Estrogenic Biphenyls. I. 2,3'-Diethyl-4-methoxybiphenyl-4'-carboxylic Acid

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In connection with the hypothesis, proposed by one of the present authors (M.O.) and Y. Urushibara[1], which postulates that the estrogenic compounds should possess not only two active hydrogens at the optimum distance apart, but the optimum thickness or width of the molecule, a number of stilbene derivatives were synthesized and found to be estrogenic[2-4]. A compound is supposed to be estrogenic from the standpoint of this hypothesis if

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1) M. Ōki and Y. Urushibara, This Bulletin, **25**, 109 (1952).

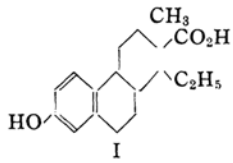
2) M. Ōki, ibid., 112.

3) idem, ibid., **26**, 66 (1953).

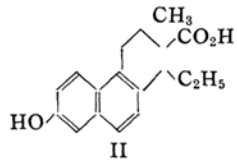
4) idem, ibid., 331.

it carries two active hydrogens at the optimum distance regardless of their nature.

Thus, doisynolic acid (I) and bisdehydrodoisynolic acid (II) are powerful estrogens[5] in spite of the facts that their



I



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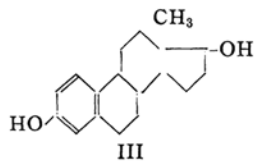
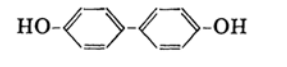


III

structures are different from that of the natural estrogen, estradiol (III), and that two active hydrogens are born by a hydroxyl and a carboxyl group.

Dodds et al.[6] reported that 4,4'-dihydroxybiphenyl (IV) was almost inactive. The fact seems probable, since this molecule is planar and the distance between two active hydrogens is less than the optimum. The authors postulate that even a biphenyl derivative can be a potent estrogen if it carries two active hydrogens at the optimum distance and possesses the optimum thickness of the molecule.

Novello et al.[7] prepared 2-alkyl-4-(*p*-hydroxyphenyl)-1-methylcyclohexanecarboxylic acids (V) and reported that they were estrogenic. Although these types of compounds are not the biphenyl derivatives, the result encouraged the authors to investigate the biphenyl series. Furthermore, while this paper was in preparation Nathan and Hogg[8] reported that 2-methyl-3-ethyl-4-(*p*-anisyl)-3-cyclohexenecarboxylic acid (VI) and its 4-unsaturated isomer (VII) were highly estrogenic.



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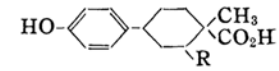

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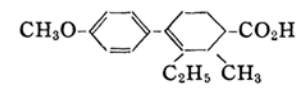
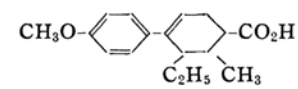
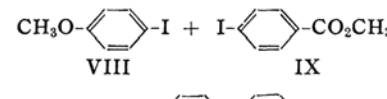


VI

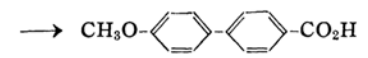


VII

4-Hydroxybiphenyl-4'-carboxylic acid (XI) possesses, from the measurement of its scale model, two active hydrogen atoms at the suitable distance and, therefore, was supposed to be an estrogen, although the activity might be slight due to its planar structure. This substance was synthesized by the method described below and, as expected, showed estrogenic activity to 60% of the tested animals (ovariectomized mice) at the dose of 500γ.

