A STUDY OF THE FLUORESCENCE OF SOME COUMARINS

A dissertation submitted to the faculty of the
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the degree of

DOCTOR OF SCIENCE

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by

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1940
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1942

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The author is grateful for the counsel and assistance of the members of the staff of the Applied Science Research Laboratory. In particular, he is indebted to Dr. William B. Reynolds, who directed this research, and to Dr. Walter Soller and Mr. Philip Berghausen, who designed the fluorometer used in this work.

TABLE OF CONTENTS

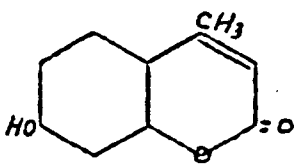
INTRODUCTION

Fluorescence is generally defined as that type of luminescence which does not have an appreciable afterglow on termination of the excitation process.[1] Excitation by light absorption is followed immediately by emission of fluorescent light.

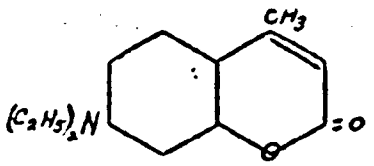
Invariably, the wavelength corresponding to the maximum intensity of fluorescence is longer than the wavelength which is absorbed most strongly by the fluorescer.[2]

In the study just completed, interest was limited to fluorescers which absorbed in the near ultraviolet and fluoresced in the blue or violet.

Since both



4-Methyl-7-hydroxy-coumarin



4-Methyl-7-diethyl-aminocoumarin

fluoresce strongly in the blue end of the spectrum, it was decided to prepare and study a group of coumarins.

Coumarins are not generally compatible with alkaline media undergoing splitting of the pyrone ring and fragmentation of the resulting side chain. Consequently,

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(1) P. Pringsheim and M. Vogel; Luminescence, Interscience Publishers (1943)

(2) G.G. Stokes; Phil. Trans. 143, 385 (1853)

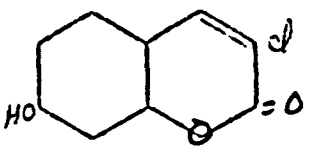
some attention was devoted to the alkaline stability of selected coumarins.

HISTORICAL

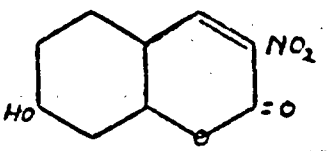
There has been little study of a comprehensive nature of the fluorescence and related phenomena of coumarins. On the whole, the frequent references to the fluorescence of coumarins are casual observations of workers primarily interested in problems of synthesis.

In 1927, T. Tasaki published a study of the ultraviolet absorptions of several benzopyrones, methyl umbelliferone being the only strong fluorescer among them.[3] In the brief study, no correlation with the fluorescence of coumarins was attempted.

Later, Chakravarti observed that a 3-chloro group enhanced the fluorescence of umbelliferone, whereas a 3-nitro group destroyed it.[4]



3-Chloro-7-hydroxy-coumarin



3-Nitro-7-hydroxy-coumarin

Previously, Privault had observed that nitro compounds

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(3) T. Tasaki; Acba Phytochimica 3, 21-9 (1927) . .

(4) D. Chakravarti; J. Ind. Chem. Soc. 12, 536 (1935)

seemed to weaken or destroy fluorescence when added to a solution of a fluorescer.[5]

In 1935, the wavelength range of the absorption and fluorescence of three 7-hydroxycoumarins was published.[6] Of course no far-reaching conclusions were possible in such a brief study.

A study of constitutional factors controlling visible fluorescence in compounds of the benzopyrone group was made by Rangaswami and Seshadri.[7]

A 7-hydroxy group seemed essential since the strongest fluorescers have that configuration. Alkyl groups in the 4- and 5- positions were thought to enhance fluorescence, whereas an 8-methyl had an inhibiting effect.

Formyl, acetyl, nitro, bromo, and hydroxyl groups in the 5-, 6-, or 8-position of a 7-hydroxycoumarin were found to inhibit fluorescence. Carbethoxy and carboxy groups in the 3-position enhance fluorescence.

Later, a brief study of some 3- substituted coumarins was made by Rangaswami, Seshadri, and Venkateswarlu[8]; again the method used was visual observation in

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(5) M. Privault; Comptes Rendu 184, 1120 (1927)

(6) W. Czapska-Norkiewicz; Bull. intern-acad. polonaise, Classe Sci. math. nat. 1935A, 445-7

(7) S. Rangaswami and T.R. Seshadri; Proc. Indian Acad. Sci. 12-A, 375 (1940)

(8) S. Rangaswami, T.R. Seshadri, and V. Venkateswarlu; Proc. Ind. Acad. Sci. 13-A, 316 (1941)

ordinary sunlight. Several 7-hydroxy and 7-methoxycoumarins with acetyl, carbethoxy, or carboxy groups as substituents in the 3-position were fluorescent. Curiously, the corresponding 3-benzoyl and 3-phenyl derivatives were not fluorescent.

Later, V. Balaiah, T.R. Seshadri, and V. Venkateswarlu continued the study showing hydrogenation of the pyrone double bond to destroy fluorescence.[9]

Cyano and phenyl groups in the three position were claimed to enhance the fluorescence of umbelliferone; this effect being absent for postiion four.

Umbelliferone-3-acetic acid does not show an enhanced fluorescence. Methylation decreases the fluorescence of a 7-hydroxycoumarin but increases that of 5- and 6-hydroxycoumarins.

METHOD OF STUDY

A careful study has been made of the absorptive and emissive properties of a graded series of coumarins.

By a graded series is meant a group each member of which differs from its nearest relative by but one feature of structure.

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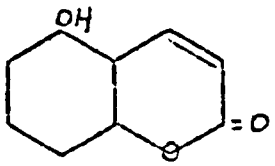
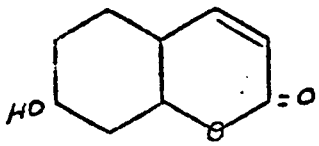
(9) V. Balaiah, T.R. Seshadri, and V. Venkateswarlu; Proc. Ind. Acad. Sci. 11-A, 68 (1942)

For example,



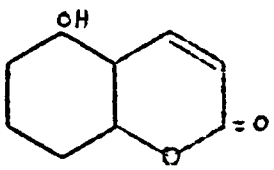
5-Hydroxy-coumarin



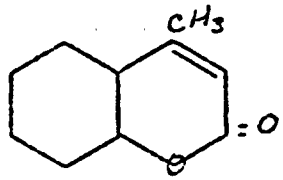
7-Hydroxy-coumarin

differ from one another by a single feature of structure.

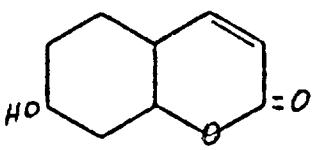
Accordingly, the members of the following series have been synthesized:



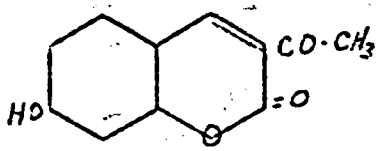
5-Hydroxy-coumarin



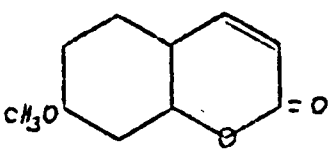
4-Methyl-coumarin



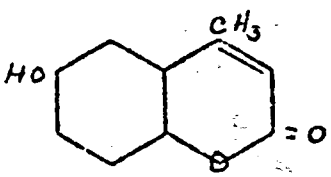
7-Hydroxy-coumarin



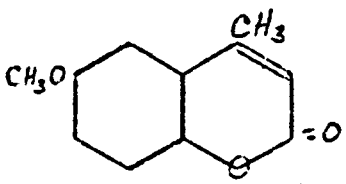
3-Acetyl-7-hydroxycoumarin



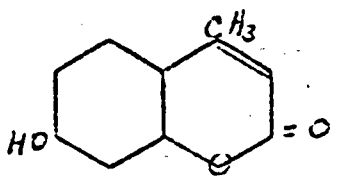
7-Methoxy-coumarin



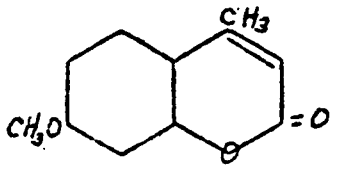
4-Methyl-6-hydroxycoumarin



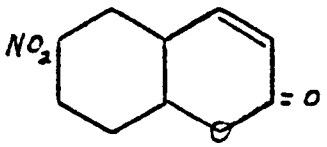
4-Methyl-6-methoxycoumarin



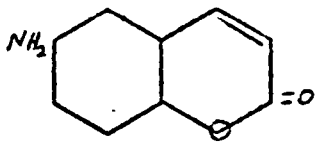
4-Methyl-7-hydroxycoumarin



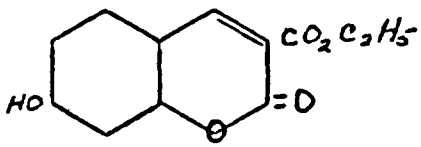
4-Methyl-7-methoxycoumarin



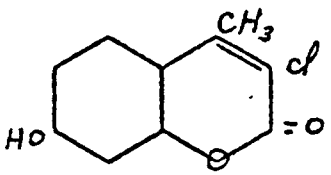
6-Nitrocoumarin



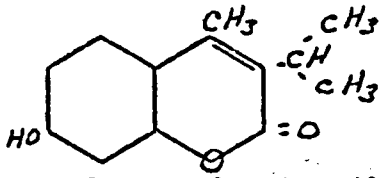
6-Aminocoumarin



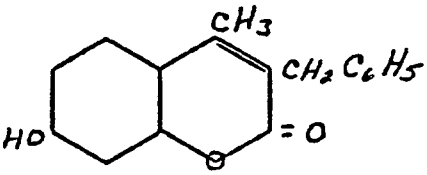
Ethyl 7-hydroxycoumarin-3-carboxylate



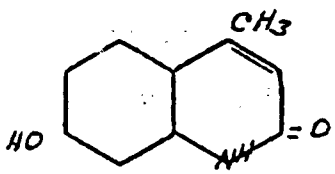
3-Chloro-4-methyl-7-hydroxycoumarin



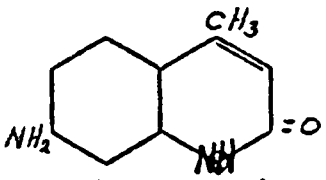
3-Isopropyl-4-methyl-7-hydroxycoumarin



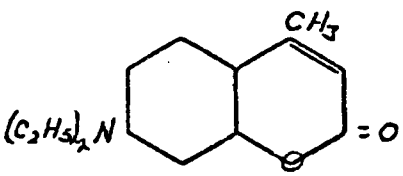
3-Benzyl-4-methyl-7-hydroxycoumarin



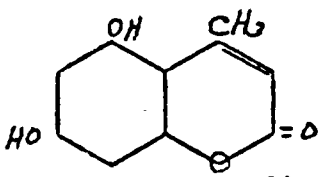
4-Methyl-7-hydroxycarbostyril



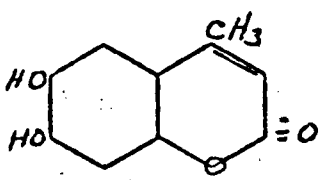
4-Methyl-7-aminocarbostyril



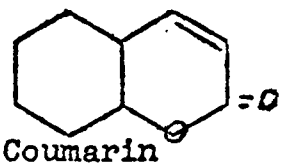
4-Methyl-7-diethylaminocoumarin



4-Methyl-5,7-dihydroxycoumarin



4-Methyl-6,7-dihydroxycoumarin



Coumarin

In this manner it was hoped that the structural gradations would be reflected in the absorptive and emissive characteristics.

Ultraviolet absorption measurements, fluorescent spectra and fluorescent intensity measurements were obtained.

SUMMARY

A total of twenty-one coumarins and carbostyrils has been synthesized. The absorptive and emissive characteristics of these compounds have been studied and certain fundamental relationships between structure and fluorescence characteristics have been observed.

A constitutional effect noticed by Chakravarti has been confirmed and several by Seshadri and associates disputed; other effects have been noted. For one, dilution behavior appears to be a characteristic of molecular configuration.

Both Coumarin and 4-Methylcoumarin have been found to fluoresce in the near ultraviolet. A 4-methyl group shifts the point of fluorescence maximum to longer wavelengths; similarly with 7-substituents whose order of efficacity is diethylamino, hydroxy, amino and methoxy. A 3-chloro or

3-carbethoxy group shifts the fluorescence maximum to longer wavelengths.

It has been shown that a 3-benzyl, a 3-isopropyl or replacement by an NH group of the heterocyclic oxygen in a 7-hydroxycoumarin confers exceptional stability of fluorescence in alkali.

SUMMARY OF DATA

A full table of data is included in order to permit further comparisons if desired.

In the discussion which follows immediately after, only that data which is considered relevant to the topic being discussed is used.

DISCUSSION AND RESULTS

Fluorescent Intensity:

The phenomenon of concentration quenching was first noticed by Stokes[10]. In the typical case, starting at very low concentrations, an increase in fluorescent intensity is associated with increased concentration up to a point beyond which the fluorescent intensity declines and is said to be quenched.

Recently Vavilov[11] has suggested that coupling between molecules occurs. At higher concentrations, the coupling is greater that at lower. In the case of the coupled molecules excitation energy may cause increased oscillation

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(10) G.G. Stokes: Ann. Physik 96, 522 (1858)

(11) S.I. Vavilov: Compt. rend. acad. Sci. U.R.S.S. 35, 100-6 (1942)

Summary of Data

Compound	Absorp. Max.	Spec. Ext.	Rel. abs. of Energy	Fluor. Bond	Fluor Max.	Film exp. time	Max. Fluor Int.	Conc. at max mg/100ml.	Spec. Fluor Int at Max	Fluor Int. at 300 mg/ 100 ml.
1. 4-Methyl-7-diethylamino-coumarin	318	17.1		4330						
	375	111.3	42.2	to	4560	2 hrs.	.703	4.7	1.496×10^5	639
	278	8.3		6060						
	243	67.3								
2. 3-Acetyl-7-hydroxy-coumarin		284	15.7							
		368	5855	27.1		None	48	.6	8.0×10^4	11
		250	25.43							
3. 7-Methoxy-coumarin	320	86.4	26.2	3640	3850	3 hrs.	None			36
				to						
				4700						

Compound	Absorp. Max.	Spec. Ext.	Rel.abs. of Energy	Fluor. Bdnd	Fluor. Max.	Film Exp.time	Max.Fluor Int.	Conc.at max mg/100ml.	Spec. Fluor Int at Max	Fluor Int. at 300 mg/ 100 ml.
4.4-Methyl-7-hydroxy-coumarin	325	89.7	24.9	3980 to 4930	4420	3/4 hr.	None			332
5.4-Methyl-7-methoxy-coumarin	320	78.3	23.8	3660 to 5930	3920	4 hrs.	None			
6.7-Hydroxy-coumarin	325	89.2	23.7	4170 to 5210	4410	1 1/2 hrs.	670	12	5.58×10^4	359
7.3-Chloro-4-methyl-7-hydroxy-coumarin	332	74.4	23.6	4070 to 5060	4580	1 1/2Hrs.	605	11	5.51×10^4 3.21×10^4	484

Compound	Absorp. Max.	Sped. Ext.	Rel.abs. of Energy	Fluor. Band	Fluor. Max.	Film Exp.time	Max.Fluor Int.	Conc.at max mg/100ml	Spec. Fluor Int at Max	Fluor Int.at 300 mg/ 100 ml.
8.4-Methyl-6,7 dihydroxy-coumarin	348 290	64.70 28.01	22.1			3 hrs.	246	4.7	5.23×10^4	239
9.5-Hydroxy-coumarin	299 249	74.5 40.7	21.2	None		19 hrs.				
10.3-Isopropyl-4-Methyl-7-m hydroxy-coumarin	323	78.1	20.0	4090 to 4930	4460	2 1/2 hrs.	189	75	2.52×10^3	125
11.4-Methyl-5.7-dihydroxy-coumarin	320 250	62.40 27.84	20.0	None		3 hrs.	42	18.8	2.23×10^3	33

Compound	Absorp. Max.	Spec. Ext.	Rel.abs. of Energy	Fluor. Band	Fluor. Max.	Film Exp.time	Max.Fluor Int.	Conc.at max mg/100ml	Spec. Fluor Int at Max.	Fluor Int.at 300 mg/ 100 ml.
12.4-Methyl-7-hydroxy carbostyril	256	30.43		3880						
	282	22.56	15.3	to	4120	3/4 hr.	535	75	7.13×10^3	450
	324	73.54		4520						
	338	67.20								
13.3-Benzyl-4-Methyl -7-3 hydroxy-coumarin	326	66.43	13.9	3970 to 4900	4480	3 hrs.	355	75	3.55×10^3	290
14.4-Methyl-coumarin	270	62.5	11.4	3510 to	3830	3 hrs.				
	310	37.7		4570	Very Faint					
15.6-Nitro-coumarin	260	122.80	10.7	None		3 Hrs.	None			

Summary of Data (cont'd)

Compound	Absorp. Max.	Spec. Ext.	Rel. abs. of Energy	Fluor. Bond	Fluor. Max.	Film exp. time	Max. Fluor. Int.	Conc. at max. mg/100 ml.	Spec. Fluor. Int. at Max.	Fluor. Int. at 300 mg/ 100 ml.
16. 4-Methyl-6-hydroxy-coumarin	270 340	59.7 25.1	9.0	4050 to 4580	4270	3 Hrs.	32	18.8	1.7×10^3	31
17. 6-Amino coumarin	370 280 240	18.16 66.75 151.95	8.9	None		19 Hrs.	24	9.4	2.55×10^3	26
18. 4-Methyl-6-methoxy-coumarin	275 342	58.3 26.5	7.8	3890 to 4930	4180	3 Hrs.	25	150	1.67×10^2	23
19. 4-Methyl-7-amino carbo-styril				3790 to 4580	4070	1½ Hrs.	423	75		379

Summary of Data (cont'd)

Compound	Absorp. Max.	Spec. Ext.	Rel. abs. of Energy	Fluor. Bond	Fluor. Max.	Film exp. time	Max. Fluor. Int.	Conc. at max. mg/100 ml.	Spec. Fluor. Int. at Max.	Fluor. Int. at 300 mg/ 100 ml.
20. Ethyl 7-hydroxy-coumarin-3-carboxylate				4370 to 6060	4540	2 Hrs.	Strongly Fluorescent			
21. Coumarin				3510 to 3520	3515	3½ Hrs.				

of the coupled molecules rather than fluorescence.

In the case of a number of substances it has been possible to show the specific fluorescent intensity to be an exponential function of concentration. The function or functions which apply to the coumarins are certainly more complex.

In form, the accompanying curves representing fluorescent intensity versus concentration fall into several classes.

First of all, 4-Methyl-6-methoxycoumarin (curve 1) is a unique case. As concentration decreases the fluorescent intensity rises gently, remains constant for a period and then declines sharply.

In the second class, which includes 7-Methoxycoumarin (curve 2) and 4-Methyl-7-hydroxycoumarin (curve 3) the decline of fluorescent intensity with concentration follows a curve similar in form to a parabola.

The third class includes 4-Methyl-6,7-dihydroxycoumarin (curve 4), 4-Methyl-6-hydroxycoumarin (curve 5), 6-Aminocoumarin (curve 6), and probably 4-Methyl-5,7-dihydroxycoumarin (curve 7). In this class, the curves show a slight decline in fluorescent intensity as the concentration decreases, then a maximum and finally the usual steep decrease in fluorescent intensity.

Finally, the fourth class included 3-Acetyl-7-hydroxycoumarin (curve 8), 4-Methyl-7-hydroxy carbostyril (curve 9), 3-Benzyl-4-methyl-7-hydroxycoumarin (curve 10),

4-Methyl-7-diethylaminocoumarin (curve 11), 4-Methyl-7-aminocarbostyril (curve 12), 3-Isopropyl-4-methyl-7-hydroxycoumarin (curve 13), 7-Hydroxycoumarin (curve 14), and 3-Chloro-4-methyl-7-hydroxycoumarin (curve 15). In all cases of this class, the fluorescent intensity curves rise to a maximum and then decline.

Inasmuch as class 3 consists mainly of 6-substituted coumarins and class 4,7-substituted coumarins, it appears that the behavior on dilution of a fluorescent coumarin is closely related to the position of asubstituent in the benzene ring. Since class 4 includes several carbostyrils and 3-substituted coumarins, it would appear that substitution in the pyrone ring does not exert a dominant influence on the dilution behavior of a coumarin. In either the 6- or 7-position a methoxy group has an anomalous influence.

At this point, it should be of interest to inspect the effect of structure on the magnitude of fluorescent intensity attained.

Comparison of 7-hydroxycoumarin (curve 14) with 4-Methyl-7-hydroxycoumarin (curve 3) indicates that a 4-Methyl group has a deleterious effect on fluorescent intensity contrary to the observation of Rangaswami and Seshadri[7].

Comparison of 7-Methoxycoumarin (curve 2) with 7-Hydroxycoumarin (curve 4) and 4-Methyl-6-hydroxycoumarin (curve 5) with 4-Methyl-6-methoxycoumarin (curve 1)

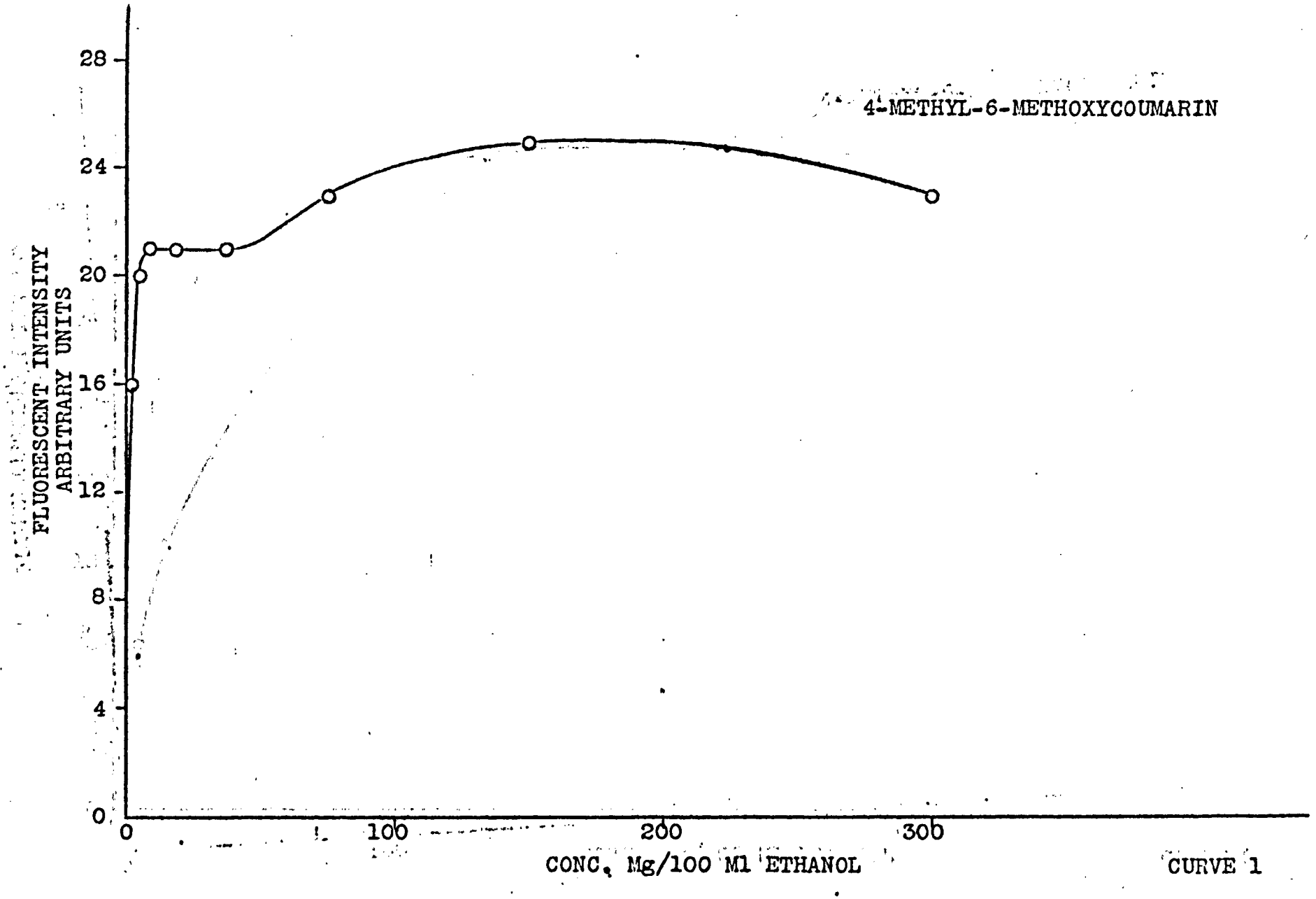
shows that methylation of a hydroxyl group decreases the fluorescent intensity in either case contrary to the observation of Balaiah, Seshadri, and Venkatesworlu[9].

Examination of 4-Methyl-7-hydroxycoumarin (curve 3), 4-Methyl-6,7-dihydroxycoumarin (curve 4), and 4-Methyl-5,7-dihydroxycoumarin confirms that a second hydroxyl group in the benzene ring of a 7-hydroxycoumarin decreases its fluorescence, slightly in the 6-position and very greatly in the 5-position.

Replacement of the lactone oxygen by an -NH- group has a favorable effect as comparison of 4-Methyl-7-hydroxycoumarin (curve 3), 4-Methyl-7-hydroxycarbostyril (curve 9), and 4-Methyl-7-aminocarbostyril (curve 12) reveals.

When substituted in the 7-position of 4-Methylcoumarin, the decreasing order of fluorescent efficacity of groups is diethylamino, hydroxy, amino, and methoxy as shown by comparison of 4-Methyl-7-hydroxycoumarin (curve 3), 4-Methyl-7-diethylaminocoumarin (curve 11), 4-Methyl-7-hydroxycarbostyril (curve 9), and 4-Methyl-7-aminocarbostyril (curve 12).

