All of the enzymes contained p.a.b., the greater part of it in a firmly bound form. Maximum yields were obtained by autoclaving in 5 *N* sodium hydroxide at 75 lb. pressure for one hour. Minimum molecular weights calculated from the p.a.b. content again far exceed accepted values, with the exception of the yeast polypeptidase. Here the calculated value of 1,050,000 is not unreasonably far from figure 700,000 given by Johnson.[5] Yeast, however, is an excellent source of p.a.b., with samples containing up to 130γ p.a.b. per g. of dry yeast or about 250–300γ per g. of protein. There has been no increase in p.a.b. content in the purification of the peptidase over the average found in the total protein of the yeast.

The question immediately arises as to whether these enzyme preparations contain foreign proteins. Northrop[10] has pointed out that crystallization is not necessarily an index of purity. All of the preparations did contain "bound" p.a.b. and many of them "bound" biotin in varying amounts. However, nothing is known about the molecular size of these p.a.b.- or biotin-containing impurities. Any foreign material present must be extremely high in p.a.b. if it is to carry all the p.a.b. present. For instance, an impurity amounting to 1% of the urease preparation would have to contain 0.21% p.a.b.

Analysis of materials for their content of various biologically active substances presents itself as a sensitive index of purity. All of the preparations tested here would be regarded as containing some impurity by this criterion.

TABLE II

P.A.B. CONTENT OF ENZYME

Enzyme	γ P. A. B. per g. H_2O 1 hr., 15 lb.	2 *N* HCl 1 hr., 15 lb.	5 *N* NaOH 1 hr., 75 lb.	Enzyme[b] per mole P. A. B., g.
Catalase, beef liver	4.3	17	19	7,200,000
Concanavalin A[a]	0.25	9.2	22	6,200,000
Rennin	1.0	7.5	19	7,200,000
Urease	1.9	11.5	21	6,500,000
Yeast polypeptidase	6.6	120	130	1,050,000
Phosphorylase	2	3.6	13	10,500,000
Rabbit muscle extract No. 1	1.7	5.0, 5.4 (6 *N*)	23	5,500,000
Rabbit muscle extract No. 2	1.7	6.0, 5.7 (6 *N*)	25	6,050,000

[a] See footnote to Table I. [b] Calculated from the p.a.b. figures in column 4.

(10) Northrop, "Crystalline Enzymes," Columbia University Press, New York, N. Y., 1939.

The authors are indebted to Miss Florence Fox for assistance in making the biotin determinations.

Summary

Six crystalline and three non-crystalline enzyme preparations and one crystalline protein not an enzyme have been analyzed for their content of biotin and *p*-aminobenzoic acid (p. a. b.). The biotin ranged from 0.32 to 11.3 micrograms per gram and the p. a. b. from 13 to 130 micrograms per gram. Assuming 1 mole of biotin or p. a. b. per mole of enzyme, the minimum molecular weights are far beyond the figures assigned to these enzymes. It is concluded that the biotin and p. a. b. are contained in impurities in the crystalline proteins rather than forming an integral part of the enzyme. Determination of these vitamins in crystalline proteins may be a useful means of detecting impurities.

MADISON, WISCONSIN RECEIVED AUGUST 24, 1943

[COMMUNICATION NO. 15 FROM THE LABORATORIES OF HOFFMANN-LA ROCHE, INC.]

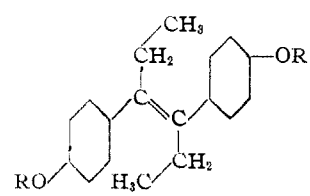
The Synthesis of Estrogenic Indene Derivatives and Remarks on the Configuration of Stilbestrol[1]

BY ULRICH V. SOLMSSEN

The discovery of a great number of estrogenic compounds not chemically related to the natural estrogens, has demonstrated an astonishing unspecificity of estrogenic activity.

