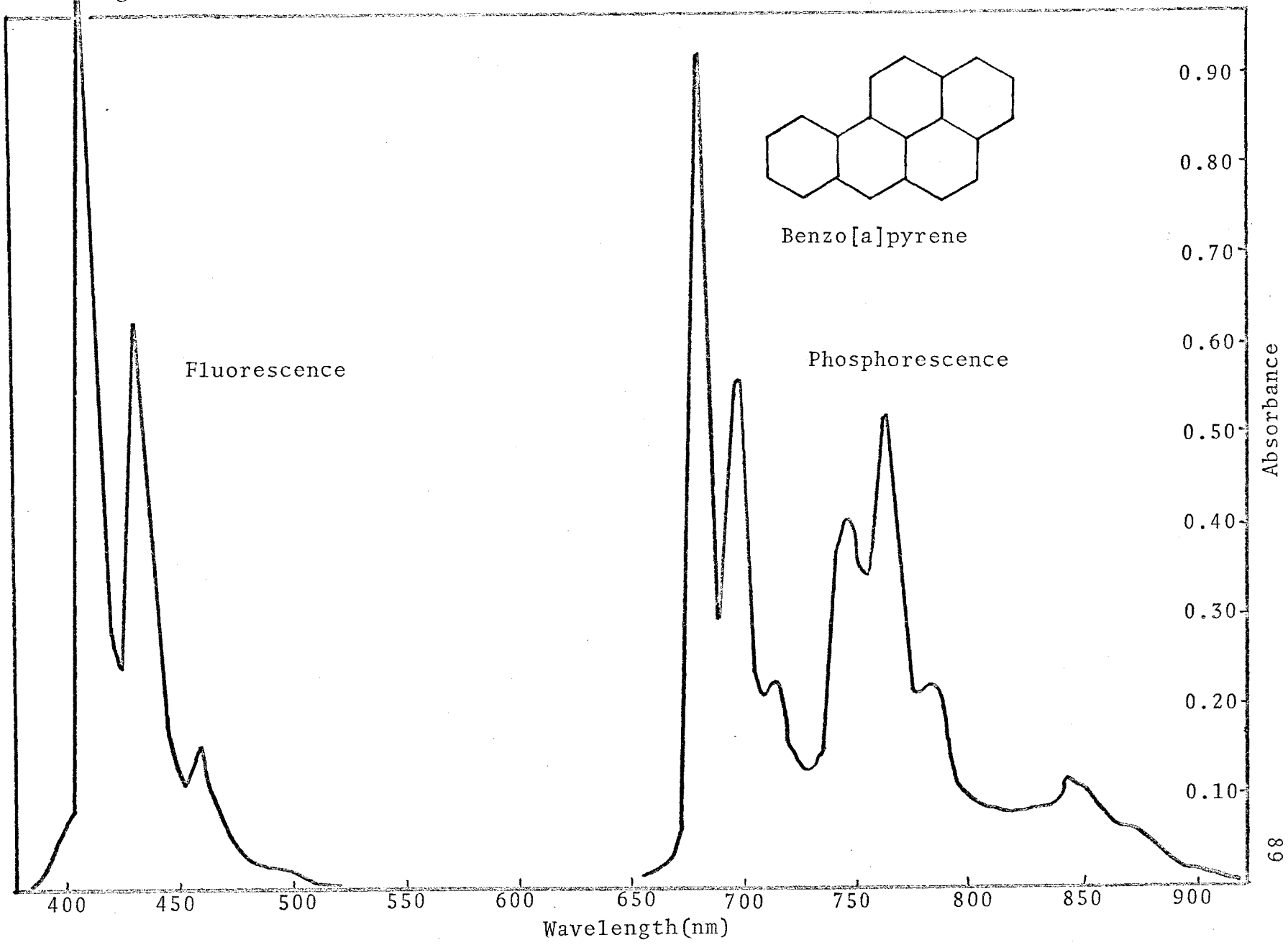


Figure 6. Cont.

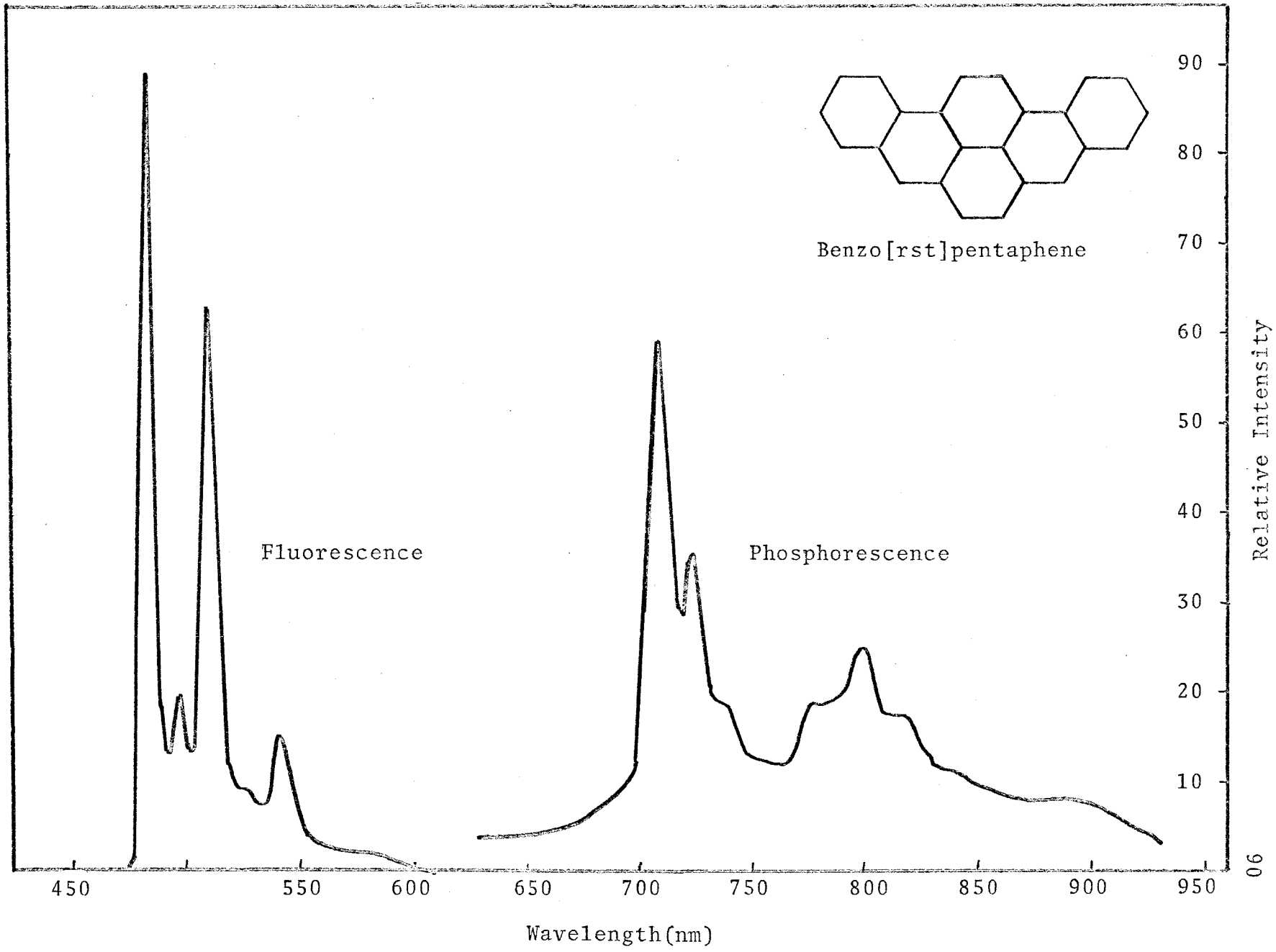


Figure 6. Cont.

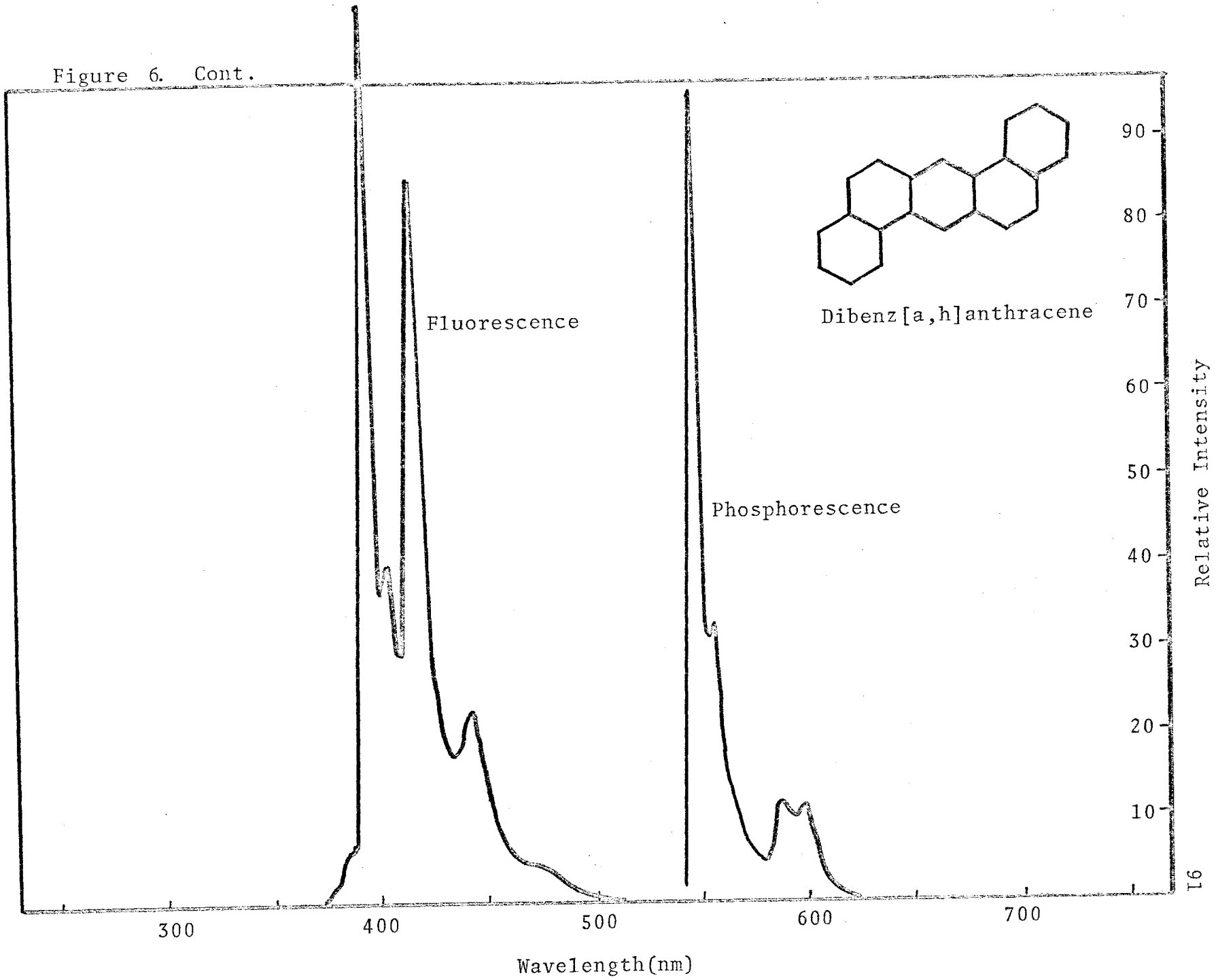


Figure 6. Cont.

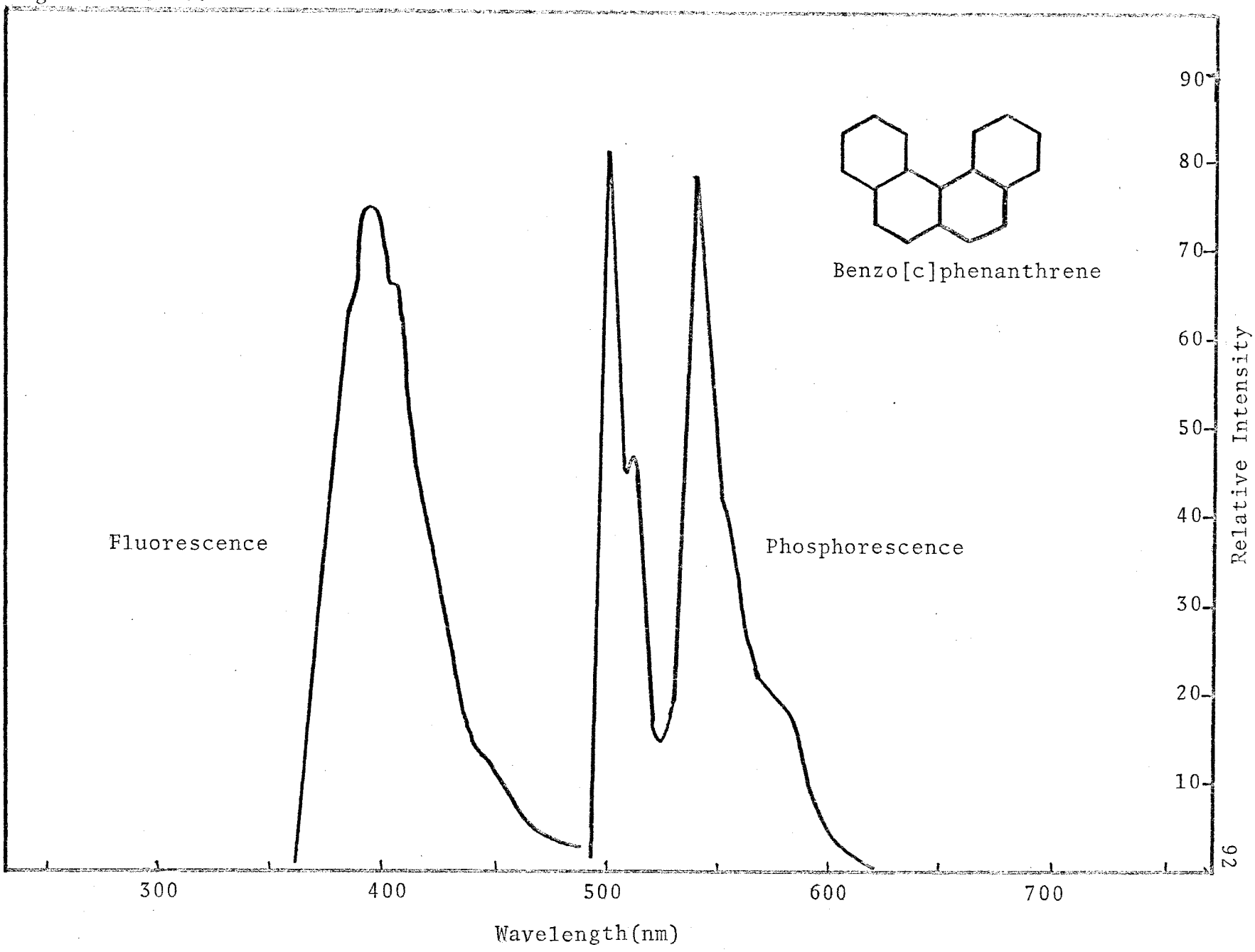


Figure 6. Cont.

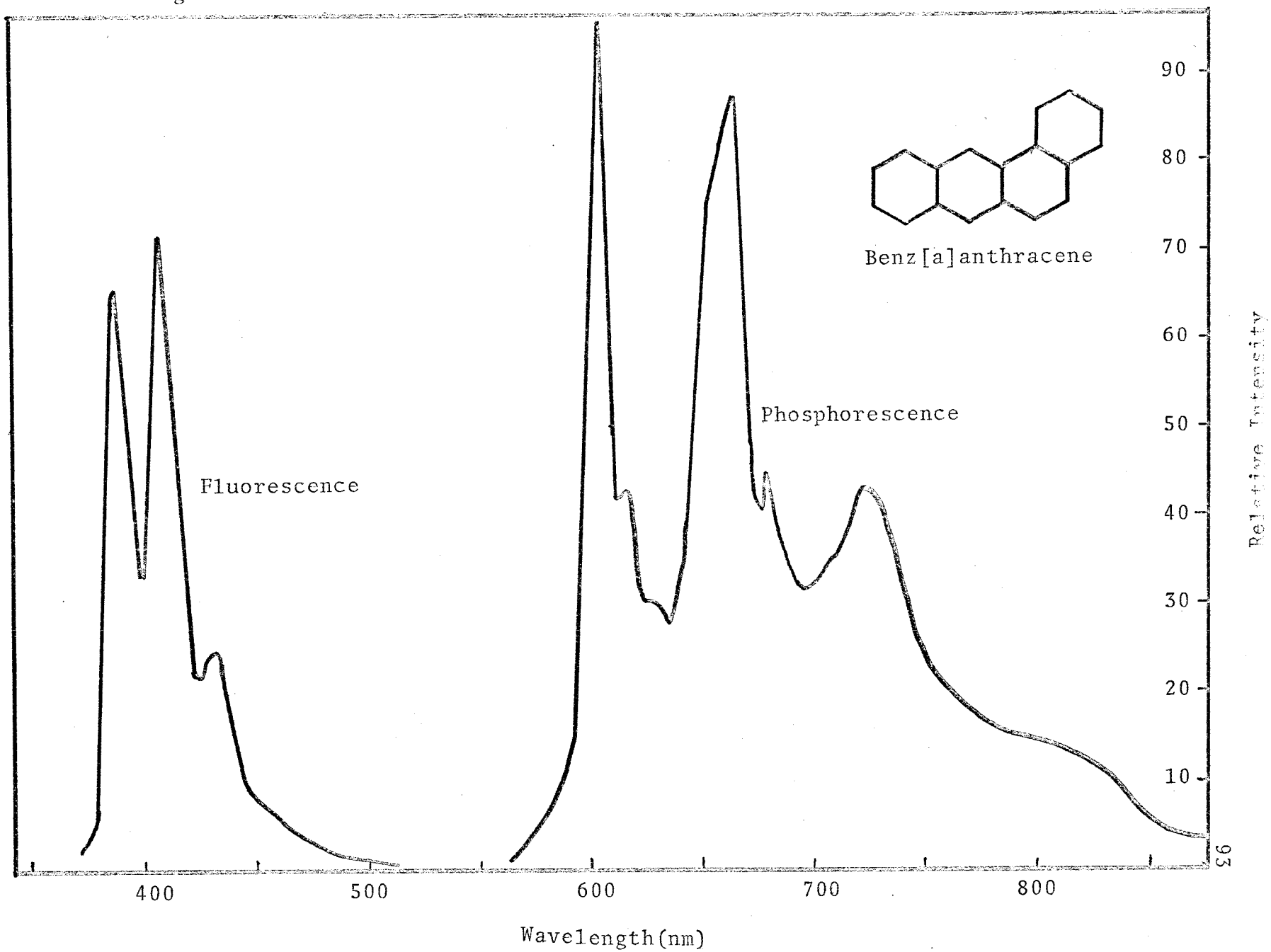


Figure 6. Cont.