Comparison of 7-hydroxycoumarin (curve 14) and 3-Acetyl-7-hydroxycoumarin shows a 3-Acetyl group to have a deleterious effect on fluorescent intensity. 4-Methyl-7-hydroxycoumarin (curve 3), 3-Benzyl-4-methyl-7-hydroxycoumarin (curve 10) show fluorescent intensities of about the same magnitude and so a 3-Benzyl group is without effect. In the case of 3-Isopropyl-4-methyl-7-hydroxycoumarin the



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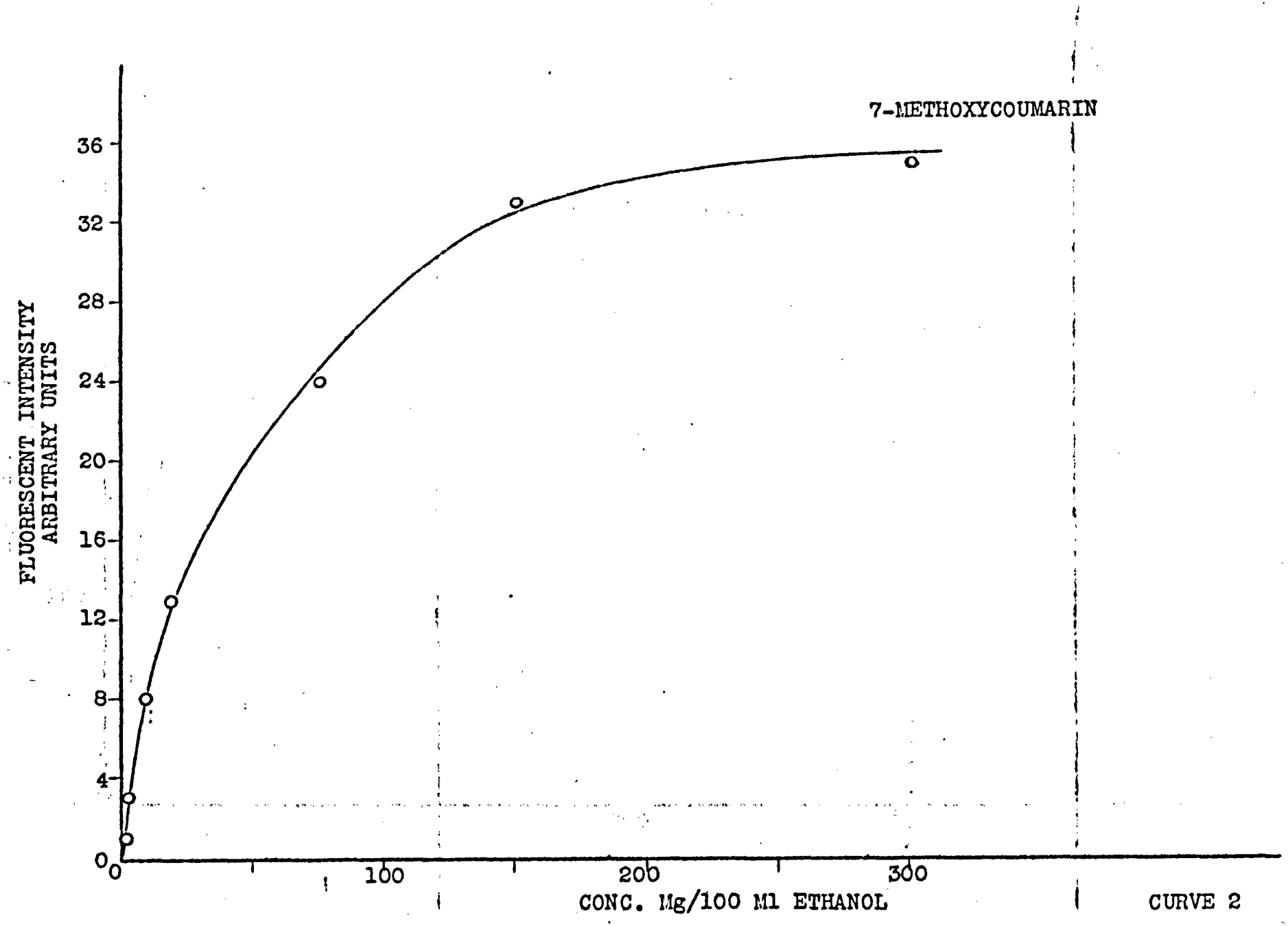

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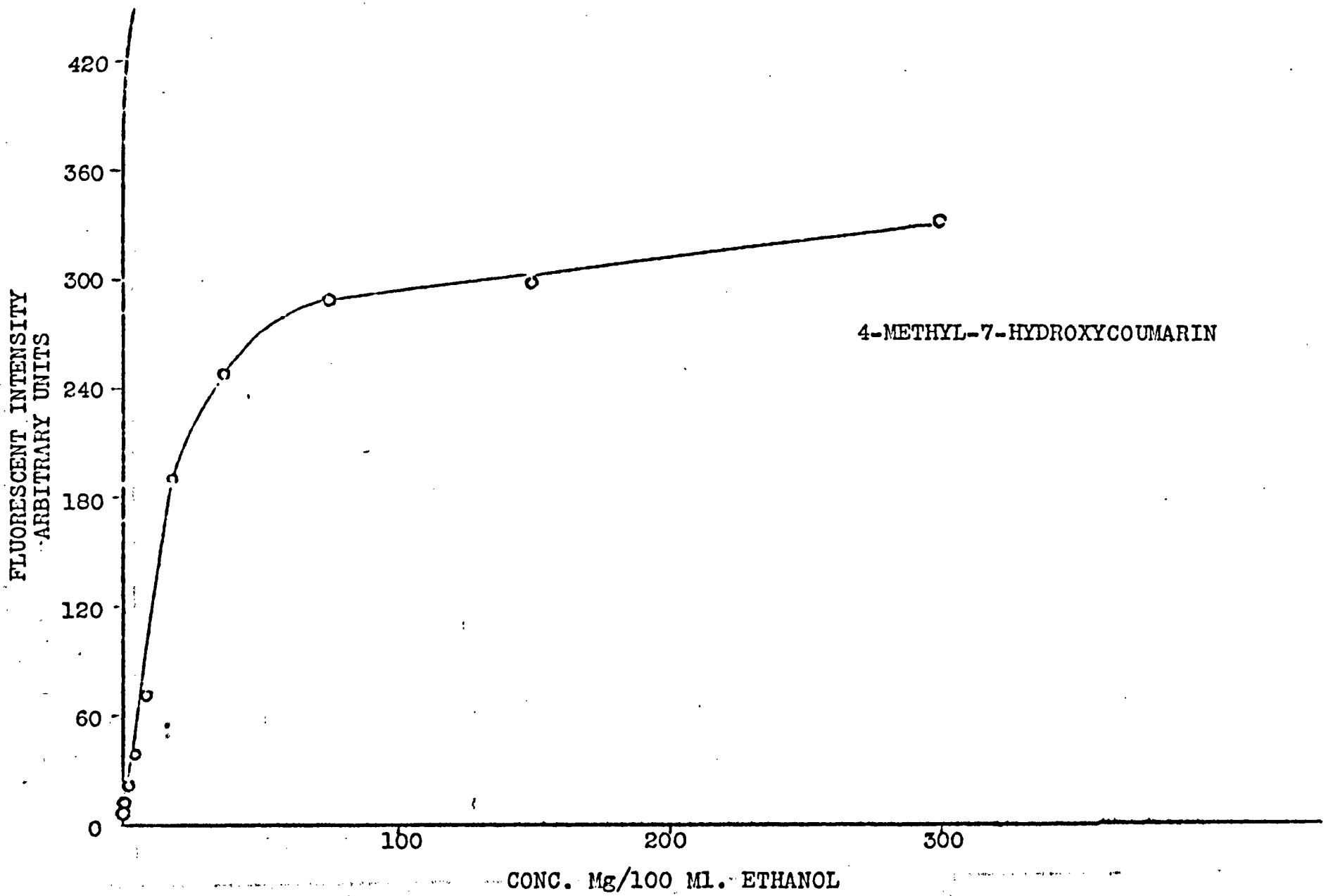

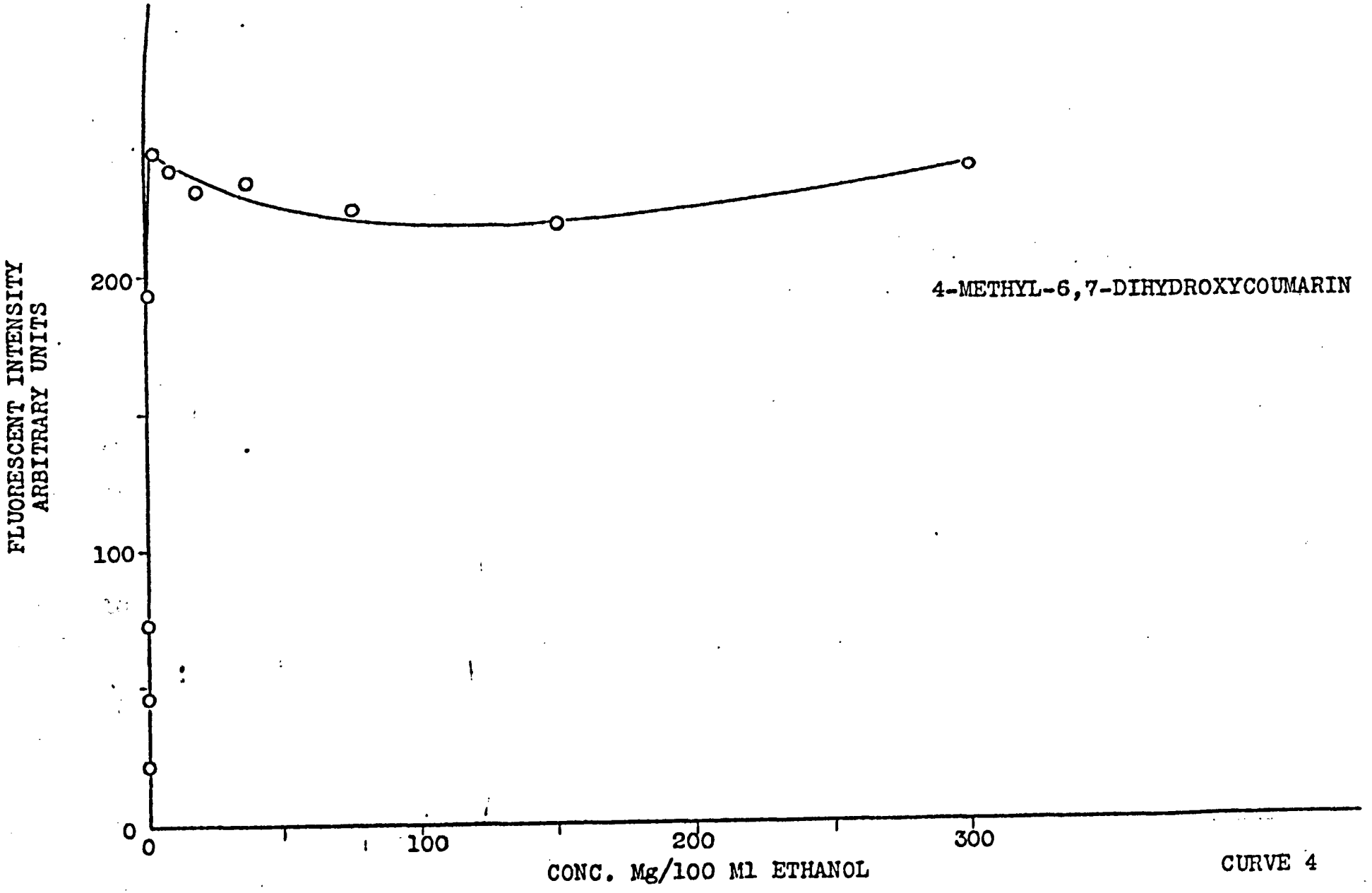

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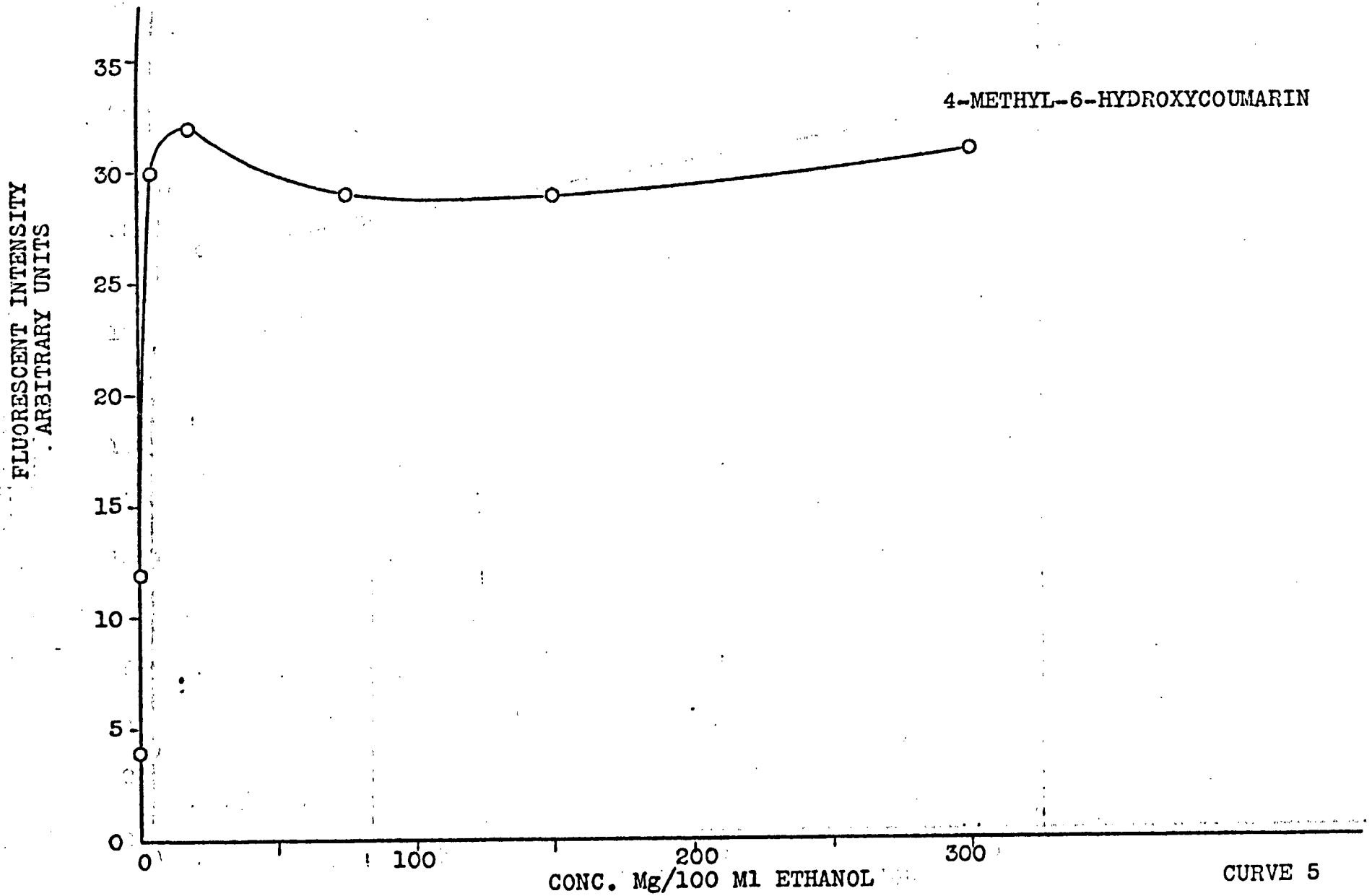

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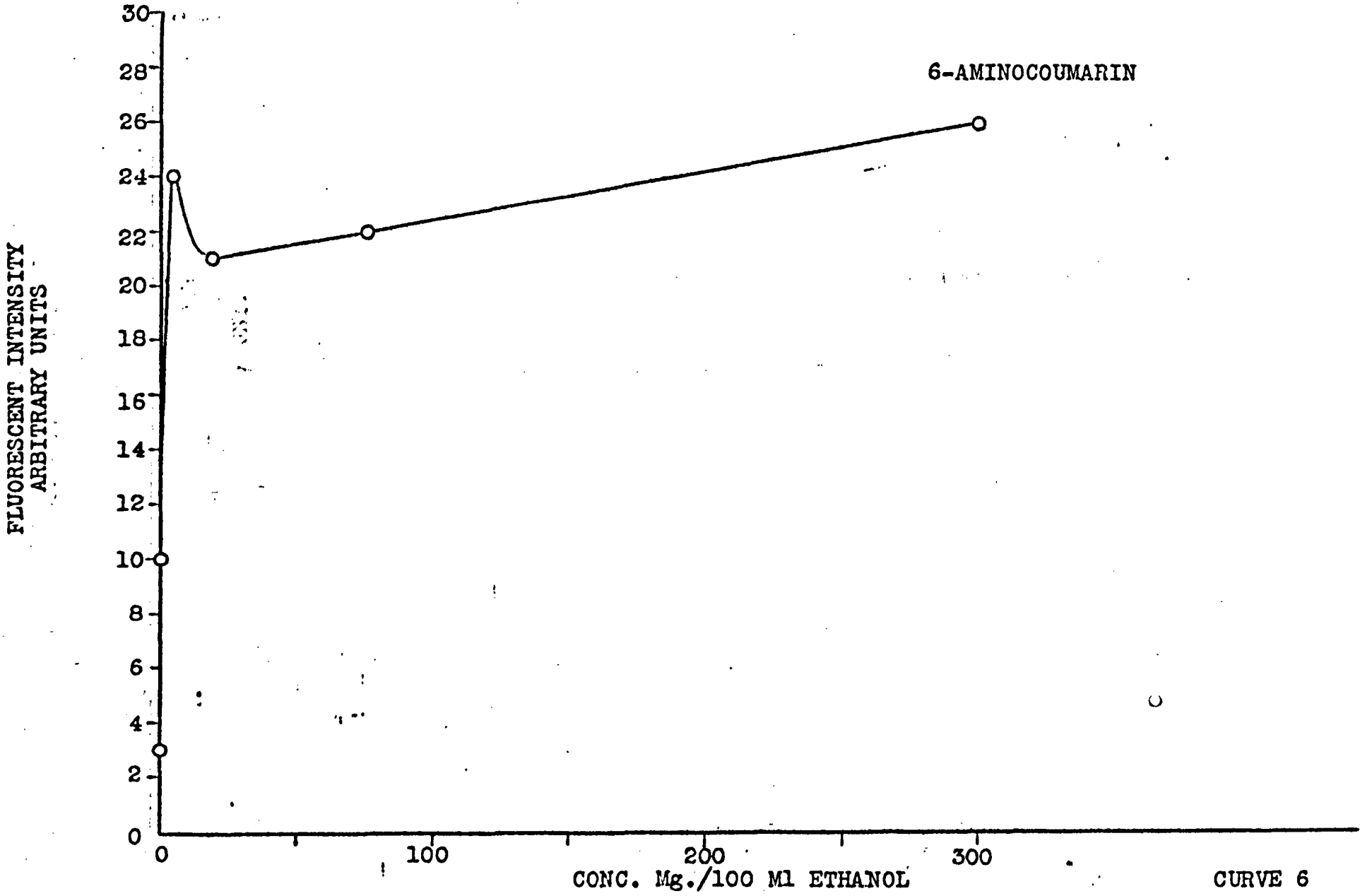
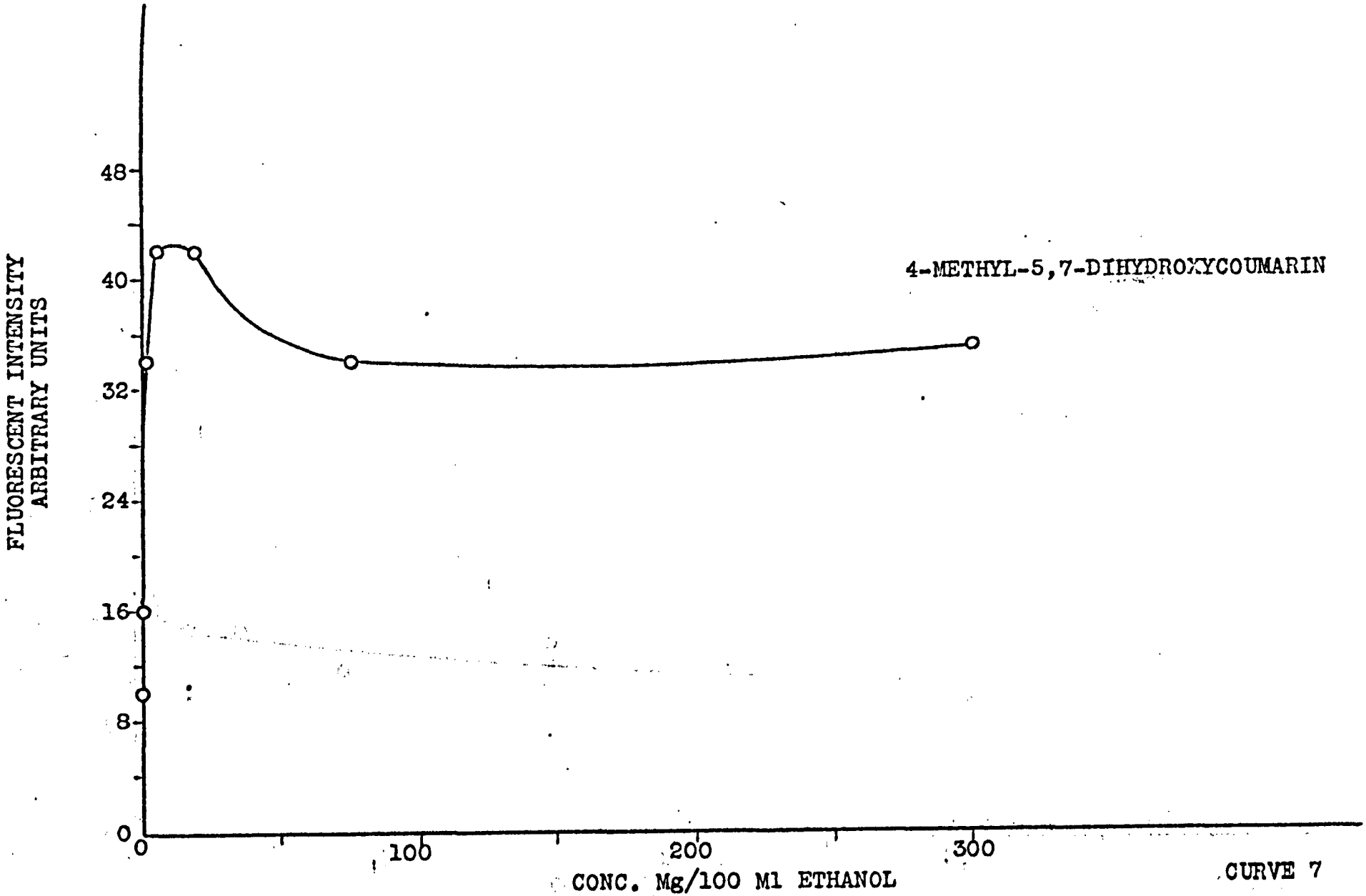
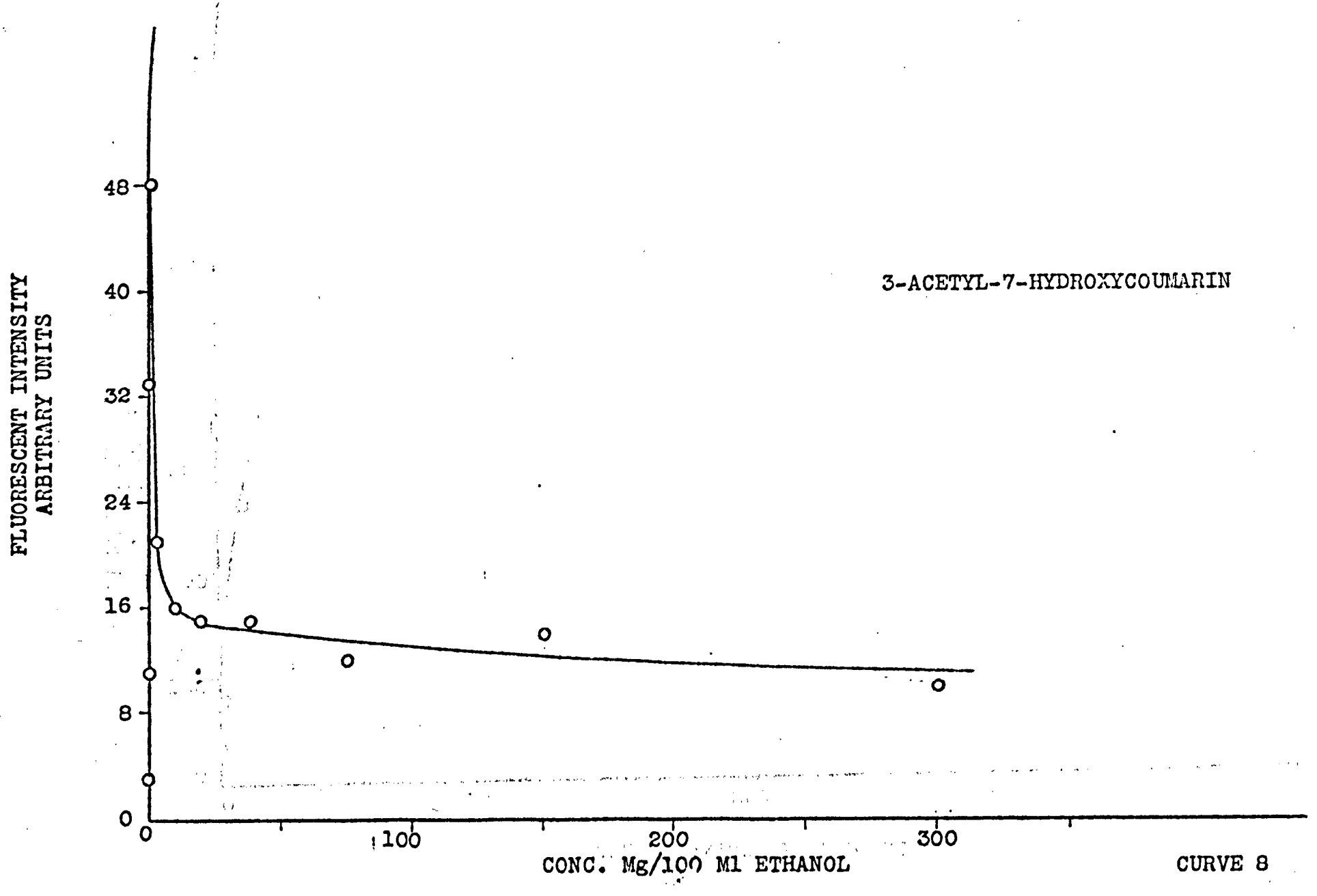


