86.5°); $n^{25}D$ 1.4535. Two hundred milligrams of palmitoleic acid in 95% ethanol was hydrogenated at atmospheric pressure in the presence of 10 mg. of platinum oxide (Adams catalyst). In 15 minutes the adsorption of hydrogen was complete and 17.0 ml. of hydrogen (corrected for catalyst) had been consumed (calculated for reduction of a single double bond fatty acid, 17.5 ml. hydrogen). The catalyst was removed by centrifugation and washed once with warm 95% ethanol. The alcohol soluble fractions were combined and concentrated to dryness *in vacuo* at 45°. The saturated acid was recrystallized once from warm 60% ethanol and once from 10% aqueous acetone; m.p. 60–61°; no depression on admixture with pure palmitic acid; neutral equivalent: calcd., 256.4, found, 255.8.

(b) **Glycerophosphate.**—The water-soluble fraction and the washings from the fatty acid isolation were combined and the glycerophosphate was isolated by the method of Folch.[16] Barium glycerophosphate: calcd., 411.7 mg., found, 345.0 mg.; 84% recovery. *Anal.* Calcd. for $C_3H_7O_6PBa$ (307.3): C, 11.73; P, 10.1; glycerol, 29.3. Found: C, 11.6; P, 10.1; glycerol,[17] 28.0. Over 99% of the water-soluble phosphorus assayed as glycerophosphate by the peroxidative method.[18]

(**Dipalmitoyl**)-L-α-**lecithin.**—One gram of dipalmitoleylglycerylphosphorylcholine in 95% ethanol was hydrogenated in the presence of 100 mg. of platinum oxide (Adams catalyst). In 20 minutes the uptake of hydrogen was complete and 58.5 ml. of hydrogen (cor. for catalyst) had been consumed (calculated for the lecithin, 59.6 ml. of hydrogen). The catalyst was removed by centrifugation and washed once with warm ethanol. The filtrates were combined and concentrated to dryness at 40° under reduced pressure in a nitrogen atmosphere. The residue was dissolved in a minimum amount of warm dioxane (60°) and upon slow cooling to room temperature, crystallization occurred. This substance was recrystallized twice more from warm dioxane, and once from diisobutyl ketone. The dipalmitoyllecithin was dried for two days at room temperature over P_2O_5 and paraffin shavings, yield 850 mg. (85% of theory).

This compound formed transparent droplets at 89–92° and upon further heating (25°/min. to 210°, then at 10°/min.), formed a meniscus at 234.8–235.0°; $[\alpha]^{25}D$ +6.62 in chloroform–methanol (1:1), *c*; $[M]_D$ +49.8; reported by Baer and Kates[5] as $[\alpha]^{23}D$ +6.6 in $CHCl_3$–methanol (1:1).

Anal.[19] Calcd. for $C_{40}H_{82}O_9NP$ (752): C, 63.9; H, 11.0; N, 1.86; P, 4.12. Found: C, 63.7; H, 11.06; N, 1.89 (Dumas), 1.82 (Kjeldahl); P, 4.15.

Five hundred milligrams of this saturated lecithin was hydrolyzed and the fatty acid isolated in a manner similar to that described for the unsaturated lecithin; yield of palmitic acid, calcd., 340 mg.; found, 330 mg. (97% of theory). The analytical composition, melting point and neutral equivalent were in excellent agreement with pure palmitic acid and with the palmitic acid isolated by hydrogenation of palmitoleic acid.

An X-ray diffraction powder pattern of the hydrogenated lecithin was made and compared with that reported for synthetic (dipalmitoyl)-L-α-lecithin.[20] The spacings of the yeast lecithin were in excellent agreement with the synthetic compound. No extraneous lines were observed.

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(19) Microanalyses were performed by Elek Micro Analytical Laboratories, 4763 West Adams Boulevard, Los Angeles, California.
(20) We are indebted to Dr. L. H. Jensen for the X-ray analysis.