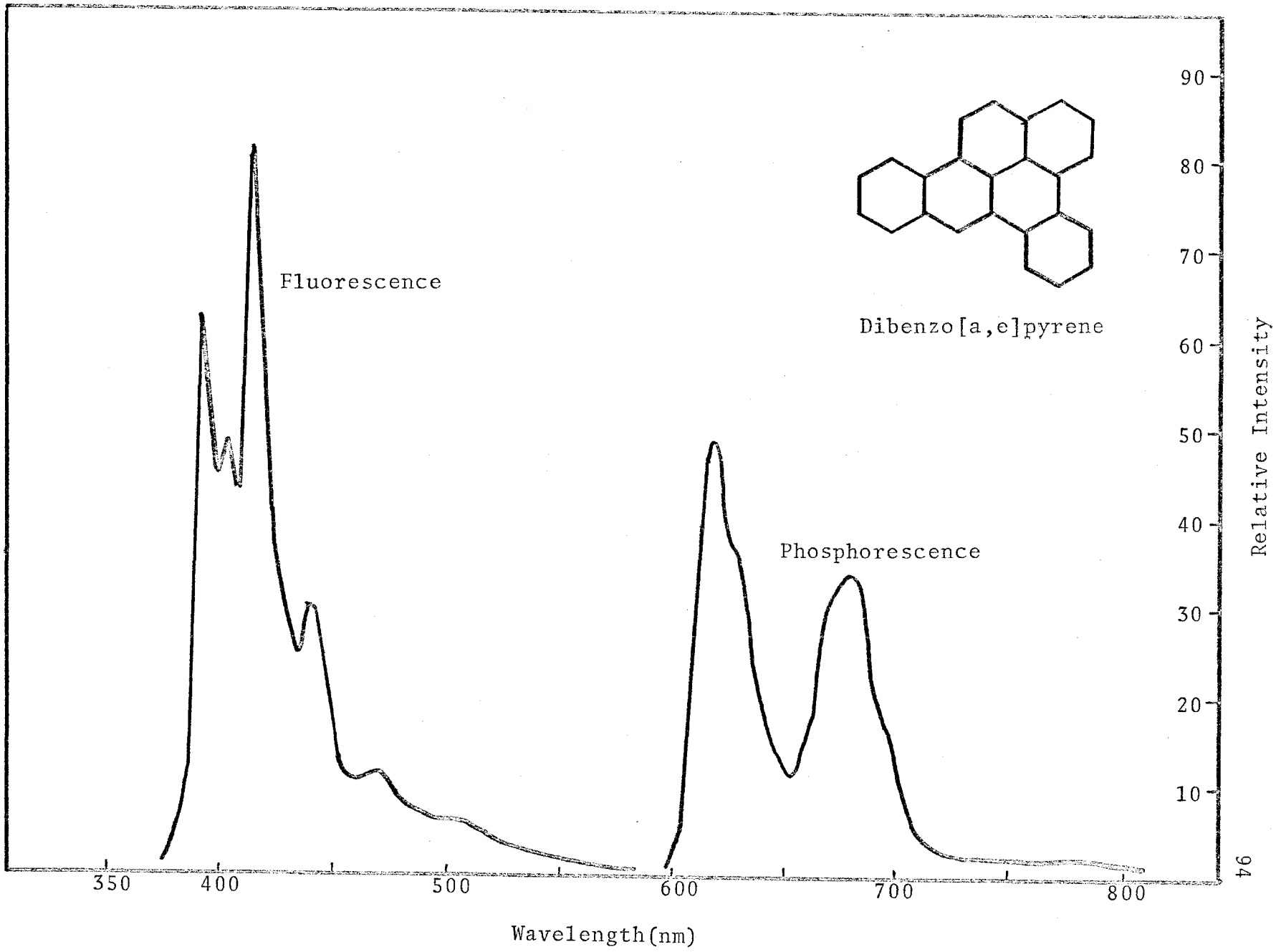


Figure 6. Cont./

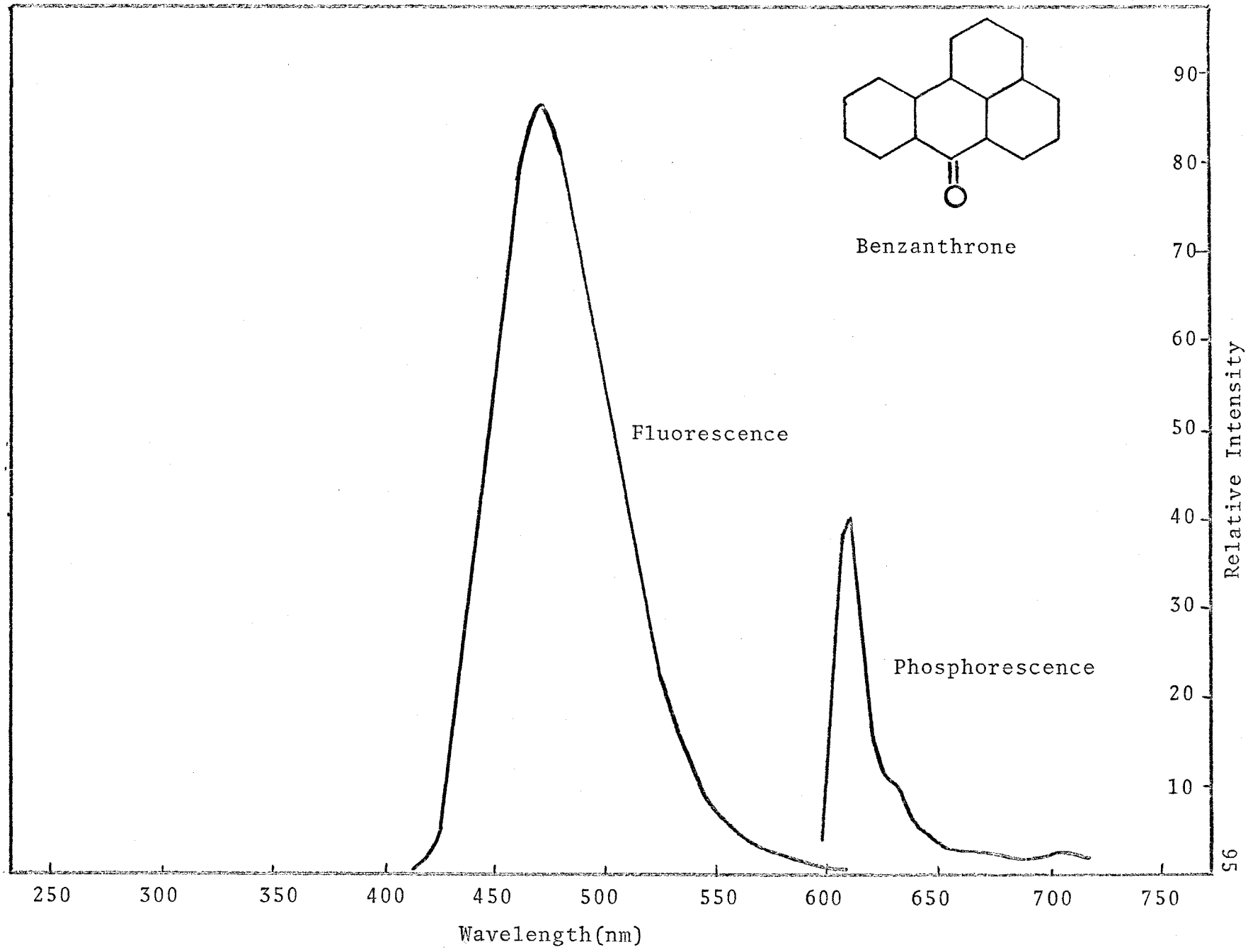


Figure 6. Cont.

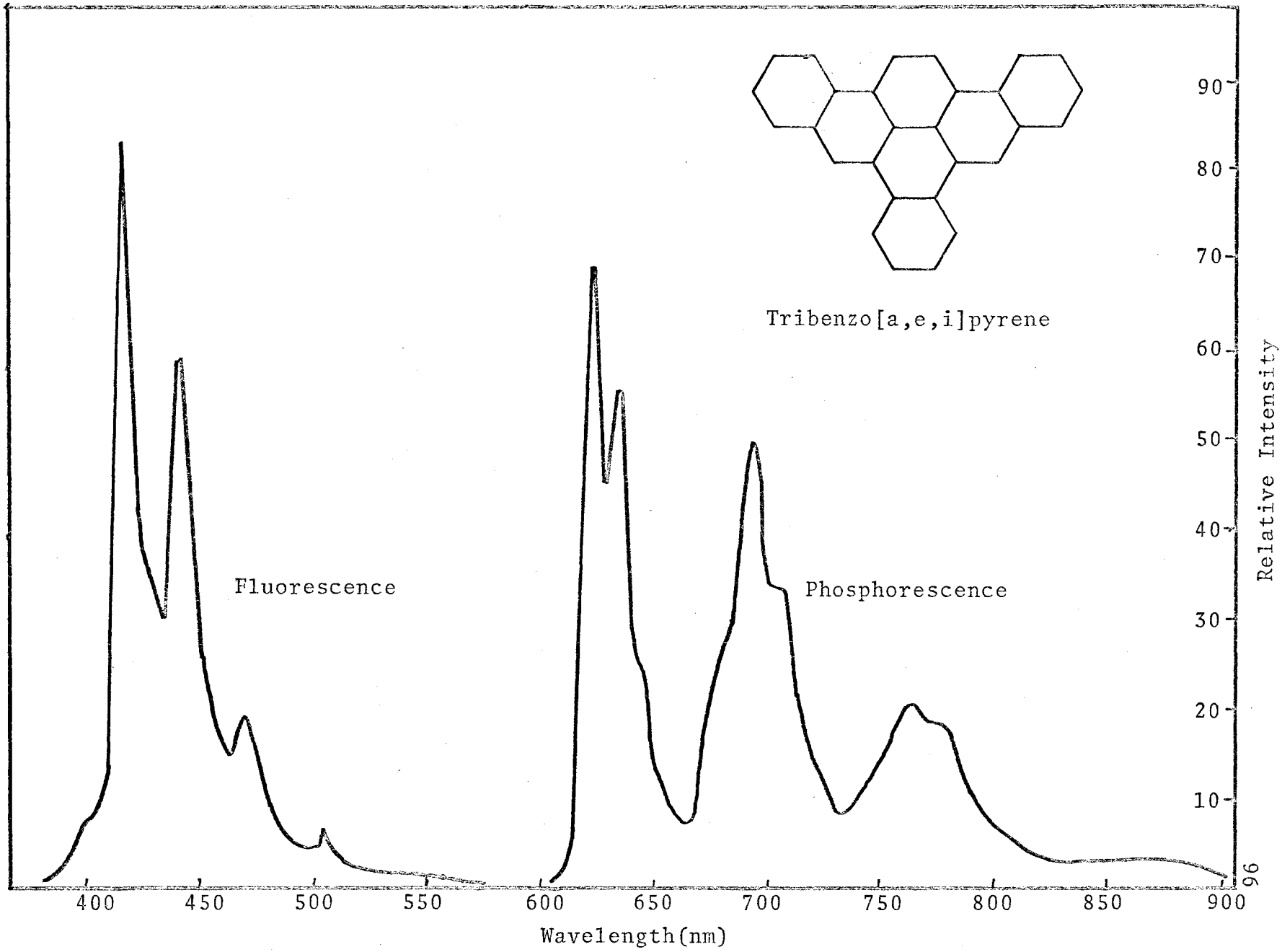


Figure 6. Cont.

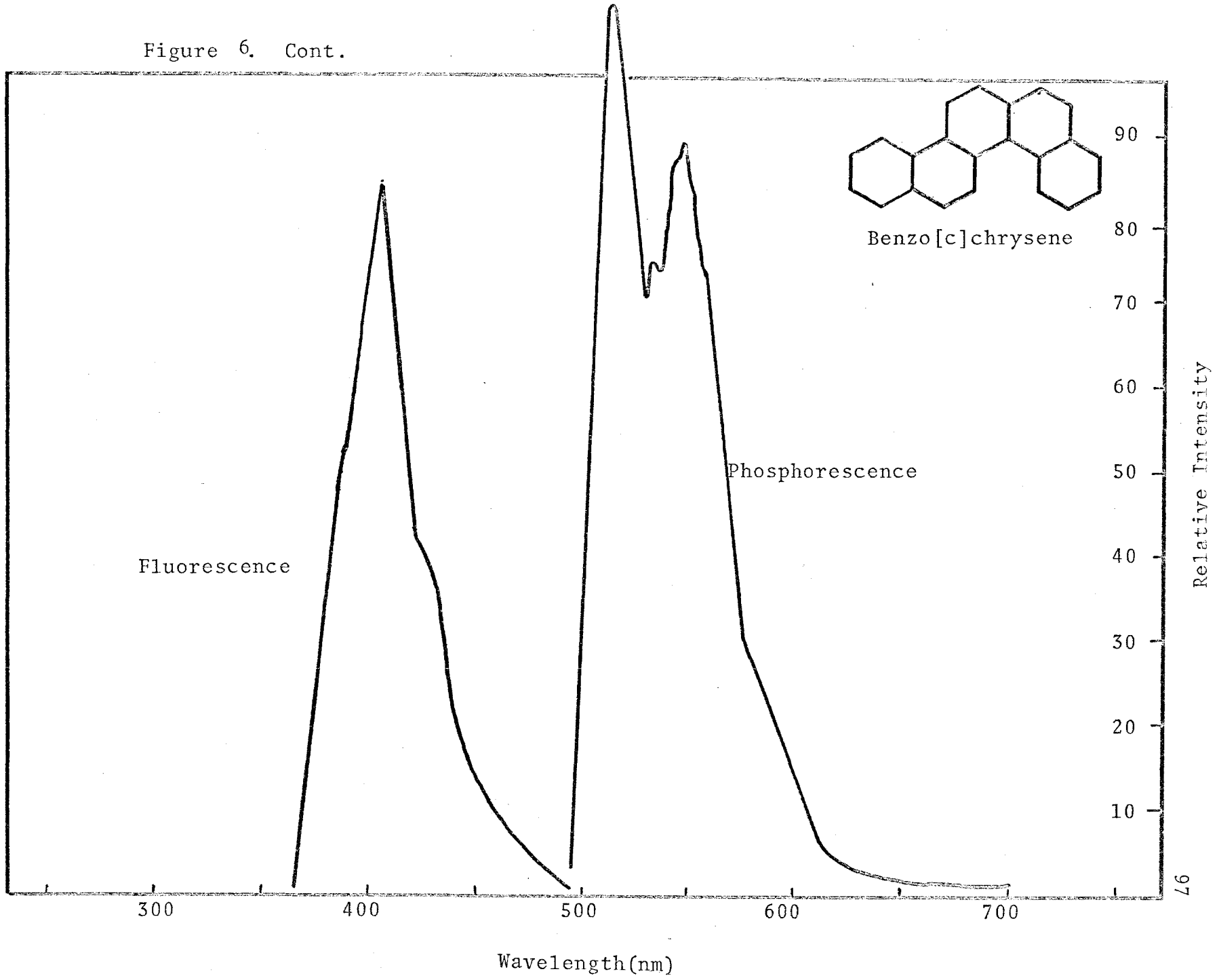


Figure 6. Cont.

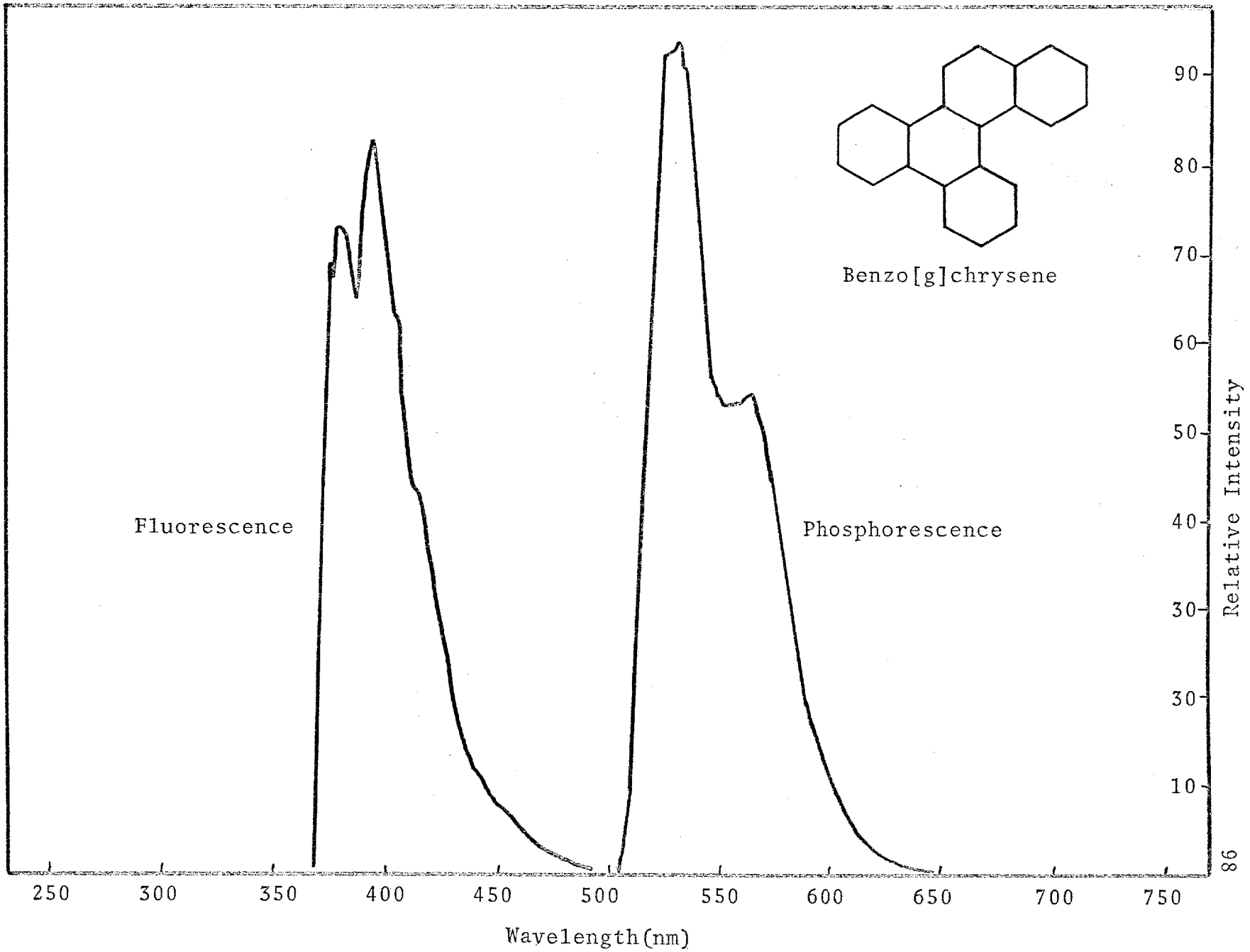


Figure 6. Cont.

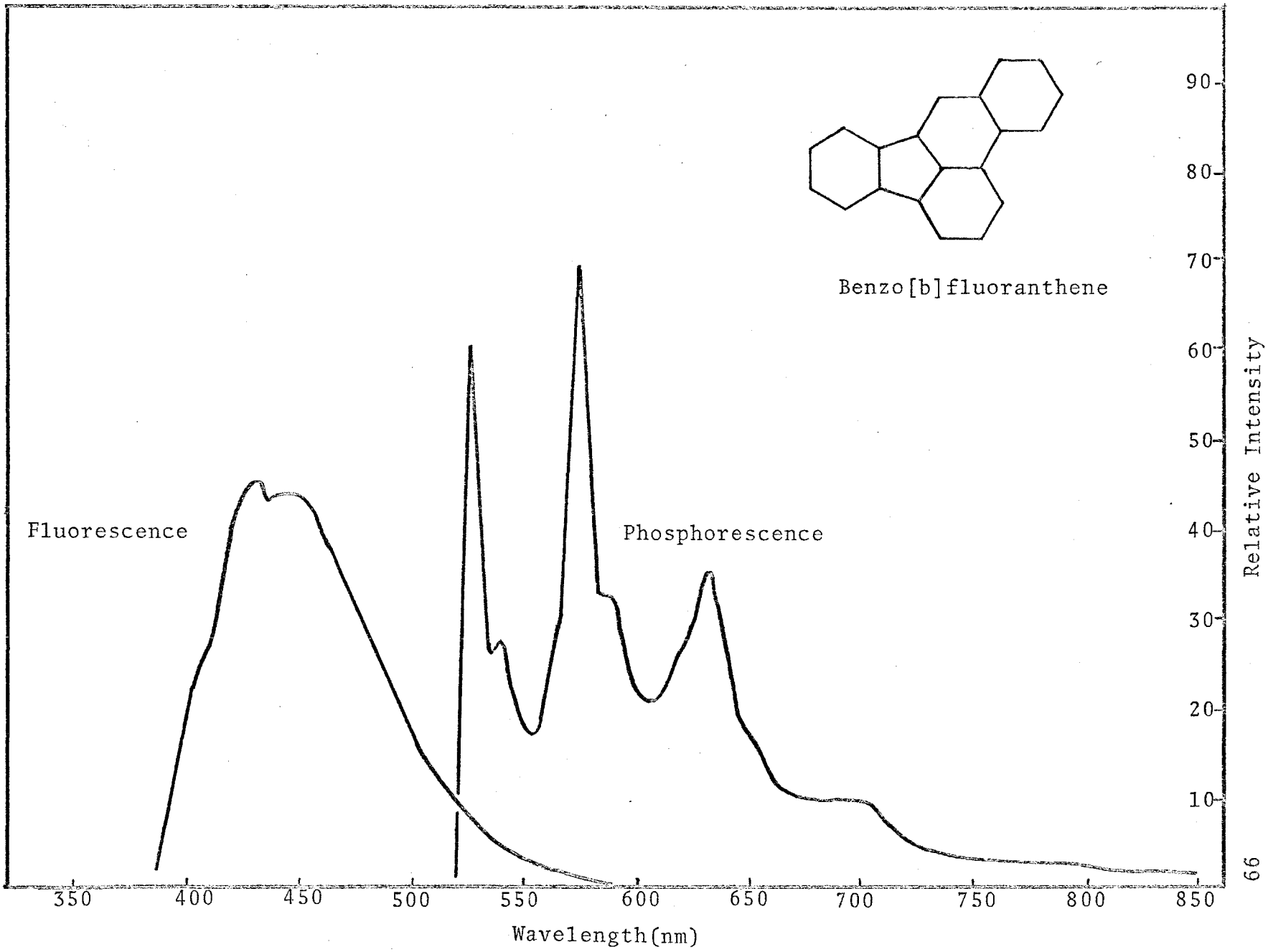


Figure 6. Cont.