CURVE 6





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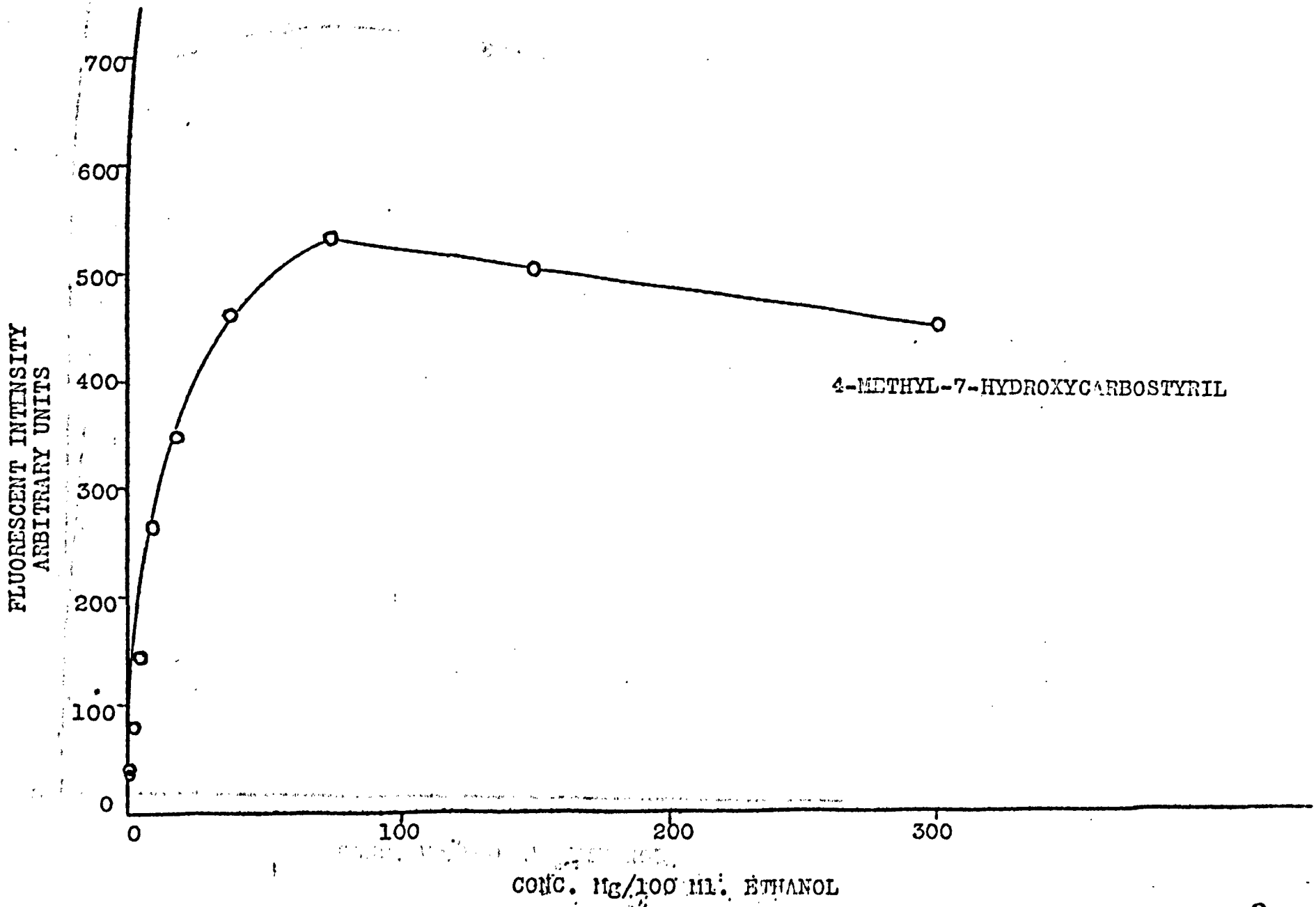
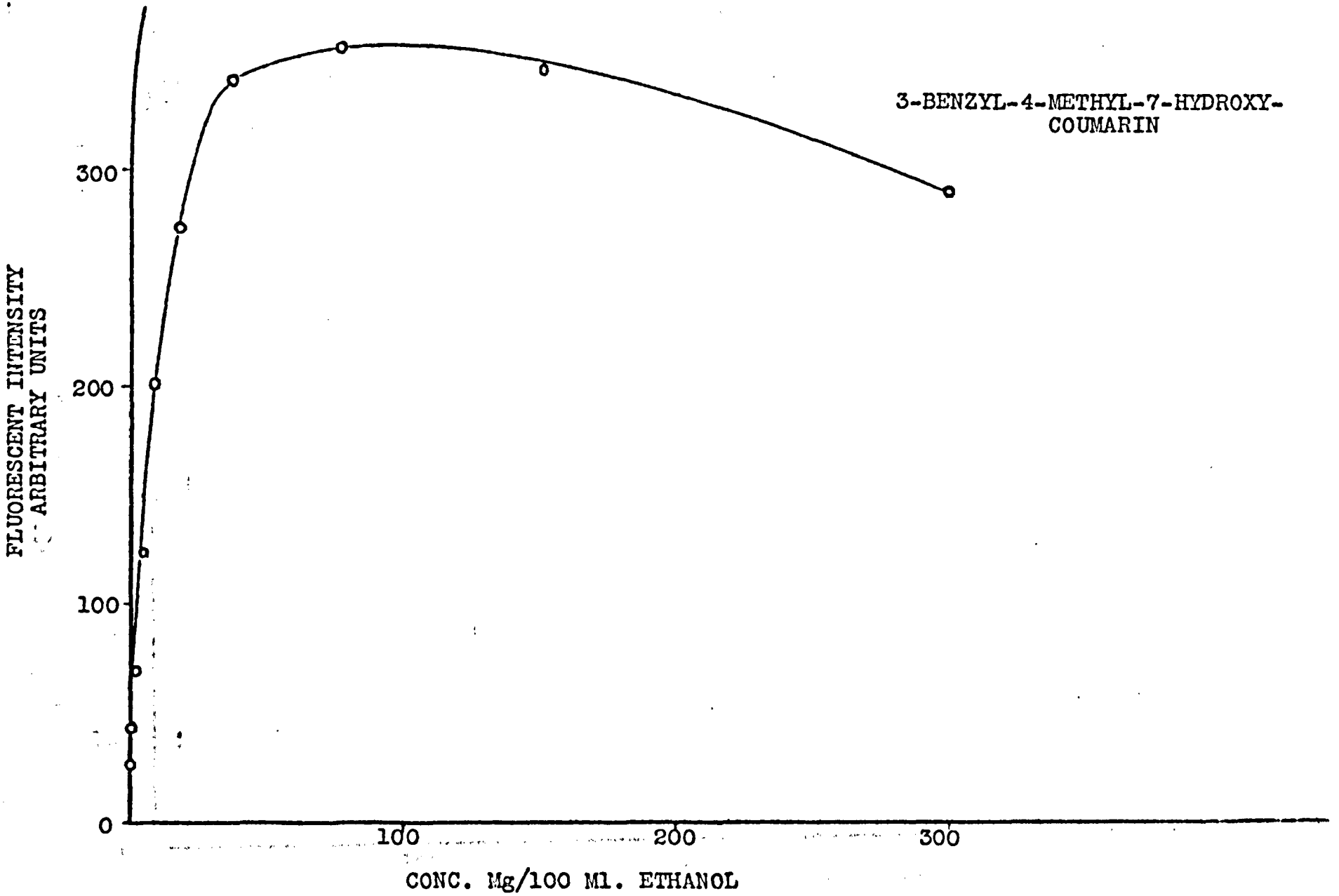
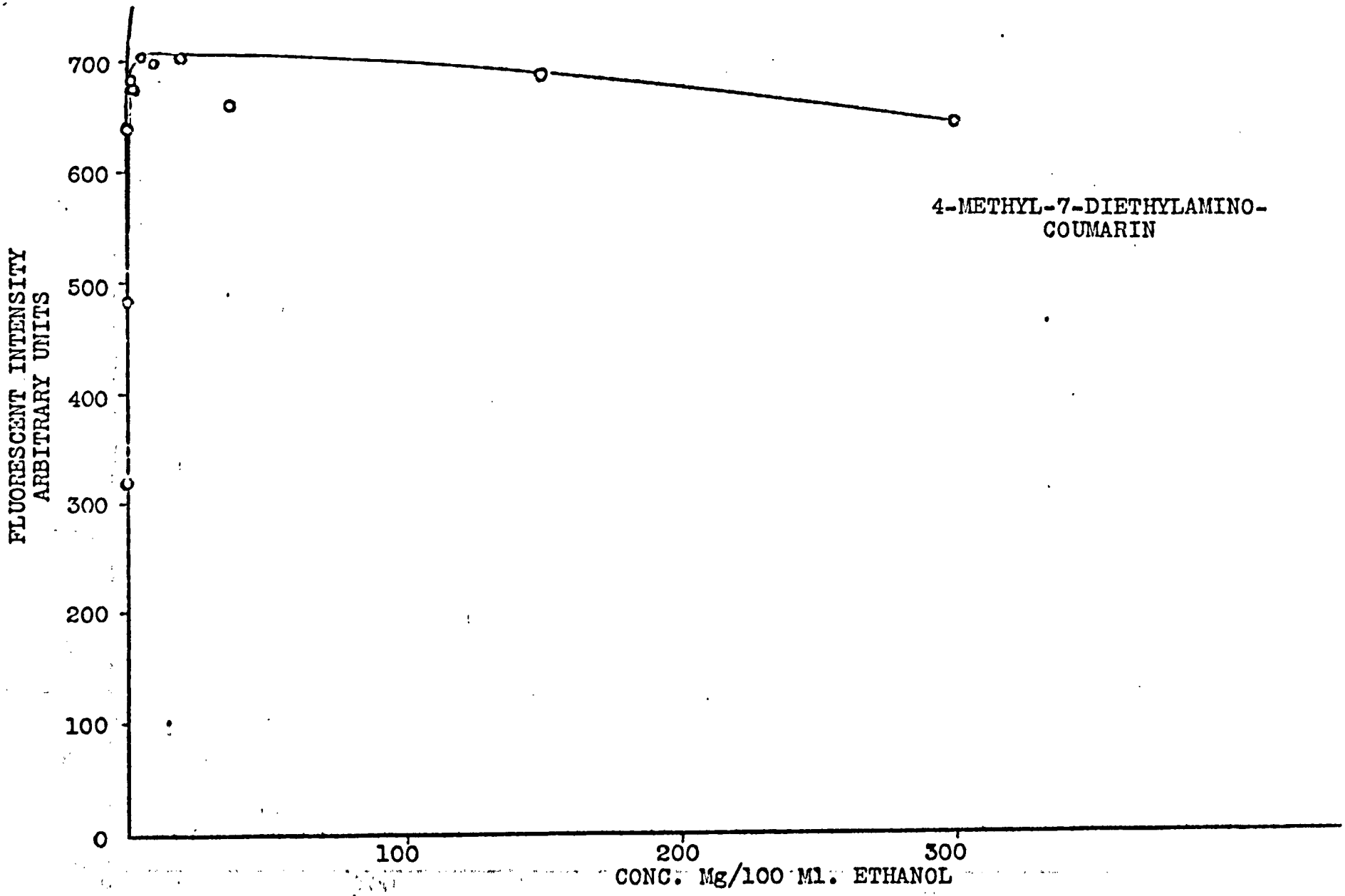
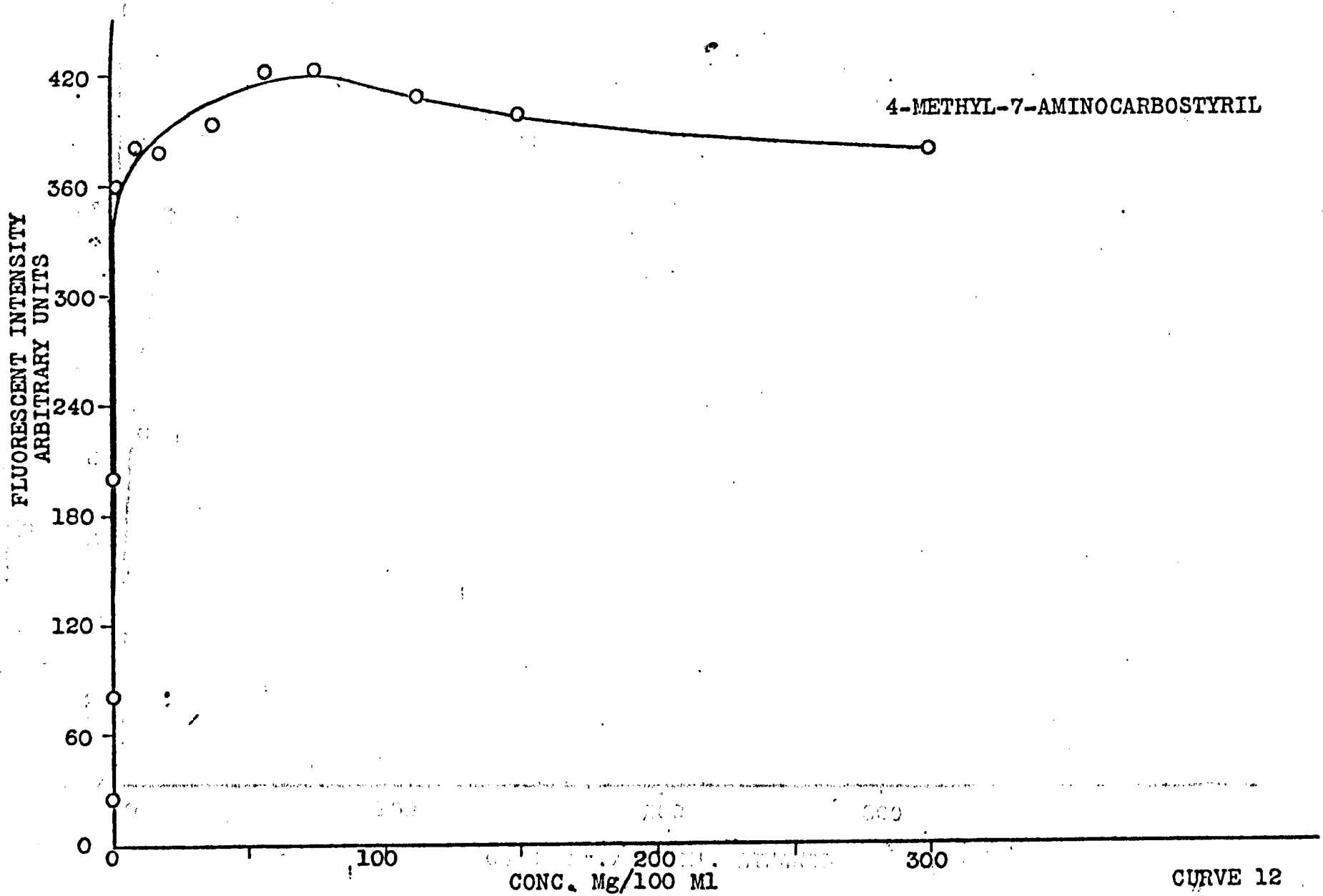
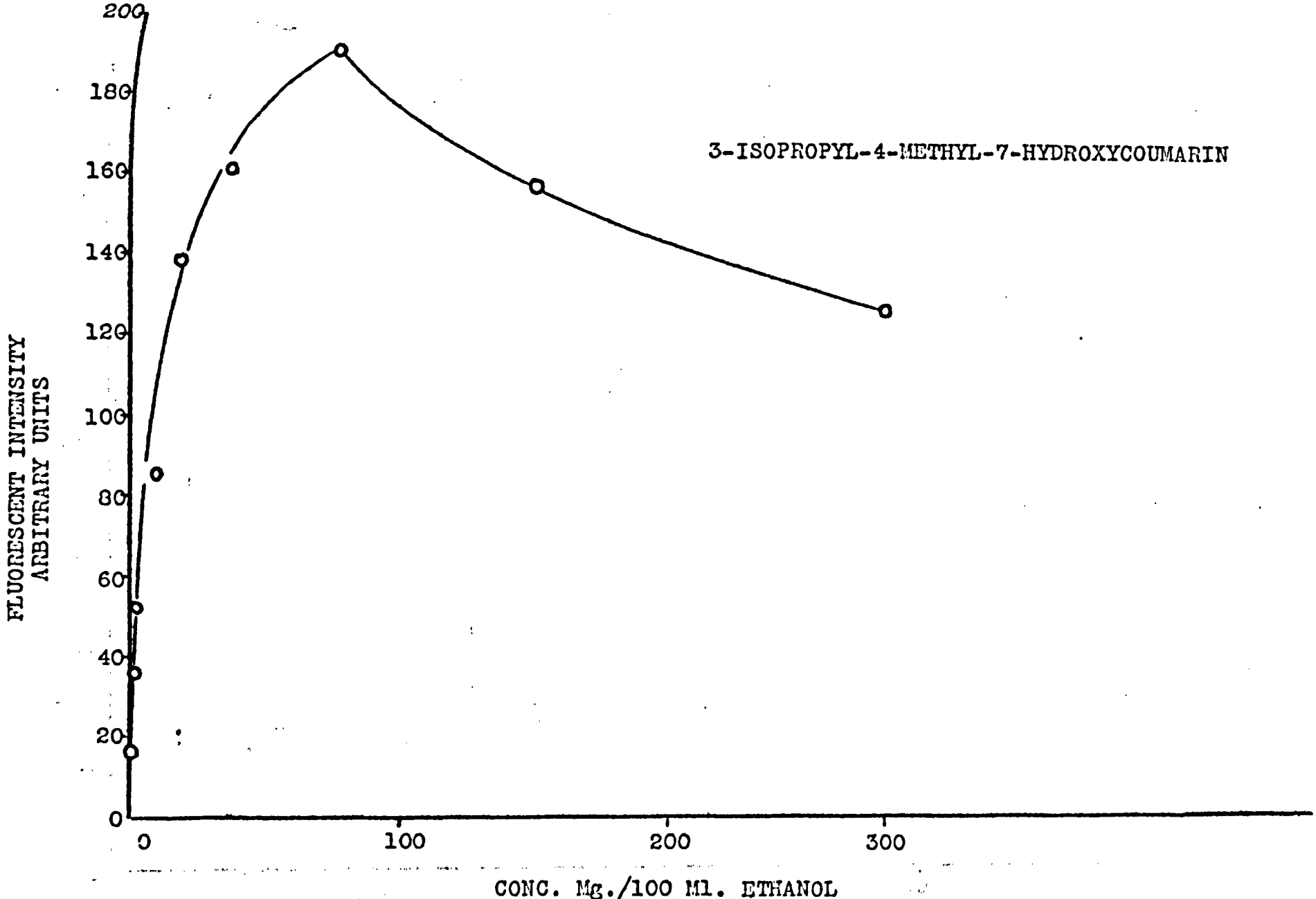


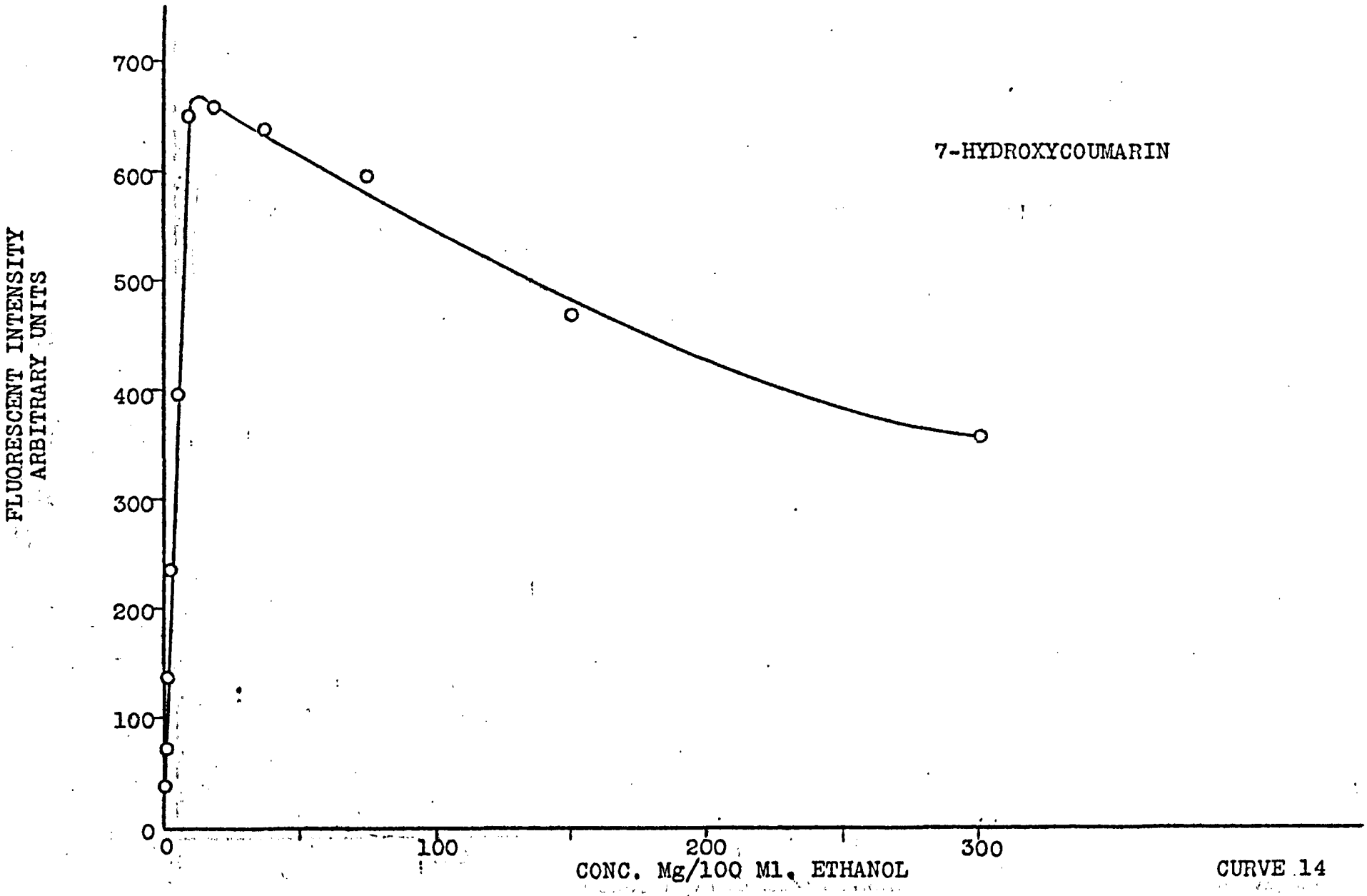




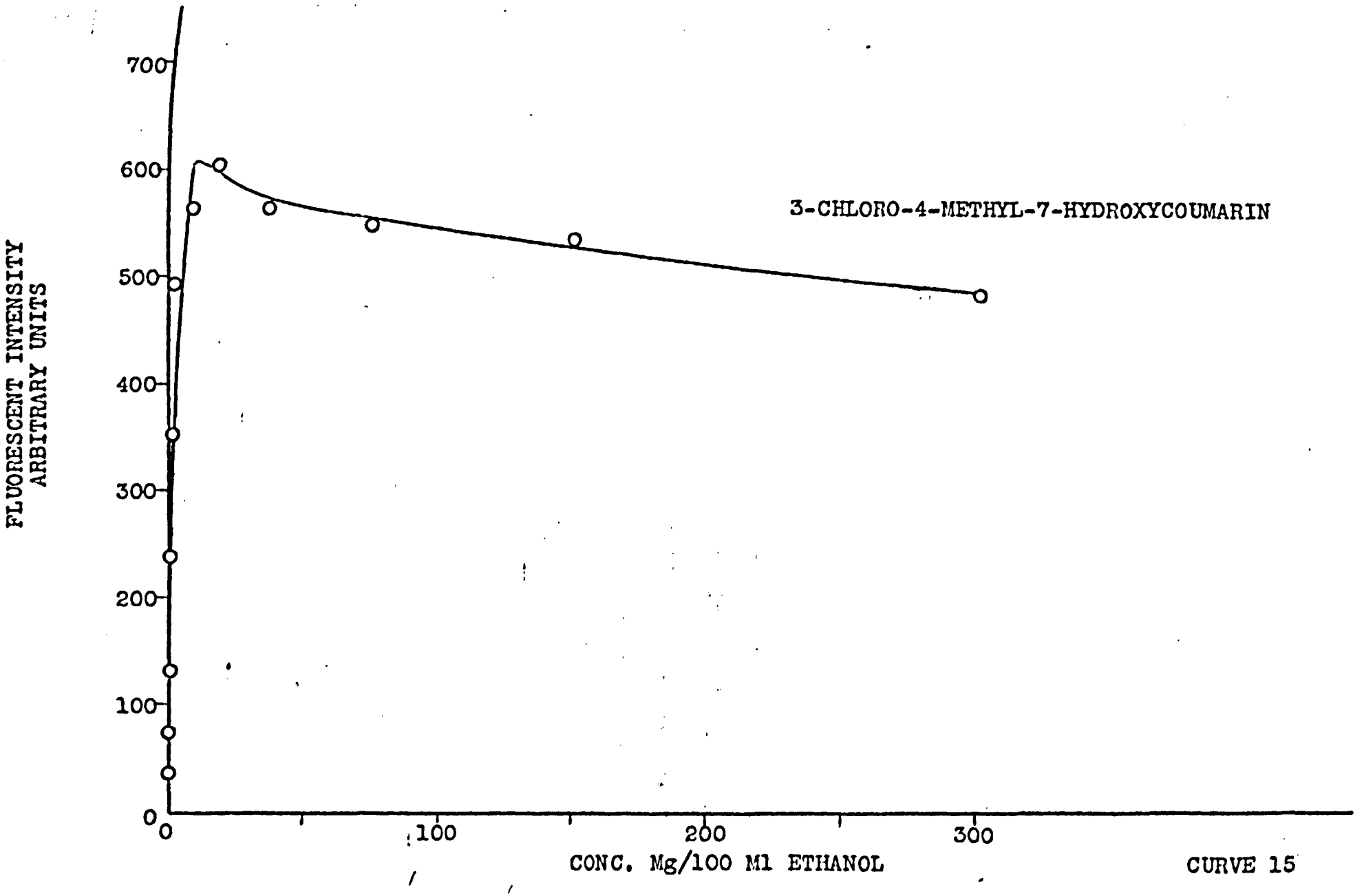


CURVE 12





CURVE 14



CURVE 15

decreased fluorescent intensity may be due to impurity. 3-Chloro-4-methyl-7-hydroxycoumarin (curve 15) shows a more intense fluorescence than 4-Methyl-7-hydroxycoumarin (curve 3) which agrees with the observation of Chakravarti .

Ultraviolet Absorption:

There is no known manner in which knowledge of the absorption characteristics of an organic molecule can be used to foretell fluorescence. In fact, Pringsheim has stated without reservation that there is no relation between the fluorescence and the ultraviolet absorption of a molecule other than the necessity that a molecule must be excited before it can fluoresce[1].

As stated by Knoblauch, the fluorescent intensity is proportional to the intensity of the exciting radiation[12]. As a corollary, the fluorescent intensity of a luminescent molecule should be related to its energy absorption.

In the preceeding section the curves Fluorescent Intensity in arbitrary units versus Concentration are given. A number of them show a maximum fluorescent intensity at some particular concentration. The specific fluorescent intensity, fluorescent intensity divided by the corresponding concentration, at this point seems a likely value for comparison of one fluorescer with another.

A Mazda B-H-4 black ray lamp was used as a source of exciting radiation in the above mentioned fluorescent

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(12) O. Knoblauch: Ann. Physik 54, 193 (1895)

intensity measurements. The spectrum of this lamp was obtained by use of a Cenco Spectrograph and the frequency and relative intensity of each line obtained by measurement on the photograph and comparison with tables of the mercury spectrum.

Then for each coumarin the ultraviolet absorption curve and the spectrum of the B-H-4 lamp was compared. For each line, the specific extinction at its wavelength is a relative measure of its absorption by the coumarin.

In this manner the relative absorption, relative intensity, and frequency of each line strongly absorbed by the coumarin from the exciting radiation was obtained. For each line, the product of these is a measure of the energy imparted to the coumarin by that line. The sum of the products for all lines absorbed is a measure of the total energy absorbed and is hereafter called the relative activation.

In Table I which follows, numbers 2, 7, and 8 are highly colored and number 6 melts over a 2.5°C. range. Consequently, these may be excluded from the discussion.

Table I shows the interesting parallel between relative activation and specific fluorescent intensity. The series arranged in order of decreasing relative activation is similarly arranged with respect to specific fluorescent intensity. It is also noteworthy that both 6- and 7-substituted coumarins appear in this series.

Table I

Compound	Relative Activation	Specific Fluor. Int.	Conc. at Max. mg/100 ml.
1. 4-Methyl-7-diethylamino-coumarin	42.2	1.5×10^5	4.7
2. 3-Acetyl-7-hydroxy-coumarin	27.1	8.0×10^4	.6
3. 7-Hydroxy-coumarin	23.7	5.6×10^4	12
4. 3-Chloro-4-methyl-7-hydroxy-coumarin	23.6	5.5×10^4	11
5. 4-Methyl-6,7-dihydroxy coumarin	22.1	5.2×10^4	4.7
6. 3-Isopropyl-4-methyl-7-hydroxy-coumarin	20.0	2.5×10^3	75
7. 4-Methyl-5,7-dihydroxy coumarin	20.0	2.2×10^3	18.8
8. 4-Methyl-7-hydroxy carbostyril	15.3	7.1×10^3	75
9. 3-Benzyl-4-methyl-7-hydroxy-coumarin	13.9	3.6×10^3	75
10. 4-Methyl-6-hydroxy coumarin	9.0	1.7×10^3	18.8
11. 6-Aminocoumarin	8.9	2.6×10^3	9.4
12. 4-Methyl-6-methoxy coumarin	7.8	1.7×10^2	150

This suggests a corollary to Knoblauch's Law: The fluorescent intensity of an emitting molecule is closely related, perhaps proportional, to the energy absorbed by the molecule. There appears to be an associated effect on susceptibility to concentration quenching of a strongly activated molecule. Both factors appear to be interrelated and influence fluorescent intensity.

Fluorescence Spectra:

The fluorescence spectra of a number of coumarins have been obtained and the position of each band determined. Generally, the band spread depends on exposure time and similar factors. Consequently, the fluorescence maximum is taken as the point of comparison.

Accompanying this section is Table II which contains all the obtained data pertinent to this subject. The positions of bands and fluorescence maxima are expressed in angstrom units. In the following remarks, the number accompanying the name of a compound refers to its position in Table II.

Both coumarin (1) and 4-Methylcoumarin (2) fluoresce in the near ultraviolet. The first has its maximum at 3515 and the latter at 3830.

It is indicated that substitution of a methyl group in the 4-position shifts the fluorescence maximum to a longer wavelength. Confirmation is obtained by comparing 7-Methoxycoumarin (3) with 4-Methyl-7-methoxycoumarin (4) and 7-Hydroxycoumarin (5) with 4-Methyl-7-hydroxycoumarin.

Further, the fluorescencemaximum of a hydroxy compound lies at a longer wavelength than the corresponding methoxy derivative. For example, compare 7-hydroxycoumarin (5) with 7-Methoxycoumarin (3), 4-Methyl-7-hydroxycoumarin (6) with 4-Methyl-7-methoxycoumarin (4), and 4-Methyl-6-hydroxycoumarin (8) with 4-Methyl-6-methoxycoumarin (9).

A hydroxy group in the 7-position shifts the fluorescence maximum to a longer wavelength than the same group in the 6-position; however, the converse is true with a methoxy group. Compare 4-Methyl-7-hydroxycoumarin (6) with 4-Methyl-6-hydroxycoumarin (8) and 4-Methyl-7-methoxycoumarin (4) with 4-Methyl-6-methoxycoumarin.

In the cases of 4-Methyl-7-hydroxycarbostyril (19) and 4-Methyl-7-aminocarbostyril (20) the fluorescence maximum of the hydroxy derivative lies at a longer wavelength than that of the amino derivative. Also, the maximum of 4-Methyl-7-diethylaminocoumarin (21) lies at a longer wavelength than that of 4-Methyl-7-hydroxycoumarin (6). It appears then when substituted in the 7-positions the various groups influence the position of the fluorescence maxima in varying degrees. The arrangement in order of decreasing wavelengths of fluorescence maxima is diethylamino, hydroxy, amino, and methoxy.