Stilbestrol (I), (4,4′-dihydroxy-α,β-diethyl-stilbene) and its hydrogenation product hexestrol (IX), (4,4′-dihydroxy-γ,δ-diphenyl-hexane) first described by Dodds and co-workers,[2,3,4] and since synthesized following various routes, have acquired wide interest as clinical substitutes for the natural estrogens.

Among the many compounds tested by various investigators, the following three indene derivatives reported were found to be inactive: 6- and 7-hydroxy-hydrindene,[5] 4- and 6-hydroxy-α-hydrindone[5] and 5-benzoxy-3-hydrindol.[6] In spite of these negative results, this Laboratory undertook to prepare 2-(*p*-hydroxyphenyl)-3-ethyl-6-hydroxy-indene (V), the structure of which is closely related to that of 4,4′-dihydroxy-α,β-diethyl-stilbene (I) and 4,4′-dihydroxy-α-methyl-β-ethyl-stilbene, if the structure formula for stilbestrol and its derivatives is written according to the British authors.



I R = H
II R = CH_3
III R = $COCH_3$
IV R = $COCH_2CH_3$

(1) Presented at the Meeting of the American Chemical Society, Pittsburgh, Pa., September 6–10, 1943.

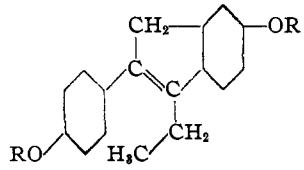
(2) Dodds, Goldberg, Lawson and Robinson, *Nature*, **141**, 247 (1938).

(3) Dodds, Goldberg, Lawson and Robinson, *Proc. Roy. Soc.* (London), **127**, 114 (1939).

(4) Campbell, Dodds and Lawson, *Nature*, **142**, 1121 (1938).

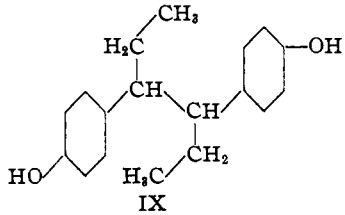
(5) Dodds and Lawson, *Proc. Roy. Soc.* (London), **125**, 222 (1938).

(6) Miyasaka, *Pharm. Soc. Japan*, **59**, 407 (1939).

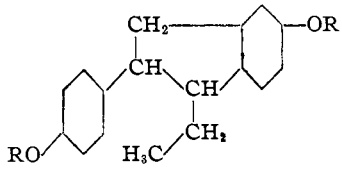


V R = H
VI R = CH_3
VII R = $COCH_3$
VIII R = $COCH_2CH_3$

In view of the high estrogenic activity of 4,4'-dihydroxy-γ,δ-diphenyl-hexane (hexestrol) (IX), the preparation of the corresponding 2-(*p*-hydroxy-phenyl)-3-ethyl-6-hydroxy-indane (X) was undertaken.