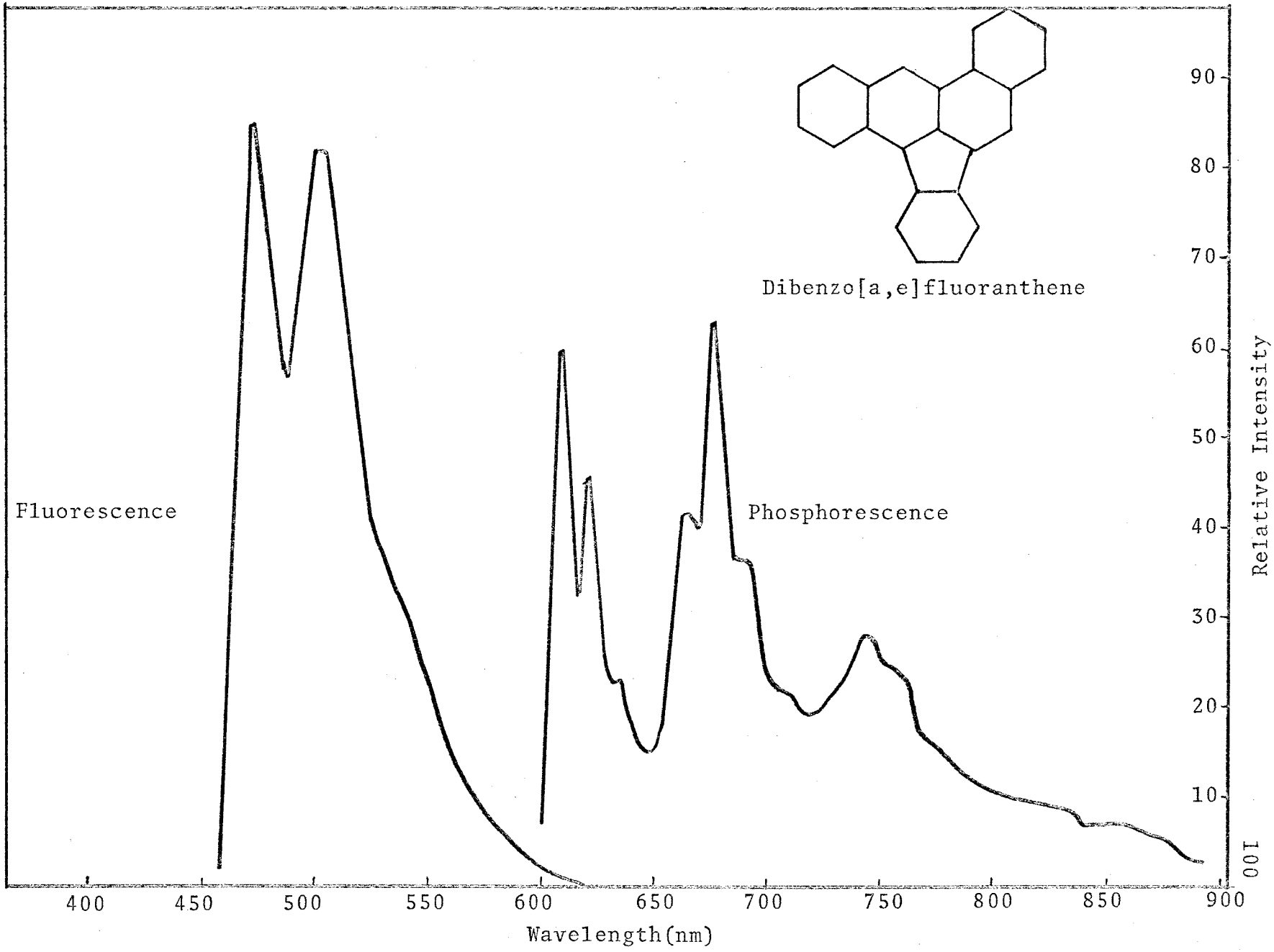


Figure 6. Cont.

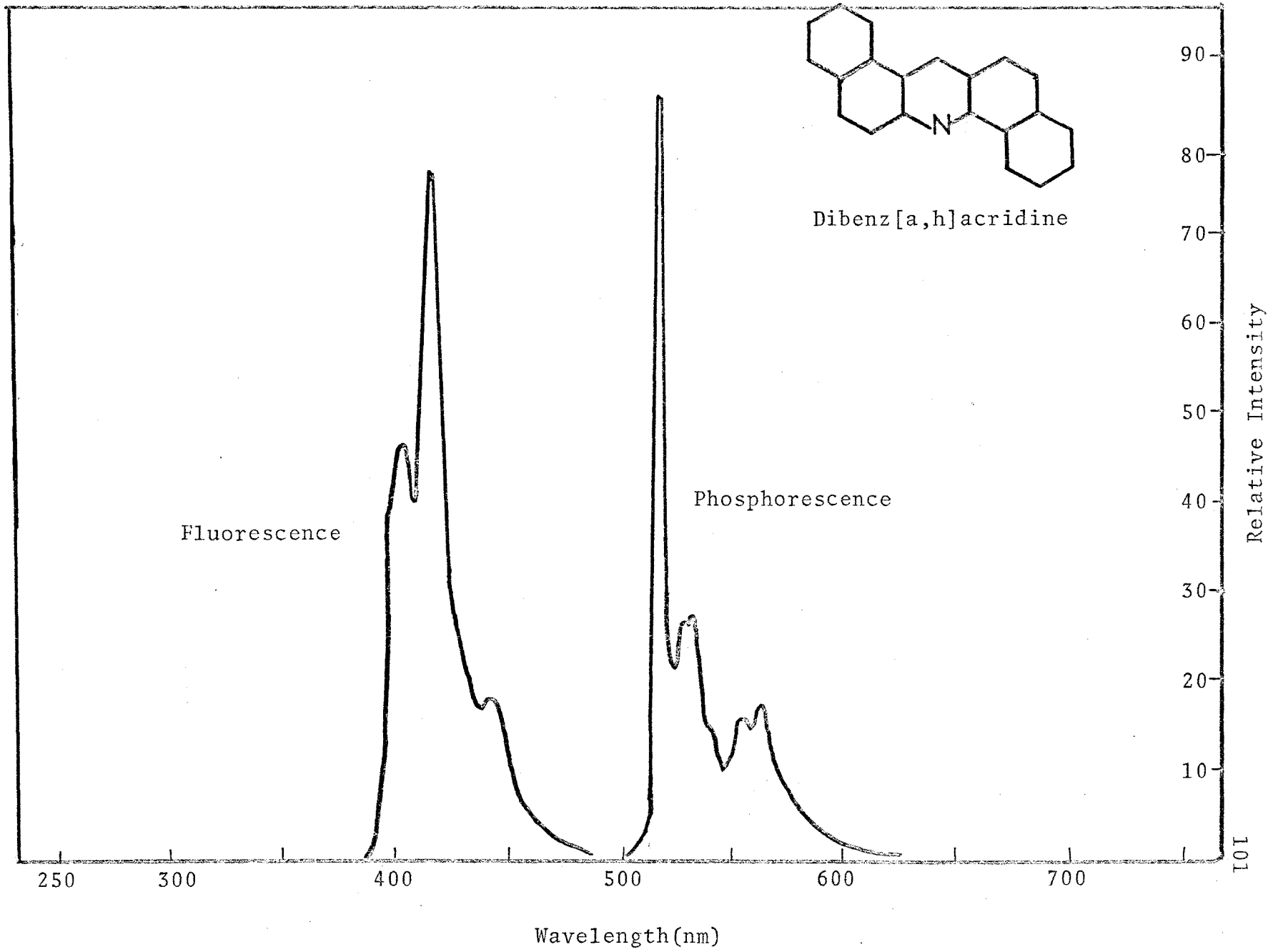


Figure 6. Cont.

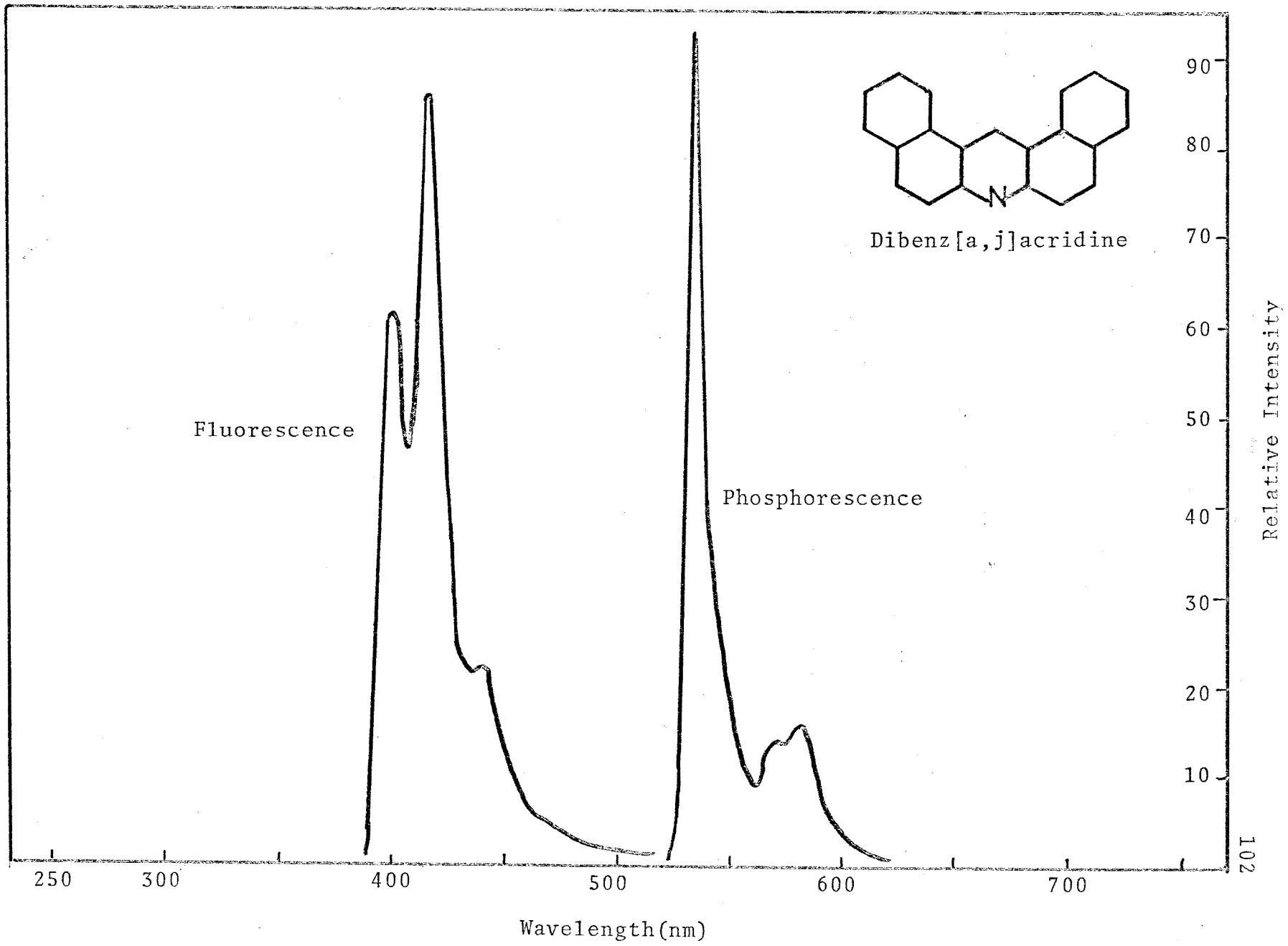


Figure 6. Cont.

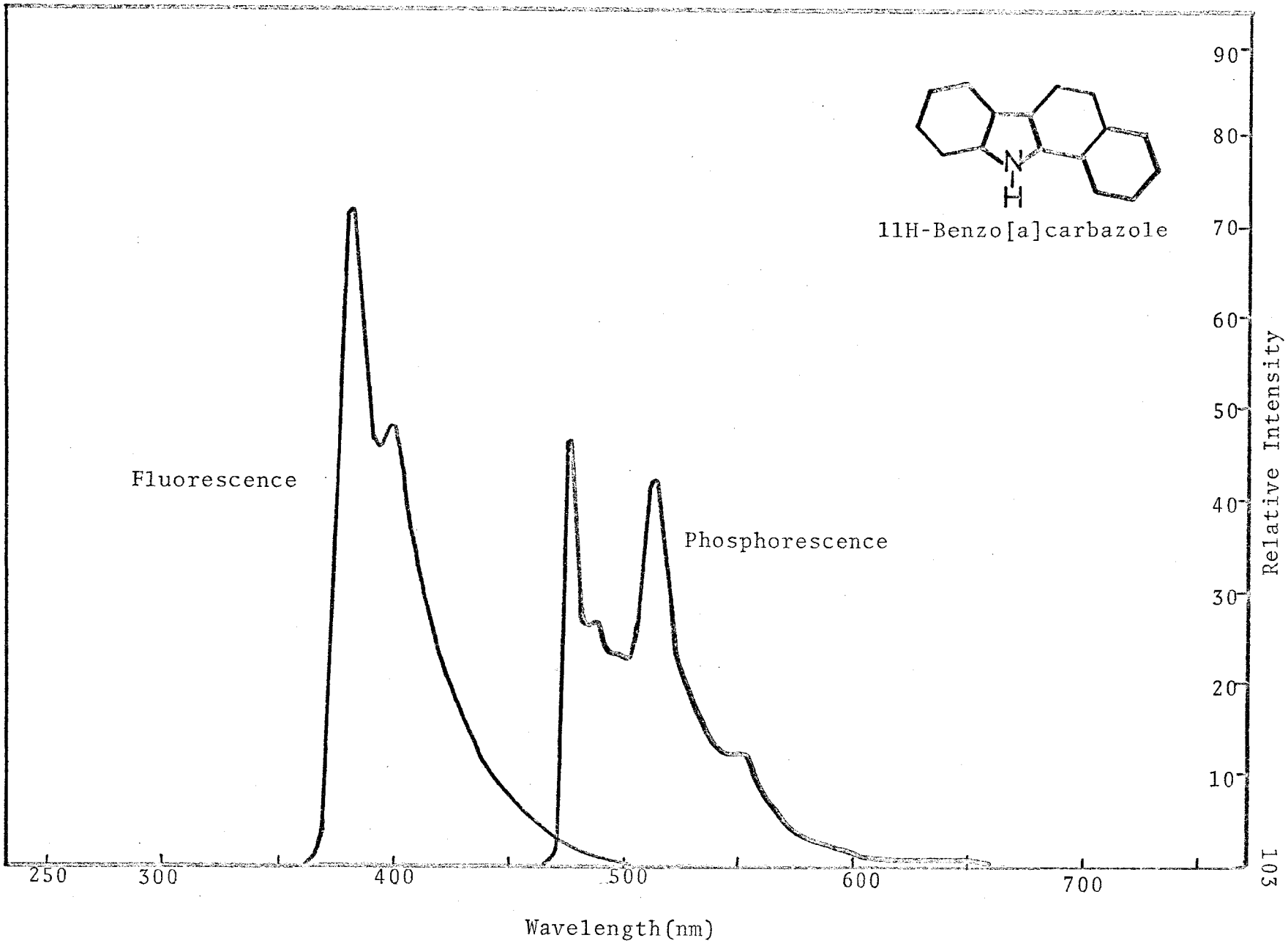


Figure 6. Cont.

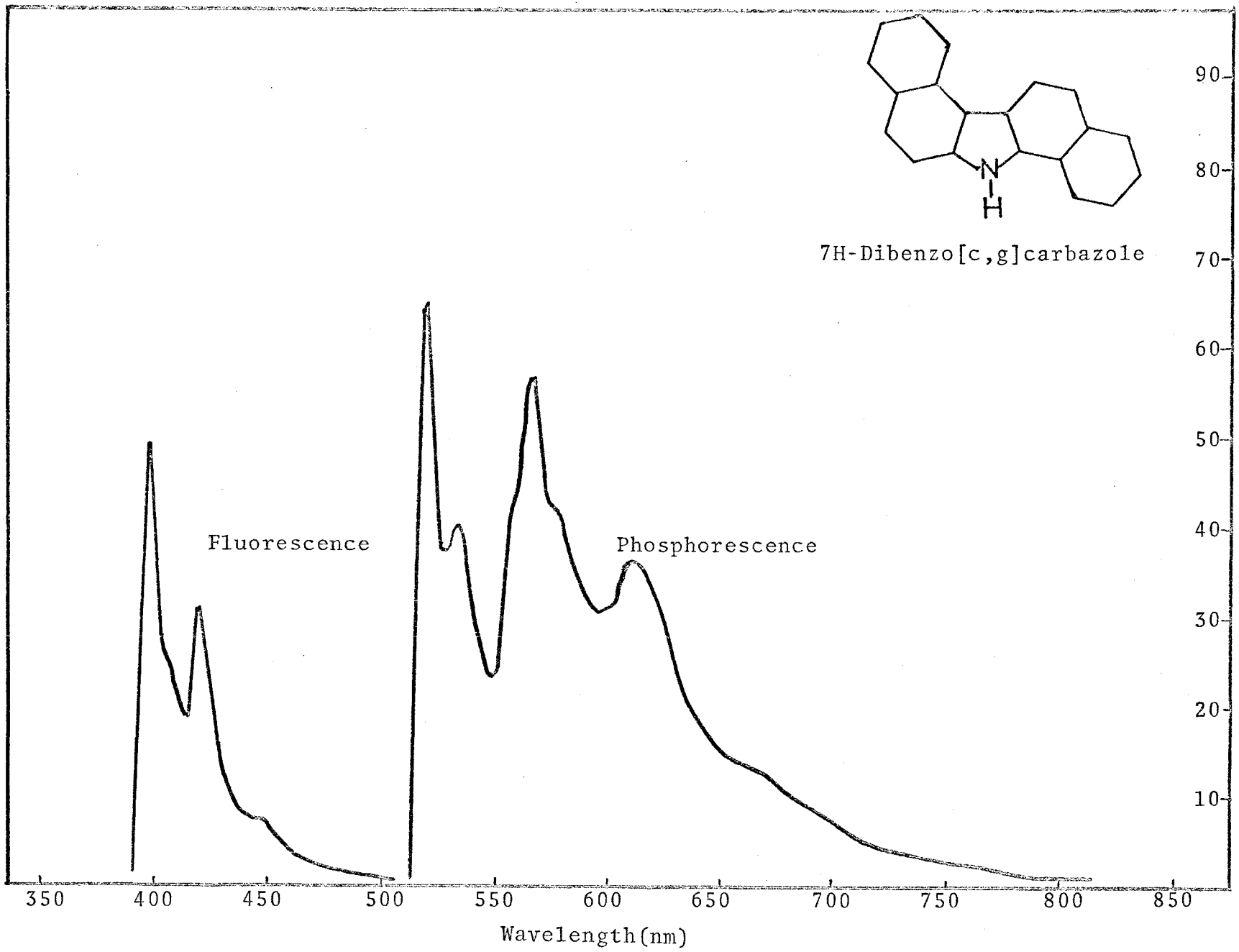


Figure 6. Cont.