Comparison of 4-Methyl-7-hydroxycoumarin (6) with 3-Isopropyl-4-methyl-7-hydroxycoumarin (15) and 3-Benzyl-4-methyl-7-hydroxycoumarin (16) indicates that an ali-

Table II

Compound	Fluorescence Band Angstrom Units	Fluorescence Maximum Angstrom Units	Exposure Time
1. Coumarin	3510 - 3520	3515	3¼ hrs.
2. Methylcoumarin	3510 - 4570	3830	3 hrs.
3. 7-Methoxycoumarin	3640 - 4700	3850	3 hrs.
4. 4-Methyl-7-methoxycoumarin	3660 - 5930	3920	4 hrs.
5. 7-hydroxycoumarin	4170 - 5210	4410	1½ hrs.
6. 4-Methyl-7-hydroxycoumarin	3980 - 4930	4420	3/4 hrs.
7. 5-hydroxycoumarin	None		19 hrs.
8. 4-Methyl-6-hydroxycoumarin	4050 - 4580	4270	3 hrs.
9. 4-Methyl-6-methoxycoumarin	3890 - 4930	4180	3 hrs.
10. 6-Aminocoumarin	None		19 hrs.
11. 6-Nitrocoumarin	None		3 hrs.
12. 3-Acetyl-7-hydroxycoumarin	None		15½ hrs.
13. 4-Methyl-5,7-dihydroxycoumarin	None		3 hrs.
14. 4-Methyl-6,7-dihydroxycoumarin	None		3 hrs.
15. 3-Isopropyl-4-methyl-7-hydroxy-coumarin	4080 - 4930	4460	2½ hrs.
16. 3-Benzyl-4-methyl-7-hydroxy-coumarin	3970 - 4900	4480	3 hrs.
17. 3-Chloro-4-methyl-7-hydroxy-coumarin	4070 - 5060	4580	1½ hrs.

Table II (cont'd.)

Compound	Fluorescence Band Angstrom Units	Fluorescence Maximum Angstrom Units	Exposure Time
18. Ethyl-7-hydroxy-coumarin-3-carboxylate	4370 - 6060	4540	2 hrs.
19. 4-Methyl-7-hydroxycarbostyril	3880 - 4520	4120	3/4 hrs.
20. 4-Methyl-7-aminocarbostyril	3790 - 4580	4070	1½ hrs.
21. 4-Methyl-7-diethylamino-coumarin	4330 - 6060	4560	

phatic group in the 3-position does not greatly influence the position of the fluorescence maximum.

3-Chloro-4-methyl-7-hydroxycoumarin (17) and Ethyl 7-hydroxycoumarin-3-carboxylate indicate that both the chloro and the carbethoxy groups displace the fluorescence maximum toward longer wavelengths; the former having a slightly greater effect than the latter.

Stability of Fluorescence in Alkali:

The Fluorescence of 7-hydroxycoumarin soon disappears on warming in alkali. As for the chemical changes which accompany and cause loss of fluorescence, little is known. Tiemann and Reimer have shown that 7-hydroxycoumarin warmed at 60° to 70°C in alkali forms trans 2,4-dihydroxycinnamic acid and on longer heating, resorcinol[13].

4-Methyl-7-hydroxycoumarin is alittle more stable. Pechmann and Duisberg have shown it to be degraded to resorcinol by strong alkali[14]. After treatment with 33% potassium hydroxide, trans 2,4-Dihydroxy-3-methylcinnamic acid is isolateable[15]. A more comprehensive work by Murty, Rao, and Seshadri indicates that the action of alkali on 4-Methylcoumarins forms the trans cinnamic acids[16]. In the example of 3-Benzyl-4-methyl-7-hydroxy-

(13) F. Tiemann and C. Reimer: Ber. 12, 994 (1879)

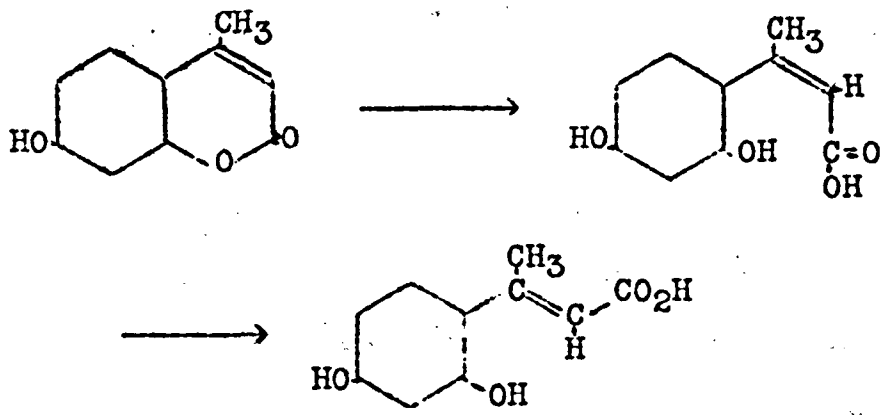
(14) H. von Pechmann and C. Duisberg: Ber. 16, 2122 (1883)

(15) K. Fries and W. Bolk: Ann. 379, 105 (1910)

(16) K.S. Murty, P.S. Rao, and T.R. Seshadri: Proc. Ind. Acad. Sci. 6A, 316 (1937)

coumarin, the action of potassium hydroxide forms 2,4-dihydroxybenzoic acid and Benzylacetone[17].

Possibly, the loss of fluorescence is associated with one of the following transformations, using 4-Methyl--7-hydroxycoumarin as an example:



In any event, the effect of substituents in the 3-position and replacement of the heterocylic oxygen by an -NH-group should be interesting.

Consequently, a number of 3-substituted coumarins and two carbostyrils were heated at 56°C. in a strong alcoholic potassium hydroxide solution and the fluorescent intensity measured at intervals. Exposure to oxygen was minimized by a nitrogen atmosphere.

The results may be summarized by the following series which is arranged in decreasing order of stability of fluorescence in alkaline solution.

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(17) S. Jacobson and B. Ghosh: J. Chem. Soc. 107, 428 (1915)

1. 4-Methyl-7-hydroxycarbostyril (curve 1)
2. 3-Benzyl-4-methyl-7-hydroxycoumarin (curve 2)
3. 3-Isopropyl-4-methyl-7-hydroxycoumarin (curve 3)
4. 4-Methyl-7-aminocarbostyril (curve 4)
5. 4-Methyl-7-hydroxycoumarin (curve 8)
6. 4-Methyl-7-diethylaminocoumarin (curve 6)
7. 7-Hydroxycoumarin (curve 7)
8. 3-Chloro-4-methyl-7-hydroxycoumarin (curve 5)
9. Ethyl 7-hydroxycoumarin-3-carboxylate (curve 9)

Most of the curves of Fluorescent Intensity in alcoholic KOH at 56°C versus time show a decrease in intensity, then a rise and finally a constant intensity or a slow decline in intensity.

Initially, 3-chloro-4-methyl-7-hydroxycoumarin (curve 5) is very weakly fluorescent. In time, the intensity rises in a nearly parabolic curve to much higher intensities; however, this is considered to be an example of poor alkali stability for the increased intensities are probably due to decomposition products.

Even though the fluorescent intensity of 4-Methyl-7-aminocarbostyril remains of the same order of magnitude over a period of many hours, it is considered to be less stable than, for example, 3-Benzyl-4-methyl-7-hydroxycoumarin (curve 2) because alkali exerts an immediate and profound influence on its fluorescence. In all other examples no change in color of fluorescence is noticed, but in this case the influence of alkali changes

the color of fluorescence from orchid to light green.

The behavior of coumarins in the presence of alkali is complicated by

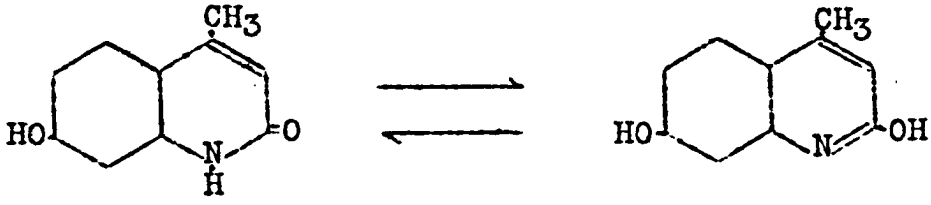
1. possible fluorescence of degradation products.

2. possibility of concentration quenching.

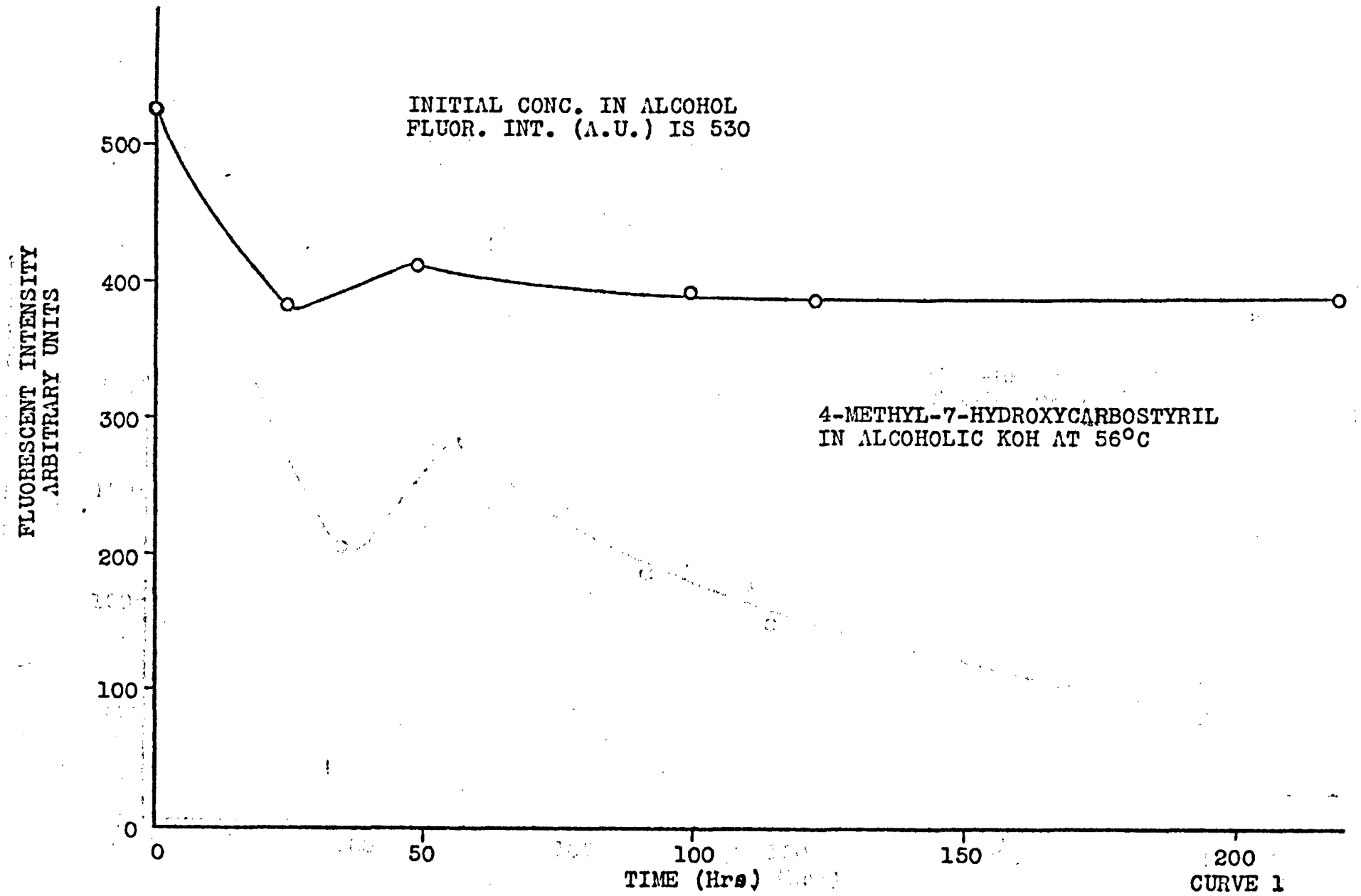
3. possibility of quenching by electrolyte.

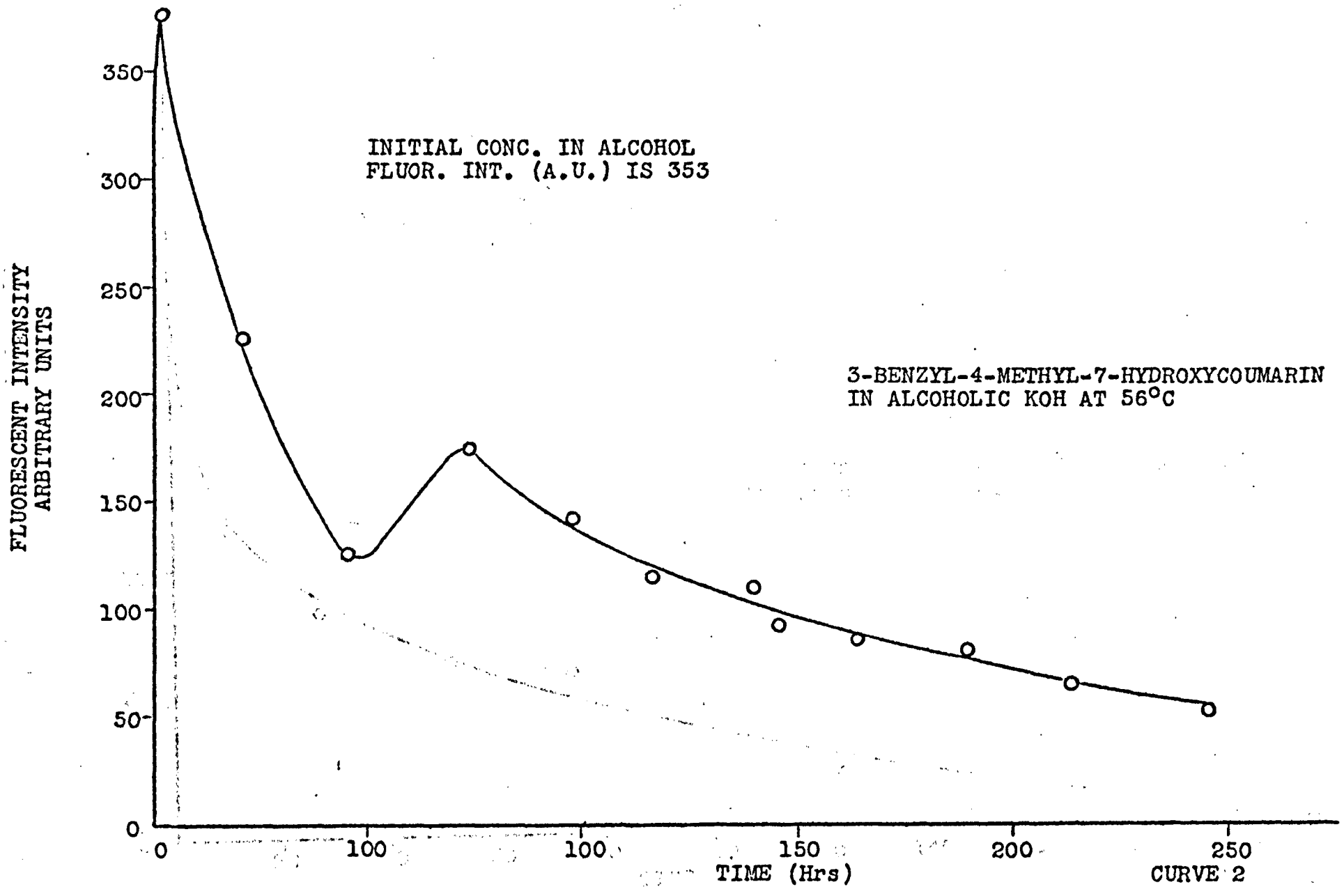
In the case of the carbostyrils there is a fourth interference.

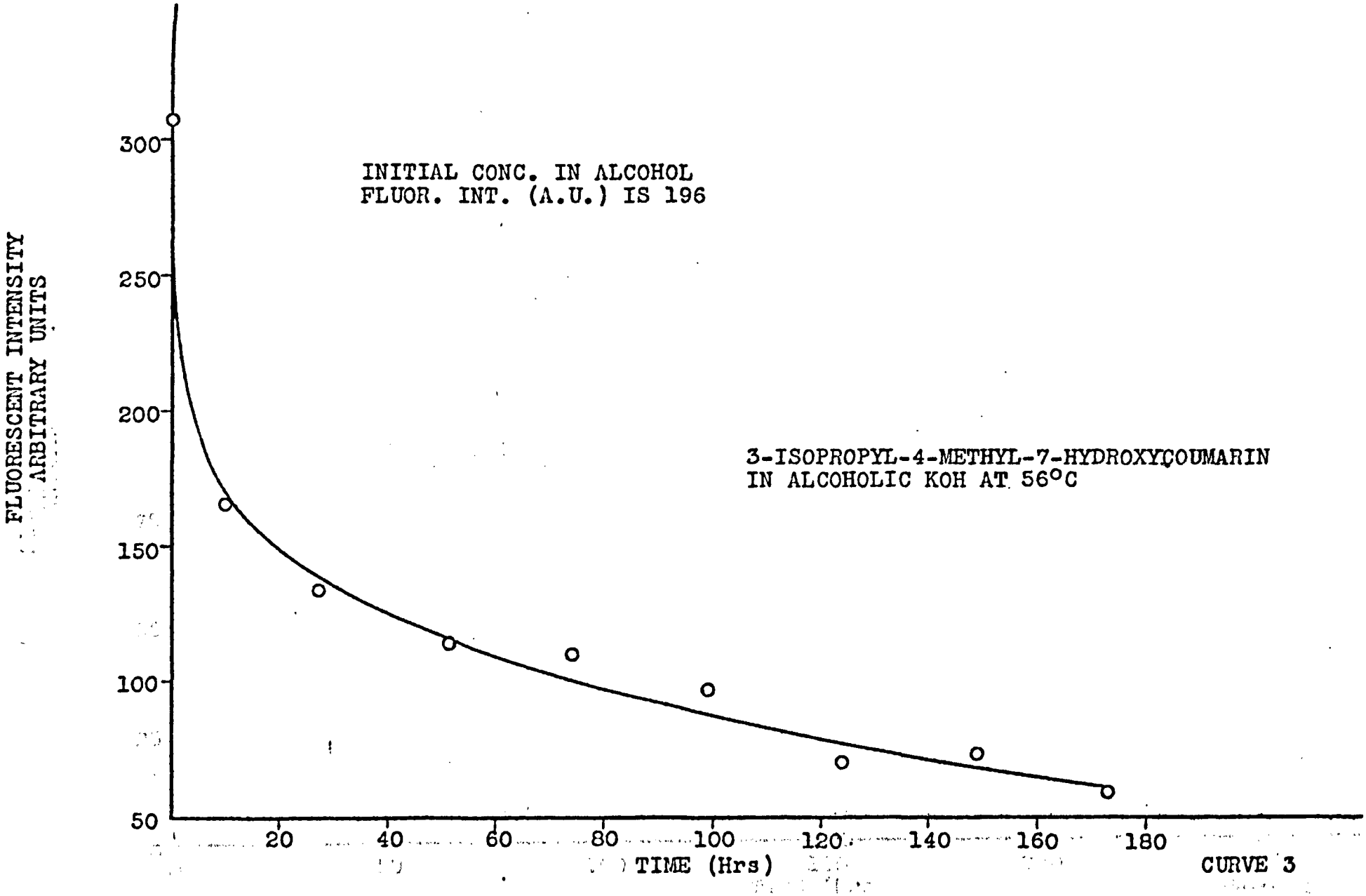
4. tautomerism.



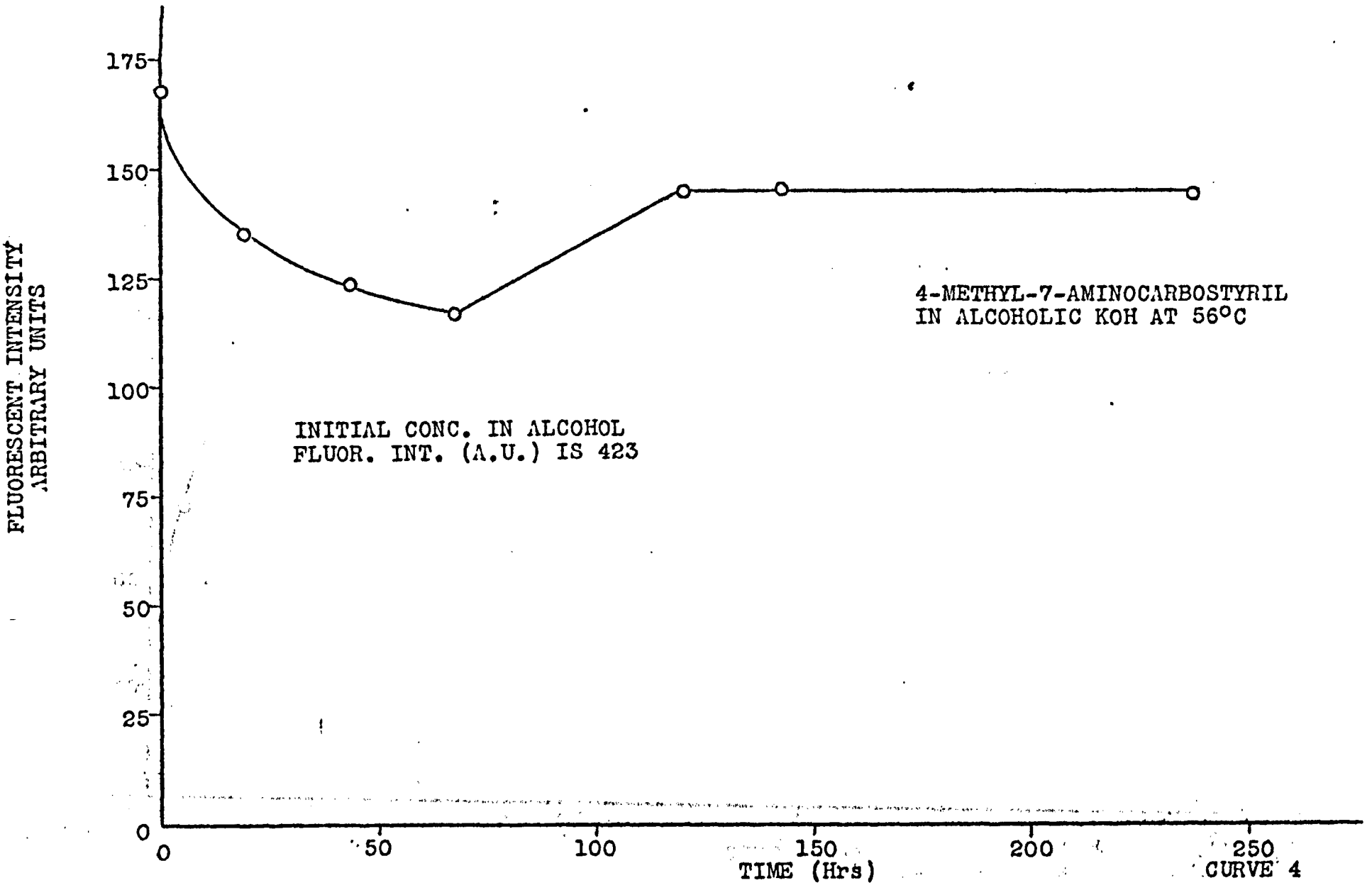
Replacement of the heterocyclicoxygen of a fluorescent coumarin by the -NH-group or introduction of a 3-Isopropyl or 3-Benzyl group has a salutary effect on the stability of the fluorescence towards alkali. Since 4-Methyl-7-hydroxycoumarin (curve 8) is more stable than 7-hydroxycoumarin, the 4-Methyl group is thought to confer greater alkali stability. A similar comparisonof 4-Methyl -7-hydroxycoumarin (curve 8) with 4-Methyl-diethylaminocoumarin (curve 6) indicates the diethylamino group to be superior to the hydroxyl in this respect. A chloro or carbethoxy group in the three position is definitely harmful.

