131. *Two Ketones of the Stilbœstrol Group.*

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The remarkable œstrogenic potency of 4 : 4′-dihydroxy-αβ-diethylstilbene (stilbœstrol) (Dodds, Golberg, Lawson, and Robinson, *Proc. Roy. Soc.*, 1939, *B*, **127**, 140, and earlier and later papers) suggested a study of related ketones in order to determine whether any progestational action can be observed.

The required compounds have been synthesised, but it appears from the report of Professor E. C. Dodds that the œstrogenic properties of these substances are of an order which would inhibit the exhibition of progestational activity.

p-Nitrophenylacetic acid (Organic Syntheses, Coll. Vol. I., 389, 398) was reduced (Robertson and Stieglitz, *J. Amer. Chem. Soc.*, 1921, **43**, 180) and converted into *p*-cyanophenylacetic acid by means of the Sandmeyer reaction. Condensation of *p*-cyanophenylacetyl chloride and anisole in the presence of aluminium chloride afforded 4-*cyano*-4′-*methoxydeoxybenzoin*, $CN{\cdot}C_6H_4{\cdot}CH_2{\cdot}CO{\cdot}C_6H_4{\cdot}OMe$ (I), which was *C*-ethylated by means of ethyl iodide and alcoholic sodium ethoxide. The 4-*cyano*-4′-*methoxy*-α-*ethyldeoxybenzoin*, $CN{\cdot}C_6H_4{\cdot}CHEt{\cdot}CO{\cdot}C_6H_4{\cdot}OMe$ (II), reacted with ethylmagnesium bromide to form 3-*anisyl*-4-p-*cyanophenylhexan*-3-*ol*, $CN{\cdot}C_6H_4{\cdot}CHEt{\cdot}CEt(OH){\cdot}C_6H_4{\cdot}OMe$ (III). This was changed by methylmagnesium bromide and hydrolysis of the product into 4-*methoxy*-4′-*acetyl*-αβ-*diethylstilbene*, $CH_3{\cdot}CO{\cdot}C_6H_4{\cdot}CEt{:}CR{\cdot}C_6H_4{\cdot}OR'$ (IV; $R = Et$, $R' = Me$), and this was demethylated by hydrobromic acid to a *phenolic ketone* (IV; $R = Et$, $R' = H$)(P3). A far more convenient process, and one that involved fewer difficulties in the purifications, was available for the preparation of the lower homologue; it consisted in treating (II) directly with methylmagnesium bromide and hydrolysing the product.

4-*Methoxy*-4′-*acetyl*-α-*methyl*-β-*ethylstilbene* (IV; R and $R' = Me$) was thus obtained and on demethylation it furnished 4-*hydroxy*-4′-*acetyl*-α-*methyl*-β-*ethylstilbene* (IV; $R = Me$, $R' = H$) (P1). A *tert.*-alcohol prepared by the action of methylmagnesium bromide on this phenolic ketone was tested (P2), but it was mixed with a compound of higher carbon content.

We are greatly indebted to Professor E. C. Dodds for permission to include the following report of his biological experiments (the indications P1, P2, P3 are given above).

Test for Progesterone Activity in Immature Rabbits.—Four immature rabbits were each injected with a total dose of 150 m.u. of œstradiol benzoate over a period of 5 days and subsequently with daily doses of one of the test substances or of progesterone (Proluton) for 6 days. The rabbits were killed 24 hours after the last injection, and the uteri dissected, fixed in Bouin's fluid, and after sectioning stained with hæmatoxylin and eosin. The progestational proliferation is expressed below in terms of the indices given by McPhail (*J. Physiol.*, 1936, **83**, 145).

Substance.	Dose (mg.).	Body weight (g.).	Response.
P_1	60	1110	Nil
P_2	30	1120	Nil
P_3	70	610	Nil
Progesterone	1	760	2·5

It is evident that in the dosages employed there was no progesterone activity in the test compounds. It was, however, noticed that the uteri of the experimental animals were larger than that of the control animal. In the case of the rabbit injected with P_3 this was obviously not due to any difference in body weight, and suggests that the substances were œstrogenic.

Test for Œstrogenic Activity.—Groups of ovariectomised female rats were injected with the test substances—six injections of sesame oil solutions being given over 3 days and the vaginas examined by smear for 3 subsequent days. The results are expressed in terms of percentage rats showing full cornification of the vaginal epithelium (*E*).