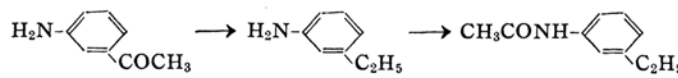


VIII IX

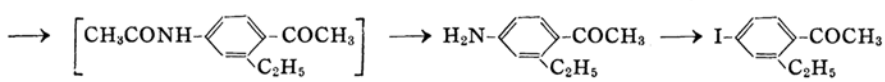


X

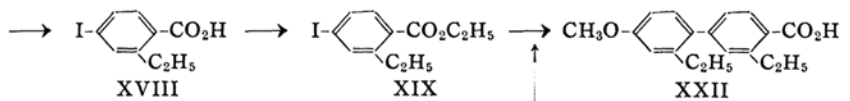
XI



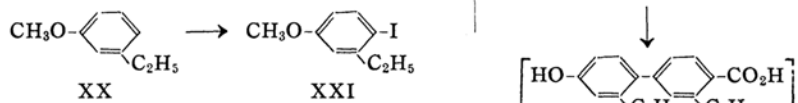
XII XIII XIV



XV XVI XVII



XVIII XIX XXII



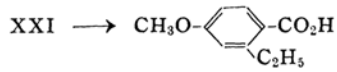
XX XXI XXIII

5) K. Miescher, *Chem. Rev.*, **43**, 367 (1948).
6) E. C. Dodds and W. Lawson, *Proc. Roy. Soc. (London)*, **B125**, 222 (1938).
7) F. C. Novello, M. E. Christy and J. M. Sprague, *J. Am. Chem. Soc.*, **76**, 738 (1954).
8) A. H. Nathan and J. A. Hogg, ibid., **78**, 6163 (1956).

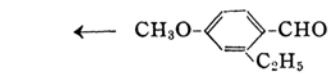
In order to give thickness and width to this molecule, the introduction of alkyl groups was attempted. Two benzene rings in biphenyl derivatives, as well known[9], cannot take coplanar structure when a substitution takes place at 2- or 2′-position. Thus thickness will be given to the molecule. As an example, 4-methoxy-2,3′-diethylbiphenyl-4′-carboxylic acid (XXII) was prepared. An ethyl group at 2-position was introduced to give steric hindrance by its bulk and to rotate benzene rings out of the coplanar structure. Another ethyl group at 3′-position was to give the optimum width of the molecule by analogy of the ethyl group next to the carboxyl group in doisynolic acid, in which, when the ethyl group is replaced by a methyl group or hydrogen, only weaker estrogenic activity is produced.

The synthesis of compound XXII is illustrated in the chart of page 509. *m*-Aminoacetophenone (XII) was reduced by modified Wolff-Kischner method[10] to *m*-ethylaniline (XIII) and the latter was acetylated. *m*-Ethylacetanilide (XIV) was treated with acetyl chloride and aluminum chloride and the product (XV) was hydrolyzed to give 2-ethyl-4-aminoacetophenone (XVI). The aminoketone (XVI) was converted into the iodo compound (XVII) through diazo reaction and the iodoketone was oxidized by sodium hypobromite to 2-ethyl-4-iodobenzoic acid (XVIII), which was esterified in the usual way. 3-Ethyl-4-iodoanisole (XXI) was prepared by direct iodination of *m*-ethylanisole (XX) in the presence of yellow mercuric oxide and submitted to the Ullmann reaction with ester XIX. Attempted demethylation of 2,3′-diethyl-4-methoxybiphenyl-4′-carboxylic acid (XXII) to 2,3′-diethyl-4-hydroxybiphenyl-4′-carboxylic acid (XXIII) with hydrogen bromide was unsuccessful.

The structural proof of 3-ethyl-4-iodoanisole was obtained in the following way. On treatment with magnesium and then with solid carbon dioxide, compound XXI yielded a carboxylic acid (XXIV). On the other hand, 2-ethyl-4-methoxybenzaldehyde (XXV)[11], prepared through modified Gattermann reaction, was oxidized by potassium permanganate to yield a carboxylic acid melting at 122–123°. These two acids were found to be identical by the mixed melting point determination.

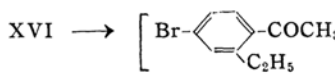


XXIV

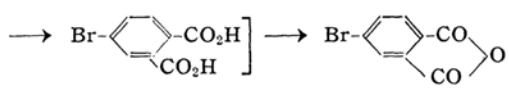


XXV

The structure of 4-amino-2-ethylacetophenone (XVI) was proven as illustrated in the following diagram. The amino group of XVI was replaced by bromine and 4-bromo-2-ethylacetophenone (XXVI) was oxidized with nitric acid and then with potassium permanganate. The dibasic acid (XXVII) was sublimed in vacuo and a produced anhydride did not depress the melting point on admixture with the authentic sample of 4-bromophthalic anhydride (XXVIII)[12].