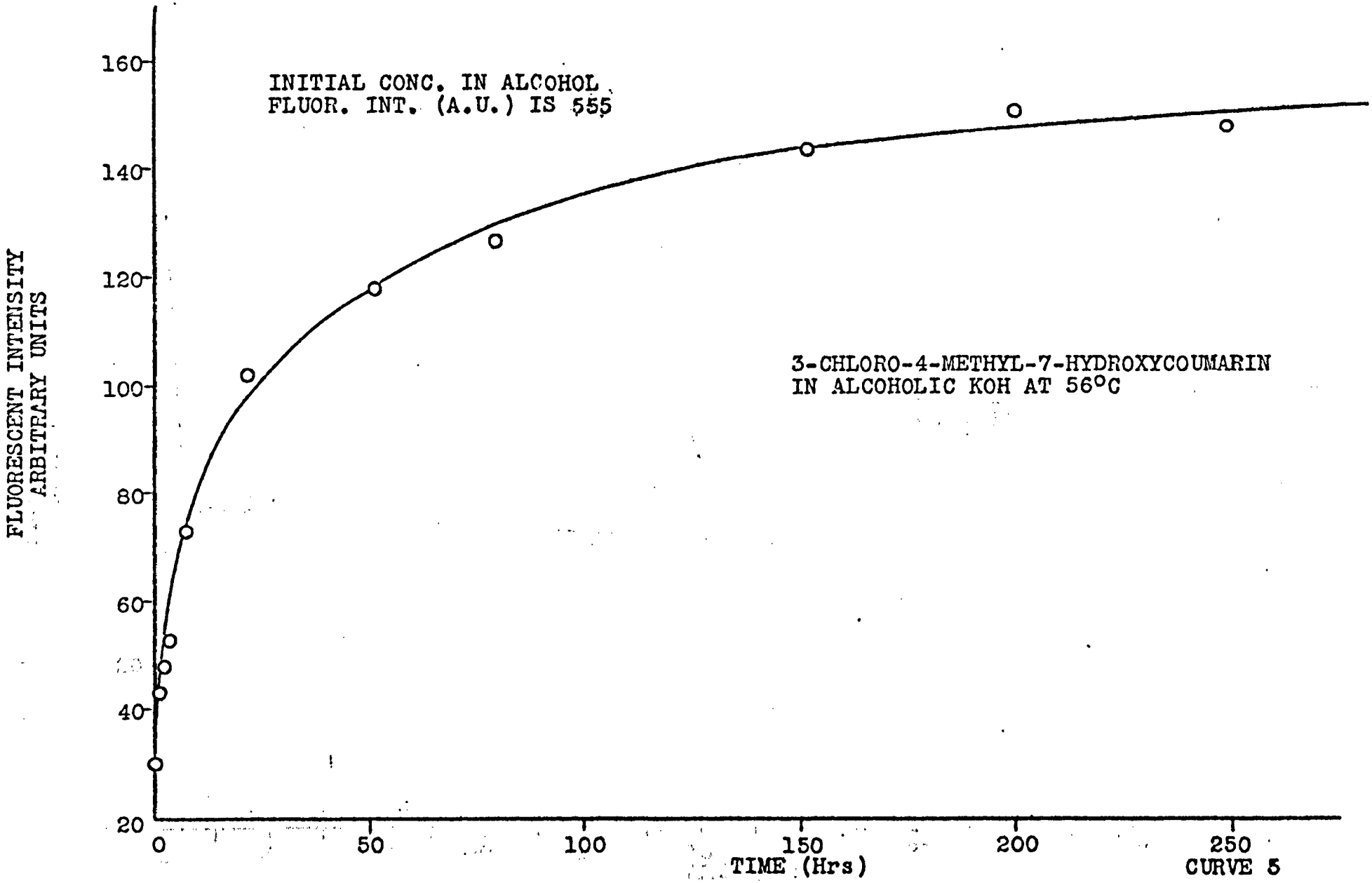




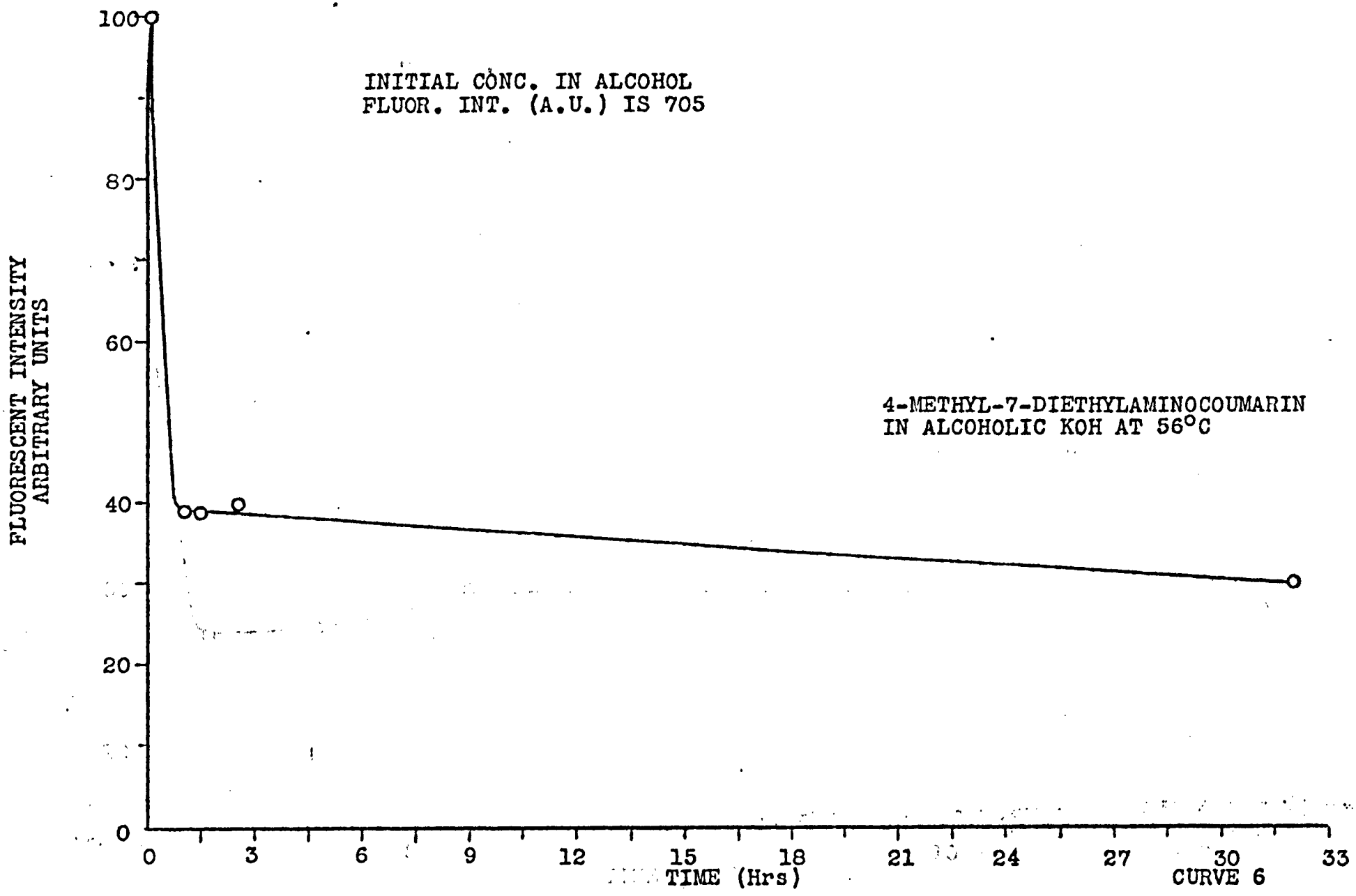


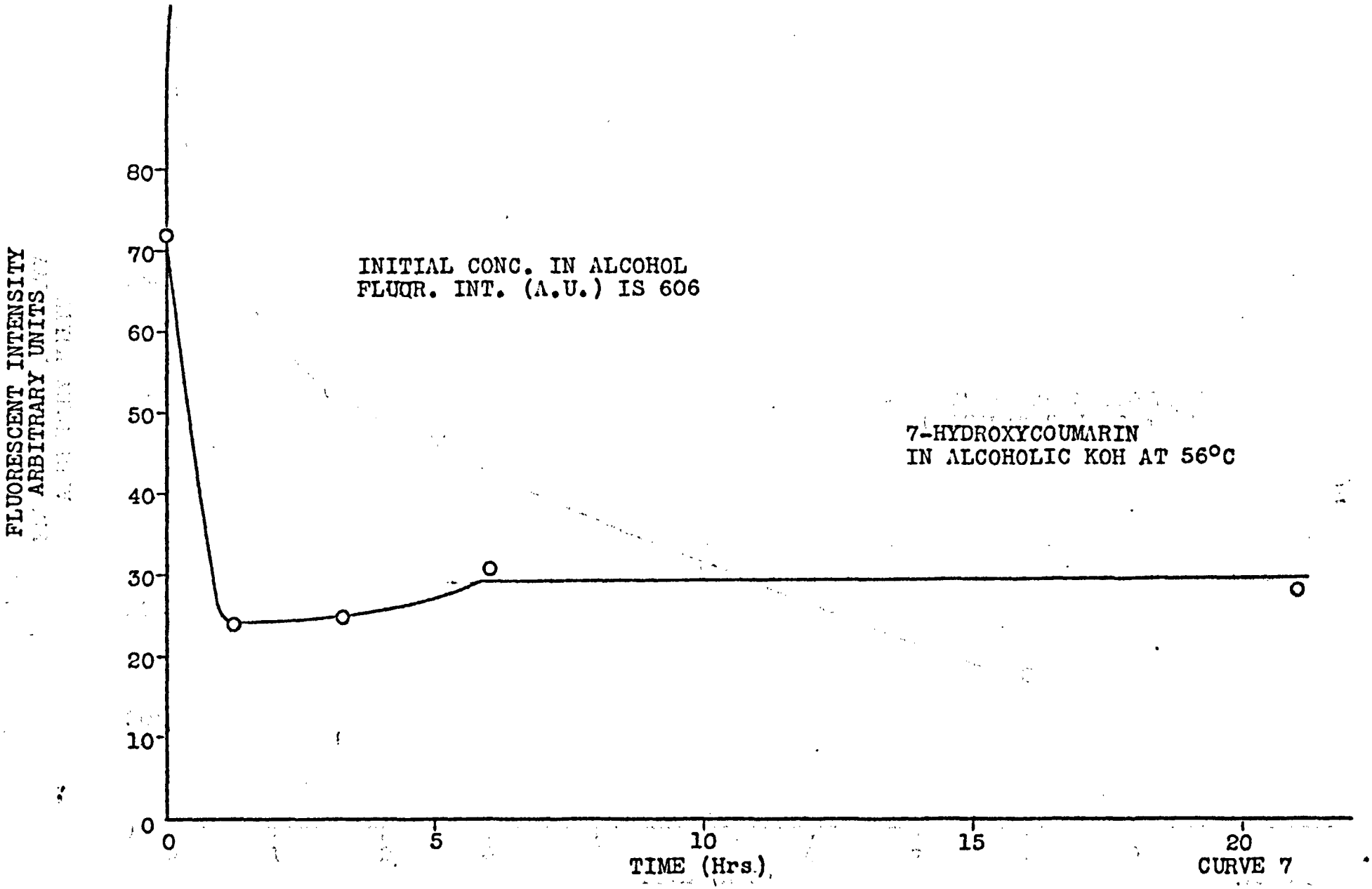
CURVE 3

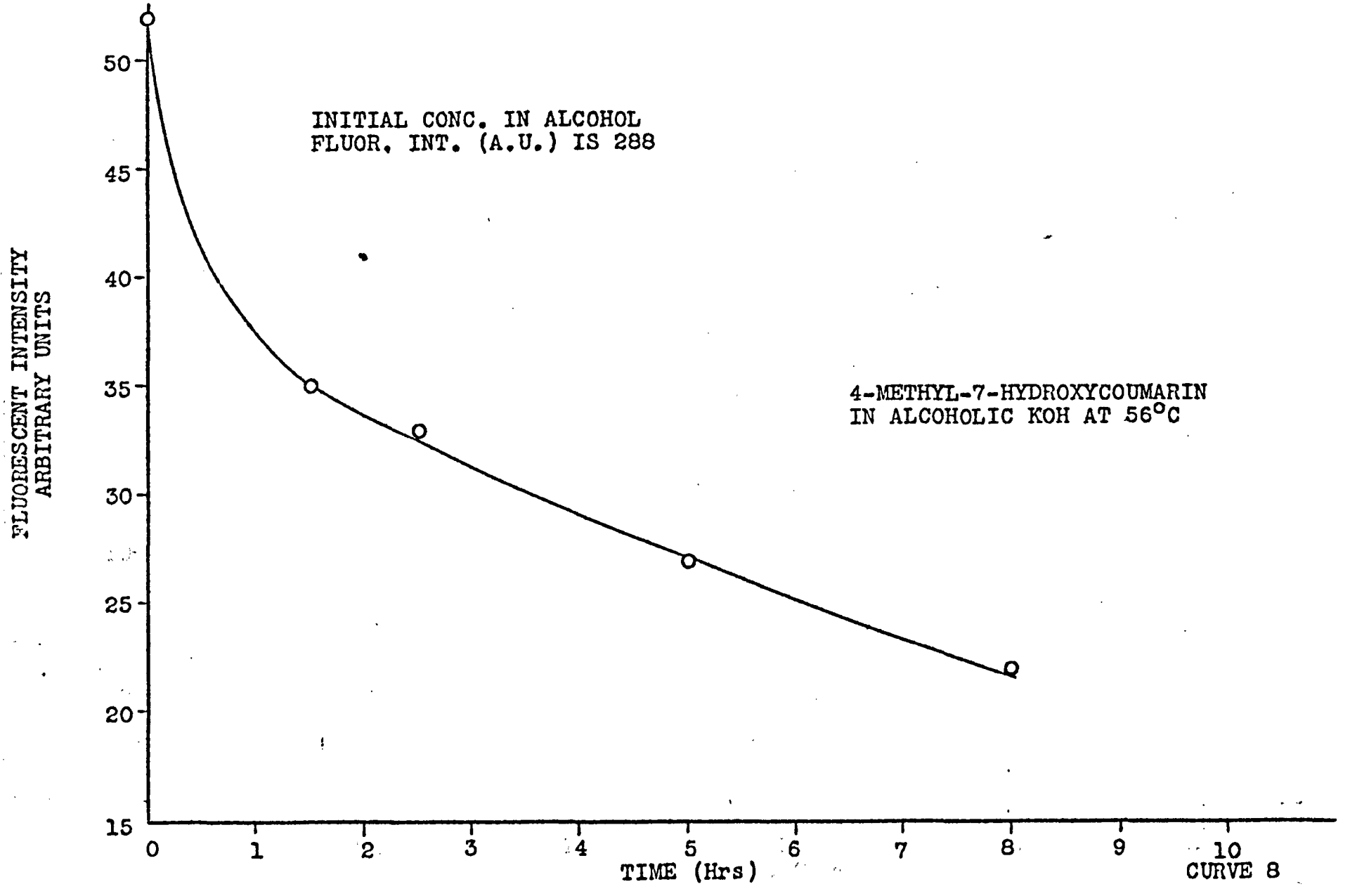




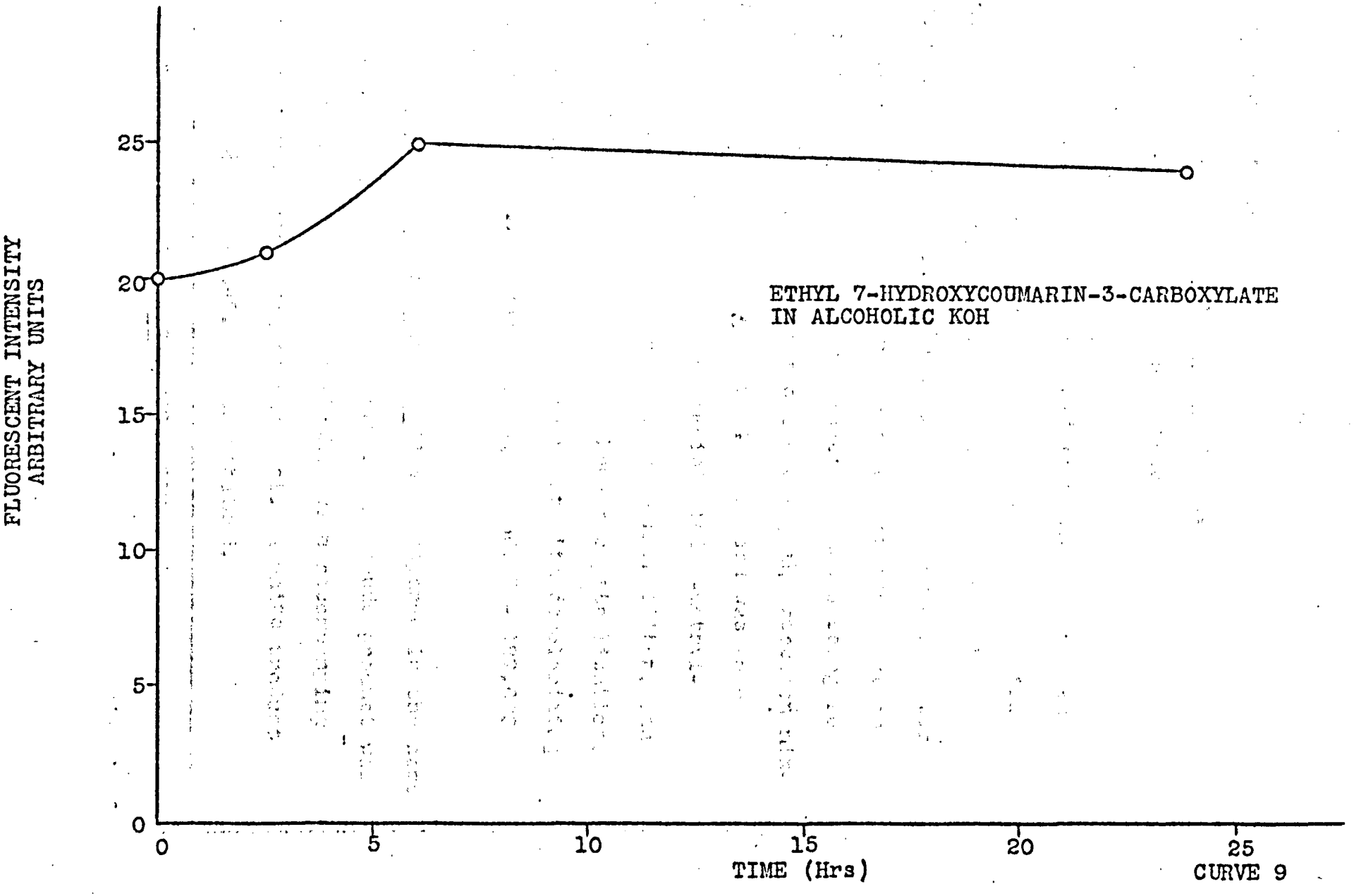
CURVE 5







CURVE 8



EXPERIMENTAL PART

Synthesis of Fluorescent Compounds:

4-Methyl-7-hydroxycoumarin is easily prepared by the von Pechmann[14] condensation of resorcinol and aceto-acetic ester.

Concentrated sulfuric acid (100 ml.) was cooled in a one liter, round bottom flask, equiped with thermometer and stirrer, and surrounded by an ice bath. A solution of resorcinol (110.1 g.) in acetoacetic ester (130.1 g.) was added at such a rate that the reaction temperature never rose above 16°C; two hours being required. After removal of the ice bath, the reaction mixture was allowed to stand for 24 hours and was then immersed in ice water. The precipitated 4-Methyl-7-hydroxycoumarin was separated on a buchner filter, and washed with cold water. After three recrystallizations from alcohol accompanied by treatment with decolorizing charcoal, a white product melting at 186.5° - 187.6°C was obtained.

4-Methyl-6-hydroxycoumarin[18] was prepared in the same manner as 4-Methyl-7-hydroxycoumarin. The crude product was recrystallized from diluted alcohol with accompanying treatment with decolorizing charcoal. A white product melting at 246.5° - 247.0°C was obtained.

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(18) W. Borsche: Ber. 40, 2732 (1907)

4-Methyl-6-methoxycoumarin[19] was prepared by methylation of 4-Methyl-6-hydroxycoumarin. A mixture of 4-Methyl-6-hydroxycoumarin (17.3 g.), potassium carbonate (74.7 g.) and dimethyl sulfate (44.7 g.) in acetone (250 ml.) were refluxed for 45 hours in a round bottom flask. The acetone filtrate was evaporated and the residue combined with the water insoluble portion of the filtration residue, dried and vacuum distilled. The fraction distilling at 175° - 184°C @ 1 mm. pressure was recrystallized from alcohol and melted at 162.6° - 163.8°C.

4-Methyl-7-methoxycoumarin was prepared in a manner similar to that of 4-Methyl-6-methoxycoumarin. The fraction distilling at 195° - 196°C @ 3 mm. was recrystallized from alcohol. The product melted at 158.5° - 160.0°C.

7-Hydroxycoumarin[20] was prepared by the condensation of malic acid and resorcinol in sulfuric acid. A mixture of resorcinol (110.1 g.) and malic acid (134.1 g.) covered with concentrated sulfuric acid (500 g.) was heated rapidly to 135°C when violent frothing began. The mixture was stirred with the flame removed until frothing had subsided. After cooling to near room temperature, the reaction mixture was immersed in ice water. The collected precipitate was recrystallized from water

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(19) G. Desai and C. Mavani: Proc. Ind. Acad. Sci. 15A, 1 (1942)

(20) H.V. Pechmann: Ber. 17, 932 (1884)

with accompanying charcoaling, dried and sublimed at 200°C @ 1 mm. pressure. Recrystallization from alcohol with accompanying treatment with charcoal gave woolly clusters melting at 232.3° - 233.0°C.

7-Methoxycoumarin was prepared by condensation of the monomethyl ether of resorcinol and malic acid. To a mixture of resorcinol (110 g.), sodium hydroxide (40 g.) and water (400 ml.) were slowly added 130 g. of dimethyl sulfate. The mixture was heated on a water bath for two hours and then refluxed for four. After standing overnight, the reaction mixture was extracted with ether and the ether soluble residue steam distilled. The monomethyl ether was separated by extracting the steam distillate with ether, extracting the ether extract with 3 N sodium hydroxide and precipitating the monomethyl ether with an excess of hydrochloric acid. The 75 g. of product, malic acid (81 g.), and sulfuric acid (300 g.) were heated rapidly to 125°C at which point vigorous frothing occurred. After frothing subsided, the mixture was allowed to cool and then poured into ice water. After standing overnight, the tacky solid was separated by filtration, recrystallized twice from dilute alcohol and then vacuum distilled. The fraction boiling at 151° - 154°C @ 1 mm. was collected as product and melted at 116.5° - 117.5°C.

6-Nitrocoumarin[21] was prepared by nitration of cou-

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(21) G. Morgan and F. Micklethwait: J. Chem. Soc. 85, 1233 (1940)

marin. A solution of coumarin (100 g.) in glacial acetic acid (120 ml.) was treated with a mixture of fuming nitric acid (56 ml.) and glacial acetic acid (40 ml.). Then concentrated sulfuric acid (100 ml.) was added to the cooled mixture. After addition and a brief period of heating on a water bath, the mixture was poured in ice water. The yellowish-white nitrocoumarin was dried by codistillation of the water with benzene and was recrystallized from benzene. The product which melted at 187.8° - 189.4°C weighed 94.1 g.

6-Aminocoumarin[21] was prepared by reduction of 6-nitrocoumarin. 6-nitrocoumarin (47 g.) was suspended in hot water (2400 ml.) acidified with hydrochloric acid (50 ml.). To the heated, stirred mixture was slowly added 94 g. of iron powder. The mixture was heated awhile longer, neutralized with an excess of sodium bicarbonate, and immediately filtered. On cooling 11.3 g. of crude product was obtained. Recrystallization from water gave a product melting 163.5° - 164.5°C.

5-Hydroxycoumarin[22] was prepared in several steps, beginning with B-Resorcylic acid.[23]

Resorcinol (104.3 g.), sodium bicarbonate (429.5 g.) and water (1.1 l.) were heated on a water bath for 5 hours and 15 minutes and then refluxed for 45 minutes while a

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(22) H. Shah and R. Shah: J. Chem. Soc. 141, 1832 (1938)

(23) Organic Syntheses: Vol. II, 557

strong stream of carbon dioxide was bubbled through the reaction mixture. Then concentrated hydrochloric acid (500 ml.) was added and the mixture allowed to cool and crystallize. After filtration and air drying, 71.7 g. of product melting at 216°C were obtained and were used directly to form Methyl β-Resorcylate[24].

B-Resorcylic acid (100 g.), methanol (350 ml.), and concentrated sulfuric acid (20 ml.) were refluxed for 22 hours, the methanol was distilled, water was added to the point of turbidity and the mixture was allowed to crystallize. After two recrystallizations from dilute methanol with accompanying treatment with charcoal, 49.3 g. of product melting at 117.5° -119.0°C were obtained. This material was used to prepare Methyl 2,4-dihydroxy-3-formylbenzoate[25].

Methyl β-Resorcylate[15] was placed in a dry one liter three neck, with all glass joints, flask, equipped with a reflux condenser, a glass stirrer, a gas inlet, and a dropping funnel. Sufficient dry ether (over sodium) was pumped into the flask to dissolve the Methyl β-Resorcylate. Then, specially prepared[26] zinc cyanide (21 g.) was added. To the stirred mixture, cooled in an ice-salt bath was added slowly a solution of Anhydrous

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(24) R. Robinson and R. Shah: J. Chem. Soc. 137, 1496 (1934)

(25) R. Shah and M. Laiwalla: Chem. Soc. 141, 1828 (1938)

(26) R. Adams and I. Levine: J.A.C.S. 45, 2373 (1923)

Aluminum Chloride (24 g.) in Anhydrous ether. A strong stream of dry hydrogen chloride was passed into the stirred, cooled mixture for 12 hours. At the end of this period, the reflux condenser was removed and 200 ml. of water slowly added. The heat of hydrolysis and evolution of HCl removed most of the other. The mixture was heated on a water bath for half an hour. The precipitate was collected and recrystallized twice from alcohol. The product melting at 134.7° - 136.5°C weighed 10.8 g. and was used to prepare β-Resorylaldehyde[27, 25].

Methyl 2,4-dihydroxy-3-formylbenzoate (10 g.) was dissolved in normal sodium hydroxide (200 ml.) and left at room temperature for two days. Acidification precipitated the acid which was triturated with benzene to remove unchanged ester and recrystallized from dilute methanol. The 2,4-dihydroxy-3-formylbenzoic (6 g.) and water (90 ml.) were heated together in a sealed tube at 100° - 110°C for 10 hours. The β-Resorcylaldehyde was extracted in ether and the residue left on evaporation of the ether was recrystallized from water.

β-Resorcylaldehyde (2 g.), anhydrous sodium acetate (3 g.), and acetic anhydride (10 g.) were heated in a sealed tube at 150° - 160°C for 3 hours and then at 175° - 180°C for an hour. After cooling, the mixture was shaken for 2 hours with water (50 ml.). The separated

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(27) K. Nakazawa: J. Pharm. Soc. Japan 59, 57 (1939)

5-acetoxycoumarin[28] was filtered off, recrystallized from water and hydrolyzed to 5-hydroxycoumarin as follows: 5-Acetoxycoumarin (1 g.) and 25% sulfuric acid (30 ml.) were refluxed for 45 minutes. The insoluble 5-hydroxy-coumarin separated and was recrystallized from dilute alcohol. A product (.3 g.) melting at 229.3° - 229.9°C was obtained.

4-Methylcoumarin[29] was prepared by condensing phenol with acetoacetic ester. To concentrated sulfuric acid (180 ml.) in a flask equipped with stirrer, thermometer and dropping funnel were slowly added a mixture of dry phenol (30 g.) and acetoacetic ester (20 g.). After an hour of stirring, the mixture was heated to 75°C on a steam bath. The reaction raised the temperature to 85°C after which the mixture cooled to room temperature and was immersed in water (1½ l.). The water solution was extracted with ether, saturated with salt and again extracted. The ether extracts were shaken with dilute sodium hydroxide until no more color was extracted. After distilling off the ether and allowing the colorless residue to congeal, the crude product was recrystallized from water. The final product melted at 78.7° - 79.8°C.

4-Methyl-7-Aminocarbostyril[30] was prepared by condensation of meta phenylene diamine and acetoacetic ester.

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(28) H. Bohme: Ber. 72B, 2130 (1939)

(29) F. Peters and H. Simonis: Ber. 41, 831 (1908)

(30) E. Besthorn and H. Byvanck: Ber. 31, 798 (1898)

Meta phenylene diamine (40 g.) and acetoacetic ester (56 g.) were heated at 130°C in a sealed tube for 5 hours. Then the crystallized contents of the tube were rinsed out with methanol, recrystallized from absolute ethanol and then from water. A malt of fine needles melting 270.4° - 272.0°C were obtained.

4-Methyl-7-hydroxycarbostyril[31,32] was prepared by a similar manner through condensation of meta aminophenol and acetoacetic ester. The crude product was triturated with 5% sodium hydroxide, filtered, and the filtrate treated with powdered dry ice. The precipitated 4-methyl-7-hydroxycarbostyril was recrystallized from dilute alcohol and dried in a vacuum oven. Woolly clusters melting at 333° -336°C were obtained.

3-Chloro-4-methyl-7-hydroxycoumarin[33] was prepared by a typical Pechmann condensation of resorcinol and α-chloro-acetoacetic ester. The product was recrystallized five times with charcoaling from dilute alcohol and melted at 243.9° - 244.7°C.

α-Chloroacetoacetic ester[34] was prepared by chlorination of acetoacetic ester as follows:
Sulfuryl chloride (135 g.) was added over a period of 3 hours to acetoacetic ester (140 g.) and warmed on a water

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(31) H. von Pechmann: Ber. 32, 3687 (1899)

(32) H. von Pechmann and O. Schwartz: Ber. 32, 3700 (1899)

(33) H. von Pechmann and E. Hanke: Ber. 34, 357 (1901)

(34) F. Allihn: Ber. 11, 567 (1878)

bath for 7 hours. The reaction mixture was vacuum distilled and the fraction (78 g.) boiling at 103° - 110°C @ .38 mm. was taken as product.

3-Isopropyl-4-methyl-7-hydroxycoumarin has not been reported in the literature and was prepared by condensation of resorcinol and α-isopropylacetoacetic ester in the usual manner. The product recrystallized twice from alcohol melted at 208.5° - 211.0°C.

Analysis calculated for $C_{13}H_{14}O_3$:

	Carbon	Hydrogen
Found	71.54%	6.47%
	70.83%	6.71%

The ultraviolet absorption curves are regarded as indicative of the structure of 3-Isopropyl-4-methyl-7-hydroxycoumarin. In all cases, tabulated below the form of the curves is nearly identical:

Compound	Maximum U.V. Absorption (mμ)	Extinction at Max.
3-Isopropyl-4-Methyl-7-Hydroxycoumarin	323	78.1
7-Methoxycoumarin	320	86.4
4-Methyl-7-hydroxy-coumarin	325	89.7
4-Methyl-7-methoxy-coumarin	320	78.3
7-Hydroxycoumarin	325	89.2

4-Methyl-5,7-dihydroxycoumarin[35] was prepared in the

(35) H. Pechmann and J. Cohen: Ber. 17, 2189 (1884)

usual manner from phloroglucinol and acetoacetic ester. The product recrystallized twice from acetic acid, melted at 271° - 271.5°C.

4-Methyl-6,7-dihydroxycoumarin[36] was prepared in a similar manner from acetoacetic ester and hydroxyhydroquinone triacetate. The crude 4-Methyl-6,7-dihydroxycoumarin (40 g.) was dissolved by warming with borax (80 g.) and water (280 ml.), on cooling of the filtrate, a complex borate separates. The borate is dissolved in water (720 ml.) and added to water (200 ml.) containing sulfuric acid (20 g.). The separated product was filtered, dried, and melted at 271° - 271.5°C.

Hydroxyhydroquinone[37] was prepared as follows: Concentrated sulfuric acid (8 g.) added to acetic anhydride (120 g.) was stirred and treated with quinone (40 g.) at such a rate that the temperature rises and remains between 40° - 50°C. The mixture is allowed to stand until cooled to room temperature and then poured into water (500 ml.), cooled to 10°C and filtered. The product recrystallized from alcohol and dried melted at 96° - 97°C.

Quinone[38] was prepared by oxidation of hydroquinone. Hydroquinone is dissolved in water (1 l.) at 50°C, cooled to 20°C and concentrated sulfuric acid (50 g.) added.

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(36) Organic Syntheses: Vol. I, 360

(37) Organic Syntheses: Vol. I, 317

(38) Organic Syntheses: Vol. I, 482

A solution of sodium dichromate (70 g.) in water (53 ml.) is added at such a rate that the stirred, cooled mixture remains between 20° - 30°C. As soon as a permanent yellowish-green color appears, the mixture is cooled to 10°C and filtered. The filtrate is extracted with benzene and the residue dissolved in benzene (250 ml.) which includes the benzene extract. The hot benzene solution is dried with calcium chloride, filtered hot, and benzene distilled until quinone precipitates. The residue was chilled in an ice bath and the quinone collected by filtration.

3-Benzyl-4-methyl-7-hydroxycoumarin[17] was prepared by a Pechmann condensation of resorcinol and α-benzylacetoacetic ester. The product was recrystallized twice from alcohol and melted at 231.7° - 233.0°C.