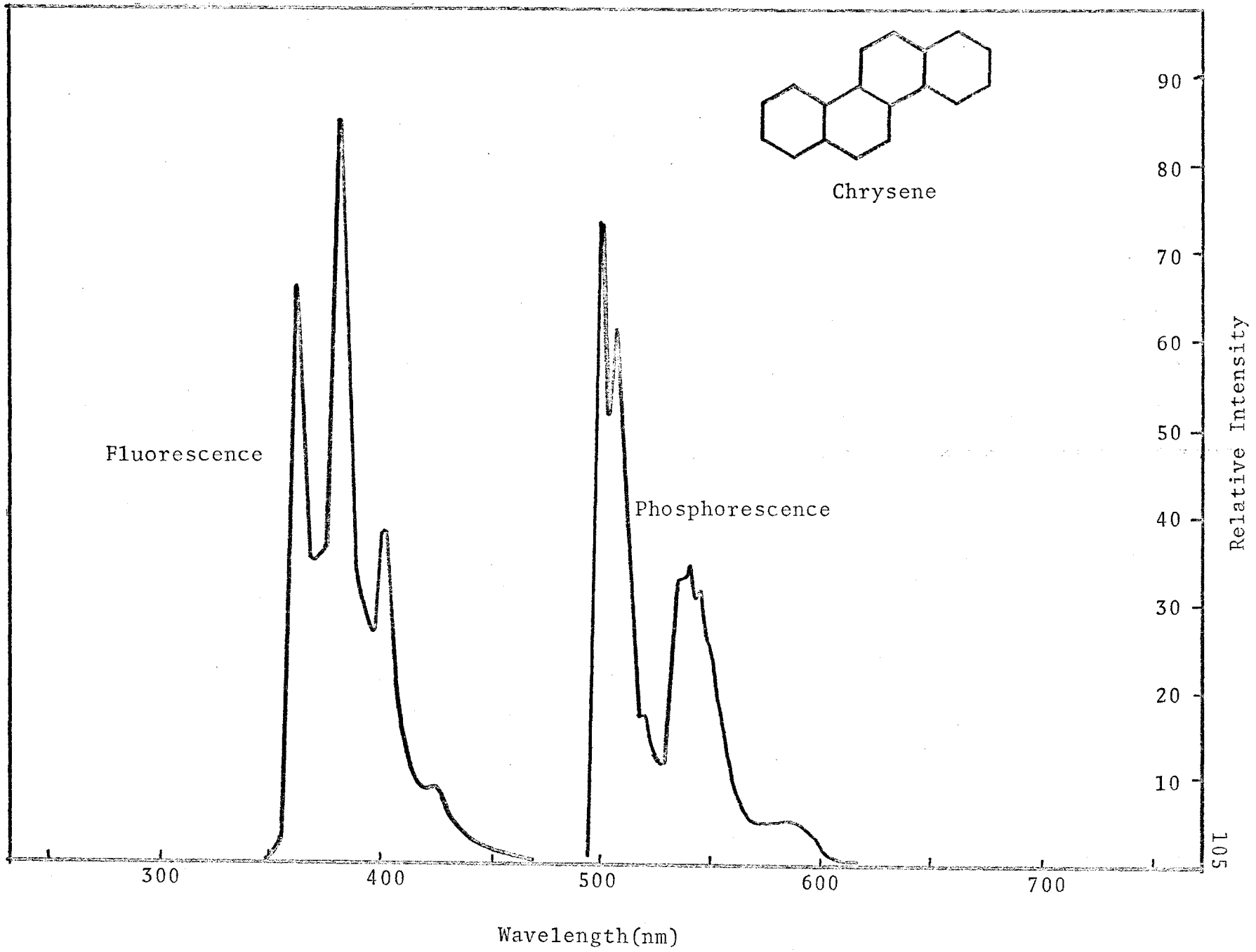


Figure 6. Cont.

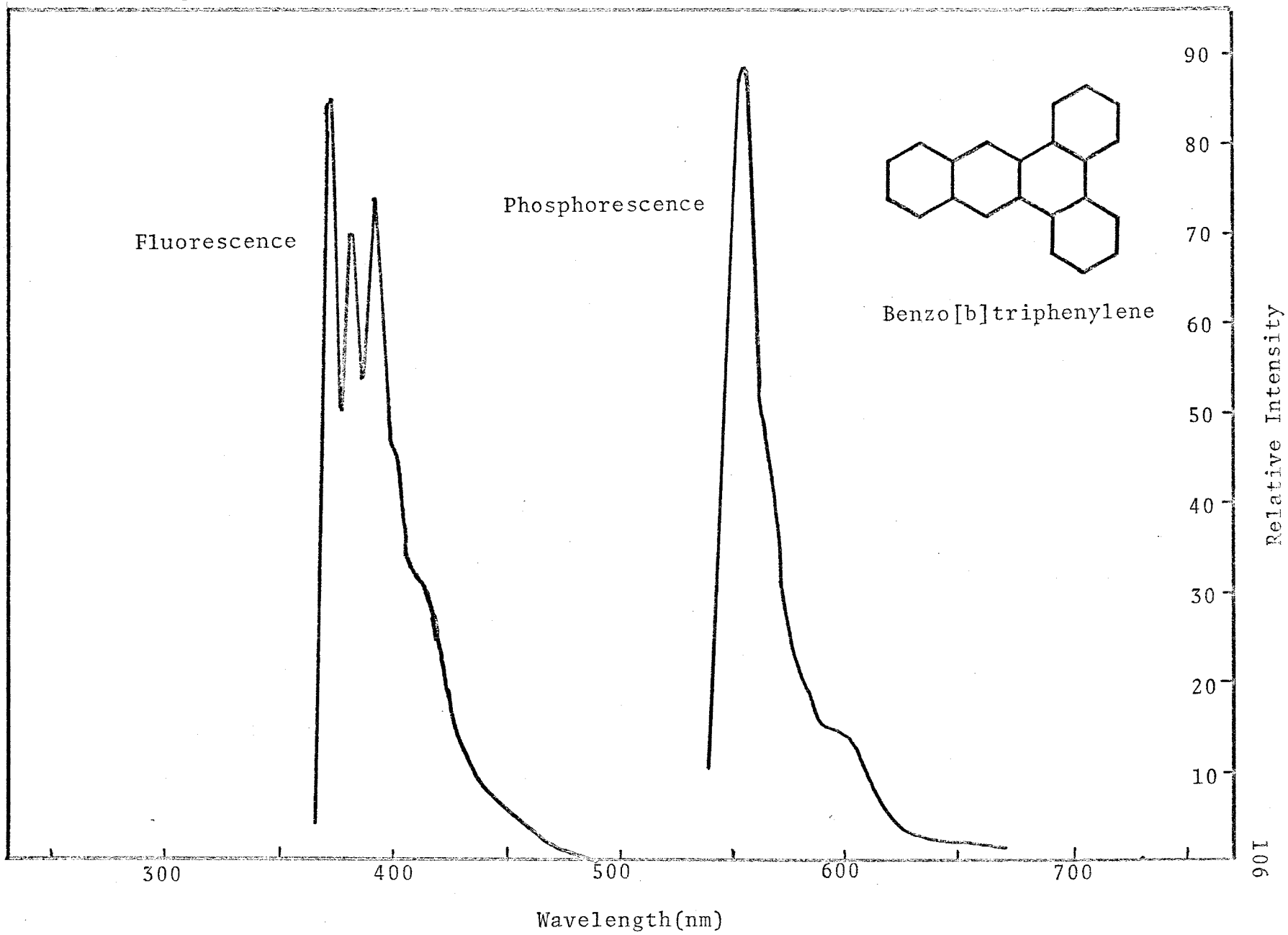


Figure 6. Cont.

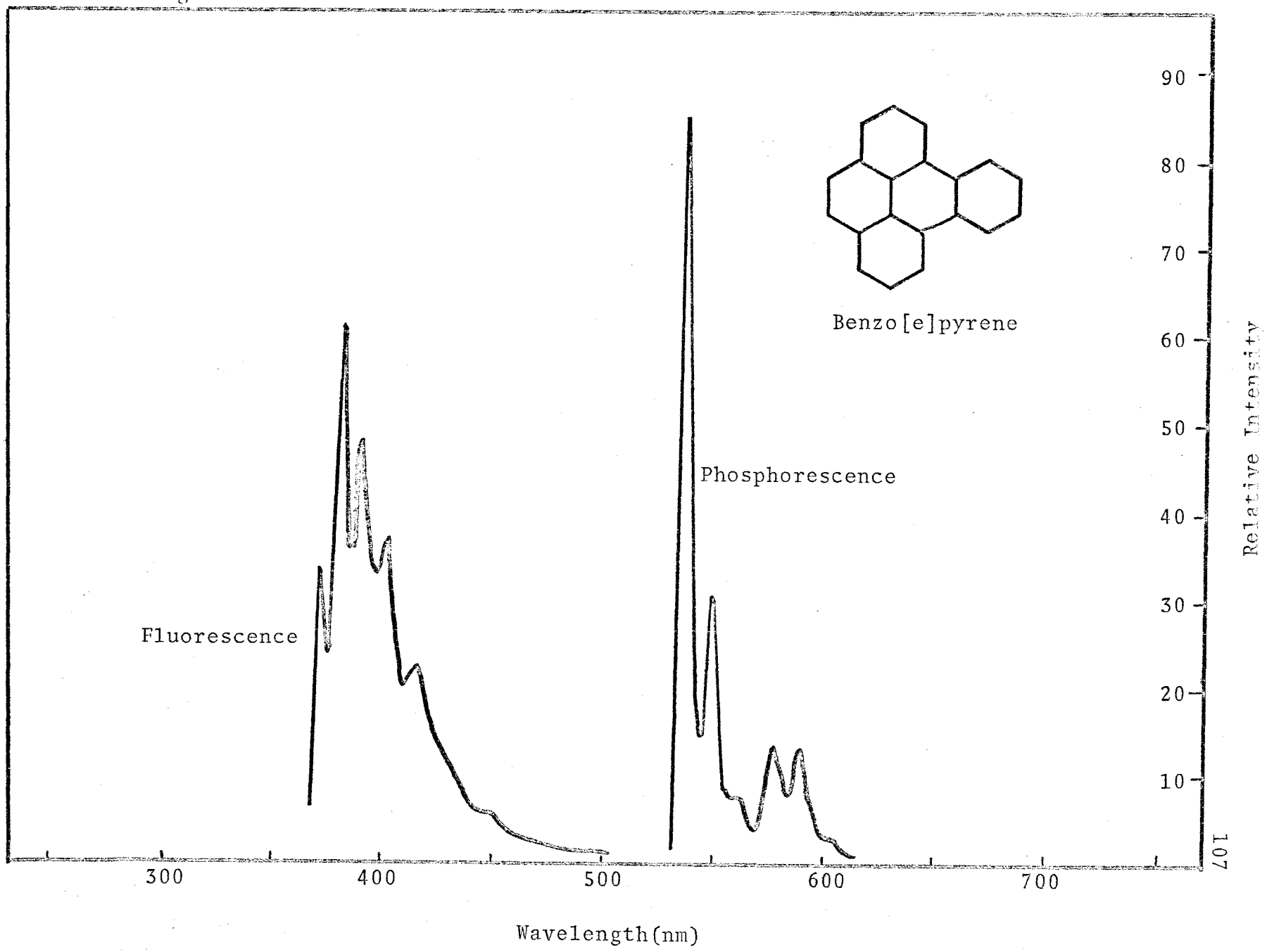


Figure 6. Cont.

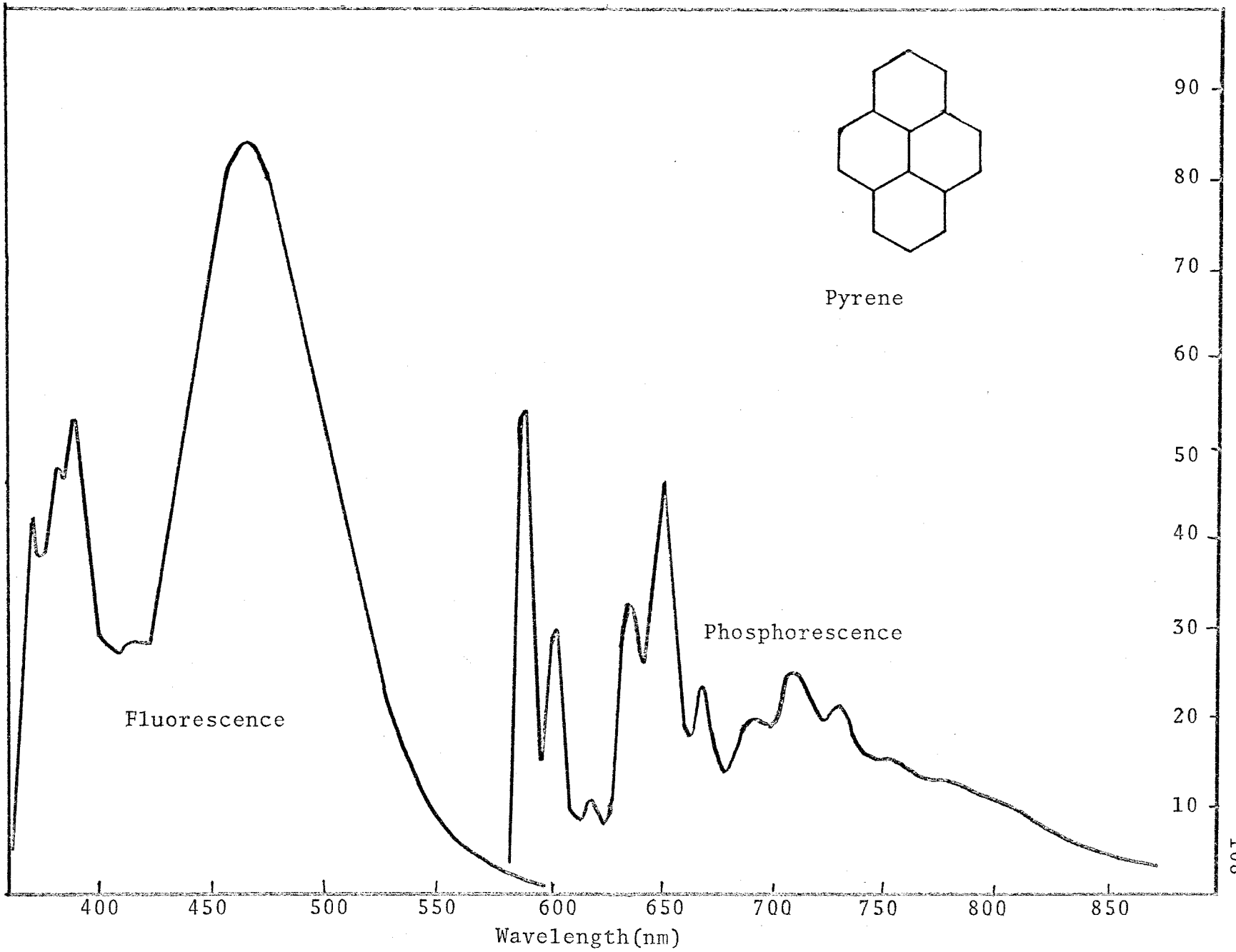


Figure 6. Cont.

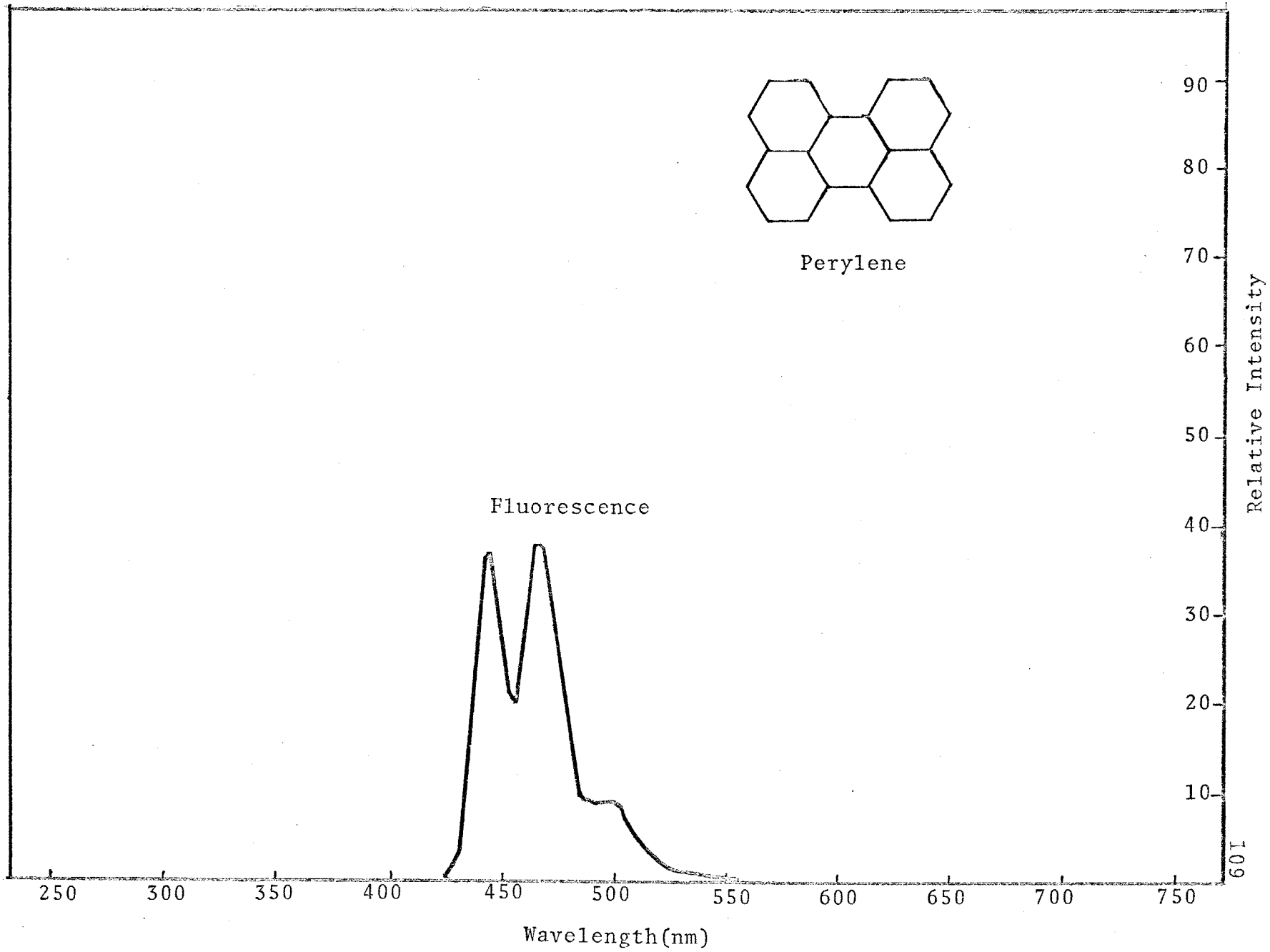


Figure 6. Cont.