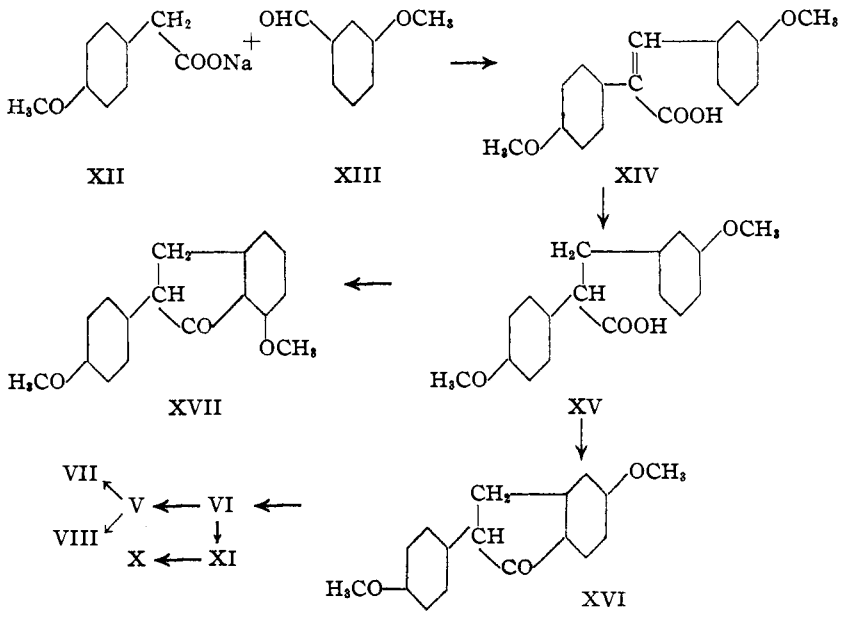


IX

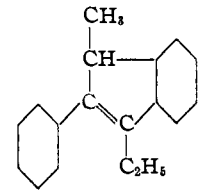


X R = H
XI R = CH_3

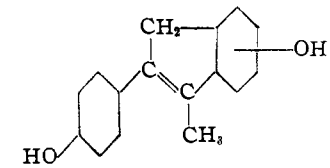
The synthesis of the desired indene derivatives has been carried out according to the scheme



The experimental work reported in this paper was begun in 1938 and completed in 1939, and publication of our results at the present time is prompted only by two publications by other investigators on similar indene derivatives. Plentl and Bogert[7,8] prepared 1-methyl-2-phenyl-3-ethyl-indene (XVIII) from sodium ethyl- phenyl-cyano-acetate and α-phenylethyl bromide by a route different from ours. No biological activity of XVIII was reported and most likely it is inactive



XVIII



XIX

due to the absence of phenolic hydroxyl groups in the molecule. These investigators proposed to apply their method to prepare the corresponding methoxy- and phenolic hydroxy compounds likely to possess estrogenic activity, but so far no further results have been published.

More recently Salzer[9,10] reported the synthesis of 2-(*p*-hydroxyphenyl)-3-methyl-4-(or 6)-hydroxy-indene (XIX) from *p*-methoxyphenyl-acetone and *m*-methoxybenzyl chloride by a third method different from both the one described in this paper and the one devised by Plentl and Bogert.[7] Salzer[9] states that it was found impossible to prepare 2-(*p*-hydroxyphenyl)-3-ethyl-6-hydroxy-indene (V) by this method since only the methyl derivative proved capable of ring closure.

Compound XIX was reported to be estrogenically active indoses of 0.2 γ[9] and 0.3–0.5 γ[10] in rats (route of administration unreported). Salzer reports that an oily hydrogenation product of the methyl derivative XIX proved inactive in doses as high as 200 γ. This low activity is strikingly different from the one reported below for the similar indane derivative (X).

The sodium salt of *p*-methoxy-phenylacetic acid (XII) was treated with *m*-methoxy-benzaldehyde (XIII), resulting in *m*-methoxy-α-(*p*-methoxy-phenyl)-cinnamic acid, m. p. 169°,[11] (XIV). As by-products, 3,4'-dimethoxy-stilbene, m. p. 108–109°, and *m*-methoxy-α-(*p*-methoxy-phenyl)-cinnamic anhydride, m. p. 119–120°, were

(7) Plentl and Bogert, THIS JOURNAL, **63**, 989 (1941).

(8) The numbering system used by these authors does not conform with the *Chemical Abstracts* and has therefore been changed here.

(9) Salzer, U. S. Patent 2,281,956.

(10) Salzer, *Z. physiol. Chem.*, **274**, 39 (1942).

(11) All melting points are uncorrected.

isolated. Hydrogenation of (XIV) gave *m*-methoxy-α-(*p*-methoxy-phenyl)-hydrocinnamic acid (XV), m. p. 106°.