	% *E*.		
Dose.	P_1.	P_2.	P_3.
10 mg.	100	100	100
1 mg.	(10)	—	40
500 µg.	0	—	0
200 µg.	0	100	0
100 µg.	0	60	0

Influence of Œstrogens on Progesterone Activity.—Simultaneous injection of œstrogen will inhibit the action of progesterone on the rabbit uterus (Hisaw and Leonard, 1930) and in the literature are the following quantitative data for the interaction, various methods of assay being used on non-pregnant animals.

Dose of progesterone.	Inhibitory dose of œstrogen.
0·75 mg.	0·01 mg. œstrone (Robson, *J. Physiol.*, 1936, **88**, 100).
1·5 mg.	0·02 mg. œstradiol (Courrier, Conférence Singer-Polignac. Ed. Brouha, 1938, *L*, p. 39).
5·0 mg.	0·04 mg. œstradiol (Allen, Hisaw, and Gardner, " Sex and Internal Secretions," 2nd Edn., 1939, 452).

Considering the variations in individual technique, these results are fairly consistent and Allen *et al.* state definitely that with higher doses of progesterone proportionally higher amounts of œstrogen have to be used to inhibit the progesterone action. To demonstrate possible progesterone action in the three compounds tests would therefore have to be performed with very large doses. The œstrogenic activity of the three substances in the doses employed in the rabbit test was obviously sufficient to inhibit any possible progesterone activity. From the œstrogenic assays performed on P1—3 and the fact that approximately 1 µg. of œstrone is needed to produce 100% œstrus by this procedure we may presume that the doses given to the rabbits were œstrogenically equivalent to approximately 12 µg., 200 µg., and 14 µg. of œstrone respectively. From the figures given for the inhibition of progesterone by œstrogen it can be assumed that 60—70 mg. of P_1 and P_3 contain less progesterone activity than 1—2 mg. of progesterone and 30 mg. of P_2 less than 10 mg. of progesterone.

EXPERIMENTAL.

p-*Cyanophenylacetic Acid.*—This substance has been obtained by Mellinghoff (*Ber.*, 1889, **22**, 3212) by semi-hydrolysis of *p*-cyanophenylacetonitrile; we have found the following method more convenient. Sodium nitrite (14 g.) in water (40 c.c.) was added to an ice-cold solution of *p*-aminophenylacetic acid (30 g.) and sodium carbonate (21·2 g.) in water (150 c.c.), and this mixture gradually added with stirring to hydrochloric acid (84 c.c., *d* 1·16) and water (84 c.c.) below 10°. The diazotised solution was slowly added to copper sulphate (48 g., hydrated) and potassium cyanide (60 g.) dissolved in water (250 c.c.), with vigorous stirring during the addition and for 1 hour afterwards. The mixture was heated at 50° for 1 hour, filtered, and acidified with hydrochloric acid. The solid was collected, boiled with water (charcoal), and crystallised from water; it formed yellow needles, m. p. 152° (yield, 50%).

4-*Cyano*-4'-*methoxydeoxybenzoin* (I).—*p*-Cyanophenylacetic acid (60 g.) was converted by means of thionyl chloride (300 g.) into its chloride, which after removal of the excess of the reagent remained in long, pale brown needles, m. p. 91—92°. Powdered aluminium chloride (75 g.) was added to a solution of the crude acid chloride and anisole (44 g.) in nitrobenzene (300 c.c.) cooled in an ice-bath and shaken from time to time. After being kept for 48 hours at room temperature, the mixture was heated for a short time at 60°, cooled, and decomposed with ice and hydrochloric acid, and the dark red oil washed with water in order to free it from soluble acids and salts. After removal of nitrobenzene by steam distillation the residual yellow oil was collected; it had b. p. 212—214°/0·2 mm. and formed white plates, m. p. 116—117°, from 90% alcohol (Found: C, 76·6; H, 5·3. $C_{16}H_{13}O_2N$ requires C, 76·5; H, 5·2%) (yield, 50—60%).

The 2 : 4-*dinitrophenylhydrazone* crystallised from acetic acid or ethyl acetate in orange needles, m. p. 212° (Found: N, 16·5. $C_{22}H_{17}O_5N_5$ requires N, 16·2%).