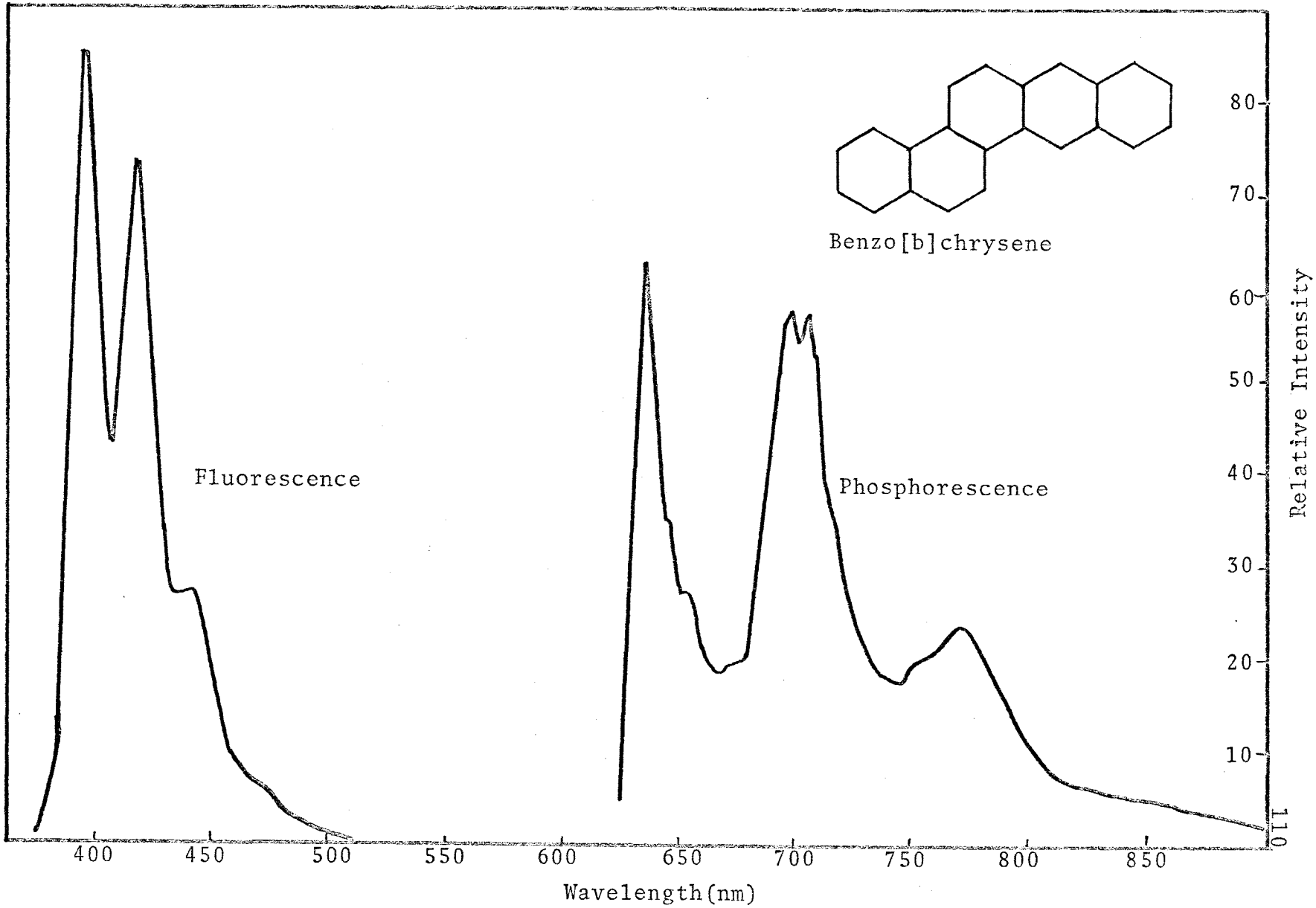


Figure 6. Cont.

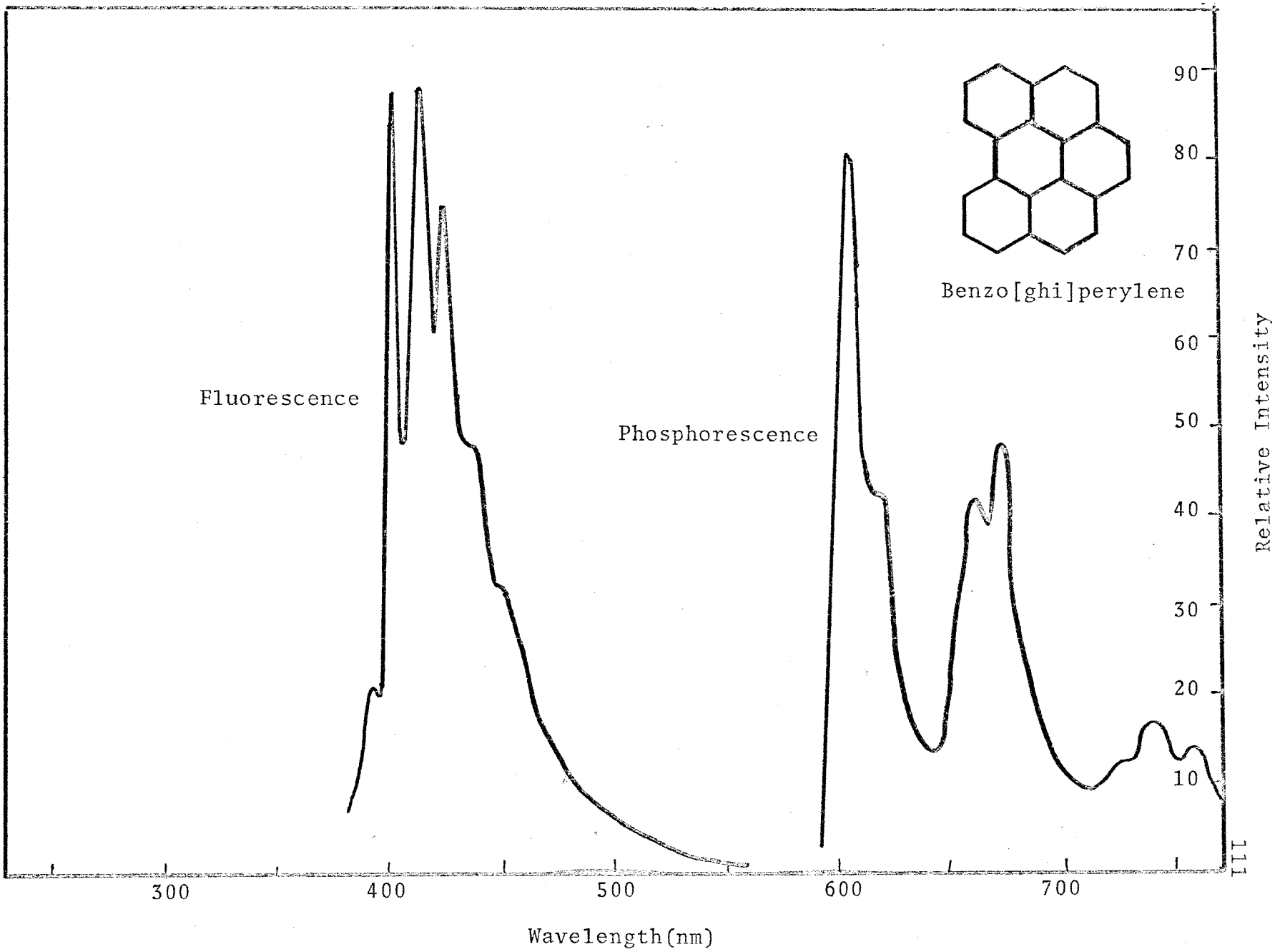


Figure 6. Cont.

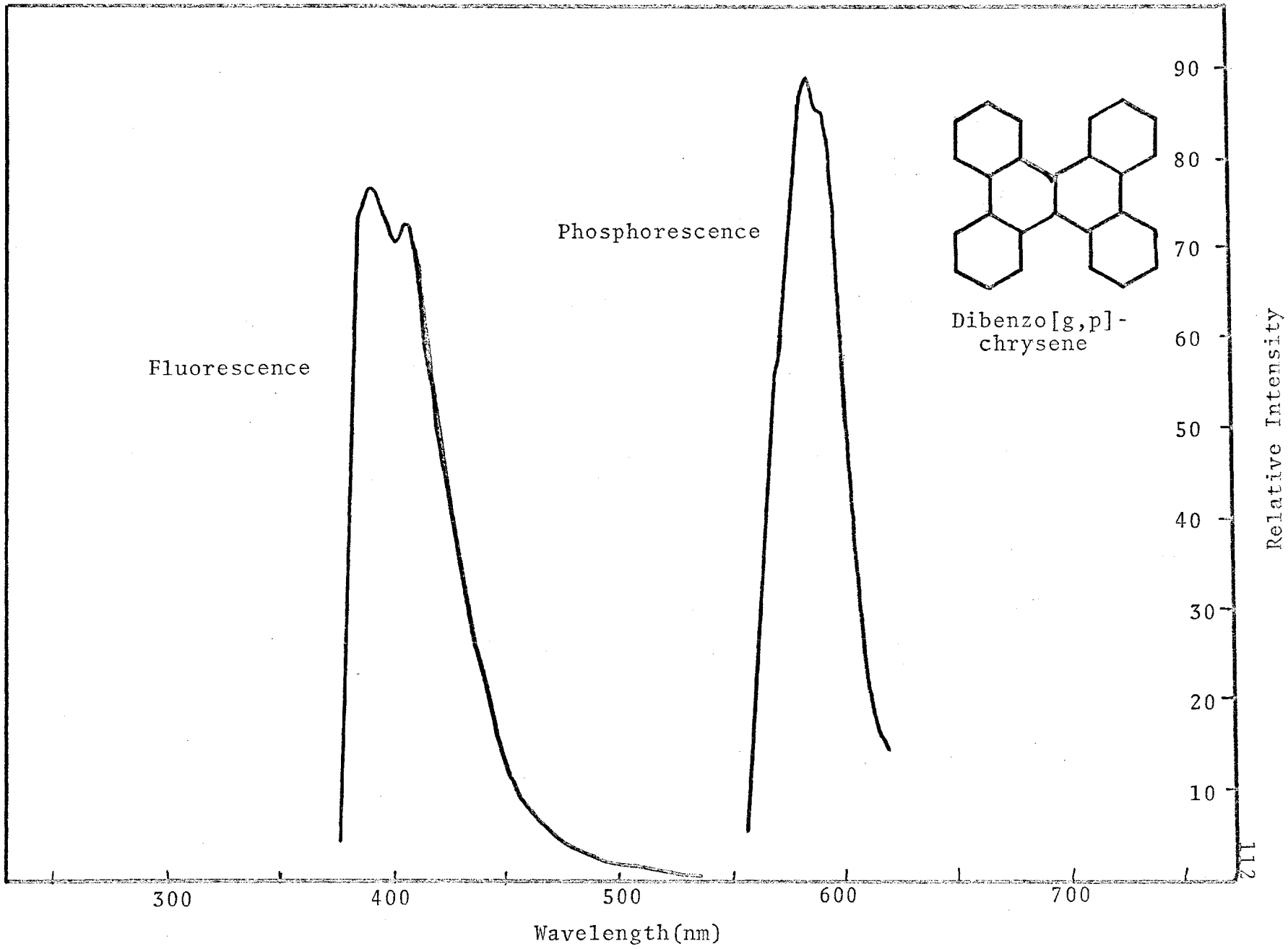


Figure 6. Cont.

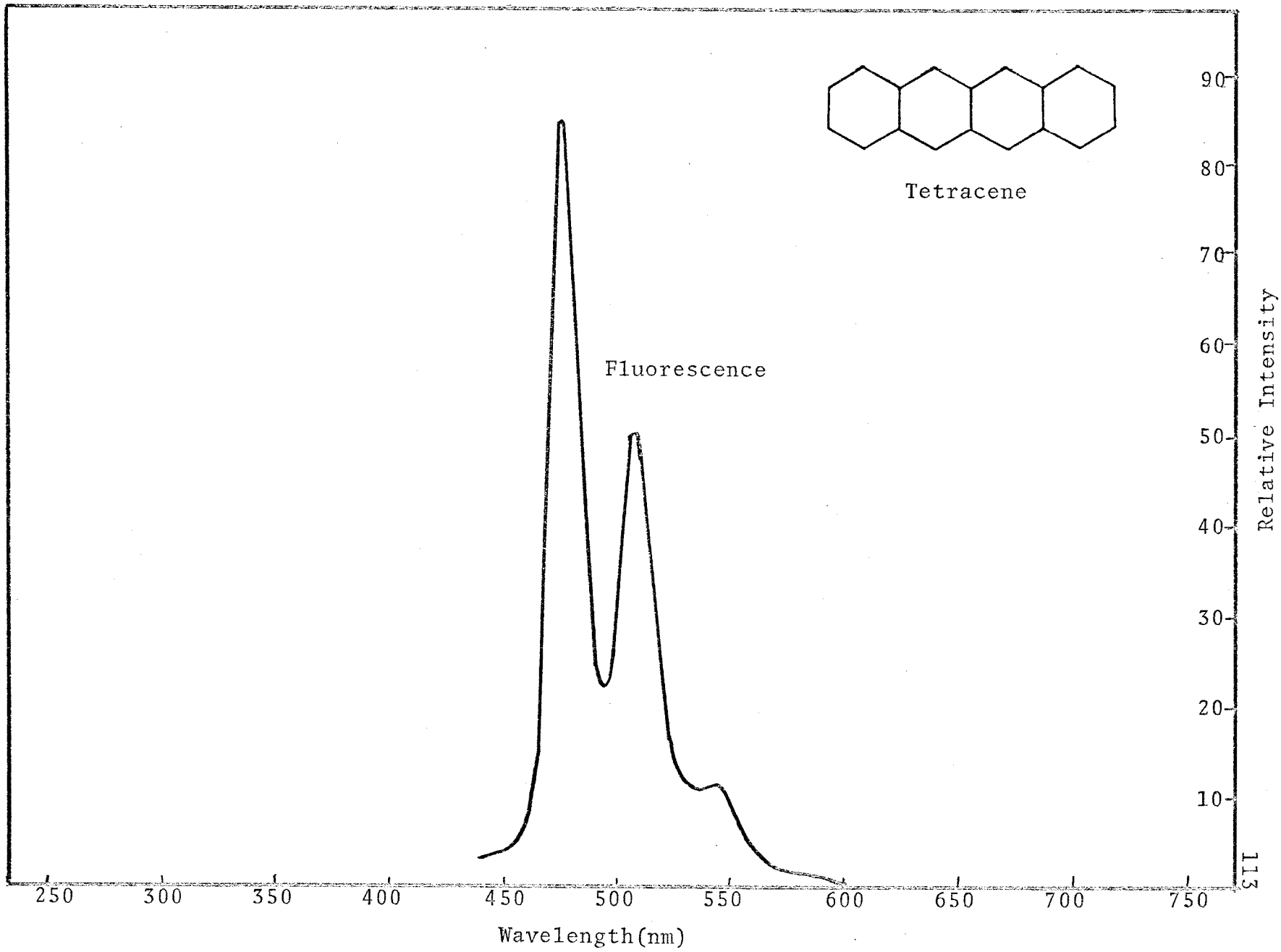


Figure 6. Cont.

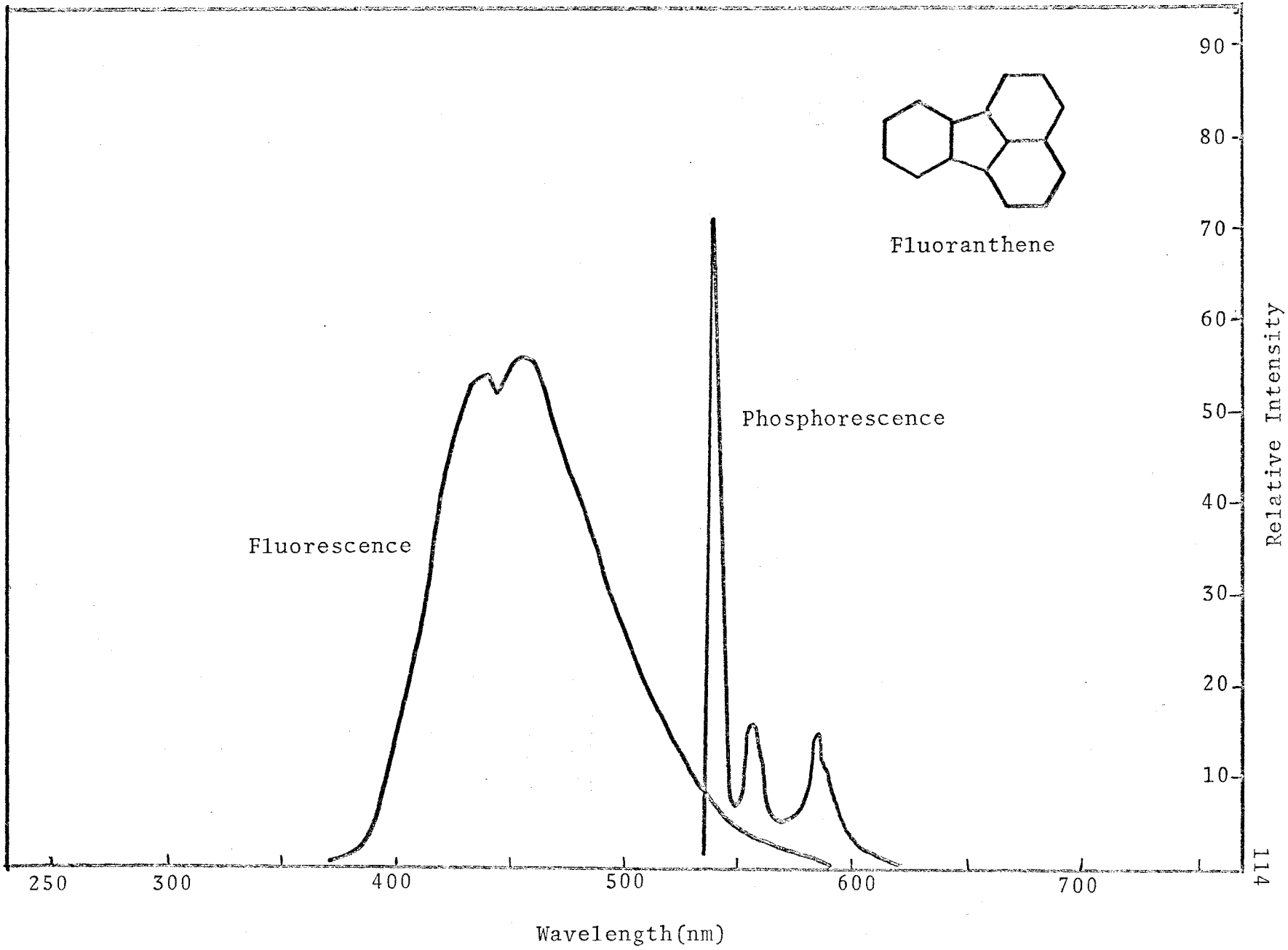


Figure 6. Cont.

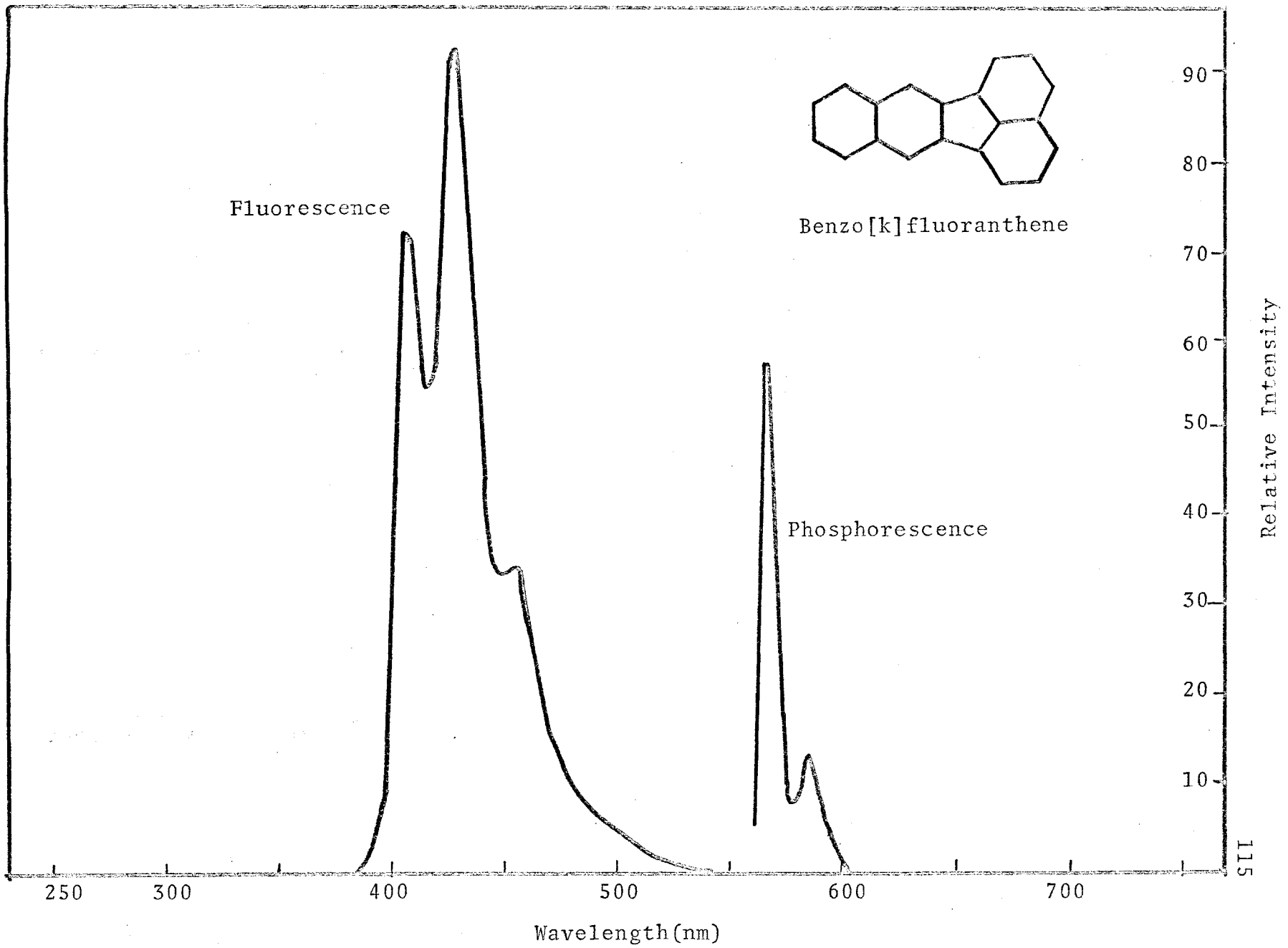


Figure 6. Cont.