SEATTLE, WASHINGTON

[CONTRIBUTION FROM THE McARDLE MEMORIAL LABORATORY, UNIVERSITY OF WISCONSIN, THE DIVISION OF PURE CHEMISTRY, NATIONAL RESEARCH COUNCIL OF CANADA, AND THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF ALBERTA[1]]

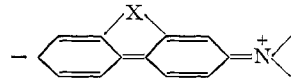
Ultraviolet Spectra and Carcinogenic Activities of Some Fluorene and Biphenyl Derivatives

BY REUBEN B. SANDIN, RUSSELL MELBY, ALLAN S. HAY, R. NORMAN JONES, ELIZABETH C. MILLER AND JAMES A. MILLER

RECEIVED JUNE 2, 1952

The ultraviolet absorption spectra of the following compounds have been determined, *viz.*, 4,4′-dinitrobiphenyl, 2,7-dinitrofluorene, 4-amino-4′-nitrobiphenyl, 2-amino-7-nitrofluorene, 4-acetylaminobiphenyl, 2-acetylaminofluorene and 2- and 2′-methyl-4-acetylaminobiphenyl. Each fluorene compound absorbs at longer wave length than its biphenyl analog without the methylene bridge, whereas the introduction of a methyl group into biphenyl has the opposite effect. The carcinogenic activities of the last four compounds have been determined and these activities have been correlated with the corresponding absorption spectra.

The interesting work of Haddow, Harris, Kon and Roe[2] on the growth-inhibitory and carcinogenic properties of 4-aminostilbene and derivatives has indicated that one of the features necessary for activity is an unbroken conjugation of the amino group with both nuclei, enabling the compound to assume a resonating quinonoid structure. Evidence, which was mainly obtained from ultraviolet spectroscopy, suggested that there was a close parallel between lack of biological activity and steric interference with the planar arrangement of the molecule. Recently Miller, Miller, Sandin and Brown[3] have considered it possible that the greater carcinogenic activity of derivatives of fluorene and its oxygen and sulfur analogs *versus* biphenyl derivatives is associated with the $-CH_2-$, $-S-$ and $-O-$ groups which help maintain a coplanar arrangement of the benzene nuclei. The molecule would then be represented to a greater extent by the resonating structures such as



X = CH_2, O, S

In this paper the absorption spectra of several pairs of biphenyl and fluorene derivatives have been compared in order to determine the effect of the methylene and methyl groups. These compounds include 4,4′-dinitrobiphenyl, 2,7-dinitrofluorene, 4-amino-4′-nitrobiphenyl, 2-amino-7-nitrofluorene, 2-acetylaminofluorene, 4-acetylaminobiphenyl and 2- and 2′-methyl-4-acetyl-

(1) Published as Contribution No. 2820 from the Laboratories of the National Research Council of Canada.
(2) A. Haddow, R. J. C. Harris, G. A. R. Kon and E. M. F. Roe, *Trans. Roy. Soc. (London)*, **A241**, 147 (1948).
(3) E. C. Miller, J. A. Miller, R. B. Sandin and R. K. Brown, *Cancer Research*, **9**, 504 (1949).

aminobiphenyl. The absorption spectra of the last four compounds have also been correlated with their carcinogenic activities for the rat.

One of us[4] has called attention to the fact that ortho-substituted methylene and dimethylene bridges such as are found in 9,10-dihydrophenanthrene and 4,5-methylene-9,10-dihydrophenanthrene hold the two aromatic rings in or near a coplanar configuration and so emphasize the biphenyl type of spectrum. The absorptions of 4,4′-dinitrobiphenyl and 4-amino-4′-nitrobiphenyl have been reported by Sherwood and Calvin[5] and their results are in agreement with the present work.[6]

Experimental

The preparation of 4,4′-dinitrobiphenyl,[7] 4-amino-4′-nitrobiphenyl,[4] 2,7-dinitrofluorene,[8] 2-amino-7-nitrofluorene,[9] 2′-methyl-4-acetylaminobiphenyl,[10] 2-methyl-4-acetylaminobiphenyl,[10] 4-acetylaminobiphenyl, m.p. 171°, and 2-acetylaminofluorene, m.p. 191°, have been described previously.

