Estrogenic Biphenyls. VII. Preparation and Estrogenic Action of Methoxyl Derivatives of 4-Methoxybiphenyl-4'-carboxylic Acid

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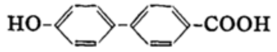
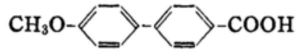
In the study on the relation between the estrogenic activity and the molecular structure it has been found that in 4-hydroxybiphenyl-4'-carboxylic acid (I) and its methyl ether (II) the introduction of alkyl groups into suitable positions of the biphenyl skeleton increases the activity[1-6]. That the introduction of alkyl groups into position 2 or 2' of 4-methoxybiphenyl-4'-carboxylic acid (II) largely modifies the otherwise planar conformation of the biphenyl skeleton is apparent from the ultraviolet absorption spectra. The enhancement of the activity caused by the alkyl groups is explained on the basis of the steric effect of the alkyl groups, which makes the molecular structure or the conformation more suitable for the development of the physiological activity.

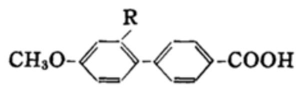
The minimum active doses of typical compounds of this series were found to be as follows: The parent compound II[3] was active only in 50% of ovariectomized mice at the dose of 1 mg., while full estrus was produced in 100% animals by a dose of 500 γ of compound III[3] and by 100 γ of either compound V[1] or compound VII[2]. The substances with a methyl or a propyl group in place of the ethyl group in compound III showed an activity similar to that of compound III in contrast to the case of the stilbene series[7]. This may be understood, when the similar ultraviolet absorption spectra of these homologous compounds are interpreted as the indication of their similar basic biphenyl skeletons which are not altered by the chain length of the straight-chain alkyl groups at position 2[2,3,8].



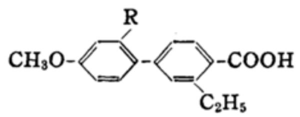
I



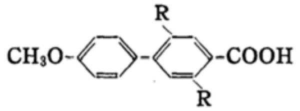
II



III $R=C_2H_5$
IV $R=OCH_3$



V $R=C_2H_5$
VI $R=OCH_3$



VII $R=C_2H_5$
VIII $R=OCH_3$

It was not explained, however, that the estrogenic activity of 2,2'-disubstituted derivatives[5,6], whose ultraviolet spectra differed markedly from those of the monoalkyl derivatives in missing the characteristic K-band in the region examined, was nearly the same as that of the monoalkyl derivatives. The fact was striking because the physiological activity such as estrogenic activity is considered to be very sensitive even to a slight deformation of the structure.

In this connection it is interesting to prepare the alkoxyl analogues in which the alkoxyl group will take the same arrangement as alkyl groups but will produce less steric hindrance. The present communication describes the preparation of three such methoxyl analogues, IV, VI and VIII. The effect of the replacement of the alkyl group by the methoxyl group on the estrogenic activity may be compared with the case of the methoxyl analogues of hexestrol. Takahashi reported that 1, 2-dimethoxy-1,2-bis-(*p*-hydroxyphenyl)-ethane was fully active at 0.5 γ while the ethoxyl analogue was so at

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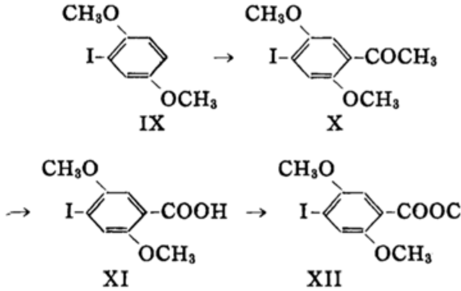
[1] M. Ōki and T. Sato, This Bulletin, **30**, 508 (1957).
[2] M. Ōki and T. Sato, ibid., **30**, 702 (1957).
[3] T. Sato and M. Ōki, ibid., **30**, 859 (1957).
[4] T. Sato and M. Ōki, ibid., **30**, 958 (1957).
[5] T. Sato, ibid., **32**, 1130 (1959).
[6] T. Sato and M. Ōki, ibid., **32**, 1289 (1959).
[7] M. Ōki, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zasshi*), **73**, 252 (1952); M. Ōki and Y. Urushibara, This Bulletin, **25**, 109 (1952).
[8] E. A. Braude, F. Sondheimer and W. F. Forbes, *Nature*, **173**, 117 (1954).

10 γ[9]. The compound in which only one of the ethyl groups of hexestrol was replaced by a methoxyl group was less active than the dimethoxyl compound[10].