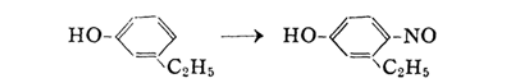


XXVI

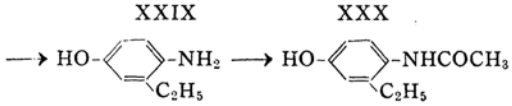


XXVII XXVIII

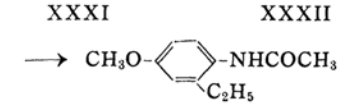
3-Ethyl-4-iodoanisole (XXI) might be synthesized through diazo reaction of 2-ethyl-4-anisidine. *m*-Ethylphenol (XXIX) was nitrosated and the product was reduced to the corresponding aminophenol (XXXI). The amino group was protected by acetylation and the hydroxyl group was methylated with dimethyl sulfate. But, before completion of this series, a more successful method than this rather lengthy one was found as described above. Therefore this method was discarded.



XXIX XXX



XXXI XXXII



XXXIII

The active dose was determined by the vaginal smear test with ovariectomized

9) R. A. Riedel, M. Orchin and L. Reggel, ibid., **70**, 199 (1948); E. A. Braude, F. Sondheimer and W. F. Forbes, *Nature*, **173**, 117 (1954).

10) Huang-Minlon, *J. Am. Chem. Soc.*, **68**, 2487 (1946); **70**, 2802 (1948); **71**, 3301 (1949).

11) B. R. Baker, ibid., **65**, 1572 (1943).

12) K. Fries and E. Hübner, *Ber.*, **39**, 435 (1906).

mice. The substance XXII was dissolved in oil and subcutaneously injected. The minimum active dose to produce estrus in 100% was found to be 100 γ.

Experimental*

***m*-Ethylaniline (XIII).**—A solution of 135 g. (1 mole) of *m*-aminoacetophenone (XII) prepared by reduction[13] of *m*-nitroacetophenone, 280 g. of potassium hydroxide and 120 g. (2 moles) of hydrazine hydrate in 700 ml. of diethylene glycol was heated to reflux for one hour. After this period the reflux condenser was removed and the low-boiling materials were collected with a downward condenser until the reaction mixture attained the temperature of 200°. The reaction mixture was refluxed for further three hours and steam-distilled. The distillate was combined with the one obtained before heating at 200° and extracted with ether. The combined extracts were dried over potassium carbonate and distilled under reduced pressure, b.p. 75-76°/4 mm. Yield 80-90%. The melting point of the acetyl derivative was 24° (reported[14] melting point is 24°). *m*-Ethylphenol was prepared[14] through diazo reaction of this compound.

3-Ethyl-4-nitrosophenol (XXX).—To a well stirred solution of 43 g. (0.34 mole) of *m*-ethylphenol (XXIX) in 200 ml. of 95% alcohol and 200 ml. of concentrated hydrochloric acid, was added 35 g. (0.5 mole) of sodium nitrite in small portions, while the temperature was maintained at 0-2°. After the addition was finished, stirring was continued for ten minutes and the mixture was poured into 2 l. of water. Crystals were collected and recrystallized from benzene. Colorless needles, m.p. 135° (decomp.). Yield, 40 g. or 74% of the theoretical.

Anal. Found: N, 9.52. Calcd. for $C_8H_9O_2N$: N, 9.27%.

4-Amino-3-ethylphenol (XXXI).—To a solution of 20 g. (0.13 mole) of the compound XXX in 200 ml. of concentrated aqueous ammonia and 300 ml. of water, was introduced hydrogen sulfide. Crystals began to separate from the solution after five to ten minutes and the color of the solution changed. Hydrogen sulfide was passed through the solution for further forty-five minutes and the crystals were collected.