The ultraviolet absorption spectra were measured on a Cary spectrophotometer.

Results and Discussion

Ultraviolet Absorption Spectra.—The spectra are shown in Figs. 1–3 and the positions and intensities of the absorption maxima are summarized in Table I. Comparison of the spectra of corresponding pairs of biphenyl and fluorene compounds shows that the presence of the methylene bridge between the two aromatic rings induces a significant shift of the absorption to longer wave length; in 2-acetylaminofluorene the band shows some fine structure but this is not observed in the spectra of any of the other fluorene derivatives. In the spectra of 2-methyl- and 2′-methyl-4-acetylaminobiphenyl shown in Fig. 3 the absorption is displaced to shorter wave length by the methyl substituent, both the 2-methyl and 2′-methyl groups having a very similar effect.

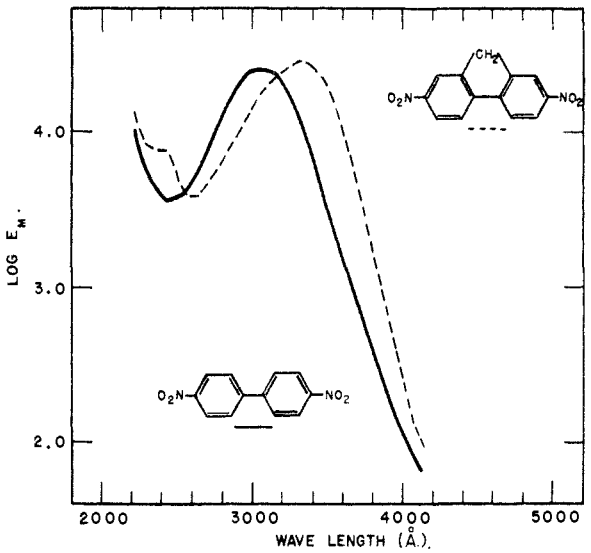


Fig. 1.

TABLE I

POSITIONS AND INTENSITIES OF THE ABSORPTION MAXIMA

Compound[a]	Maxima: Wave length, Å.	Maxima: Intensity, log E_{molar}
4,4′-Dinitrobiphenyl	3060	4.40
2,7-Dinitrofluorene	3310	4.43
4-Amino-4′-nitrobiphenyl	2475, 3790	4.10, 4.19
2-Amino-7-nitrofluorene	2610, 4000	4.09, 4.28
4-Amino-4′-nitrobiphenyl hydrochloride	2210, 3010	4.10, 4.22
2-Amino-7-nitrofluorene hydrochloride	2340, 3230	4.05, 4.28
4-Acetylaminobiphenyl	2740	4.42
2-Acetylaminofluorene[b]	2880	4.46
2-Methyl-4-acetylaminobiphenyl	2600	4.34
2′-Methyl-4-acetoaminobiphenyl	2600	4.35

[a] Solvent ethanol; the hydrochlorides were prepared by dissolving the free bases in 0.1 *N* HCl in 90% ethanol. [b] This compound showed fine structure with subsidiary maxima at 2780 (*E* 4.42); 3050 (*E* 4.26); and 3140 (*E* 4.17).

TABLE II

CARCINOGENIC ACTIVITIES OF 2-ACETYLAMINOFLUORENE AND CERTAIN BIPHENYL DERIVATIVES[a]

Compound	Sex	No. rats studied	No. of rats with tumors in: Liver	Mammary gland	Ear duct	Small intestine
2-Acetylamino-fluorene	M	26	22	0	10	13
	F	25	0	21	20	5
4-Acetylamino-biphenyl	M	15	0	0	0	0
	F	15	0	11	2	0
2-Methyl-4-acetyl-aminobiphenyl	M	9	0	0	0	0
	F	9	0	0	0	0
2′-Methyl-4-acetyl-aminobiphenyl	M	9	0	0	0	0
	F	9	0	0	0	0

[a] Final tumor incidences in rats fed 1.62 mmoles of compound per kg. of a grain diet for 8 months and the grain diet alone for an additional 2 months.