Ring closure was effected by shaking a benzene solution of the hydrocinnamic acid with phosphoric anhydride. Two isomeric reaction products, 2-(*p*-methoxy-phenyl)-6-methoxy-indanone-(3) and 2-(*p*-methoxyphenyl)-4-methoxy-indanone-(3) were obtained and separated by crystallization or chromatographic adsorption. The one isomer has a melting point of 172°, the other 96°. Both isomers were subjected to the Grignard reaction with ethylmagnesium iodide. Whereas the substance with m. p. 96° reacted readily, the other isomer was found to be completely inert toward the Grignard reagent, even under forced conditions.[12] Though no structural proof has been attempted and therefore the two possible structural formulas cannot be assigned definitely to the two isomers, it is believed that the inert isomer, m. p. 172°, has the methoxy group in ortho-position to the keto group, preventing the Grignard reaction due to steric hindrance. Therefore, it has been tentatively assumed that the isomer, m. p. 96°, used for the next steps in the synthesis has the methoxy group in 6-position as represented by formula XVI. The crude reaction product with ethylmagnesium iodide was treated with sulfuric acid in order to complete the apparently spontaneous dehydration and 2-(*p*-methoxy-phenyl)-3-ethyl-6-methoxy-indene (VI) was obtained in peach-colored crystals, m. p. 87–88°, characterized by a very strong blue fluorescence under the ultraviolet lamp. The possibility has been considered of the double bond being formed in 1,2-position rather than 2,3-position, but in analogy with all similar 2,3-substituted indene derivatives reported in the literature, the more likely 2,3-position of the double bond has been assumed to be correct. Furthermore there is physiological evidence in favor of the 2,3-unsaturation. Linnel and Sharma[13] showed that 3,4′-dihydroxy-α,β-diethylstilbene is only active in doses of 4.5 mgm. This indicates that this compound is $^1/_{45,000}$ as active as stilbestrol. The high activities reported below for compounds V and VII made the 2,3-unsaturation the more likely one since a 1,2-unsaturated compound would correspond to 3,4′-dihydroxy-α,β-diethylstilbene, rather than to stilbestrol (I).

The demethylation of the methoxy groups was effected by treatment with hydrobromic acid and 2-(*p*-hydroxy-phenyl)-3-ethyl-6-hydroxy-indene (V) was obtained as a slightly colored crystalline substance, m. p. 136°. This substance proved to be unstable, being partly decomposed with discoloration after brief exposure to air. An unsuccessful attempt was made to purify the dihydroxy derivative over the stable esters. By treatment with acetic anhydride, the diacetyl derivative (VII), m. p. 118–120°, and by treatment with *n*-propionic anhydride the corresponding diproproxy derivative (VIII), m. p. 88–89°, were obtained, both substances being perfectly stable on exposure to air.

By hydrogenation of the di-methoxy derivative (VI) 2-(*p*-methoxy-phenyl)-3-ethyl-6-methoxy-indane (XI) was obtained as an oil, and after demethylation 2-(*p*-hydroxy-phenyl)-3-ethyl-6-hydroxy-indane (X) as a colorless crystallized substance, m. p. 164–165°.

Configuration of Stilbestrol.—Apart from the further problem of synthesizing and biologically testing these various indene derivatives, this project offered another interesting aspect. Stilbestrol may have the *cis*- or *trans*-configuration and Dodds and co-workers[1] have reported the isolation of stilbestrol, m. p. 171° (I), and of ψ-stilbestrol, m. p. 140–142°. These investigators stated that the biologically less active compound probably has a *cis*-configuration and the more active stilbestrol a *trans*-configuration "being more closely related stereochemically to estradiol." This hypothesis has been widely accepted, but in spite of a number of studies[14,15,16,17,18,19] of this problem no unequivocal proof for the *trans*-configuration of stilbestrol has as yet been offered. This is mainly due to the fact that difficulties were encountered in preparing ψ-stilbestrol in a pure form. Consequently no direct and conclusive comparison between the two isomers was possible.

Only recently Walton and Brownlee[20] reported a further purification of ψ-stilbestrol, m. p. 140–142°, of Dodds and co-workers,[1] yielding stilbestrol m. p. 171° and apparently pure ψ-stilbestrol m. p. 151°. The latter was converted into stilbestrol by heating with alcoholic hydrochloric acid. Whereas the structure of stilbestrol was established[16] by ozonization, the structure of ψ-stilbestrol remains to be proved.