4-*Cyano*-4'-*methoxy*-α-*ethyldeoxybenzoin* (II).—Ethyl iodide (25 g.) was added in one portion to a solution of cyanomethoxydeoxybenzoin (40 g.) in alcoholic sodium ethoxide (3·7 g. of sodium in 65 c.c.) previously heated on the steam-bath. The violent reaction, necessary for the best results, was controlled by cooling. After refluxing for 10 minutes, more alcoholic sodium ethoxide (1·5 g. of sodium in 25 c.c.) and ethyl iodide (10·2 g.) were added, and the mixture refluxed for 6 hours. After dilution with water and removal of the alcohol under diminished pressure, an ethereal extract of the product was washed with aqueous sodium thiosulphate and water, dried, and distilled. The product was a yellow oil (35 g.), b. p. 202—205°/0·3 mm.; it crystallised on trituration with ether and separated from ethyl alcohol in colourless plates, m. p. 60—62° (Found: C, 77·7; H, 6·1. $C_{18}H_{17}O_2N$ requires C, 77·4; H, 6·1%). The dinitrophenylhydrazone was a viscous red tar and could not be induced to crystallise.

3-*Anisyl*-4-p-*cyanophenylhexan*-3-*ol* (III).—A solution of cyanomethoxyethyldeoxybenzoin (6 g.) in ether (25 c.c.) was added dropwise with stirring to an ice-cold solution of ethylmagnesium bromide (from 3·6 g. of ethyl bromide and 0·85 g. of magnesium) in ether (40 c.c.); stirring and cooling were continued for 1 hour and the mixture was kept for 12 hours. After decomposition with ice and ammonium chloride the product was isolated by means of ether; it formed a pale yellow oil (5 g.), b. p. 192—198°/0·2 mm. (Found: C, 77·8; H, 7·3; N, 4·6. $C_{20}H_{23}O_2N$ requires C, 77·7; H, 7·4; N, 4·5%).

4-*Methoxy*-4'-*carboxy*-αβ-*diethylstilbene*.—A solution of anisylcyanophenylhexanol (1 g.) in methyl alcohol (40 c.c.) was saturated with hydrogen chloride and refluxed for 8 hours. Methyl alcohol (20 c.c.) was added, and the liquid again saturated with hydrogen chloride and refluxed for 8 hours. The resulting ester was isolated in the known manner as a viscous oil and hydrolysed by means of boiling aqueous potassium hydroxide (10%). The *acid* was liberated as a gum; it crystallised from acetic acid (50%) (charcoal) in needles (0·1 g.), m. p. 167° (Found: C, 77·1; H, 6·8. $C_{20}H_{22}O_3$ requires C, 77·4; H, 7·1%). Attempts to improve the yield by different procedures were unsuccessful. The action of a boiling mixture of acetic acid, sulphuric acid, and a little water resulted in extensive decomposition and tar formation.

When the nitrile (2 g.) was refluxed for 24 hours with water (20 c.c.), alcohol (60 c.c.), and potassium hydroxide (10 g.), an oily acid could be isolated from the product. This slowly solidified in part and after draining on a porous tile crystallised from aqueous alcohol in colourless, fluffy needles, m. p. 142°. The yield was only 10% and was increased to 30% by heating the nitrile (2 g.) with water (10 c.c.), alcohol (65 c.c.), and potassium hydroxide (10 g.) at 165° for 2 hours in a closed steel tube. The purified acid, m. p. 142°, was evidently 3-*anisyl*-4-p-*carboxyphenylhexan*-3-*ol* (Found: C, 73·1; H, 7·5. $C_{20}H_{24}O_4$ requires C, 73·2; H, 7·3%).

These two acids were prepared with the idea of hydrogenating the nucleus bearing the carboxyl, but the unfavourable yields induced us to seek a solution of this problem in a different way. Similar compounds, the preparation of which will, it is hoped, be placed on record in a short time, have, however, shown considerable resistance to hydrogenation.