It was the most convenient way to use this crude material for the next reaction, since this compound was unstable and did not keep well. The analytical sample was obtaind in colorless plates by recrystallization from water, m.p. 169.5-171° (decomp.). Yield 15.5 g. or 85% of the theoretical.

Anal. Found: N, 10.33. Calcd. for $C_8H_{11}ON$: N, 10.20%.

4-Acetamido-3-ethylphenol (XXXII).—Fifteen grams (0.11 mole) of the crude aminophenol (XXXI) was treated with 25 g. of acetic anhydride. On recrystallization from water, were obtained colorless plates which melted at 137.5-139°. This compound took a molecule of water of crystallization, as analysis indicated, solidified over the first melting point and remelted at 173°. Yield 17 g. or 77% of the theoreticlal.

Anal. Found: N, 7.25. Calcd. for $C_{10}H_{13}O_2N \cdot H_2O$: N, 7.07%.

4-Acetamido-3-ethylanisole (XXXIII).—To a solution of 40 g. (0.2 mole) of the compound XXXII in 10 g. (0.25 mole) of sodium hydroxide and 200 ml. of water, was dropwise added 30 g. (0.24 mole) of dimethyl sulfate at 30-40°, while the mixture was well stirred. The crystals were collected and recrystallized from aqueous alcohol (1 : 1). Colorless needles, m.p. 118-119°. Yield 35 g. or 89% of the theoretical.

Anal. Found: N, 7.28. Calcd. for $C_{11}H_{16}O_2N$: N, 7.21%.

4-Methoxybiphenyl-4'-carboxylic Acid (X).—Methyl *p*-iodobenzoate (IX) was prepared[15] from *p*-iodobenzoic acid and methanol and *p*-iodoanisole (VIII)[16] from anisole and iodine. A mixture of 2.5 g. (0.0095 mole) of the compound IX and 5.5 g. (0.0235 mole) of VIII was preheated at 230° and 8.0 g. (0.125 atom) of copper bronze activated by Kleiderer and Adams' method[17] was added in portions with good stirring, while the temperature was maintained at 230-240°. The addition required twenty minutes. Then the temperature was raised to 280° and the reaction mixture was stirred for forty minutes at that temperature. After cooling, the mixture was extracted with acetone, the solvent was evaporated and the residue was refluxed with 10% sodium hydroxide solution in aqueous alcohol (1 : 1) for two hours. After dilution with 500 ml. of water active charcoal was added and filtered off to remove adherent oil. The filtrate was acidified with hydrochloric acid. The crystals were collected, boiled with 30 ml. of acetic acid and the mixture was filtered to remove insoluble material. The filtrate was concentrated and gave, on cooling, crystals melting at 247°. The compound gave no depression in the mixed melting point with the authentic 4-methoxybiphenyl-4'-carboxylic acid[18] prepared by oxidation of the corresponding acetyl compound. The reported melting point is 248-249°[18,19].

4-Hydroxybiphenyl-4'-carboxylic Acid (XI).—The compound X was demethylated according to the method of Fieser and Bradsher[18]. m.p. 259°.

3-Ethyl-4-iodoanisole (XXI).—To a solution of 44 g. (0.33 mole) of *m*-ethylanisole (XX),

* All boiling and melting points are uncorrected.

13) K. Buchka, *Ber.*, **10**, 1714 (1877); C. Engler, ibid., **11**, 930 (1878); L. C. King, M. McWhirter and D. M. Barton, *Ann.*, **67**, 2091 (1945).

14) Mm, A. Behal and E. Choay, *Bull. soc. chim. France*, (3), **11**, 206 (1894).

15) H. Schmidt and G. Schultz, *Ann.*, **207**, 320 (1881)

16) M. P. Brenans, *Bull. Soc. chim. France*, (3), **25**, 819 (1901).

17) E. C Kleiderer and R. Adams, *J. Am. Chem. Soc.*, **55**, 4225 (1933).

18) L. F. Fieser and C. K. Bradsher, ibid., **58**, 1738 (1936).