The new phenyl-indene derivatives offered another approach to this problem. Wiegand and Merkel[21] showed that the absorption curve of 2-phenyl-indene in dioxane is very similar to that of *trans*-stilbene, whereas that of *cis*-stilbene is entirely different.

The absorption spectra of stilbestrol (I) and its di-propionate (IV) have been reported previously.[22,23] However, no reference was made to their significance regarding the configuration of stilbestrol.

(12) Marvel, Blomquist and Vaughn, THIS JOURNAL, **50**, 2810 (1928).

(13) Linnel and Sharma, *Quart. J. Pharm.*, **14**, 259 (1941).

(14) Von Wessely and Kleedorfer, *Naturwissenschaften*, **27**, 567 (1939).

(15) Von Wessely, *et al.*, *Monatsh.*, **73**, 127 (1940).

(16) Von Wessely, *Angew. Chem.*, **53**, 197 (1940).

(17) Von Wessely and Welleba, *Naturwissenschaften*, **28**, 780 (1940).

(18) Von Wessely and Welleba, *Ber.*, **74**, 777 (1941).

(19) Giacomello and Bianchi, *Gazz. chim. ital.*, **71**, II, 667 (1941).

(20) Walton and Brownlee, *Nature*, **151**, 305 (1943).

(21) Wiegand and Merkel, *Medicine and Chemistry*, **3**, 320 (1936).

(22) Elvidge, *Quart. J. Pharmacol.*, **12**, 347 (1939); **13**, 219 (1940).

(23) Kharasch and Kleiman, THIS JOURNAL, **65**, 11 (1943).

The absorption spectrum of 2-(*p*-acetoxyphenyl)-(3)-ethyl-6-acetoxy-indene (V)[24] in ethanol has been compared with the spectra of *trans*-stilbene (m. p. 120°), *cis*-stilbene (m. p. +1°) (prepared according to Weygand and Rethberg[25]), stilbestrol (I) (m. p. 169°), and stilbestrol diacetate (III) (m. p. 124°), prepared from stilbestrol and acetic anhydride.

As expected the absorption curves No. 5 for 2-(*p*-acetoxyphenyl)-3-ethyl-6-acetoxy-indene (V) and No. 1 for *trans*-stilbene are very similar with a characteristic maximum at 295 and 297 mμ, respectively (Fig. 1). These curves closely conform with those reported by Wiegand and Merkel[22] for *trans*-stilbene and 2-phenylindene. The curves No. 3 for stilbestrol and No. 4 for its diacetate are entirely different from the curve for *trans*-stilbene. The intensity of absorption of stilbestrol depends, to a certain extent, on its dilution in the solvent, but stilbestrol (I) in all dilutions measured, shows a marked inflection at 280 mμ where the curve No. 2 for *cis*-stilbene has its characteristic maximum. The absorption curves of stilbestrol and its dimethyl ether reported by Kharasch and Kleiman,[23] both show the inclination at 280 mμ. The curve No. 4 for stilbestrol diacetate does not show this inflection. On the other hand, in the case of 2-phenyl-indene and 2-(*p*-acetoxyphenyl)-3-ethyl-6-acetoxy-indene (V) substitution of the aromatic rings with acetoxy groups has not materially influenced the absorption spectrum. A comparison of the absorption curves of pure ψ-stilbestrol and its diacetate with the curves reported here might furnish more conclusive evidence. It would also be interesting to compare the spectra of *cis*- and *trans*-diethylstilbene and *cis*- and *trans*-*p*,*p*'-dihydroxy-stilbene. In spite of these reservations the absorption curves reported here cannot easily be reconciled with a *trans*-configuration of stilbestrol.