2, 4-Dimethoxybiphenyl-4′-carboxylic acid (IV) was prepared by the Ullmann reaction of 2, 4-dimethoxyiodobenzene and ethyl 4-iodobenzoate. The 2-methoxyl analogue (VI) of 2, 3′-diethyl-4-methoxybiphenyl-4′-carboxylic acid (V), one of the most active members of the alkyl series, was prepared from 2, 4-dimethoxyiodobenzene and ethyl 2-ethyl-4-iodobenzoate[1] through the same method. 2′, 4, 5′-Trimethoxybiphenyl-4′-carboxylic acid (VIII), the totally methoxylated analogue of the diethyl compound VII, was obtained from 4-iodoanisole and ethyl 4-iodo-2, 5-dimethoxybenzoate (XII), which was prepared by the following reaction sequence:



2, 5-Dimethoxyiodobenzene (IX), prepared either from hydroquinone dimethyl ether by direct iodination or from 2, 5-dimethoxyaniline by diazo reaction, was treated with acetyl chloride and aluminum chloride in carbon disulfide. 4-Iodo-2, 5-dimethoxyacetophenone (X), thus produced, was oxidized with sodium hypobromite to give the corresponding benzoic acid XI, which was esterified to XII in the usual manner. Attempted Friedel-Crafts reaction of 2, 5-dimethoxyacetanilide in carbon disulfide with the aim of obtaining 4-acetamido-2, 5-dimethoxyacetophenone, which might be another intermediate for the preparation of XII, failed and the starting material was recovered.

The estrogenic activities were determined by the vaginal smear test with ovariectomized mice, substances in oil solution being injected subcutaneously. Compound IV was found active at 400 γ, while the corresponding ethyl compound (III) at 500 γ, as stated above. Compound VI was active only to 20% of the test animals at 100 γ, while the corresponding diethyl compound (V) was fully estrogenic at this level. The same situation is also observed in 2′, 5′-disubstituted compounds. Namely, while the 2′, 5′-diethyl compound (VII) was active equally to compound V, the 2′, 5′-dimethoxyl analogue (VIII) was active to 40% of the animals even at 200 γ. Although these 2-methoxyl compounds may be represented by thinner structural models than the corresponding alkyl compounds, the weaker activities of the former may be attributed to the thinner structures only with a reserve, since the direct physiological effect of the methoxyl group itself is obscure.

Experimental*

2, 4-Dimethoxybiphenyl-4′-carboxylic Acid (IV).—With good stirring, 15 g. (0.057 mol.) of 2, 4-dimethoxyiodobenzene, prepared by iodination of resorcinol dimethyl ether with iodine and yellow mercuric oxide according to Kauffmann and Kieser[11], and 5 g. (0.018 mol.) of ethyl 4-iodobenzoate were heated to 230°C and 15 g. (0.24 atom) of activated copper bronze[12] was added in small portions over a period of twenty minutes, while the temperature was maintained at 230~250°C. When the addition was over, the reaction mixture was heated to 280~290°C and kept at that temperature for thirty minutes. The acetone extract was subjected to vacuum distillation and the distillate up to 120°C/5 mm. was discarded. The residue was hydrolyzed with aqueous-alcoholic alkali and tetramethoxybiphenyl was removed by filtration. Acidification of the aqueous solution gave a crude product, which was extracted with hot acetic acid, and the substance obtained from the extract was recrystallized from ethanol to yield 1 g. of colorless plates melting at 199~200°C.

Anal. Found: C, 69.39; H, 5.99. Calcd. for $C_{15}H_{14}O_4$: C, 69.75; H, 5.46%.

3′-Ethyl-2, 4 - dimethoxybiphenyl - 4′ - carboxylic Acid (VI).—In the same way as described for the preparation of compound IV, 11 g. (0.042 mol.) of 2, 4-dimethoxyiodobenzene and 5.5 g. (0.018 mol.) of ethyl 2-ethyl-4-iodobenzoate[1] were condensed with 15 g. (0.24 atom) of activated copper bronze. The acetone extract was concentrated until the temperature of the distilling vapor reached 152°C at 5 mm. The crude acid, obtained by hydrolysis of the product, was extracted with hot benzene. Repeated recrystallization from ethanol gave 200 mg. of colorless needles melting at 187~187.5°C.

Anal. Found: C, 71.24; H, 6.10. Calcd. for $C_{17}H_{18}O_4$: C, 71.31; H, 6.34%.