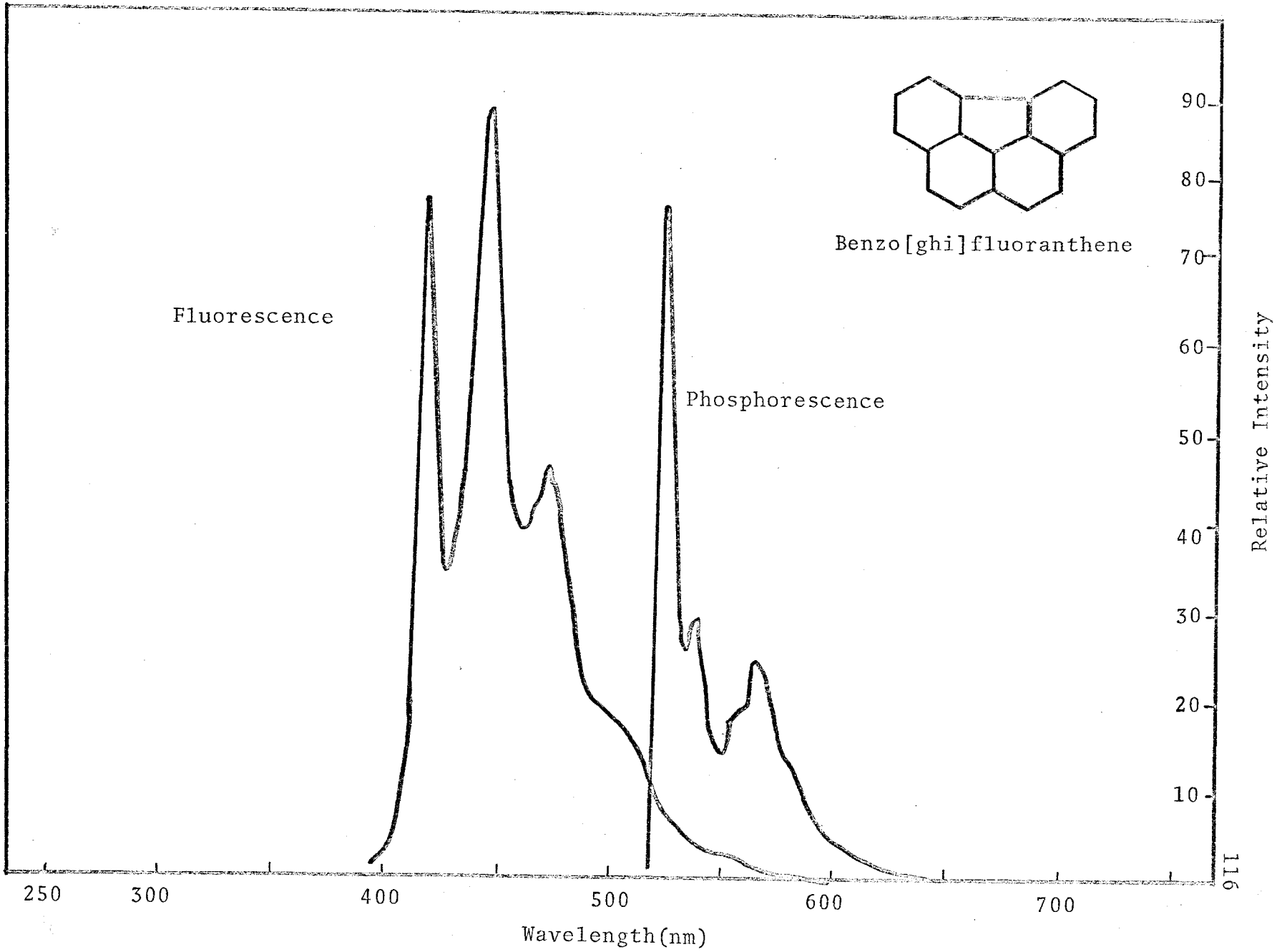


Figure 6. Cont.

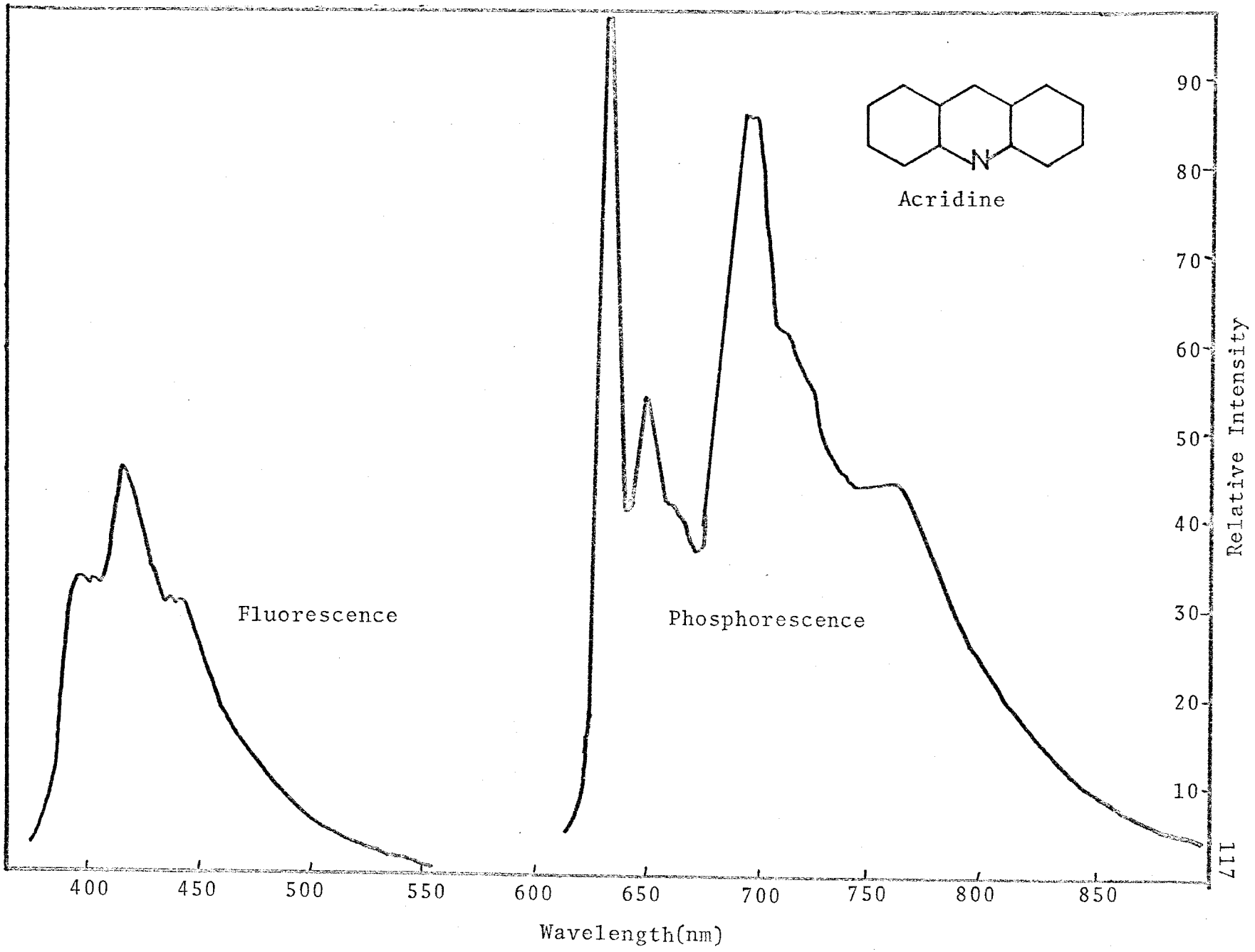


Figure 6. Cont.

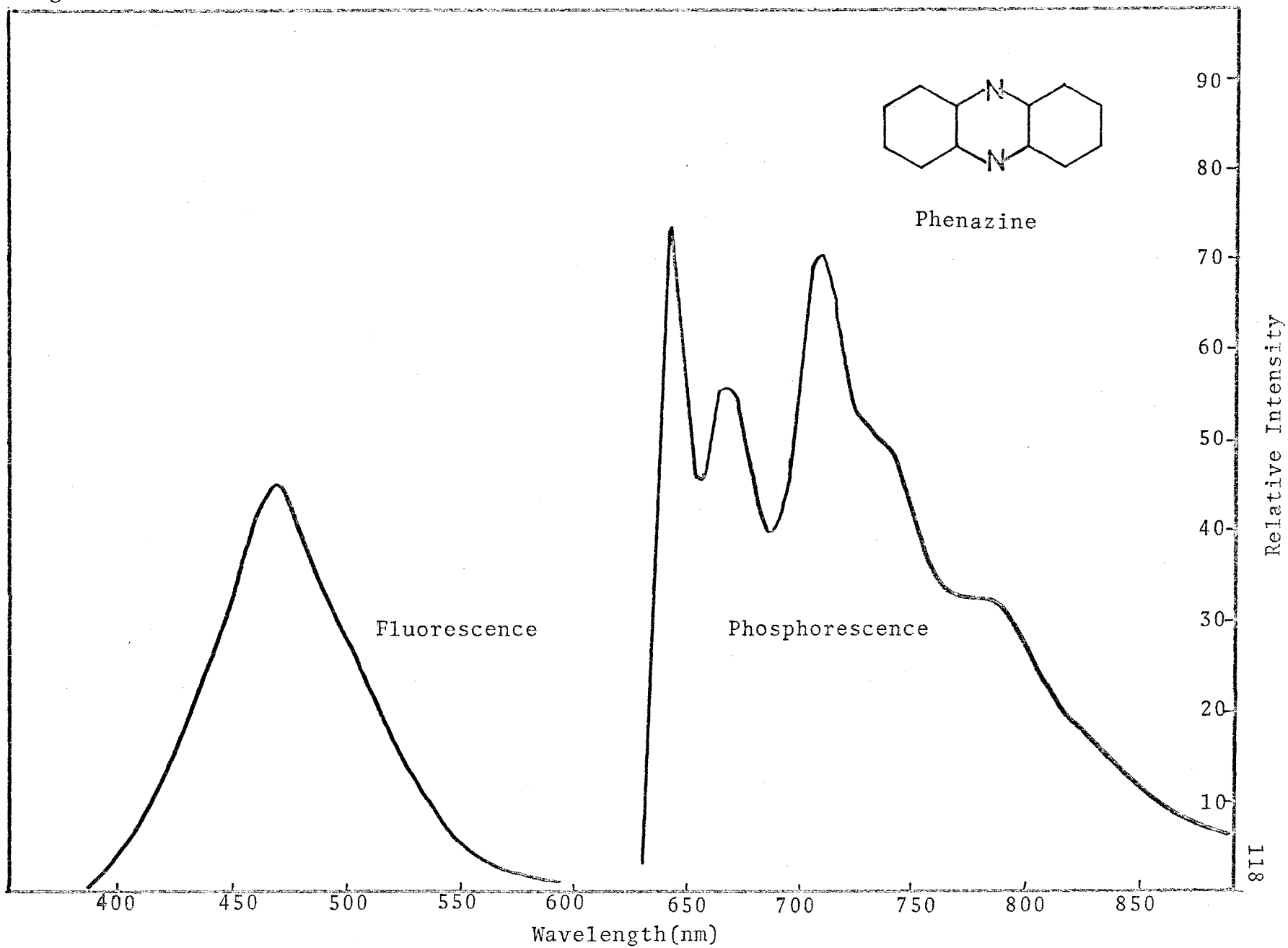


Figure 6. Cont.

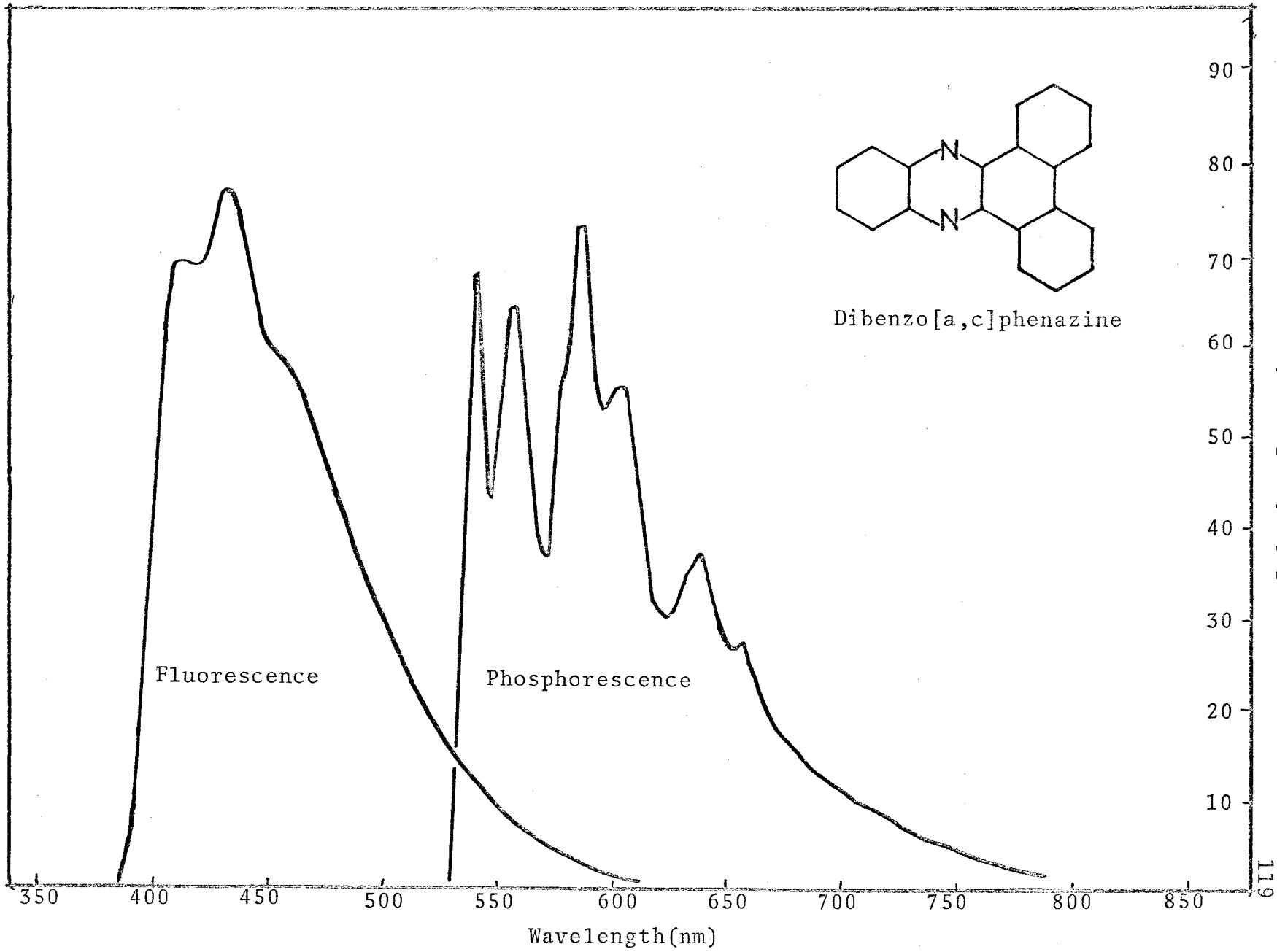


Figure 6. Cont.

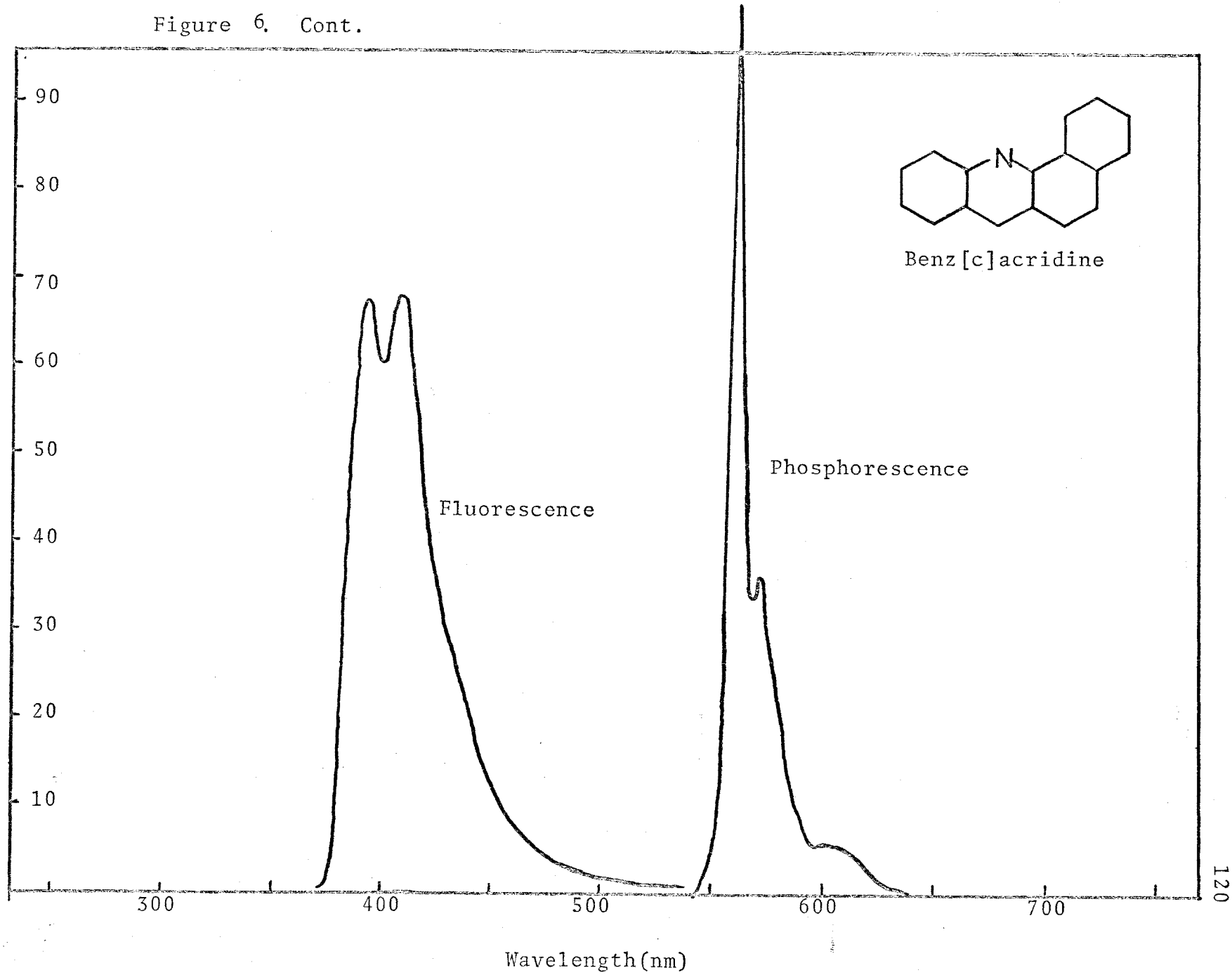


Figure 6. Cont.