Estrogenic Activities.—The new phenylindene derivatives were assayed in castrated female mice in the Pharmacological Laboratories of Hoffmann-La Roche, Inc., by Dr. R. H. K. Foster, to whom we are greatly indebted for this work. The results are summarized in Table I.

It will be noticed that the acetylated phenylindene derivative (VII) was found to be more active than the free dihydroxy derivative (V). This is probably due to the instability of the non-acetylated compound, which is also demonstrated by a decreased biological activity after the preparation had been kept for some time. The free hydroxy compound, if assayed immediately after being prepared, might have equal or higher activity than the acetylated compound which is more stable. The latter has one-twelfth of the activity of stilbestrol or roughly the same as that reported for 4,4'-dihydroxy-α-methyl-β-ethyl-stilbene,[2] to which of the estrogenic stilbene derivatives it is most closely related. The ratio of oral to subcutaneous dosages for 2-(*p*-acetoxyphenyl)-3-ethyl-6-acetoxy-indene is 25 and therefore less advantageous than the ratio of 4.75 found for stilbestrol. Hydrogenation of the 2,3 double bond in the phenyl-indene derivatives (VII) decreases the activity, whereas hydrogenation of stilbestrol results in equal or increased activity.[4] It is surprising that the activity found for 2-propoxyphenyl-3-ethyl-6-propoxy-indene (VIII) is markedly lower than that of the acetylated compound (VII).

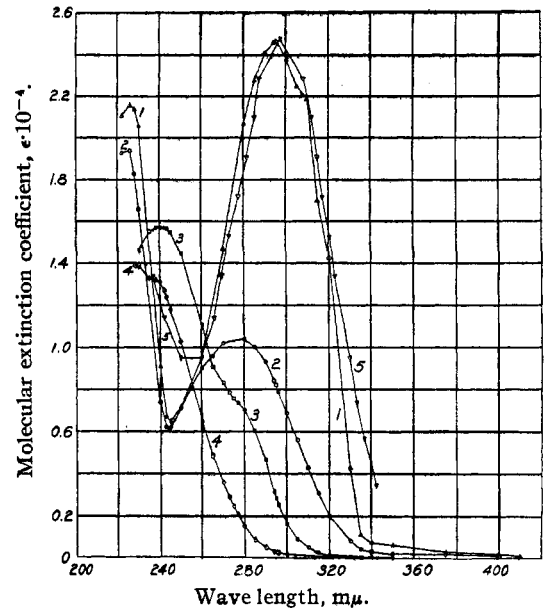


Fig. 1.—1, *trans*-Stilbene; 2, *cis*-stilbene; 3, stilbestrol (concn. $1.107 \cdot 10^{-4}$ mole/liter); 4, stilbestrol diacetate; 5, 2-(*p*-acetoxy-phenyl)-3-ethyl-6-acetoxy-indene.

TABLE I

Substance	Route of administration	No. of animals	E. P. 50[a]	Unit dose rel. to stilbestrol
2-(*p*-Acetoxyphenyl)-3-ethyl-6-acetoxy-indene (VII)	Subcutaneous	153	0.93γ	11.6
2-(*p*-Propoxyphenyl)-3-ethyl-6-propoxy-indene (VIII)	Subcutaneous	22	30.00γ	375.0
2-(*p*-Hydroxyphenyl)-3-ethyl-6-hydroxy-indene (V)	Subcutaneous	12	1.20γ	15.0
2-(*p*-Hydroxyphenyl)-3-ethyl-6-hydroxy-indene (X)	Subcutaneous	48	1.87γ	23.3
Estrone	Subcutaneous	38	0.095γ	1.2
Stilbestrol	Subcutaneous	45	0.08γ	1.0
Stilbestrol	Oral	27	0.38γ	1.0
2-(*p*-Acetoxyphenyl)-3-ethyl-6-acetoxy-indene (VII)	Oral	20	23.00γ	60.5

[a] Defined as the dose producing estrus in 50% of the mice.