4-*Methoxy*-4'-*acetyl*-αβ-*diethylstilbene* (IV; R = Et, R' = Me).—A solution of anisylcyanophenylhexanol (5 g.) in ether (25 c.c.) was slowly added to one of methylmagnesium bromide (2·5 g. of magnesium) in ether (15 c.c.) with cooling and stirring (cf. Butenandt and Schmidt-Thomé, *Ber.*, 1939, **72**, 182). The mixture was refluxed for 12 hours, cooled, and cautiously decomposed by the addition of acetic acid (30 c.c.) and water (20 c.c.). The ether was evaporated, and the residue refluxed for 15 minutes. The neutral product, collected by means of ether, distilled as a pale yellow oil (4·5 g.), b. p. 186—190°/0·2 mm. (Found : C, 82·6; H, 7·9%). This material was sufficiently pure for most purposes, but several preparations gave the high value for carbon content cited. A mixture with Girard's reagent T (4·5 g.) (*Helv. Chim. Acta*, 1936, **19**, 1095, 1107), ethyl alcohol (45 c.c.), and acetic acid (4·5 c.c.) was refluxed for 1 hour. After cooling, it was added to an ice-cold solution of sodium carbonate (3·8 g.) in water (450 c.c.) and shaken with ether. The emulsion formed separated after several hours; the aqueous layer was acidified, and the recovered ketone collected by means of ether; b. p. 162—172°/0·4 mm. (Found : C, 82·1; H, 8·0. $C_{21}H_{24}O_2$ requires C, 81·8; H, 7·8%). The 2 : 4-dinitrophenylhydrazone showed little tendency to crystallise, but separated from benzene–light petroleum (b. p. 40—60°) (1 : 10) as a red crystal powder, m. p. 102°.

4-*Hydroxy*-4′-*acetyl*-αβ-*diethylstilbene* (IV; R = Et, R′ = H).—A mixture of methoxyacetyldiethylstilbene (4 g.), acetic acid (30 c.c.), and hydrobromic acid (12 c.c., *d* 1·5) was refluxed (bath at 170°) for 1—1½ hours. The product, freed from acids, was collected by means of ether and boiled with 2N-sodium hydroxide; unchanged material was recovered and submitted again to the demethylation process. The *phenol* was liberated from the alkaline solution, isolated in the usual manner, and distilled (2 g.). It formed a pale yellow glass, b. p. 202—206°/0·2 mm. (Found : C, 81·2; H, 7·6. $C_{20}H_{22}O_2$ requires C, 81·6; H, 7·5%). There was considerable loss due to the formation of condensation or polymerisation products or both.

4-*Methoxy*-4′-*acetyl*-α-*methyl*-β-*ethylstilbene* (IV; R and R′ = Me).—4-Cyano-4′-methoxy-α-ethyldeoxybenzoin (5 g.) was treated with methylmagnesium bromide (from 5 g. of magnesium) under the conditions described above in the case of cyanomethoxydiphenylhexanol. The colourless oil (4 g.) obtained had b. p. 191—194°/0·3 mm. (Found : C, 81·7; H, 7·6. $C_{20}H_{22}O_2$ requires C, 81·6; H, 7·5%). The 2 : 4-dinitrophenylhydrazone crystallised from benzene–light petroleum in small, red prisms, m. p. 115°.

4-*Hydroxy*-4′-*acetyl*-α-*methyl*-β-*ethylstilbene* (IV; R = Me, R′ = H).—This was prepared from the foregoing by the method described for the diethyl analogue. The pale yellow glass (yield, 50%) had b. p. 210—218°/0·4 mm. (Found : C, 81·3; H, 7·1. $C_{19}H_{20}O_2$ requires C, 81·4; H, 7·1%). The *acetyl* derivative, formed by refluxing with acetic anhydride and anhydrous sodium acetate, was obtained as a colourless oil by distillation in a high vacuum. It solidified and then crystallised from alcohol in white needles, m. p. 102° (Found : C, 77·9; H, 7·1. $C_{21}H_{22}O_3$ requires C, 78·4; H, 6·9%).

Treatment of the phenolic ketone (3 g.) with an excess of methylmagnesium bromide afforded a product (1·8 g.), b. p. 190—198°/0·3 mm., which was far more mobile than the starting material, from which it was freed by means of Girard's reagent T. The analytical results (Found : C, 83·7, 83·7; H, 8·0, 8·1. $C_{20}H_{22}O$ requires C, 86·4; H, 7·9%. $C_{20}H_{24}O_2$ requires C, 81·1; H, 8·1%) suggest an approximately 1 : 1 mixture of 4-hydroxy-4′-α-hydroxy*iso*propyl-α-methyl-β-ethylstilbene and the *iso*propenyl derivative derived by dehydration.

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