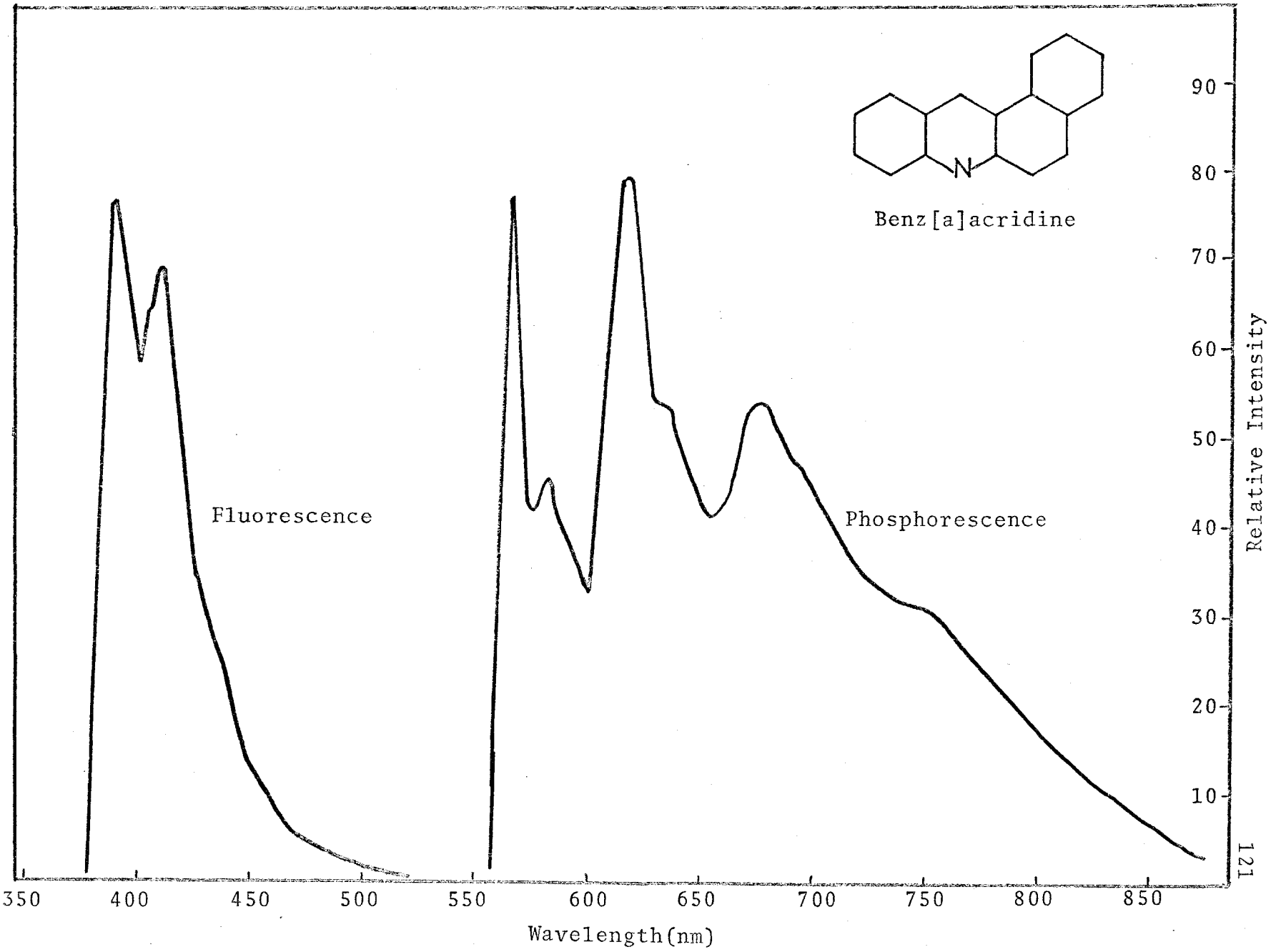


Figure 6. Cont.

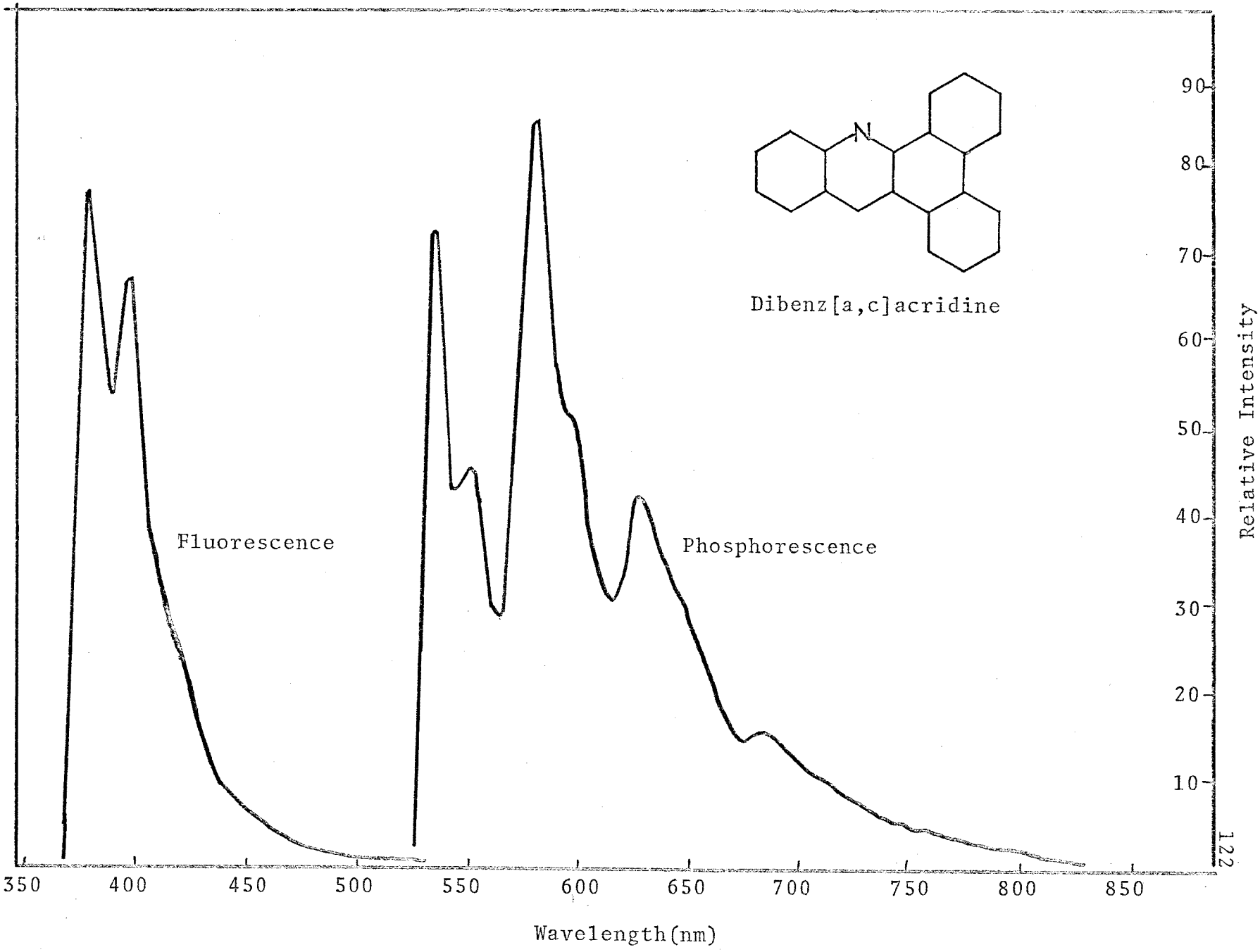


Figure 6. Cont.

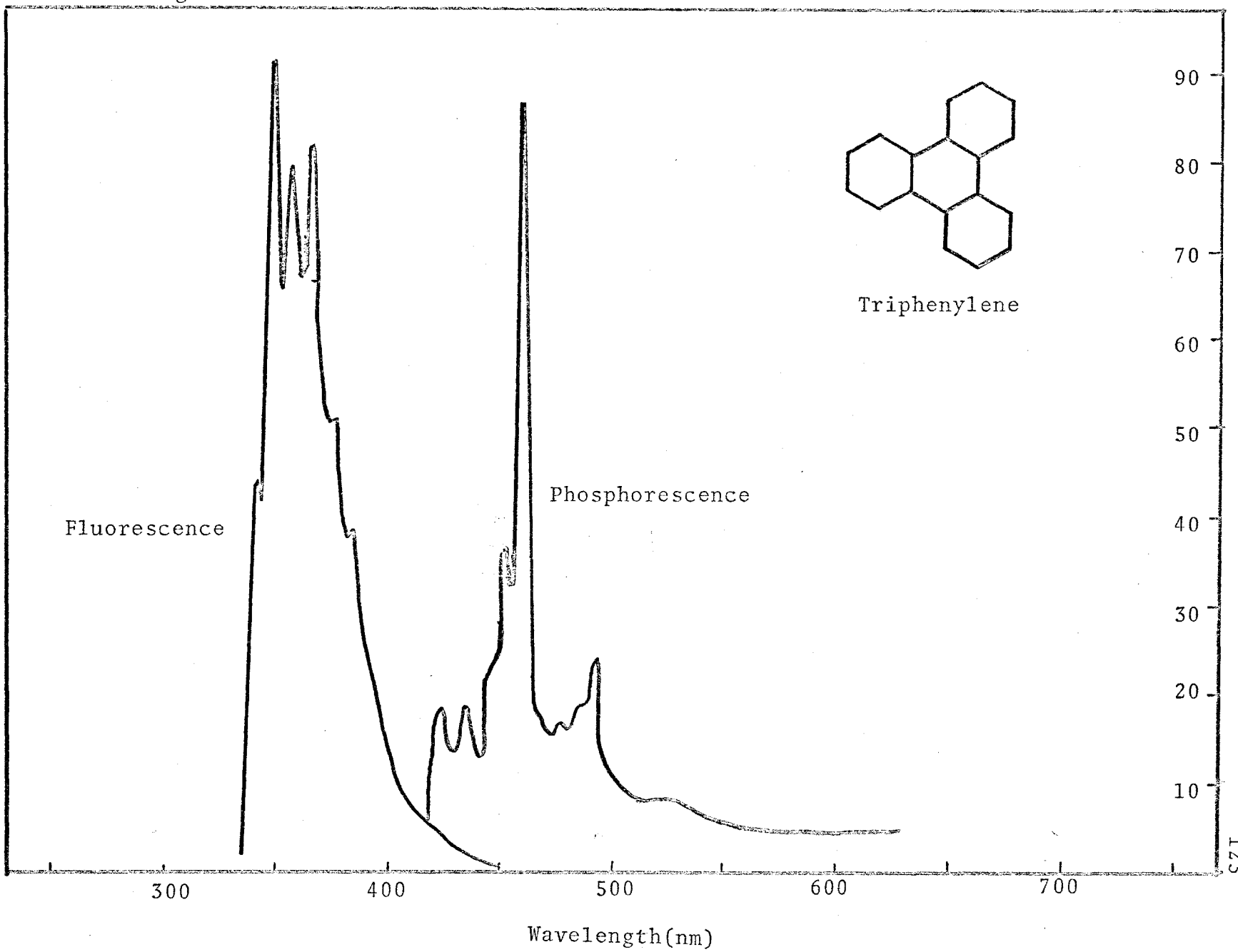
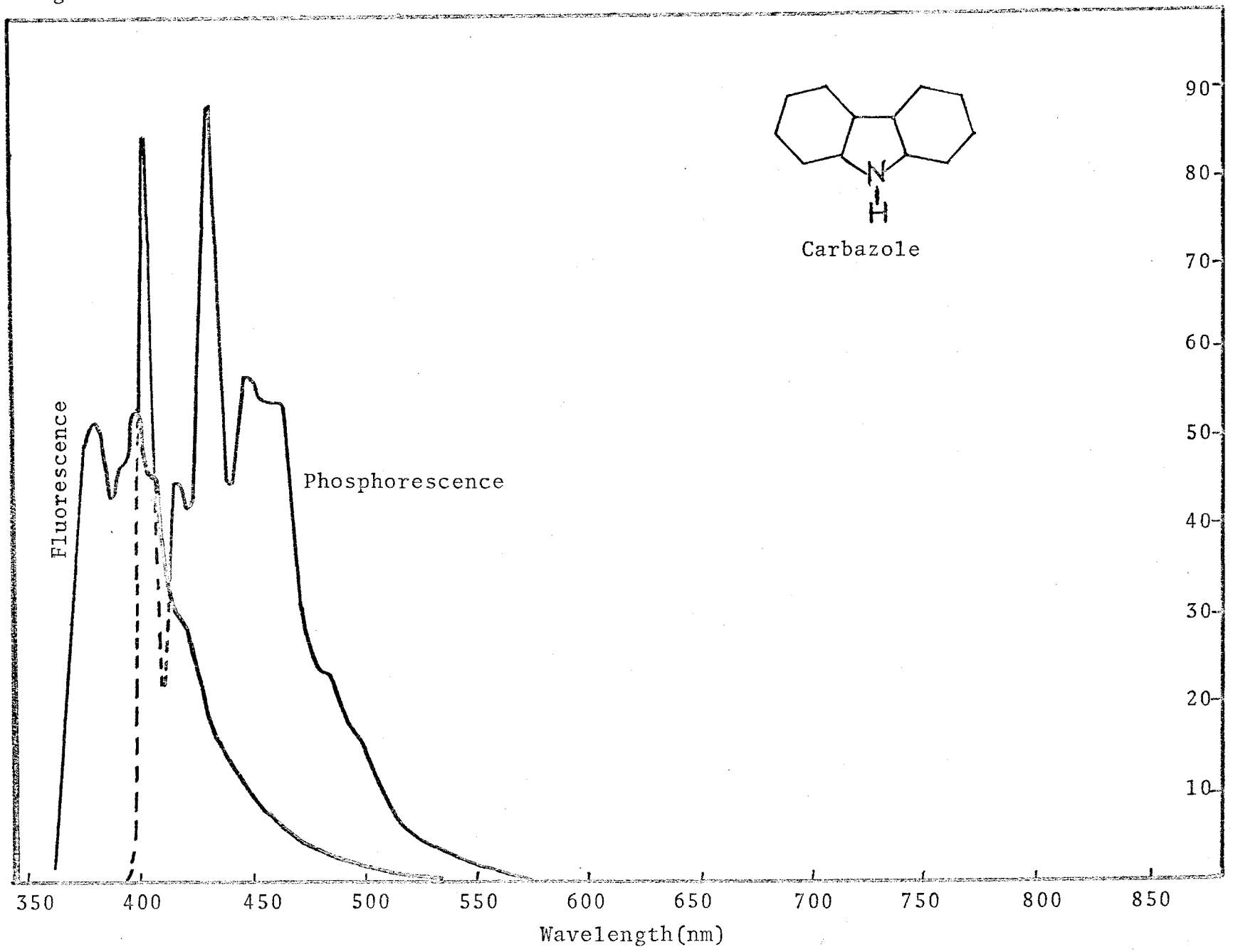


Figure 6. Cont.



D. Electronic Emission Spectral Index for Carcinogenic Hazard

The statistical method developed by Cornfield [20], the so-called relative odds method [21], was used to analyze the E_s, E_t, $\Delta E_{s,t}$, τ_f, τ_p, and τ_t values in Table 1. No analyses were preformed on the Φ_f and Φ_{st} values because of their uncertainty. Cornfields' method will give the prevalence of carcinogenic compounds amoung a group of compounds which have a certain range of some property. The reliability of the results obtained by Cornfields' method were then subjected to significance tests. The significance tests used were the χ^2 (chi squared) test (if data for more than 50 compounds were available), and Fishers' exact test (if data for less than 50 compounds were available)[22,23]. The results are given in Tables 3 through 8 respectively.

With the exception of the fluorescence lifetimes, none of the photophysical properties gave a significant correlation with the known carcinogenic activities of the compounds studied. The fluorescence lifetimes gave a good correlation, however there is a high degree of uncertainty in these values and the correlation must be viewed with caution. In view of these results there does not appear to be a convenient and easily applied index for determining carcingoenic hazard of PNA's or their mixtures based on an emission spectral index.

Table 3. Statistical analysis of the correlation between singlet state energy and carcinogenicity.

Cornfields' analysis:

Carcinogenic Activity	Singlet State Energy $E_s^* \leq 74.6$ Kcal/mole	$E_s > 74.6$ Kcal/mole	Totals
Yes	13	4	17
No	22	20	42
Totals	35	24	59

$$\text{Prevalence} = (13 \times 20)/(4 \times 22) = 2.96$$

The prevalence of carcinogenic compounds amoung compounds with $E_s \leq 74.6$ Kcal/mole is 2.96 times as high as amoung those compounds with $E_s > 74.6$ Kcal/mole.

Significance test:

$$\chi^2 = 2.00 \qquad 10\% < P < 20\%$$

Conclusion: No significant correlation exists between singlet state energies and carcinogenic activity.

*Value which gives the best statistical fit.

Table 4. Statistical analysis of the correlation between triplet state energy and carcinogenicity.

Cornfields' analysis:

Carcinogenic Activity	Triplet State Energy $E_t^* \leq 48.4$ Kcal/mole	$E_t > 48.4$ Kcal/mole	Totals
Yes	7	10	17
No	10	32	42
Totals	17	42	59

$$\text{Prevalence} = (7 \times 32)/(10 \times 10) = 2.24$$

The prevalence of carcinogenic compounds amoung compounds with $E_t \leq 48.4$ Kcal/mole is 2.24 times as high as amoung those compounds with $E_t > 48.4$ Kcal/mole.

Significance test:

$$\chi^2 = 1.03 \qquad 30\% < P < 40\%$$

Conclusion: No significant correlation exists between triplet state energies and carcinogenic activity.

*Value which gives the best statistical fit.

Table 5. Statistical analysis of the correlation between singlet-triplet splitting energy and carcinogenicity.

Cornfields' analysis:

	Singlet-Triplet Splitting Energy		
Carcinogenic Activity	$\Delta E^{*}_{s,t} \leq 27.0$ Kcal/mole	$\Delta E_{s,t} > 27.0$ Kcal/mole	Totals
Yes	16	1	17
No	32	10	42
Totals	48	11	59

Prevalence = (16x10)/(1x32) = 5.00

The prevalence of carcinogenic compounds amoung compounds with $\Delta E_{s,t} \leq 27.0$ Kcal/mole is 5.00 times as high as amoung those compounds with $\Delta E_{s,t} > 27.0$ Kcal/mole.

Significance test:

$\chi^2 = 1.52$ $\quad 20\% < P < 30\%$

Conclusion: No significant correlation exists between singlet-triplet splitting energies and carcinogenic activity.

*Value which gives the best statistical fit.

Table 6. Statistical analysis of the correlation between fluorescence lifetime and carcinogenicity.

Cornfields' analysis:

Carcinogenic Activity	Fluorescence Lifetime $\tau_f^* \geq 9.96$ ns	$\tau_f < 9.96$ ns	Totals
Yes	6	2	8
No	2	11	13
Totals	8	13	21

Prevalence = (6x11)/(2x2) = 16.5

The prevalence of carcinogenic compounds amoung compounds with $\tau_f \geq 9.96$ ns is 16.5 times as high as amoung those compounds with $\tau_f < 9.96$ ns.

Significance test:

Fisher exact test P = 0.3%

Conclusion: A significant correlation exists between fluorescence lifetimes and carcinogenic activity.

*Value which gives the best statistical fit.

Table 7. Statistical analysis of the correlation between phosphorescence lifetime and carcinogenicity.

Cornfields' analysis:

	Phosphorescence Lifetime		
Carcinogenic Activity	$\tau_p^* \geq 1.0$ sec	$\tau_p < 1.0$ sec	Totals
Yes	10	7	17
No	10	15	25
Totals	20	22	42

Prevalence = (10x15)/(10x7) = 2.14

The prevalence of carcinogenic compounds amoung compounds with $\tau_p \geq 1.0$ sec is 2.14 times as high as amoung those compounds with $\tau_p < 1.0$ sec.

Significance test:

Fisher exact test P = 10%

Conclusion: No significant correlation exists between phosphorescence lifetimes and carcinogenic activity.

*Value which gives the best statistical fit.

Table 8. Statistical analysis of the correlation between triplet lifetime and carcinogenicity.

Cornfields' analysis:

Carcinogenic Activity	Triplet Lifetime $\tau_t^* \leq 0.53$ ms	$\tau_p > 0.53$ ms	Totals
Yes	4	1	5
No	5	7	12
Totals	9	8	17

Prevalence = (4x7)/(1x5) = 5.6

The prevalence of carcinogenic compounds amoung compounds with $\tau_t \leq 0.53$ ms is 5.6 times as high as amoung those compounds with $\tau_t > 0.53$ ms.

Significance test:

Fisher exact test P = 16.3%

Conclusion: No significant correlation exists between triplet lifetimes and carcinogenic activity.

*Value which gives the best statistical fit.

E. Photodynamic Bioassay

Photodynamic action is the destruction of protoplasm by the combined action of light, a photosensitizer and oxygen. The overall effect is the destruction of cellular components by a photosensitized oxidation.