Experimental

Sodium Salt of *p*-Methoxy-phenylacetic Acid (XV).—The sodium salt was prepared by dissolving *p*-methoxyphenylacetic acid in dry ether and adding one equivalent of

(24) The absorption curve for 2-(*p*-acetoxyphenyl)-3-ethyl-6-acetoxy-indene was kindly taken for us by Dr. S. Bergstrom, Columbia University, College of Physicians and Surgeons. For measuring all other spectra we are indebted to Mr. A. Motchane of these Laboratories.

(25) Weygand and Rethberg, *Ber.*, **73**, 771 (1940)

sodium dissolved in absolute alcohol. The almost colorless precipitate was filtered off and dried, m. p. 197°.

m-Methoxy-α-(p-methoxyphenyl)-cinnamic Acid (XIV).—99 g. of sodium *p*-methoxyphenylacetic acid and 71.5 g. of *m*-methoxybenzaldehyde (XIII) in 500 cc. of glacial acetic acid were refluxed for six hours in an oil-bath at 175°. After refluxing for two hours the reaction mixture became almost clear, but, nearing the end of the reaction, crystalline reaction products began to precipitate. The reaction mixture was poured into water and allowed to stand for several hours. The crystalline precipitate was filtered off, washed with water and then treated with aqueous sodium carbonate solution on a steam-bath. After cooling, the mixture was shaken with ether several times, the ether layer separated, washed with water and worked up as described below. The alkaline aqueous solution was acidified with hydrochloric acid and the resulting crystalline precipitate recrystallized from acetic acid; yield 87 g.(92.2%); m. p. 169°.

Anal.[26] Calcd. for $C_{17}H_{16}O_4$: C, 71.8; H, 5.6. Found: C, 71.9; H, 5.8. The above-mentioned ether extract was evaporated and the crystalline residue recrystallized from ethanol: 8.8 g. of a fraction, m. p. 109–110°, was obtained, which according to the following analysis is 3,4'-di-methoxy-stilbene.

Anal. Calcd. for $C_{16}H_{16}O_2$: C, 80.0; H, 6.7. Found: C, 80.1; H, 6.7.

From the mother liquor another fraction was obtained by evaporation. After recrystallization it had a melting point of 120–121°.

Anal. Calcd. for $C_{34}H_{30}O_{17}$: C, 74.2; H, 5.5. Found: C, 74.1; H, 5.8.

1.3 g. of this compound was refluxed for one and a half hours with 50 cc. of 5% aqueous sodium hydroxide. The almost clear solution was extracted with ether, the alkaline layer filtered, acidified with hydrochloric acid and the resulting crystalline precipitate identified as *m*-methoxy-α-(*p*-methoxyphenyl)-cinnamic acid by its melting point and mixed melting point. Therefore the substance, m. p. 120–121°, is *m*-methoxy-α-(*p*-methoxyphenyl)-cinnamic anhydride.

m-Methoxy-β-(p-methoxy-phenyl)-hydrocinnamic Acid (XV).—10.6 g. of *m*-methoxy-α-(*p*-methoxyphenyl)-cinnamic acid dissolved in glacial acetic acid was hydrogenated at atmospheric pressure in the presence of palladium when the equivalent of 1 mol. of hydrogen was taken up rapidly. The catalyst was filtered off and the solvent evaporated. The crystalline residue was dissolved in ethanol and hot water added to the hot alcoholic solution until turbidity; yield 8.5 g. (79.8%); m. p. 106°, colorless crystals.

Anal. Calcd. for $C_{17}H_{18}O_4$: C, 71.3; H, 6.3. Found: C, 71.1; H, 6.0.