19) W. S. Johnson, C. D. Gutsche and R. O. Offenhauer, ibid., **68**, 1648 (1946).

prepared by methylation[20] of *m*-ethylphenol, in 70 ml. of alcohol were alternatively added 86 g. (0.4 mole) of yellow mercuric oxide and 84 g. (0.33 mole) of iodine in small portions with good swirling, while the temperature was maintained below 30°. The reaction mixture was finally heated on a water bath for ten minutes and filtered after cooling. Water was added to the filtrate and oil was extracted with ether. The combined extracts were sucessively washed with water, aqueous potassium iodide, and again with water, and dried over calcium chloride. After evaporation of the solvent, the residue was fractionated under diminished pressure. The main portion boiled at 147-150°/18 mm. and the pure compnund was obtained on redistillation, b. p. 120-120.5°/5 mm. Yield 60 g. or 76 % of the theoretical.

Anal. Found: I, 48.63. Calcd. for $C_9H_{11}OI$: I, 48.39 %.

2-Ethyl-4-methoxybenzoic Acid (XXIV). (A) From 3-Ethyl-4-iodoanisole (XXI).—A mixture of 5 g. (0.019 mole) of 3-ethyl-4-iodoanisole (XXI), 0.7 g. (0.029 atom) of magnesium and 50 ml. of ether was heated under reflux for one hour. To the Grignard reagent, a piece of solid carbon dioxide was added and, when the mixture attained room temperature, hydrochloric acid was added to decompose the complex. The aqueous layer was extracted with ether and the combined extracts were concentrated. The ethereal layer was extracted with 50 ml. of 10 % aqueous sodium hydroxide; the aqueous layer was treated with active charcoal and acidified with hydrochloric acid. The crystals were collected and recrystallized from aqueous alcohol. Colorless plates, m.p. 120-121°. Yield 1.5 g. or 43 % of the theoretical.

(B) From 2-Ethyl-4-methoxybenzaldehyde (XXV).—A suspension of 2-ethyl-4-methoxybenzaldehyde (XXV)[11] in excess aqueous potassium permanganate was heated on a water bath for thirty minutes. Sulfur dioxide was introduced to the suspension and crystals were collected. On recrystallization of the alkali soluble part from aqueous alcohol, there were obtained colorless plates melting at 122-123°.

Anal. Found: C, 66.32; H, 6.82. Calcd. for $C_{10}H_{12}O_3$: C, 66.65; H, 6.71 %.

The melting point did not depress when both samples were mixed.

4-Amino-2-ethylacetophenone (XVI).—To a well stirred solution of 41 g. (0.25 mole) of *m*-ethylacetanilide (XIV) and 35 g. (0.45 mole) of acetyl chloride in 200 ml. of carbon disulfide, was added 116 g. (0.87 mole) of powdered aluminum chloride in portions of ca. 20 g. After the addition, the reaction mixture was heated under reflux for one hour and left to stand at room temperature for two hours. The supernatant carbon disulfide layer was decanted and the lower layer was decomposed with ice and hydrochloric acid. The aqueous layer was decanted off and the lower layer was hydrolyzed by heating with 200 ml. of dilute hydrochloric acid (1 : 1) for two hours. The cooled hydrolysate was basified with sodium hydroxide and the organic base was extracted with ether. The ethereal extracts were dried over potassium carbonate and fractionated. A fraction which boiled at 150-162°/5 mm. gave a pure sample on refractionation (b.p. 159-161°/5 mm.). The distillate solidified on cooling and the solid was recrystallized from benzene-petroleum ether. Colorless needles, m.p. 62-63°. Yield 16.5 g. or 40 % of the theoretical.

Anal. Found: N, 8.66. Calcd. for $C_{10}H_{13}ON$: N, 8.58 %.