α-Benzylacetoacetic ester[39] was prepared as follows: In a one-liter reflux apparatus protected from moisture and equipped with stirrer and dropping funnel were placed dry benzene (300 ml.) and sodium (23 g.). Then to the stirred refluxing mixture was added acetoacetic ester (130 ml.) over a period of several hours. After solution of the sodium, the mixture was filtered into a similar apparatus, and to the refluxing solution benzyl chloride (140 ml.) was slowly added. Refluxing and stirring were continued

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(39) F. Ehrlich: Ann. 187, 12 (1877)

until the mixture was no longer alkaline. Then it was filtered from the salt, washed, stripped, and vacuum distilled. The fraction boiling at 148° - 153°C @ 9 mm. was taken as product (85 g.).

α-Isopropyl acetoacetic ester[40] was prepared similarly. Dry alcohol prepared by refluxing absolute alcohol over magnesium and iodine for several hours and distilling directly into the reaction vessel was used as solvent. Isopropyl bromide was used. The fraction boiling at 195° - 197°C was taken as product.

Ethyl-7-hydroxycoumarin-3-carboxylate[41] was prepared from 2,4-dihydroxybenzaldehyde (5 g.), malonic ester (6 g.), and a drop of piperidine. After standing a week the solid paste was washed out with dilute hydrochloric acid. The precipitate was recrystallized from dilute alcohol. The product melted at 171.9° - 172.3°C.

3-Acetyl-7-hydroxycoumarin[9] was prepared from 2,4-dihydroxybenzaldehyde (5 g.), acetoacetic ester (7.5 g.), and a drop of piperidine. The product was recrystallized from alcohol and melted at 237° - 237.6°C.

4-Methyl-7-diethylaminocoumarin was prepared as follows: Distilled metadiethylaminophenol (20 g.), acetoacetic ester (22 g.), zinc chloride (10 g.), and absolute alcohol (40 g.)

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(40) E. Frankland and B. Duppa: Ann. 204, 179 (1880)

(41) H. von Pechmann and E. Graeger: Ber. 34, 385 (1901)

were refluxed for 30 hours. The reaction mixture was poured into water (400 ml.) containing a few drops of sulfuric acid. After several days, the separated oil crystallized and was filtered, dried, and vacuum distilled. The fraction boiling at 240° - 243°C @ 6.5 mm. was taken as product; it melted at 70.1° - 71°C.

Fluorescence Intensity

In order to measure the fluorescent intensity of coumarins in alcohol, it was necessary to build an instrument. The sketch and wiring diagram which follow show the salient features of the instrument designed by Messrs. Soller and Berghausen.

In use, a fluorescent solution was irradiated in the black box with ultraviolet passed by a Wood's filter, which has a sharp cut-off at about 4100A°. After passing through a 2% sodium nitrite solution to remove ultraviolet frequencies, the fluorescent light fell on a photocell. The relative positions of photocell, sample cell, and ultraviolet source are indicated in the vertical view of the sketch of the black box.

For each fluorescent coumarin, the fluorescent intensities, expressed in arbitrary units, of solutions varing in concentration from 0-300 mg/100 ml. of alcohol were obtained as follows:

When there is no fluorescent solution in the black box, the dark current after amplification can be balanced by a small resistance. Then the fluorescent solution is placed in

the sample cell and the resulting photelectric current is balanced after amplification by dial box resistances. The fluorescent intensity is proportional to the applied resistance. Since the line voltage can fluctuate, the fluorescent solution was alternated with a standard fluorescent solution and an average fluorescent intensity obtained for each. Then each average fluorescent intensity (in ohms of dial box resistance) was multiplied by a factor which would reduce the fluorescent intensity of the standard to a previously accepted value. The fluorescent intensity of the fluorescent solution was then said to be expressed in arbitrary units. As used in this work, an arbitrary unit is such that a solution of 4-Methyl-7-hydroxycoumarin, 300 mg/100 ml in alcohol in a 4 cms by 2 cm by 5 cm volume (20 ml.) has a fluorescent intensity of 360 arbitrary units.

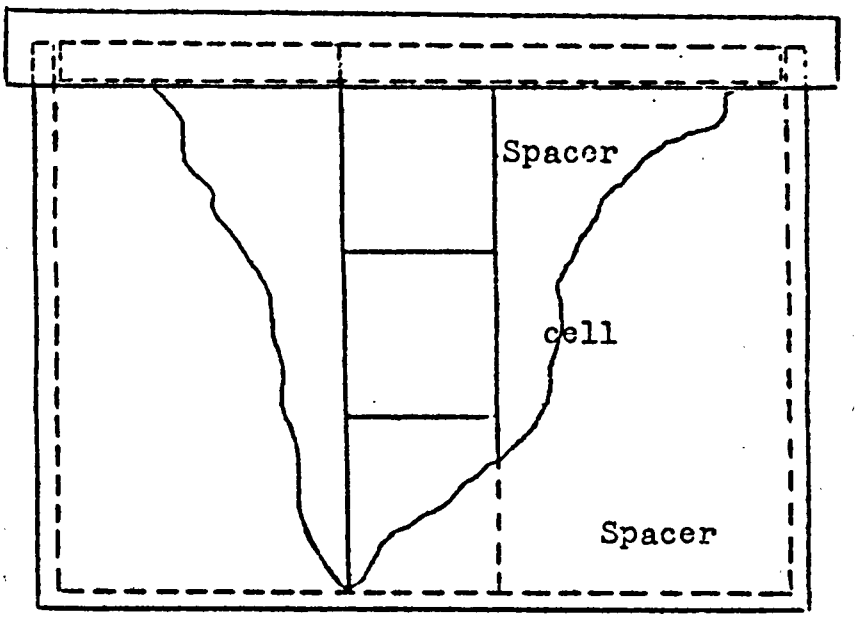
4-Methyl-7-hydroxycoumarin does change in fluorescent intensity on standing in alcohol as the following shows:

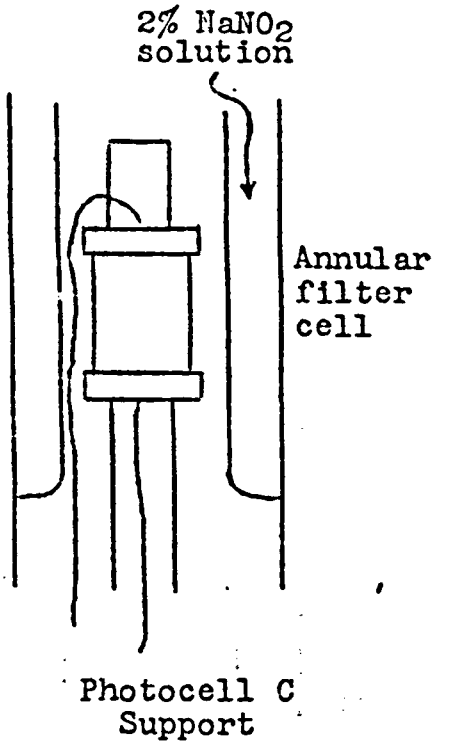
Compound in Alcohol	Initial Fluor. Int. (Arbirtary Units)	Final Fluor. Int.	Time (Hrs.)
4-Methyl-7-hydroxy-coumarin	360	165	2,280
3-Benzyl-4-Methyl-7-hydroxycoumarin	308	301	192

Either of the two compounds indicated is a satisfactory standard over a period of a few days. 3-Benzyl-4-Methyl-7-hydroxycoumarin under nitrogen in a sealed cell was used as a standard.

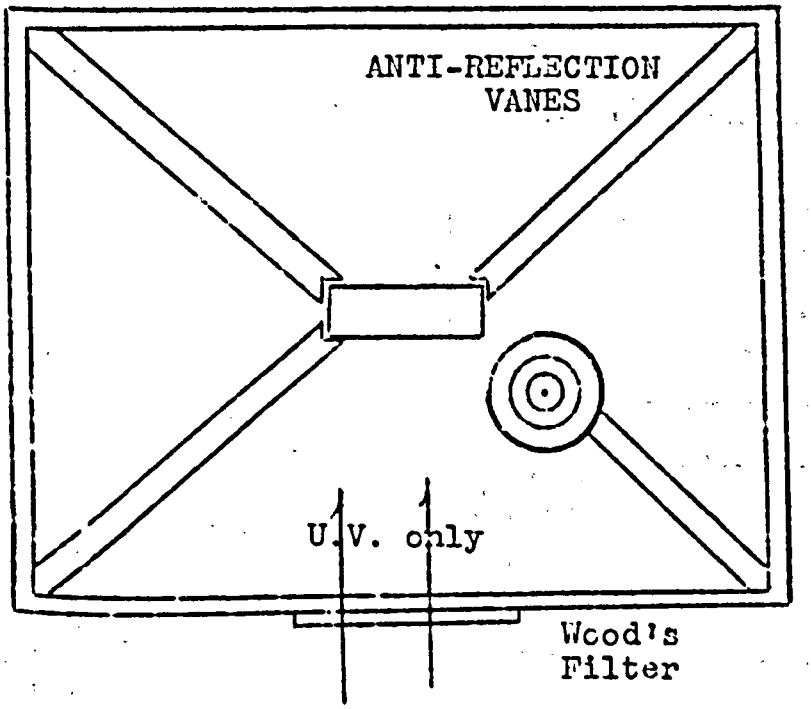
HORIZONTAL VIEW

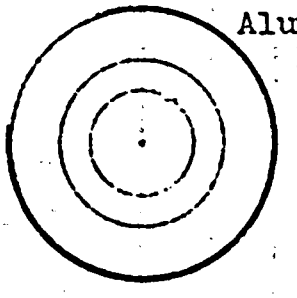
FLANGED LIGHT-TIGHT COVER



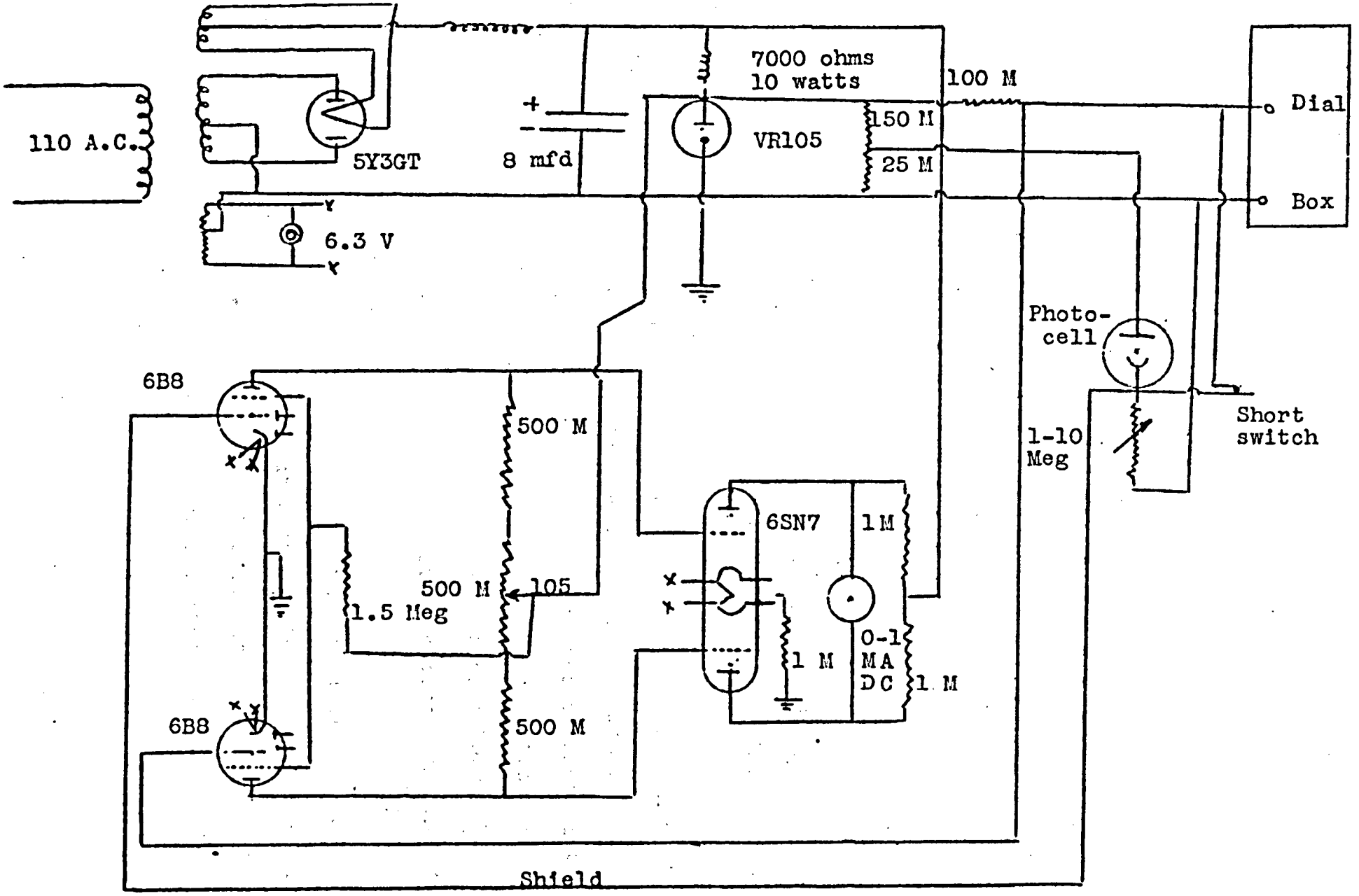


VERTICAL VIEW
ALL SURFACES BLACK





LIGHT-TIGHT BLACK BOX



AMPLIFIER CIRCUIT

Ultraviolet Absorption

The ultraviolet absorption curves for the series studied were plotted from data obtained from a Beckmann spectrophotometer.

Briefly, in each case a solution of the coumarin in pure alcohol was prepared. The instrument is so arranged that a light beam of constant intensity can be passed alternately through a set of matched cells (equal in length); one containing the solution the other alcohol. In this manner, an instrumental comparison of the intensity of the beam incident upon the coumarin molecules and the transmitted beam (incident less absorbed) is possible.

The law which applies to light absorption is:

$$E = kcd \log Io/I$$

Where E is called the extinction, Io the incident intensity, I the transmitted intensity, c the concentration of the solution, d its thickness, and k a constant characteristic of the absorbing molecules. Thus, E obtained directly from the instrument divided by the product of cell length by solution concentration is the specific extinction which is of general comparative value.

In a complete determination, the specific extinction of a compound is obtained at each wavelength. In the Beckmann instrument, the wavelength selector is located between the sample and the photocell rather than between the light source and the sample.

The curves, specific extinction versus wavelength, were used to obtain a comparative value of the energy absorbed by each compound.

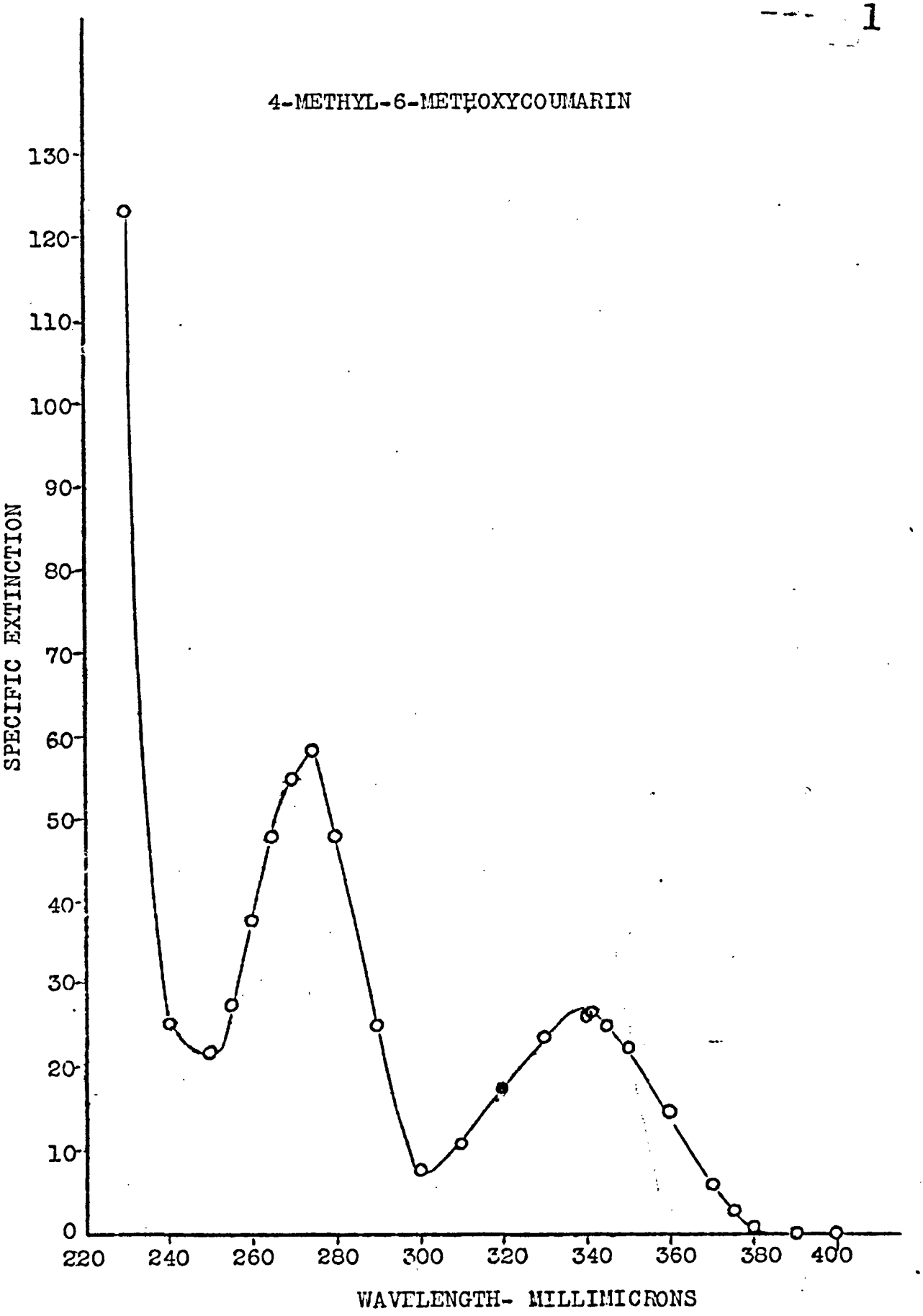
In the fluorescent intensity measurements, a Mazda B-H-4 lamp was used as a source of exciting radiation. From a photograph of its spectrum and tables, the frequency and intensity of each line could be determined. This photograph also included the spectrum of a sodium vapor lamp as reference.

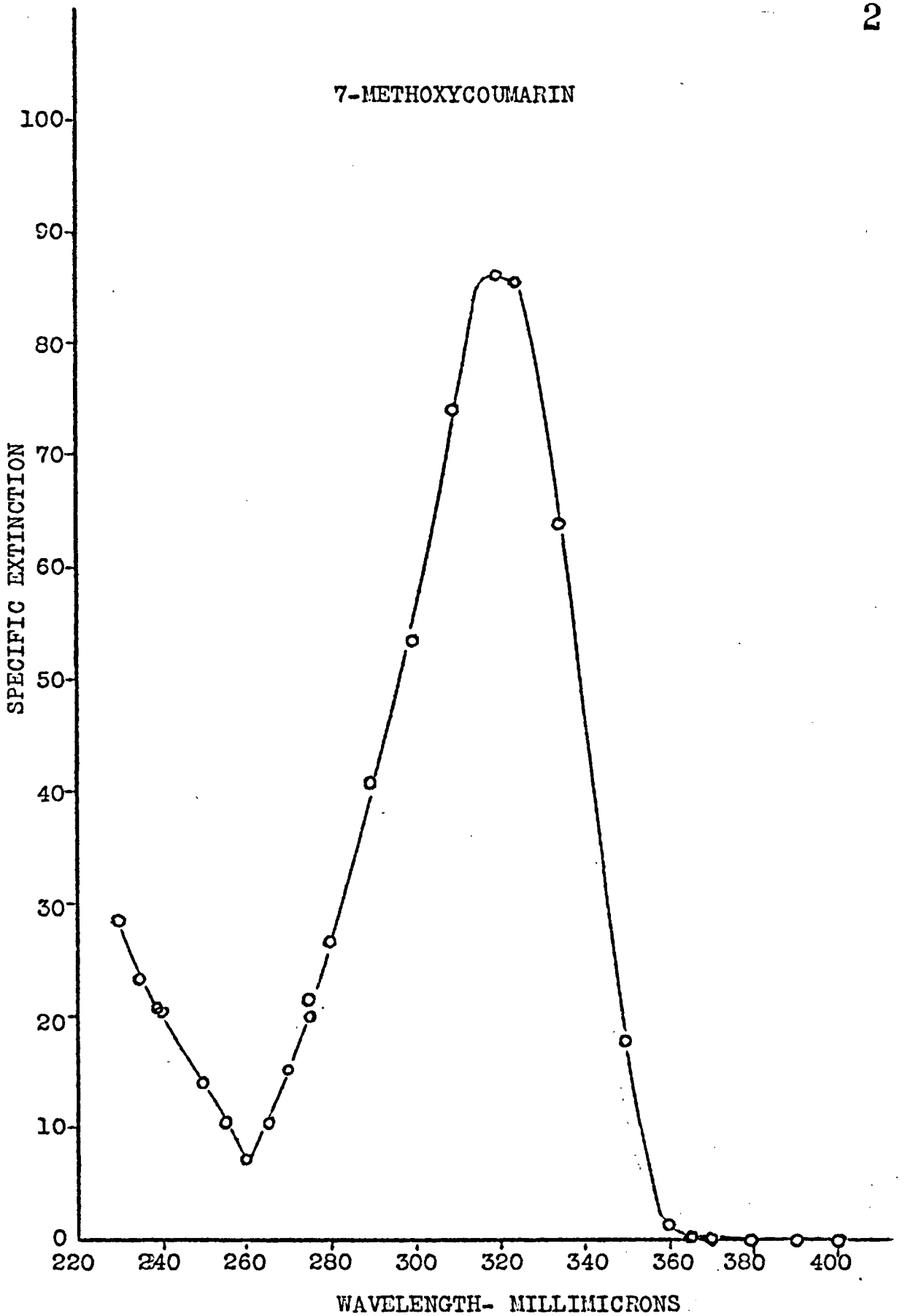
In turn, each line could be compared with the specific extinction versus wavelength curve for a selected compound; the specific extinction at that wavelength being a measure of its relative absorption. Then the product of frequency by relative absorption by relative intensity is a measure of the energy absorbed from the line by the compound. The sum of these, the relative activation being a measure of the energy absorbed from the exciting radiation.

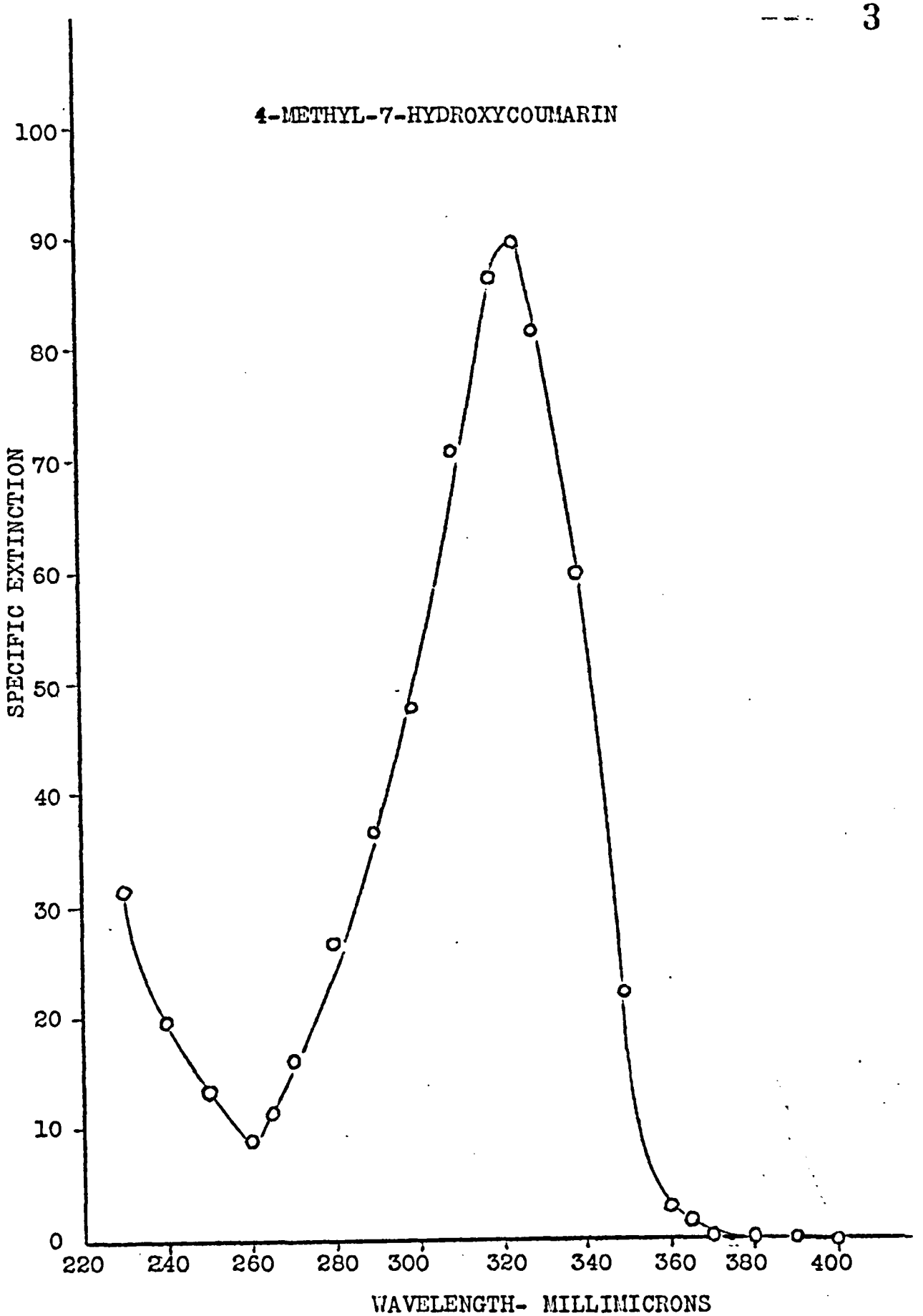
A tabulation of the lines of the spectrum of a Mazda B-H-4 lamp follow together with the ultraviolet absorption curves of the series studied.