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[9] T. Takahashi, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zasshi*), **73**, 765 (1952); Y. Urushibara and T. Takahashi, This Bulletin, **23**, 53 (1950).

[10] T. Takahashi, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zasshi*), **73**, 629 (1952).

* All boiling and melting points are uncorrected. The author is indebted to Mr. T. Mizushima for carrying out the microanalyses.

[11] H. Kauffmann and F. Kieser, *Ber.*, **45**, 2333 (1912).

[12] E. C. Kleiderer and R. Adams, *J. Am. Chem. Soc.*, **55**, 4219 (1933).

2,5-Dimethoxyiodobenzene. — Hydroquinone dimethyl ether was iodinated with iodine and mercuric oxide[13]. It was also prepared[14] from 2,5-dimethoxyaniline by diazotization followed by decomposition in the presence of potassium iodide.

4-Iodo-2, 5-dimethoxyacetophenone (X).—To a well stirred mixture of 100 ml. of carbon disulfide and about one fifth of 33 g. (0.25 mol.) of powdered aluminum chloride was added dropwise a solution consisting of 16 g. (0.20 mol.) of acetyl chloride and 50 g. (0.19 mol.) of 2, 5-dimethoxyiodobenzene in the course of thirty minutes, while the rest of the aluminum chloride was added occasionally through another opening of the flask. The reaction mixture was gently refluxed for one hour and then poured into ice water containing hydrochloric acid. The carbon disulfide layer was concentrated and the residue was extracted with ether. The ethereal solution was successively washed with water, aqueous alkali, sodium thiosulfate and again water. Evaporation of the solution afforded 15 g. (26% of the theoretical) of light brown needles melting at 98~99°C on recrystallization from ethanol.

Anal. Found: C, 39.28; H, 3.60. Calcd. for $C_{10}H_{11}O_3I$: C, 39.23; H, 3.62%.

The 2, 4-Dinitrophenylhydrazone was obtained as deep-orange plates, m. p. 210~212°C, on recrystallization from ethyl acetate.

Anal. Found: N, 11.72. Calcd. for $C_{16}H_{15}O_6N_4I$: N, 11.53%.

4-Iodo-2, 5-dimethoxybenzoic Acid (XI).—To a sodium hypobromite solution, prepared by dropping 21 g. (0.13 mol.) of bromine into 15 g. (0.38 mol.) of sodium hydroxide in 130 ml. of water below 0°C, was added 14 g. (0.046 mol.) of compound X dissolved in 50 ml. of dioxan over a period of one hour, the temperature being kept below 20°C. After the excessive hypobromite was decomposed with sodium bisulfite, the whole was steam-distilled and the remainder was acidified with hydrochloric acid. Colorless needles melting at 175~177°C were obtained on recrystallization of the product from ethanol. Yield, 10 g. or 71% of the theoretical.

Anal. Found: C, 35.25; H, 3.00. Calcd. for $C_9H_9O_4I$: C, 35.09; H, 2.94%.

Ethyl 4-Iodo-2, 5-dimethoxybenzoate (XII).—A solution of 5 g. (0.016 mol.) of compound XI in 50 ml. of ethanol, saturated with dry hydrogen chloride, was heated under reflux for five hours. After the solvent was removed the residue was diluted with water and extracted with ether. The ethereal solution was successively washed with water, sodium bicarbonate solution, sodium thiosulfate solution and again water. Colorless plates melting at 65~66°C were obtained on recrystallization from petroleum ether. Yield, 5 g. or 93% of the theoretical.

Anal. Found: C, 39.30; H, 3.95. Calcd. for $C_{11}H_{13}O_4I$: C, 39.30; H, 3.90%.

2′, 4, 5′-Trimethoxybiphenyl-4′-carboxylic Acid (VIII).—The Ullmann reaction was carried out in the same way as described for the preparation of compound IV, using 8 g. (0.034 mol.) of 4-iodoanisole, 5 g. (0.015 mol.) of compound XII and 15 g. (0.24 atom) of activated copper bronze. The acidic part was extracted with hot benzene and the extract was concentrated to yield 200 mg. of colorless plates melting at 142~143°C.

Anal. Found: C, 66.84; H, 5.48. Calcd. for $C_{16}H_{16}O_5$: C, 66.66; H, 5.59%.

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[13] H. Kauffmann and I. Fritz, *Ber.*, **41**, 4413 (1908).

[14] F. Ullmann et al., *Ann.*, **332**, 38 (1904).