4-Bromophthalic Anhydride (XXVIII).—To a diazonium solution obtained from 7.0 g. (0.043 mole) of 4-amino-2-ethylacetophenone (XVI), 20 ml. of 48 % hydrobromic acid and 3.5 g. (0.051 mole) of sodium nitrite, was added a small amount of copper bronze. The mixture was heated on a water bath to complete the reaction and extracted with ether. The extracts were washed with aqueous sodium hydroxide and ether was evaporated. The residue was refluxed with dilute nitric acid, which was composed of 10 ml. of nitric acid (d; 1.38) and 40 ml. of water, for five hours. After cooling, the crystalline material was collected and further oxidized by aqueous potassium permanganate. A dicarboxylic acid, thus obtained, was sublimed under reduced pressure. Colorless plates, m.p. 105-106°. The compound did not depress the melting point on admixture with the authentic specimen of 4-bromopthalic anhydride (XXVIII) prepared through oxidation[21] of 1,6-dibromo-2-naphthol followed by sublimation[12].

2-Ethyl-4-iodoacetophenone (XVII).—A solution of 3-ethyl-4-acetylbenzenediazonium sulfate prepared from 33 g. (0.2 mole) of the compound XVI, 42 g. (0.4 mole) of concentrated sulfuric acid, 100 ml. of water and 14 g. (0.2 mole) of sodium nitrite in 30 ml. of water, was added to a hot solution of 50 g. (0.3 mole) of potassium iodide in 200 ml. of water. The organic material was extracted with ether and the ethereal extracts were successively washed with aqueous sodium hydroxide, aqueous sodium thiosulfate and water. After drying over calcium chloride, the solvent was evaporated and the residue was distilled under reduced pressure. Colorless oil, b.p. 166°/20 mm. Yield 33 g. or 60 % of the theoretical.

Anal. Found: I, 46.30. Calcd. for $C_{10}H_{11}OI$: I, 46.80 %.

2,4-Dinitrophenylhydrazone of XVII was prepared in the usual way. Scarlet needles, m.p. 152-153°.

Anal. Found: N, 12.61. Calcd. for $C_{16}H_{15}O_4N_4I$: 12.34.

2-Ethyl-4-iodobenzoic Acid (XVIII).—To a sodium hypobromite solution prepared from 79.9 g. (0.5 mole) of bromine, 63 g. (1.6 moles) of sodium hydroxide and 400 ml. of water, was dropwise added a solution of the compound XVII in 170 ml. of dioxan over a period of one hour, during which time stirring was applied and the

20) c. f. G. S. Hiers and F. D. Hager, "*Org. Synth.*," Coll. Vol. 1, (1940), p. 58.

21) c. f. A. F. Smith, *J. Chem. Soc.*, **35**, 789 (1879).

temperature was maintained below 20°. The reaction was completed by heating on a water bath for 10 minutes and the excess of hypobromite was decomposed with sodium bisulfite. Bromoform was removed by steam distillation and the remainder was acidified with hydrochloric acid. The solid was collected and recrystallized from aqueous alcohol. Colorless needles, m.p. 130-131°. Yield 25 g. or 83% of the theoretical.

Anal. Found: I, 46.23. Calcd. for $C_9H_9O_2I$: I, 45.99%.

Ethyl 2-Ethyl-4-iodobenzoate (XIX).—A solution of 5.0 g. of the acid (XVIII) and 5 ml. of concentrated sulfuric acid in 25 ml. of ethanol was heated under reflux for four hours. After cooling, the mixture was diluted with 100 ml. of cold water and extracted with ether. The ester boiled at 166-168°/17 mm. Yield 5.0 g. or 90% of the theoretical.

Anal. Found: I, 42.03. Calcd. for $C_{11}H_{13}O_2I$: I, 41.76%.

2,3'-Diethyl-4-methoxybiphenyl-4'-carboxylic Acid (XXII).—The Ullmann reaction was carried out in the same way as described in the preparation of X, using 5.0 g. (0.016 mole) of the compound XIX, 10 g. (0.038 mole) of XXI and 10.0 g. (0.157 atom) of activated copper bronze. The acidic portion was dissolved in ether and passed through an alumina column. On recrystallization from aqueous alcohol of the compound from the eluent, colorless prisms melting at 136.5–137° were obtained . Yield 0.5 g. or 10% of the theoretical based on the amount of the compound XIX.

Anal. Found: C, 76.34; H, 7.21. Calcd. for $C_{18}H_{20}O_3$: C, 76.03; H, 7.09%.

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