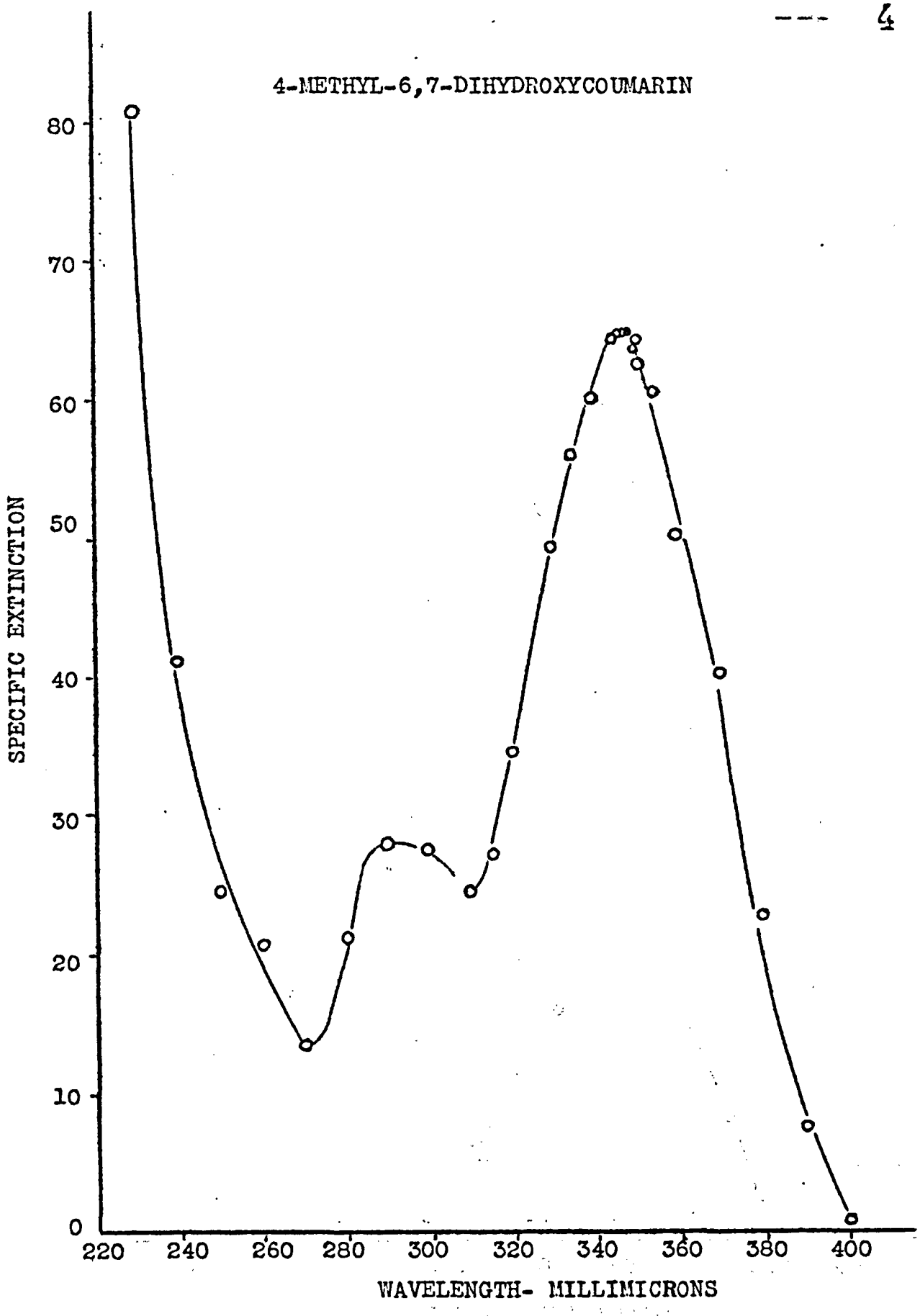
Ultraviolet Spectrum of Mazda B-H-4 Lamp

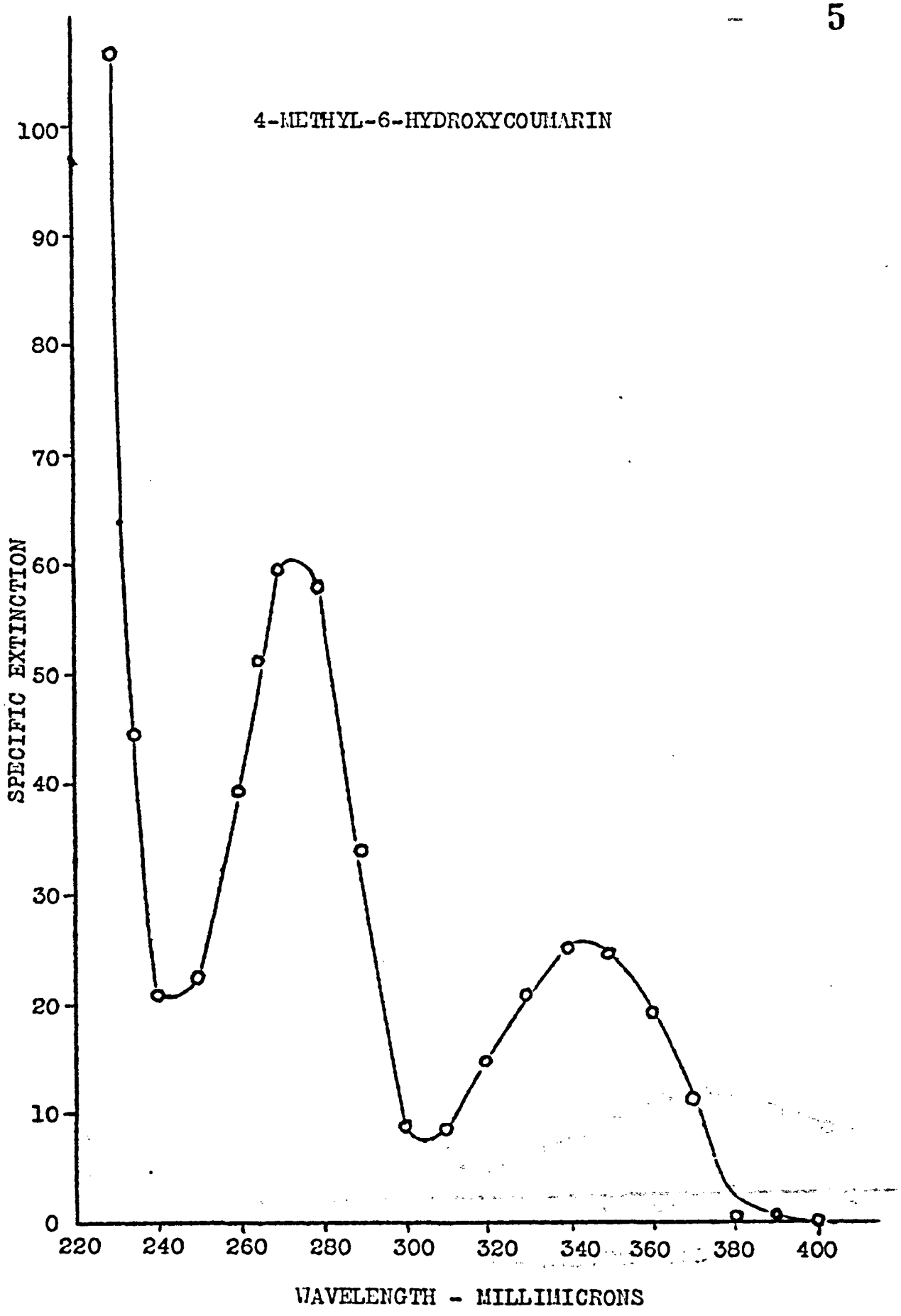
Wavelength	Frequency x 10^{-21}	Rel. Intensity of Line
2967.28	1.011	5
3021.5	.993	5
3125.6	.960	8
3131.56	.958	7
3131.84	.956	7
3341.48	.898	6
3380	.862	1
3530	.851	1
3550	.846	1
3650.15	.822	10
3654.83	.821	7
3662.87	.819	4
3663.27	.819	6
3730	.804	1
3770	.796	3
3800	.789	2
3840	.782	2
3906.4	.768	6

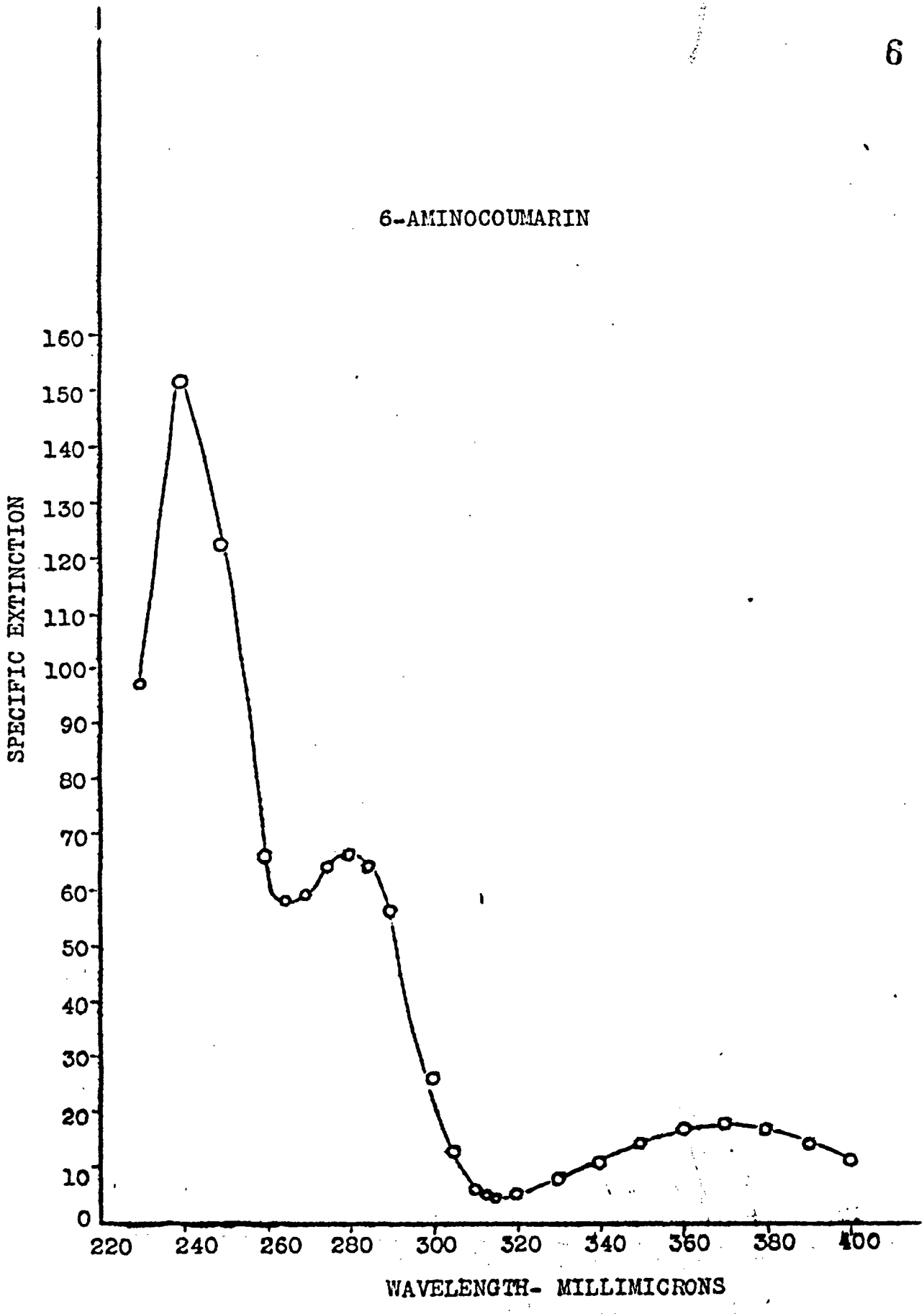




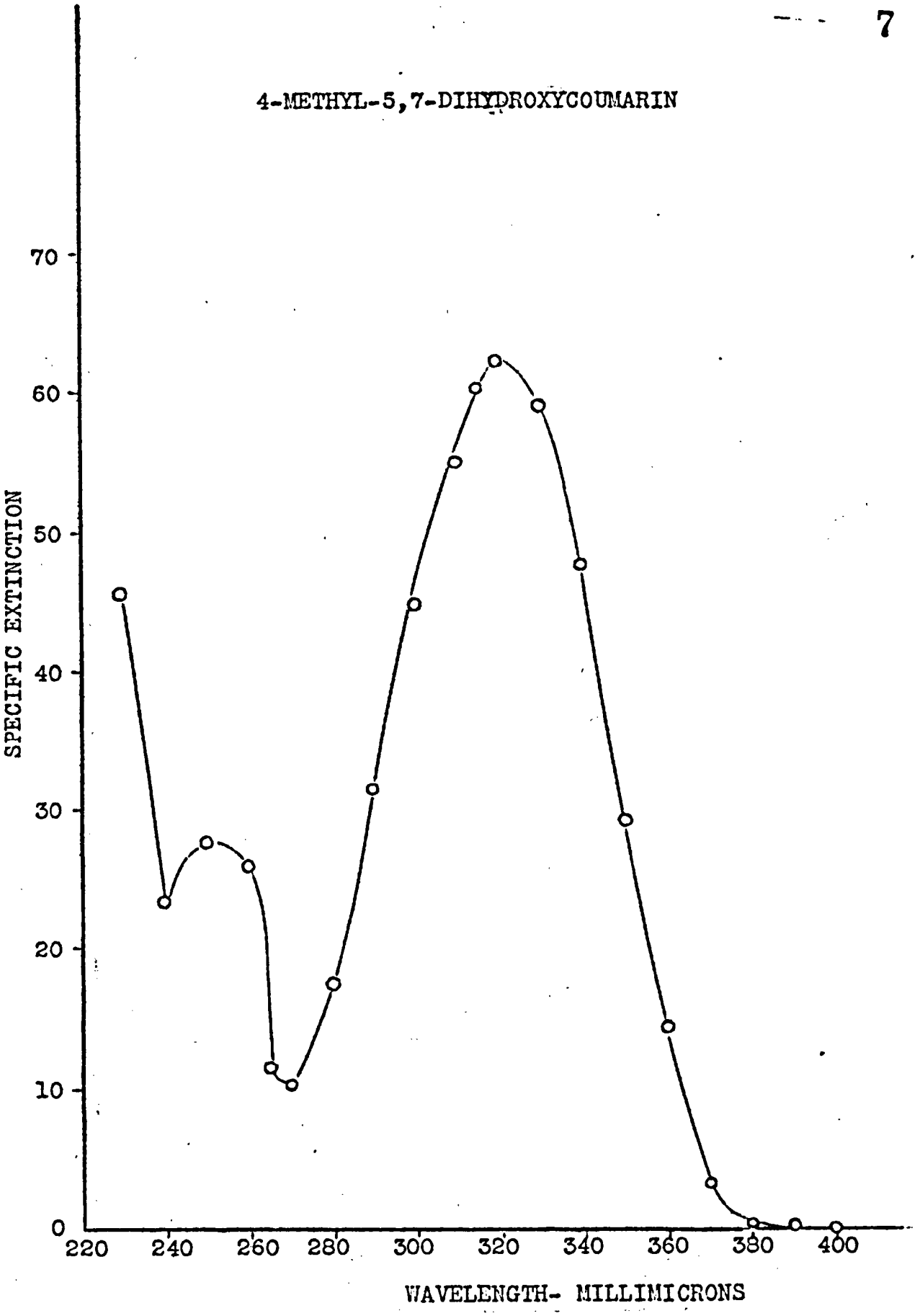


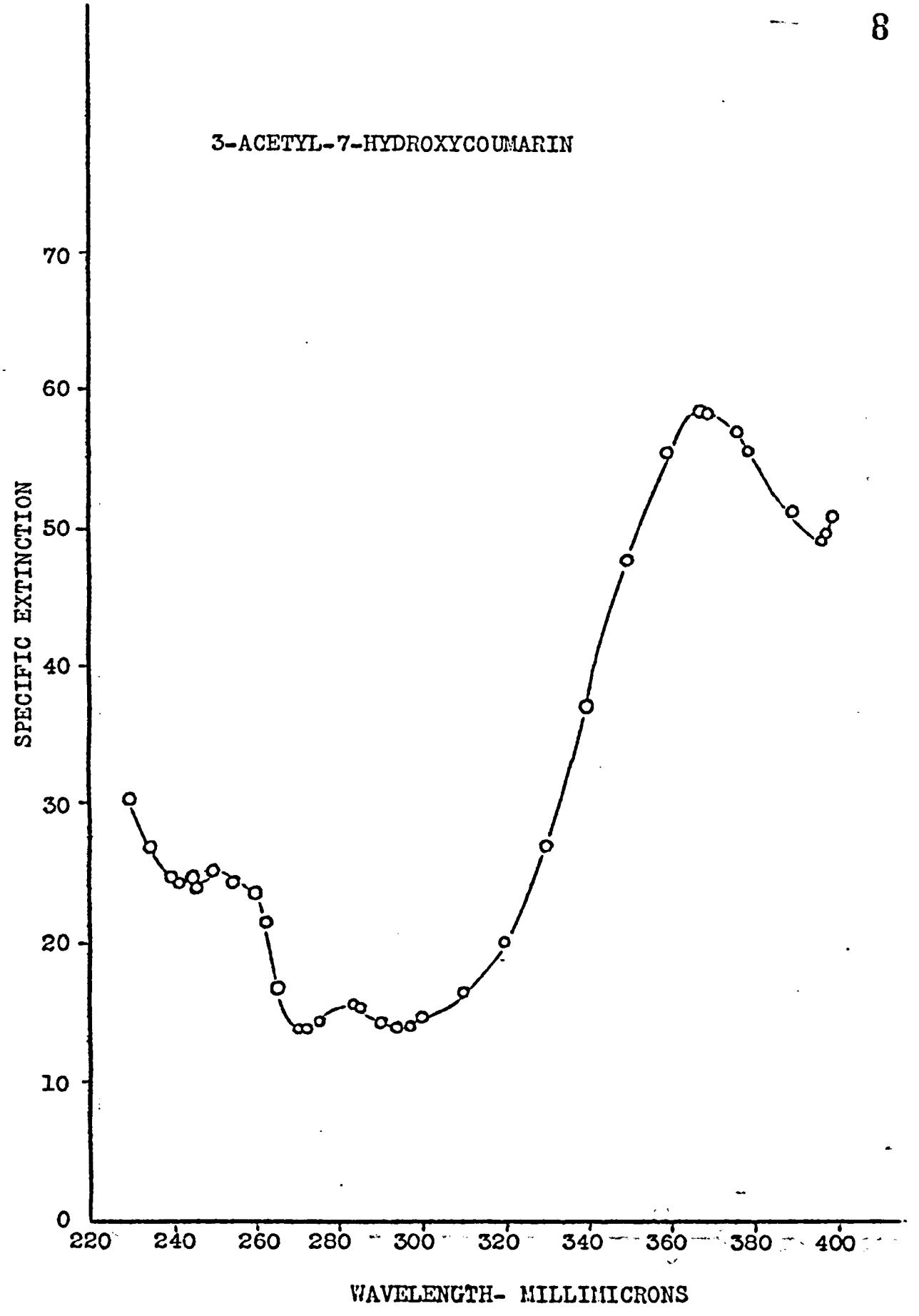


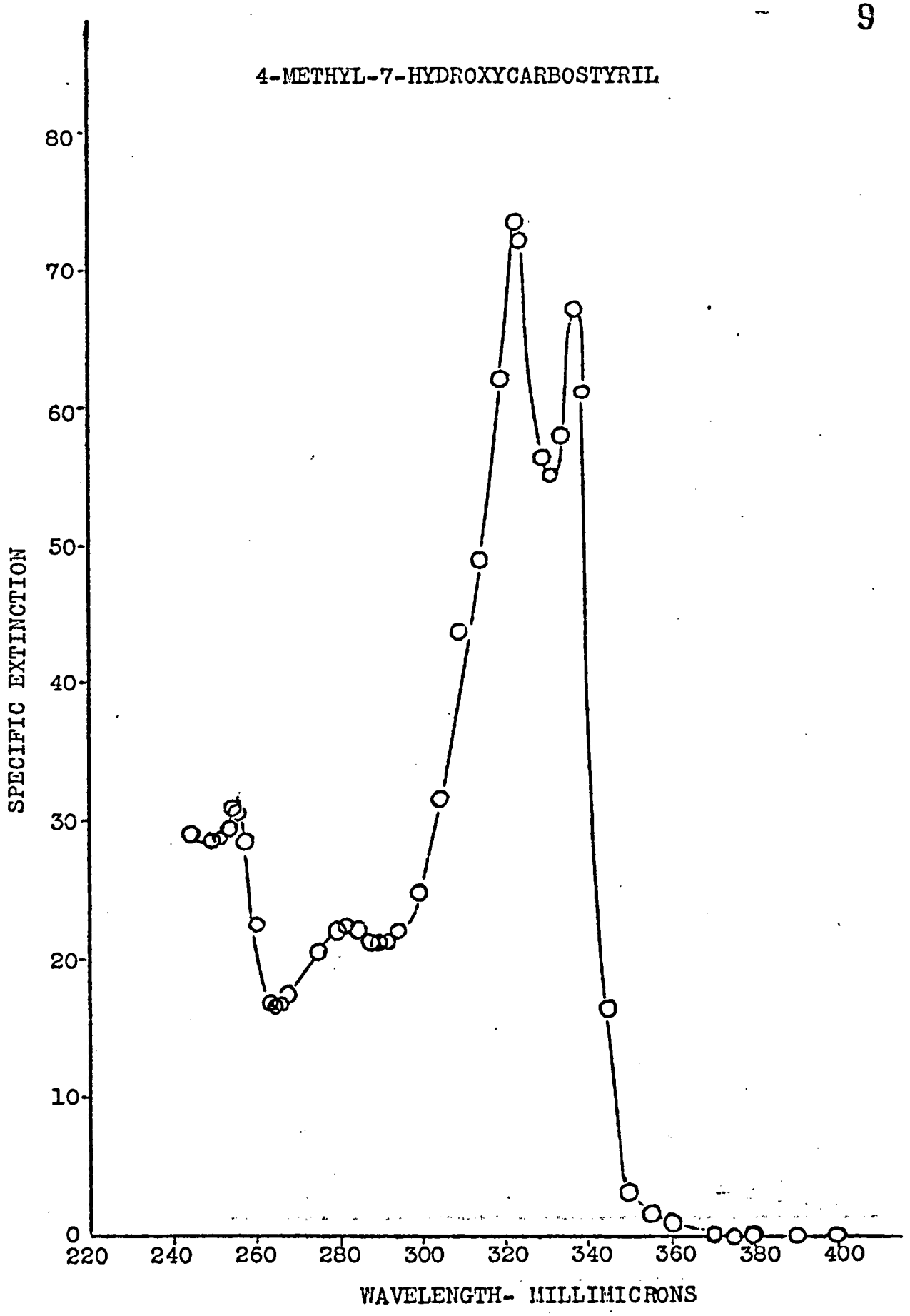


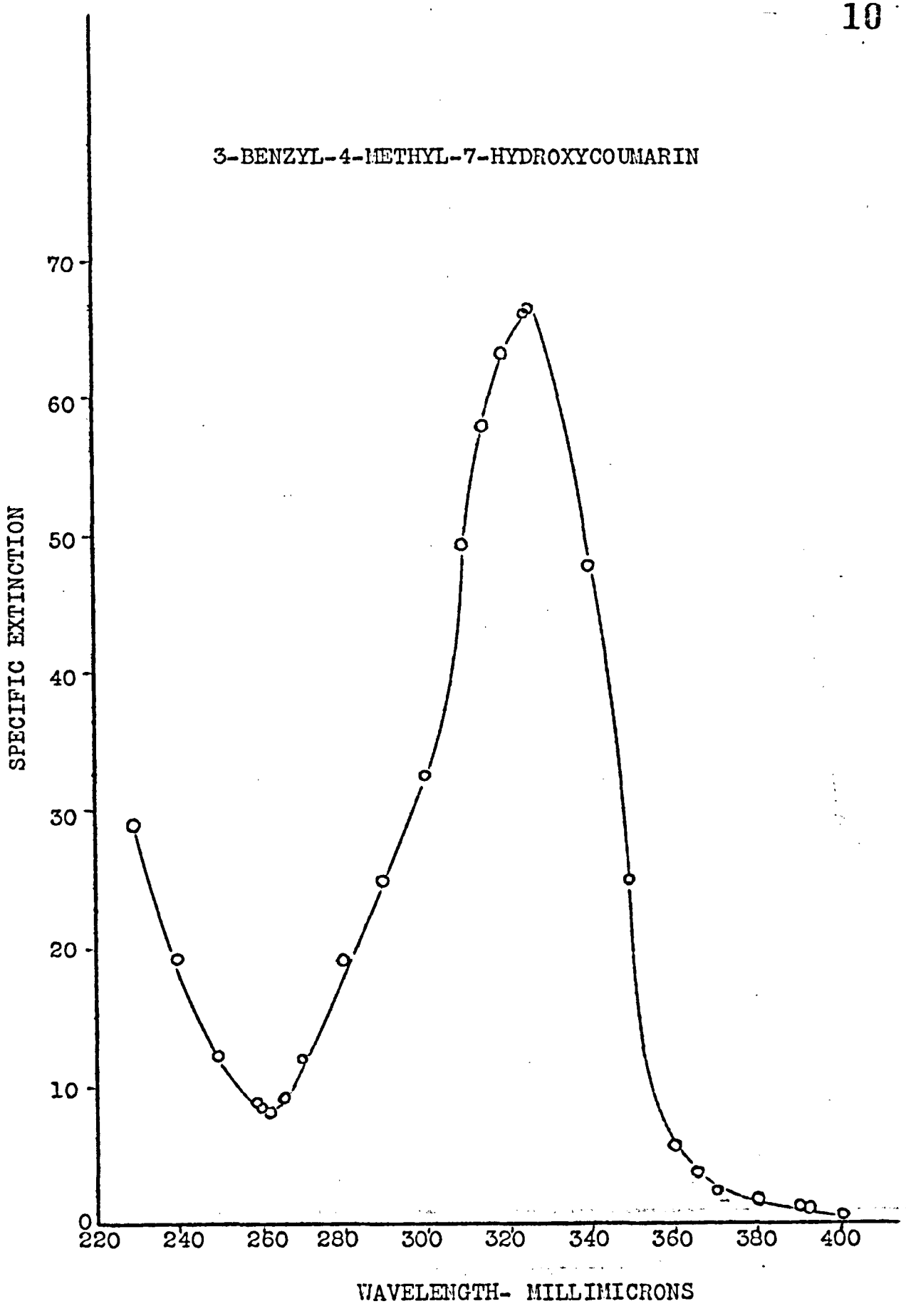


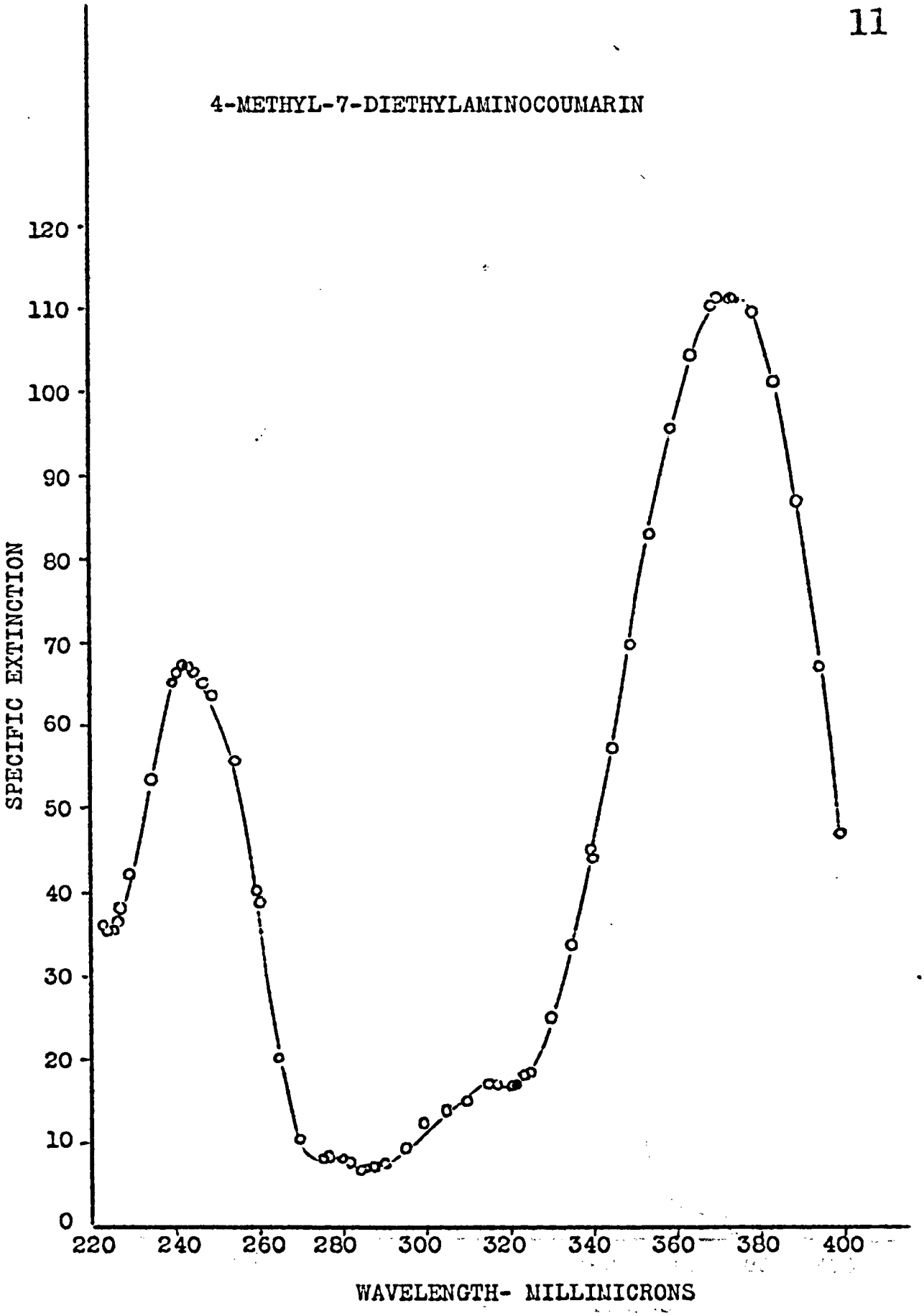
4-METHYL-5,7-DIHYDROXYCOUMARIN











decreased fluorescent intensity may be due to impurity. 3-Chloro-4-methyl-7-hydroxycoumarin (curve 15) shows a more intense fluorescence than 4-Methyl-7-hydroxycoumarin (curve 3) which agrees with the observation of Chakravarti .