One recently developed theory [24] of carcinogenesis has implicated the excited states of polynuclear aromatic hydrocarbons (PNA's) and another theory [25] has implicated excited oxygen in the cancer inducing mechanism. Experiments on mice have shown that there is an enhancement in carcinogenesis in animals exposed to both light and carcinogens but such irradiation has no effect on non-carcinogens [25,26,27]. These findings indicate that a more detailed study on the role of light and PNA excited states in carcinogenesis is definitely needed to properly evaluate the potential hazard to man oxposed to PNA containing atmospheres.

Epstein *et al.* [28] have determined the photodynamic activity (P.A.) of 117 polynuclear aromatic compounds of known degrees of carcinogenic activity. The method of Cornfield (see section D) applied to the 117 compounds show that the prevalence of carcinogenic compounds amoung compounds with a high P.A. is 4 times as great as amoung those with a low P.A.

In view of the above, a bioassay based on the photodynamic activities of PNA's could potentially serve as a rapid and convenient means of evaluating the carcinogenic hazard of both pure compounds and their mixtures.

We have measured the photodynamic activities of 34 polynuclear aromatic and heterocyclic compounds using the crustacean *Artemia salina* as a test organism. *Artemia salina* has been shown by Anderson [29] to undergo four stages of development during the first 70 hours after hatching. A newly hatched A. *salina* is called a nauplius and during the first stage of development, which lasts approximately 20 hours, the organism increases in length from approximately 450 μ to 600 μ. The first stage nauplii are less transparent, and consequently less permeable to radiation, than the latter stage nauplii.

Work has been conducted on nauplii which have an average age of 24 hours and 48 hours respectively. Both age groups contain nauplii at different stages of development because the eggs begin to hatch after approximately 24 hours and are continuing to hatch up until the time

they are harvested. We have found that samples containing more early stage (less transparent) nauplii are less susceptible to photodynamic immobilization because fewer photons are penetrating the organisms.

Experiments were run on nauplii harvested after 48 hours (average age of 24 hours) and nauplii hatched for 48 hours and then separated from unhatched eggs and maintained an additional 24 hours (average age of 48 hours). The sensitizer used was benz[c]acridine and some typical results are given in Table 9. Plots of average number of nauplii immobilized as a function of photons absorbed were found to be linear for nauplii of both age groups. The slopes of such plots are a measure of the rate of immobilization (number of nauplii immobilized per einstein of radiation absorbed). It was found that the "old", more transparent nauplii, are immobilized approximately seven times faster than the "young" ones. The standard deviations are given in column three of Table 9. Nauplii in both age groups have an average standard deviation of approximately $\pm$ 3 nauplii. There is no improvement in the precision of the photodynamic bioassay when the "old" nauplii are used. However the significant increase in the immobilization rate when using the "old" nauplii makes it advantageous to use them in the bioassay for two reasons: (1) Most polynuclear aromatic hydrocarbons

Table 9. Effect of age on the rate of photodynamic immobilization of *A. salina* nauplii by benz[c]acridine.[a]

	Iat x 10^7 (einsteins)	ANI[b]	Standard Deviation
"Young" Nauplii:[c]			
	11.86	2.58	1.78
	15.26	5.42	3.15
	16.95	9.00	3.54
	18.64	10.00	3.64
	20.34	11.92	3.00
	22.04	14.42	3.73
"Old" Nauplii:[d]			
	1.08	8.00	3.63
	1.72	15.73	4.31
	2.15	18.64	4.65
	2.58	23.54	2.25
	3.01	25.27	2.49
	3.44	27.00	2.68

(a) Concentration of benz[c]acridine was 6.4 x 10^{-8}M. Samples were incubated in the dark for 2 hours prior to irradiation.

(b) Average number of nauplii immobilized. Twelve samples, each containing thirty nauplii, were used for both age groups.

(c) Nauplii harvested after 48 hours.

(d) Nauplii harvested after 48 hours and maintained in the absence of unhatched eggs an additional 24 hours.

are extremely insoluble in sea water. To offset the small amounts of hydrocarbon present it is desirable to have nauplii which are as sensitive to photodynamic immobilization as possible. This will make it more likely that a measurable rate of immobilization can be observed. (2) Polynuclear aromatics undergo photochemical decomposition to some extent when irradiated. It is therefore advisable to have nauplii at their most photodynamically labile age to minimize the importance of sensitizer photodecomposition.

The procedure we have developed for determining the photodynamic activities of individual PNA's involves the following steps: (1) The hatching of the *A. salina* eggs. (2) Separation of the nauplii from the egg shells and unhatched eggs. (3) Preparation of solutions of sensitizers (PNA's) of known concentration. (4) Preparation and incubation of nauplii in sensitizer-sea water solutions. (5) Irradiation of nauplii-sensitizer solutions. (6) Calculation of the relative photodynamic activity of each PNA. The details of each of these steps follow.

(1) The hatching of the *A. salina* eggs.

The synthetic sea water used for hatching and maintaining the *A. salina* nauplii contains the following chemicals: sodium chloride, 23.4 g; magnesium sulfate, 5.6 g; calcium

chloride, 0.9 g; and potassium chloride, 0.8 g. The salts are dissolved in one liter of demineralized water. The demineralized water is obtained by passing tap water through a Barstead, mixed resin, demineralizing column. The *A. salina* are hatched by placing approximately 0.25 g of the eggs in 500 ml of synthetic sea water and bubbling a stream of compressed air through the solution for 48 hours.

(2) Separation of the nauplii from the egg shells and unhatched eggs.

After 48 hours the nauplii, unhatched eggs and egg shells are transferred to a one liter beaker. A small light is then placed near one side of the beaker. The nauplii are attracted by the light and congregate at the wall of the beaker nearest the light. The nauplii are then pipeted out of the beaker and placed in 500 ml of fresh sea water. A slow stream of air is then allowed to bubble through the contents of the beaker for 24 hours.

(3) Preparation of solutions of sensitizers (PNA's) of known concentration.

In the early part of this work, sensitizer solutions were prepared by shaking a small quantity of PNA with sea water for two hours. This procedure was found to

be unsatisfactory because impurities were leached off the wall of the flask and because only a few of the smaller PNA's went into solution.

We have observed that the *A. salina* nauplii can survive in sea water solutions containing 1% acetone. Stock solutions of the appropriate PNA were made up in spectrograde acetone. Sensitizer-sea water solutions were then made by placing approximately 90 ml of sea water in a 100 ml volumetric flask and adding to this 1 ml of PNA-acetone solutions. The contents of the volumetric flask were then diluted to volume with sea water. No precipitation of PNA occurred when the concentration of the stock solution was such that the addition to the sea water did not result in a PNA concentration exceeding 10^{-6} M.

(4) Preparation and incubation of nauplii in sensitizer-sea water solutions.

The nauplii, which have been maintained in the absence of unhatched eggs for 24 hours, are again congregated to one side of the beaker as described above. The nauplii are then pipeted out and placed in a fine mesh net to remove excess sea water. The sea water free nauplii are then placed in the appropriate sensitizer-

sea water solution. Five 10 ml beakers are then prepared, each containing approximately 2 ml of sensitizer-sea water solution and 30 nauplii. The samples are then incubated, in the dark, for 2 hours.

(5) Irradiation of nauplii-sensitizer solutions.

The beakers containing the nauplii are placed in a merry-go-round photochemical reactor and irradiated with the 366 nm light from a filtered mercury arc. The samples are removed from the reactor at time intervals and the number of nauplii alive, in each beaker, counted by viewing the sample under a low power microscope. The number alive and the irradiation time are then recorded on data sheets for each sample. The samples are then returned to the reactor for further irradiation.

(6) Calculation of the relative photodynamic activity of each PNA.

The light intensity incident on each nauplii sample was determined by potassium ferrioxalate actinometry. The number of einsteins of radiation absorbed per unit of time by each nauplii sample can be calculated by using the equation:

$$I_a = I_o(1-10^{-\varepsilon lc})$$

which for the very dilute solutions encountered in this work reduces to:

$$I_a = 2.303\ I_o\ \varepsilon l c$$

where I_a = the number of einsteins of radiation absorbed per unit of time (ein/min)

I_o = the light intensity incident on the samples

ε = molar absorptivity of the PNA at the irradiating wavelength (1/mole-cm)

l = pathlength of the irradiated sample (cm)

c = molar concentration of the PNA (mole/l)

Data for a typical run are given in Table 10. Two types of controls are used. One, the dark control, contains nauplii and a PNA but is not exposed to the 366 nm radiation. The other, the sea water control, contains nauplii but no PNA and is exposed to light. Inspection of the control data in Table 10 shows that the sensitizers are not toxic to the nauplii in the absence of light and that the organisms are not immobilized when exposed to light in the absence of a sensitizer. The bioassay is, therefore, a

Table 10. Typical data from a photodynamic bioassay.[a]

Compound:	time(min)	$I_a t \times 10^7$	ANI[b]
Benz[c]acridine	16.4	6.12	5.0
	20.0	7.46	12.5
	23.0	8.58	15.2
	27.0	10.1	19.8
slope = 3.583			
Benzo[c]phenanthrene	35.0	2.66	3.2
	55.0	4.19	7.0
	75.0	5.71	20.5
	95.0	7.23	28.2
slope = 5.809			
Dark Control	0.0	-	0.0
	120.0	-	0.0
Sea Water Control	0.0	-	0.0
	120.0	-	1.25

(a) Concentrations of benz[c]acridine and benzo[c]-phenanthrene were 6.57×10^{-7} M and 3.89×10^{-6} M respectively.

(b) Five samples, each containing 30 nauplii, were used for each compound and each control group.

valid procedure to evaluate the phototoxicity of the respective sensitizers.

Next a tabulation of the average number of *A. salina* nauplii immobilized after exposure to an increasing number of einsteins of radiation absorbed by the respective PNA's (I_a x time = einsteins absorbed) with which they are in contact is then made (Table 10). The data (ANI versus $I_a t$) is then fitted to a straight line by a least squares treatment of the data points. The more photodynamically active a compound the greater the slope of these curves. Also the more active a compound the fewer will be the number of einsteins required to produce 50% immobilization of the nauplii. Both the slopes and the reciprocals of the number of einsteins producing 50% immobilization (Q_{50}^{-1} values) are used as a measure of photodynamic activity.

Two major sources of error that enter into the bioassay procedure are: the variations in the resistance of individual nauplii to photodynamic immobilization; and errors indetermining the light intensity (i.e., actinometer errors). Not much can be done about the former except perhaps using a larger number of samples;

but the errors in determining absolute light intensities can be eliminated by taking relative values for the photodynamic bioassay index. To do this a standard must be used that is common to all the bioassay runs. We have used benz[c]acridine as a standard and assigned to it a relative activity of 1.0. Thus the slope of the immobilization curve for benz[c]acridine, which is a measure of the rate of immobilization, is assigned a value of 1.0 and the other PNA's are then related to this value and expressed as the relative rate of photodynamic immobilization (RRPI values). The Q_{50}^{-1} values are also placed on a relative scale again using benz[c]-acridine as the standard. The RRPI and relative Q_{50}^{-1} (RQ_{50}^{-1} values) for the 34 carcinogenic and non-carcinogenic PNA's studied are given in Table 11.

The RRPI and RQ_{50}^{-1} values in Table 11 were analyzed in the same manner as the photophysical properties (see Section D), and the results are given in Tables 12 and 13 respectively. The RRPI values correlate extremely well with carcinogenicity. The RQ_{50}^{-1} values give only a fair correlation. The photodynamic bioassay, using RRPI values, appears to be a very good method for determining the carcinogenicity of a pure compound. The assay does not, in general, seem to be sensitive

Table 11. Relative photodynamic activities of carcinogens and non-carcinogens

Compound	RRPI	RQ_{50}^{-1}
Carcinogens:		
Benz[a]anthracene	163	171
Benzo[a]pyrene	3.56	106.3
11H-Benzo[a]carbazole	2.36	7.49
Dibenz[a,j]acridine	2.19	2.79
Benzo[g]chrysene	1.72	3.47
Benzo[c]phenanthrene	1.62	1.71
Benzo[c]chrysene	1.34	0.609
7H-Dibenzo[a,g]carbazole	0.793	0.262
Benzanthrone	0.687	0.654
Dibenz[a,h]acridine	0.271	0.036
Dibenzo[a,e]fluoranthene	0.173	0.175
Benzo[b]fluoranthene	0.162	0.137
Benzo[rst]pentaphene	0.024	0.005
Dibenz[a,h]anthracene	0.0	0.0
Dibenzo[a,e]pyrene	0.0	0.0
Tribenzo[a,e,i]pyrene	0.0	0.0
Non-carcinogens:		
Benzo[ghi]fluoranthene	3.02	2.71
Benz[c]acridine	1.00	1.00
Dibenzo[a,c]phenazine	0.668	1.49

Table 11 continued

Benzo[e]pyrene	0.593	0.326
Tetracene	0.533	1.08
Dibenzo[g,p]chrysene	0.431	0.341
Fluoranthene	0.311	2.96
Chrysene	0.202	0.316
Benz[a]acridine	0.157	0.095
Benzo[b]triphenylene	0.118	0.169
Benzo[b]chrysene	0.103	0.047
Pyrene	0.096	0.133
Dibenz[a,c]acridine	0.076	0.107
Benzo[k]fluoranthene	0.062	0.124
Perylene	0.036	0.072
Benzo[ghi]perylene	0.030	0.058
Acridine	0.023	0.022
Phenazine	0.005	0.005

Table 12. Statistical analysis of the correlation between RRPI and carcinogenicity.

Cornfields' analysis:

	Photodynamic Activity		
Carcinogenic Activity	RRPI* $\geq$ 1.01	RRPI < 1.01	Totals
Yes	7	9	16
No	1	17	18
Totals	8	26	34

Prevalence = (7x17)/(1x9) = 13.2

The prevalence of carcinogenic compounds amoung compounds with RRPI $\geq$ 1.01 is 13.2 times as high as amoung those compounds with RRPI < 1.01.

Significance test:

Fisher exact test P = 1.13%

Conclusion: A significant correlation exists between RRPI values and carcinogenic activity.

*Value which gives the best statistical fit.

Table 13. Statistical analysis of the correlation between RQ_{50}^{-1} and carcinogenicity.

Cornfields' analysis:

	Photodynamic Activity		
Carcinogenic Activity	RQ_{50}^{-1} $\geq$ 1.09*	RQ_{50}^{-1} < 1.09	Totals
Yes	6	10	16
No	2	16	18
Totals	8	26	34

Prevalence = (6x16)/(10x2) = 4.80

The prevalence of carcinogenic compounds amoung compounds with $RQ_{50}^{-1} \geq 1.09$ is 4.8 times as high as amoung those compounds with $RQ_{50}^{-1} < 1.09$.

Significance test:

Fisher exact test P = 6.8%

Conclusion: A fair correlation exists between RQ_{50}^{-1} values and carcinogenic activity.

*Value which gives the best statistical fit.

to the larger ring carcinogens. Benzo[ghi]fluoranthene is the only non-carcinogen that had an RRPI greater than 1.0 and this compound could act as an interferent if it were present in a mixture.

F. Summary

Photophysical properties, i.e., singlet state energies, triplet state energies, singlet-triplet splitting energies, fluorescence lifetimes, phosphorescence lifetimes, triplet lifetimes, fluorescence efficiencies, and triplet formation efficiencies, were determined for a number of polynuclear aromatic hydrocarbons and their heterocyclic analogs. Statistical analysis of the results indicated that there was no significant correlation, with the exception of fluorescence lifetimes, between these properties and the known carcinogenic activities of the compounds studied. An electronic emission spectral index for carcinogenic hazard based on these photophysical properties does not exist for the compounds studied. An index based on fluorescence lifetimes could potentially be used to evaluate the carcinogenic hazard of an untested pure compound, but such an index would not be applicable to mixtures.

The photodynamic activities of 34 polynuclear aromatic hydrocarbons and their heterocyclic analogs were determined using the crustacean *Artemia salina* as a test organism. There was found to be a good statistical correlation between photodynamic activity and carcinogenicity in the compounds studied. Thus a photodynamic

bioassay using *A. salina* provides a simple and convenient method for determining the carcinogenic hazard of an untested pure compound. The method, although untested on mixtures, should be applicable to air samples and other PNA mixtures.

References

1. *J. Am. Chem. Soc.*, 82, 5545 (1960).

2. (a) J. L. Hartwell editor, "Survey of Compounds which have been Tested for Carcinogenic Activity," Public Health Service Publication No. 149, Washington, D.C., 1951; (b) P. Shubik and J. L. Hartwell editors, *ibid.*, Supplement 1, 1957; (c) S. S. Epstein, M. Small, H. L. Falk and N. Mantel, *Cancer Research*, 24, 855 (1964); (d) E. Sawicki, T. W. Stanley and W. C. Elbert, *Talanta*, 11, 1433 (1964); (e) S. S. Epstein, N. Mantel and T. W. Stanley, *Environmental Science and Technology*, 2, 132 (1968).

3. B. Schiedt, *Ber.*, 71, 1250 (1938).

4. A. Zinke, R. Ott and O. Schuster, *Mh. Chem.*, 83, 1100, 1497 (1952).

5. E. L. Martin, *J. Am. Chem. Soc.*, 58, 1438 (1936).

6. (a) N. P. Buu-Hoi, D. Lavit and J. Lamy, *J. Chem. Soc.*, 1848 (1959); (b) G. D. Johnson, *Organic Synthesis* Collective Volume 4, 900 (1963); (c) E. A. Braude, A. G. Brook and R. P. Linstead, *J. Chem. Soc.*, 3569 (1954).

7. C. Djerassi and R. R. Engle, *J. Am. Chem. Soc.*, 75, 3838 (1953).

8. E. Clar, Guye Vuillene and J. F. Stephen, *Tetrahedron*, 20, 2108 (1964).

9. J. L. Adelfang and N. Cromwell, *J. Org. Chem.*, 26, 2368 (1961).

10. (a) *Chem. Zbl.*, II, 2262 (1937); (b) *ibid.*, 5053 (1937); (c) J. Thiele and F. Heule, *Ann.*, 347, 296 (1906); (d) A. Sieglitz, *Ber.*, 52B, 1513 (1919).

11. K. Tada, R. Takitani and S. Iwasaki, *Kyoritsu Yakka Daigaku Kenkgu Nempo*, 5, 16 (1961).

12. (a) E. Ghighi, *Gazz. Chim. Ital.*, 60, 194 (1930); (b) W. Bousche, A. Witte and W. Bothe, *Ann.*, 359, 49 (1907).

13. E. Clar, "Polycyclic Hydrocarbons," Volumes 1 & 2, Academic Press., New York, N.Y., 1964.

14. John B. Birks, "Photophysics of Aromatic Molecules," Wiley-Interscience, John Wiley and Sons Ltd., New York, N.Y., 1970.

15. M. Zander, "Phosphorimetry," Academic press, New York, N.Y., 1968.

16. Isadore Berlman, "Handbook of Fluorescence Spectra of Aromatic Molecules," Academic Press, New York, N.Y., 1965.

17. N. Turro, Jr., "Molecular Photochemistry," W. A. Benjamin Inc., New York, N.Y., 1967.

18. C.A. Parker, "Photoluminescence of Solutions," Elsevier Publishing Co., New York, N.Y., 1968.

19. Acknowledgement is given to Dr. Tom V. Atkinson for the use of his computer programs and for invaluable discussions during the course of this work.

20. J. Cornfield, *J. Nat. Cancer Institute*, 11, 1269 (1951).

21. S. S. Epstein, I. Bulon, J. Koplan, M. Small and N. Mantel, *Nature*, 204, 750 (1964).

22. R. Langley, "Practical Statistics," Dover Publications, Inc., New York, 1971.

23. K. A. Brownlee, "Statistical Theory and Methodology," John Wiley & Sons, Inc., New York, 1965.

24. N. P. Buu-Hoi and S. S. Sung, *Naterwissenschafften*, 57, 135 (1970).

25. A. U. Khan and M. Kasha, *Ann. New York Acad. Sci.*, 171, 24 (1970).

26. L. Santamaria, G. G. Giordano, M. Alfisi and F. Cascione, *Nature*, 210, 824 (1966).

27. E. Cavalieri and M. Calvin, *Photochem. and Photobiology*, 14, 641 (1971).

28. S. S. Epstein, M. Small, H. L. Falk and N. Mantel, *Cancer Research*, 24, 855 (1964).

29. D. T. Anderson, *Aust. J. Sool.*, 15, 47 (1967).