These curve displacements are all in accord with anticipations based on the spectrographic studies of the biphenyl and fluorene hydrocarbons discussed previously,[4] and they suggest that the principal effect of the methylene bridge in the fluorene derivatives is to increase charge transfer between the two rings as a consequence of the greater planarity of the ring system.

In the one series tested, carcinogenic activity was associated with absorption maxima at longer wave lengths and more planar structures. Thus, 2-acetylaminofluorene, which induces a high incidence of tumors at each of four tissue sites, absorbs maximally at a longer wave length than 4-acetylaminobiphenyl, which is active as a mammary carcinogen but essentially inactive at other sites.

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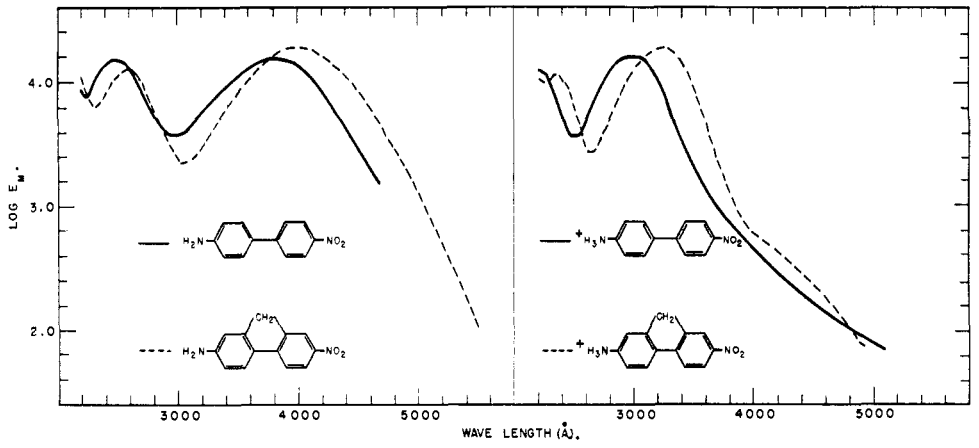

Fig. 2.

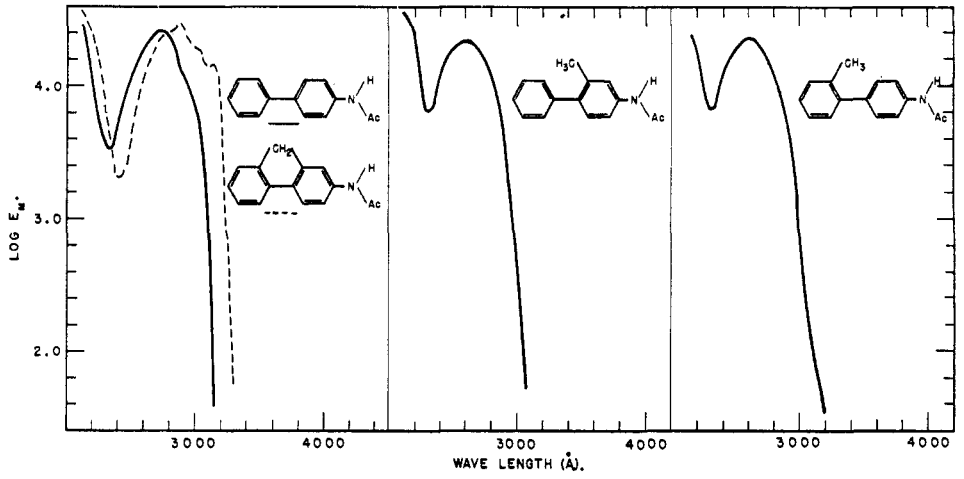

Fig. 3.

Acknowledgments.—The technical assistance of Mr. D. S. Keir and Mrs. M. A. MacKenzie in the determination of the spectra is gratefully acknowledged. One of us (R.B.S.) is indebted to the Canadian Cancer Society for financial aid in support of this work. The carcinogenicity tests were made under a grant from the National Cancer Institute, U. S. Public Health Service.

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