Ultraviolet Absorption:

There is no known manner in which knowledge of the absorption characteristics of an organic molecule can be used to foretell fluorescence. In fact, Pringsheim has stated without reservation that there is no relation between the fluorescence and the ultraviolet absorption of a molecule other than the necessity that a molecule must be excited before it can fluoresce[1].

As stated by Knoblauch, the fluorescent intensity is proportional to the intensity of the exciting radiation[12]. As a corollary, the fluorescent intensity of a luminescent molecule should be related to its energy absorption.

In the preceeding section the curves Fluorescent Intensity in arbitrary units versus Concentration are given. A number of them show a maximum fluorescent intensity at some particular concentration. The specific fluorescent intensity, fluorescent intensity divided by the corresponding concentration, at this point seems a likely value for comparison of one fluorescer with another.

A Mazda B-H-4 black ray lamp was used as a source of exciting radiation in the above mentioned fluorescent

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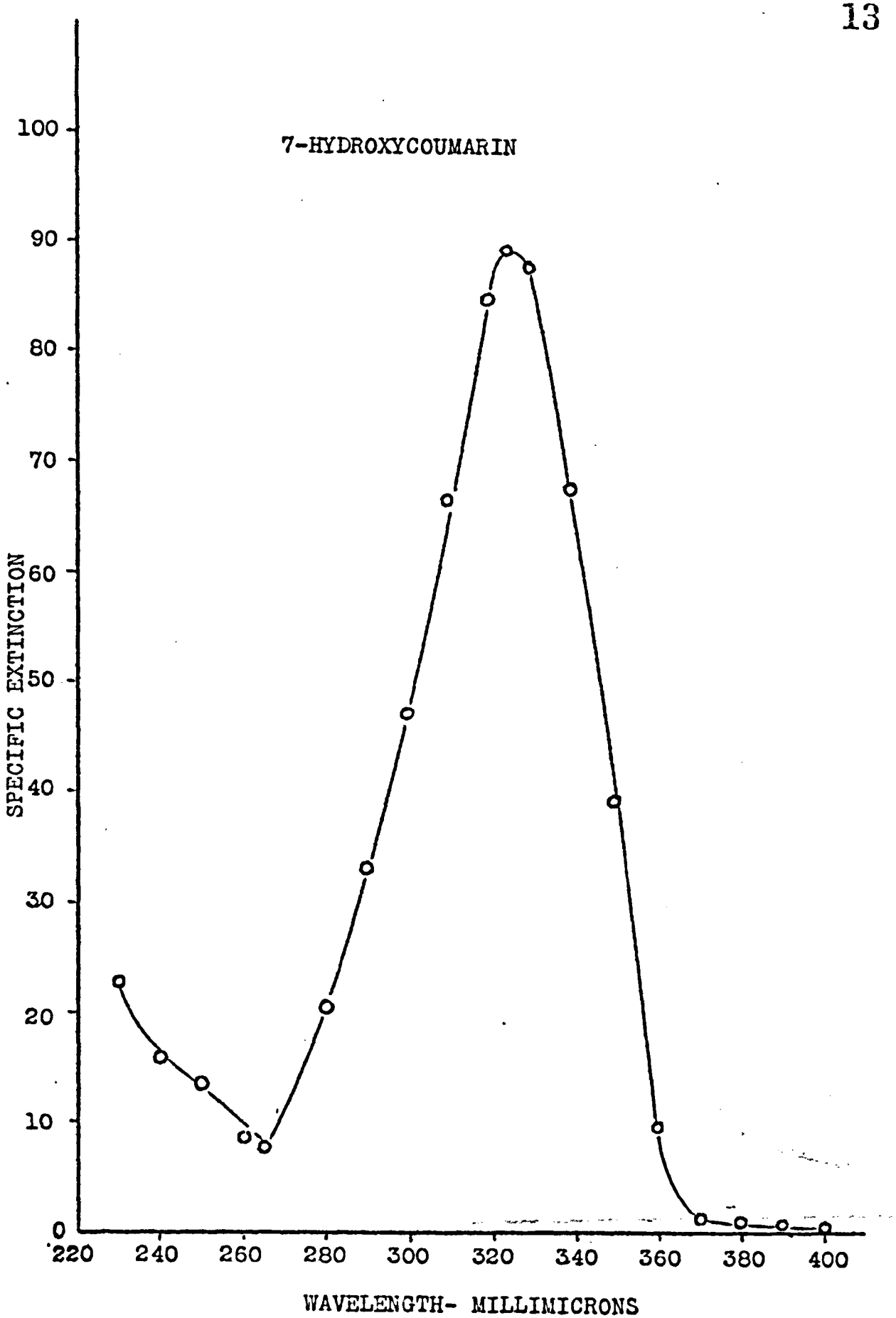
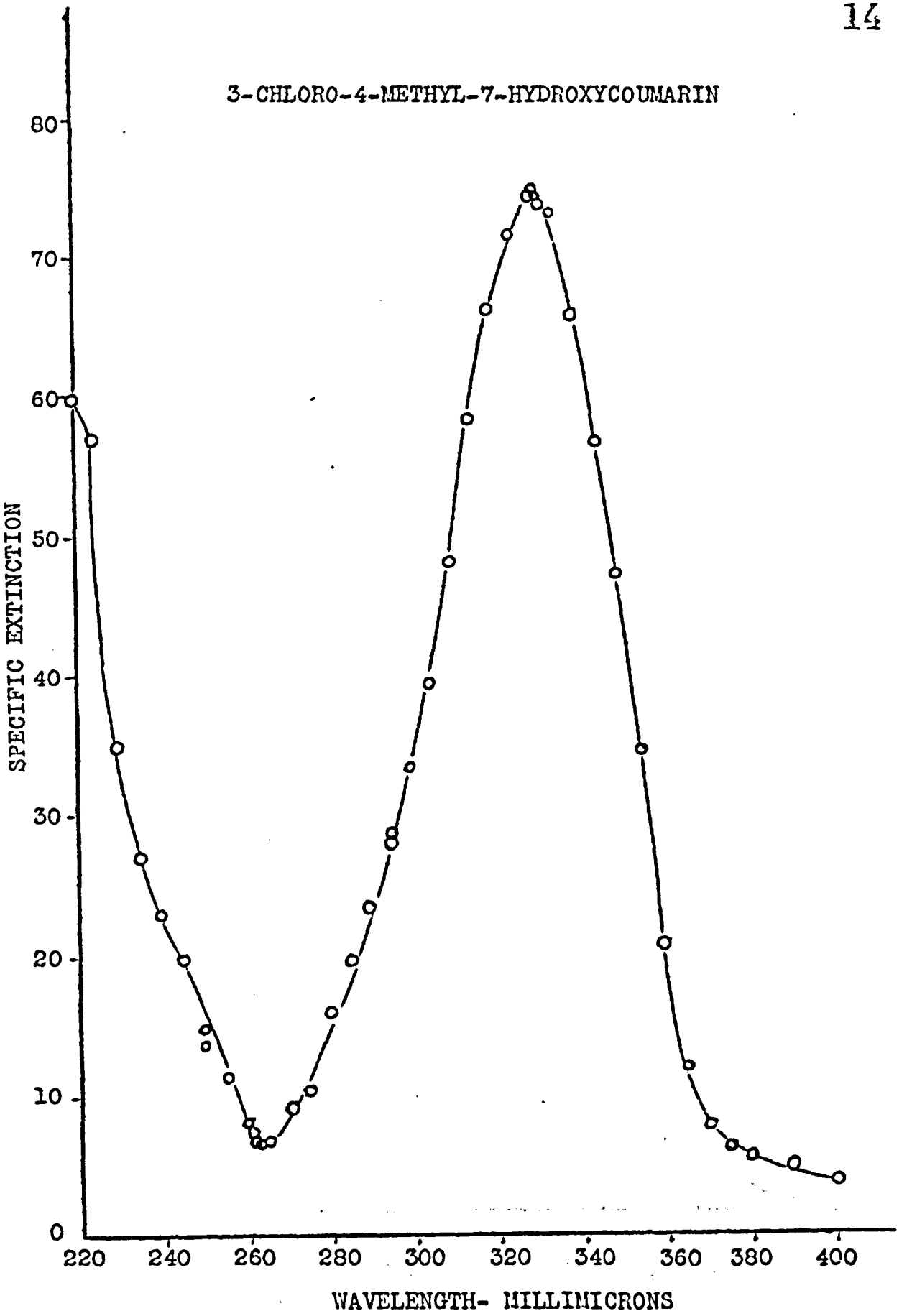
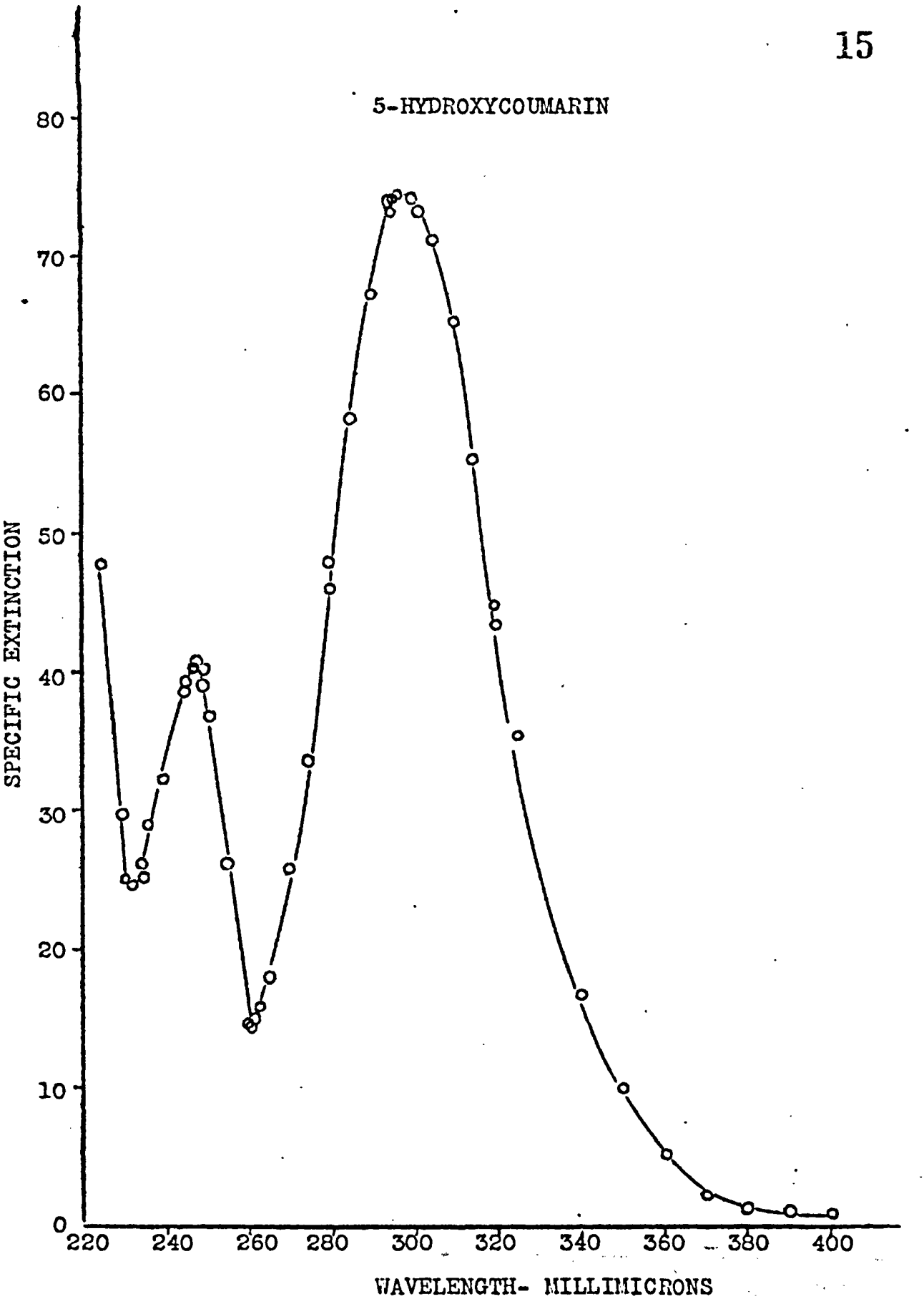
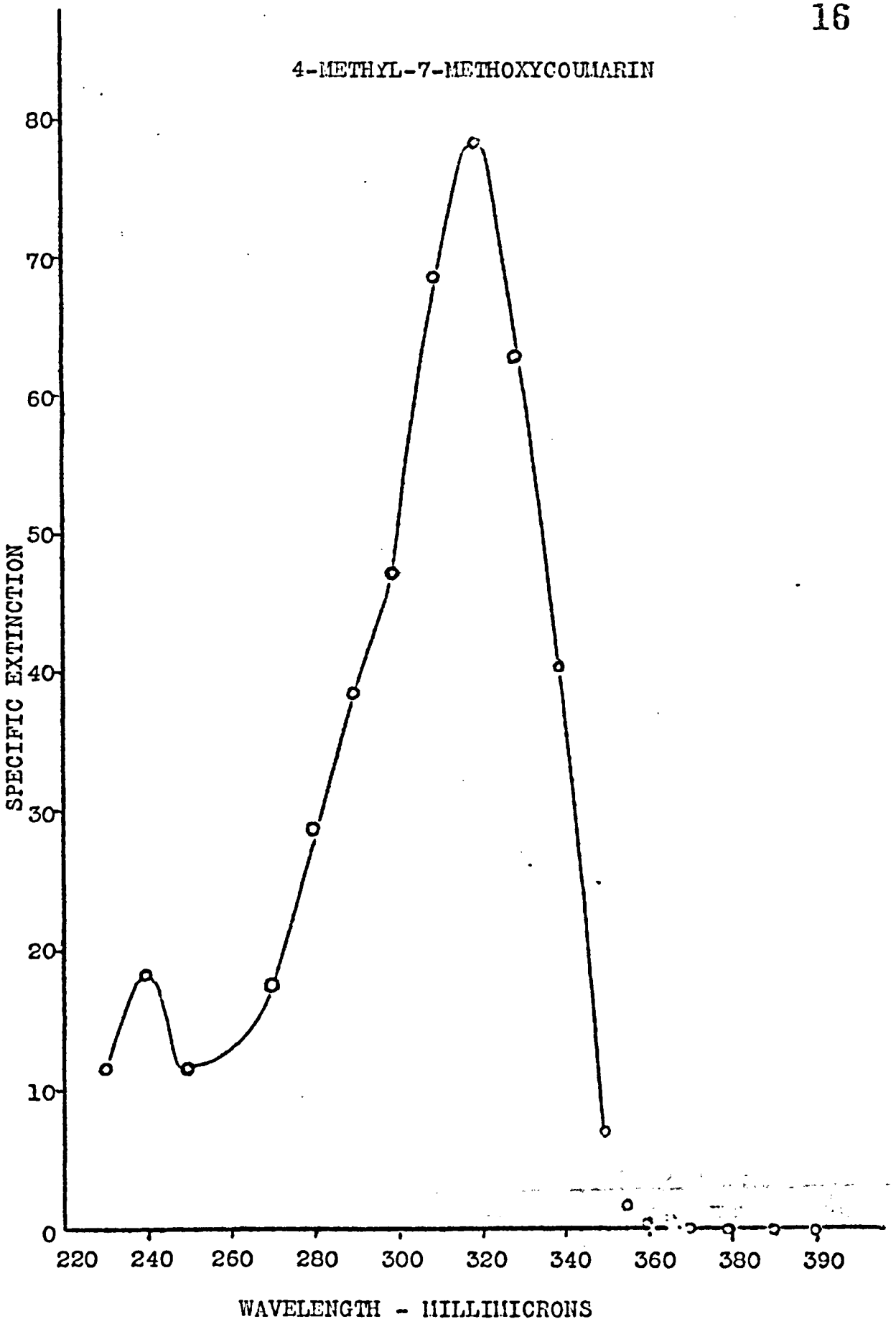
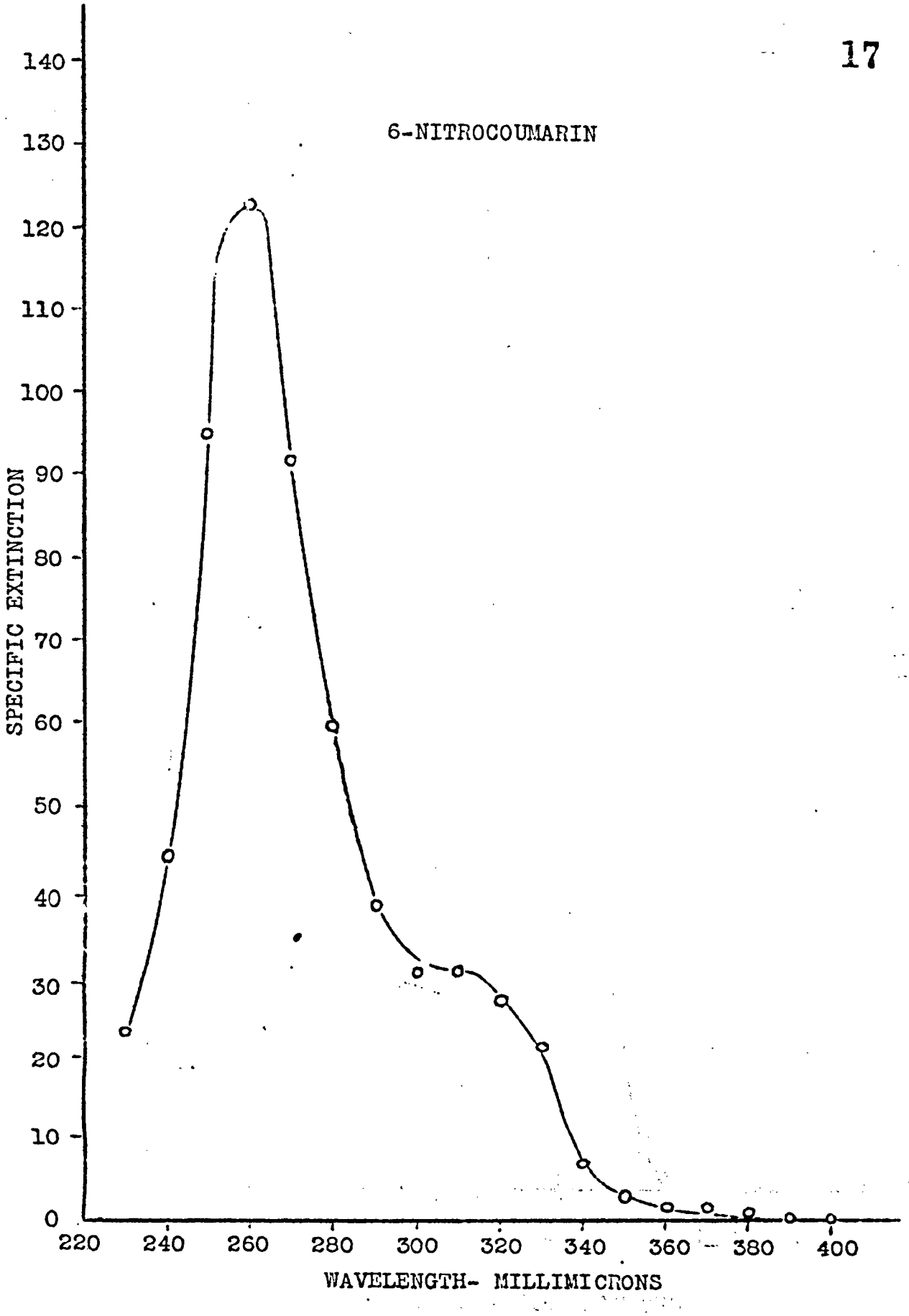
(12) O. Knoblauch: Ann. Physik 54, 193 (1895)



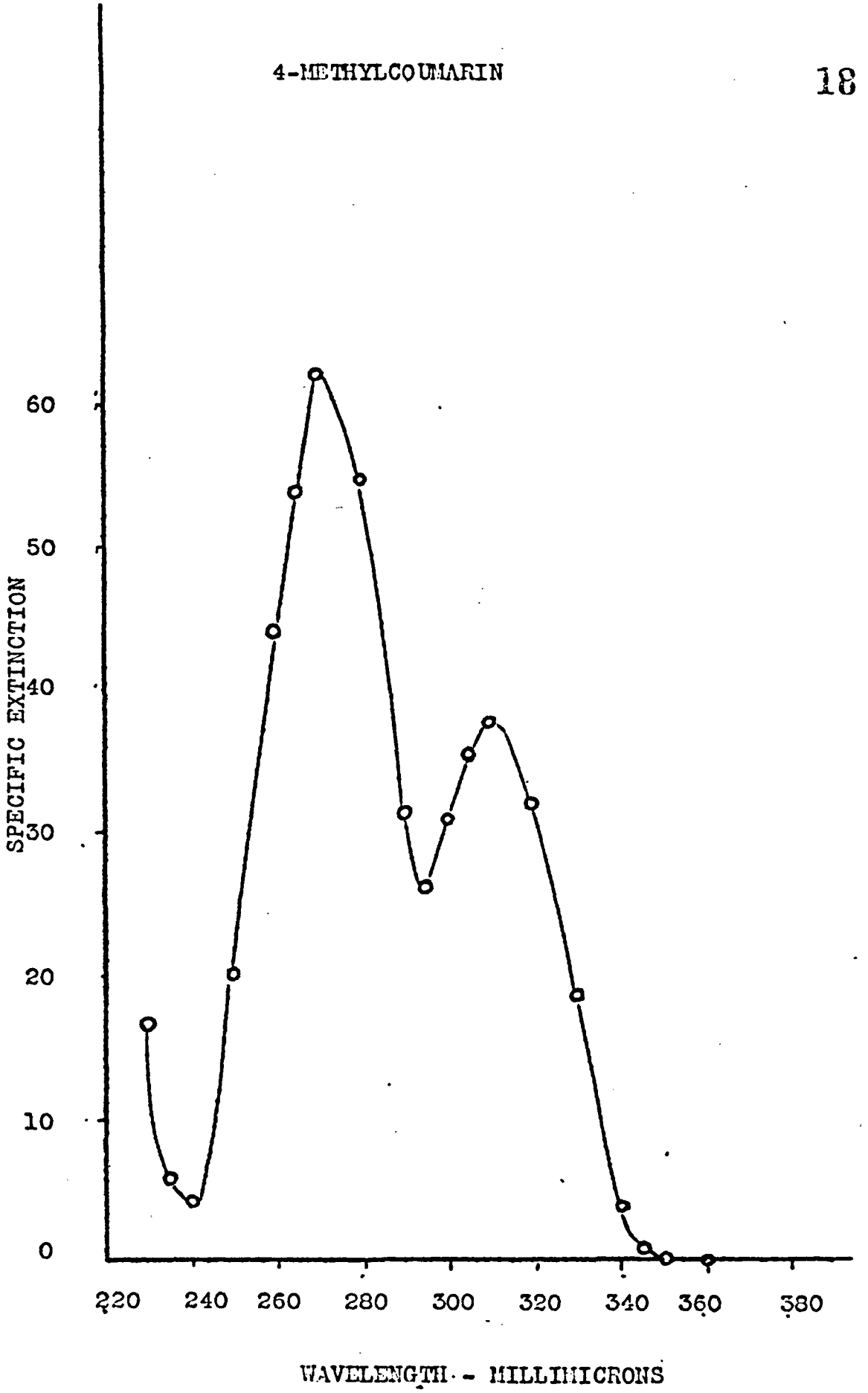








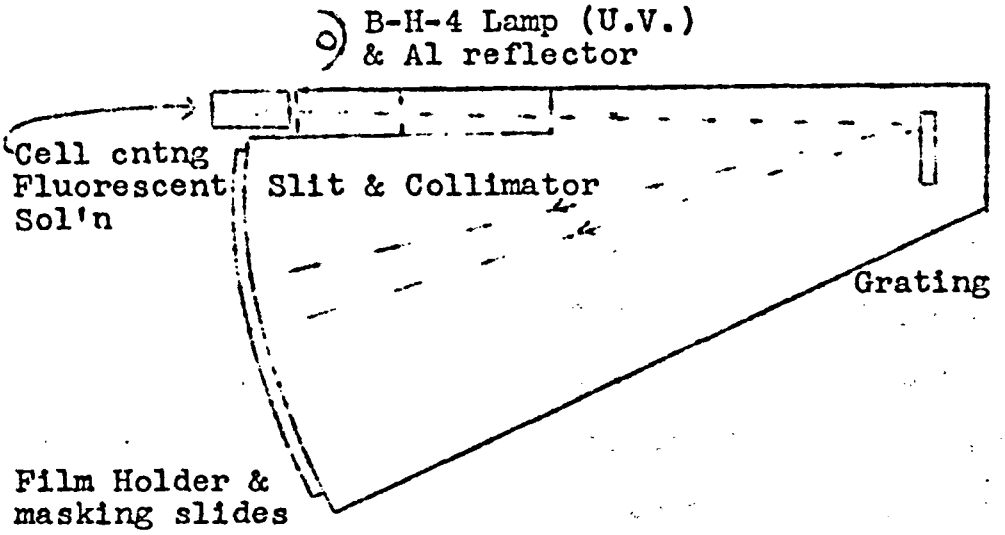
4-METHYLCOUMARIN



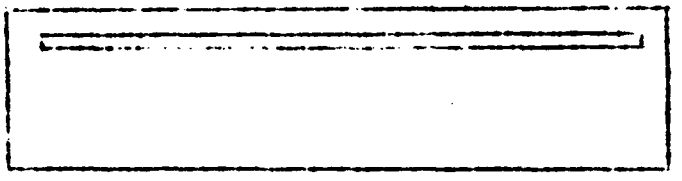
Fluorescent Spectra

A Cenco Grating Spectrograph was used to obtain photographs of fluorescent spectra where possible.

CENCO GRATING SPECTROGRAPH
(Top view)



FILM HOLDER



Strip of Film Exposed
by Masking Slide

Usually, about four spectra were printed on each film. As reference, the spectrum of the exciting lamp (1 min. exposure) and that of a sodium vapor lamp (15 min. exposure) were taken. Then, two exposures to the fluorescence were obtained, one of about 3 hours; the other of about 20 hours.

The fluorescence exposures were taken as follows: A solution of the fluoresoer (.3 g/100 ml.) in pure alcohol in a square glass cell was placed against the widened slit of the spectrograph. A Mazda B-H-4 lamp equipped with an aluminum reflector was placed as close as possible to the solution of the fluoresoer without placing the lamp in such a position that light from it could reach the slit either directly or by reflection from a glass surface.

To aid in the study of the photographs of fluorescence spectra, a scale was made as follows: A photo of the B-H-4 spectrum and the sodium vapor lamp spectrum was made. The position of each line was determined by use of a hand spectroscope and tables. The spectrograph is designed so that the wavelength of a line is linearly related to its distance from a point of reference.

In each case where a print of the fluorescence spectrum was obtained, the band edges and maximum were estimated and their positions determined by use of the scale.

Fluorescence Stability in Alkali

Alcohol which had been refluxed over potassium hydroxide was distilled under nitrogen into a flask con-

taining alcohol washed potassium hydroxide pellets.

After becoming saturated at room temperature and clearing, a portion (100 ml.) of this solution was placed in a constant temperature bath and allowed to come to a temperature of 56°C.

The details of the bath are described in the accompanying sketch. The alkaline solution was kept at 56°C in a vessel suspended in acetone vapor.

To the alkaline solution under nitrogen was added a portion (30 ml.) of fluorescer (.3 g/100 ml.) in aldehyde free alcohol. The mixture was stirred by bubbling nitrogen through and then a sample pipetteful (9.054 ml./pipette) was removed, placed in a glass cell in a nitrogen atmosphere and diluted with aldehyde free alcohol. The bath chamber was filled with nitrogen and closed.

In the usual manner, by alternation in the fluorometer of sample and standard and adjustment of the average values, the fluorescent intensity in arbitrary units of the sample was obtained. Exposure to the atmosphere was minimized by a nitrogen atmosphere.

At intervals of time, the indicated procedure was repeated. Of course, in all measurements of fluorescent intensity, all vessels must be scrupulously clean for traces of impurities may have a profound effect on fluorescent intensity.

In each case, fluorescent intensity in arbitrary units was plotted against time in hours. The complexity of the

degradation process does not permit any but a qualitative comparison of the effect of structure on the durability of fluorescence in alkali.