2-(p-Methoxy-phenyl)-6-methoxy-indanone-(3) (XVI) and 2-(p-Methoxyphenyl)-4-methoxy-indanone-(3) (XVII).—Fourteen grams of *m*-methoxy-α-(*p*-methoxyphenyl)-hydrocinnamic acid (VI) was dissolved in 450 cc. of dry benzene in a 1-liter glass-stoppered bottle; 70 g. of phosphoric pentoxide was added and the mixture shaken for ninety minutes. The reaction mixture was then poured on ice, ether added, the benzene–ether layer separated, washed with water, with dilute sodium carbonate solution and then again with water. On evaporation of the solvents, a crystalline residue was obtained which was separated into two components by crystallization in the following manner. The residue was dissolved in acetone with addition of norit, filtered and petroleum ether added carefully so that no precipitate appeared while hot, but crystallization started when cooling. After one hour in the cold, the precipitate was filtered off, and once more crystallized from acetone–petroleum ether, m. p. 172°; yield 4.5 g. (34.4%).

Anal. Calcd. for $C_{17}H_{16}O_3$: C, 76.0; H, 6.0; OCH_3, 23.1. Found: C, 75.6, 75.4; H, 6.2, 6.3; OCH_3, 23.2.

(26) Microanalyses were carried out by Dr. Al Steyermark of these Laboratories.

The mother liquor was evaporated to one-third of its volume and overnight in the ice-box the second crystalline fraction was obtained; after recrystallization from methanol, m. p. 96°; yield 4.5 g. (34.4%).

Anal. Calcd. for $C_{17}H_{16}O_3$: C, 76.0; H, 6.0. Found: C, 76.2; H, 6.4.

The isomer, m. p. 96°, was distilled at 240–250° bath temperature (2 mm.). The distillate was a light red oil which on triturating with ethanol gave the same crystals, m. p. 96°. The two isomeric indanone derivatives were also purified by chromatographic adsorption on aluminum oxide using a mixture of one part benzene and two parts petroleum ether as the solvent. The pure substances appear in the middle part of the column showing a bright green-blue fluorescence under the ultraviolet lamp. They were eluted from the adsorbent by a mixture of hot methanol and benzene.

2-(p-Methoxy-phenyl)-3-ethyl-6-methoxy-indene (VI).—Six grams of substance (XVI) was dissolved in 100 cc. of anhydrous benzene and diluted with 100 cc. of anhydrous ether. This solution was added to the Grignard reagent prepared from 0.8 g. of magnesium, 5.3 g. of ethyl iodide and 50 cc. of anhydrous ether. The reaction began spontaneously and was completed by refluxing for one hour, whereafter the ether was distilled off and the remainder refluxed for two more hours. After cooling, the mixture was poured on ice with addition of ammonium chloride, the benzene layer separated, dried and evaporated *in vacuo;* 6 g. of an orange-colored oil was obtained which was refluxed for two hours with 100 cc. of 5% aqueous sulfuric acid. The oil, which then showed somewhat deeper color, was taken up in benzene, separated from the acid layer, washed with water and dried over sodium sulfate. The benzene solution was then passed under suction through a tube (4 × 70 cm.) filled with aluminum oxide (Merck, ignited). A mixture of one part benzene and two parts petroleum ether was used for developing the chromatogram, the progress of which was followed under the ultraviolet lamp until the following zones were clearly discernible.

(a) 8 cm. wide. Various narrow zones—greenish fluorescence (top)
(b) 35 cm. wide. Uniform blue fluorescence
(c) 1 cm. wide. Slightly lighter blue fluorescence (bottom)

The zones were separated mechanically and eluted on the Büchner funnel with a mixture of methanol and benzene until the aluminum oxide showed only slight and the filtrate hardly any fluorescence. The methanol was removed by washing with water and the benzene solution evaporated *in vacuo*. The contents of zones (b) and (c) proved to be identical when the residues from the benzene solution were recrystallized from hot methanol, giving a total of 4.5 g. (71.4%) of the desired 2-(*p*-methoxy-phenyl)-3-ethyl-6-methoxy-indene, m. p. 87–88°, peach-colored crystals.

Anal. Calcd. for $C_{19}H_{20}O_2$: C, 81.4; H, 7.2. Found: C, 81.7; H, 7.3.

2-(p-Hydroxy-phenyl)-3-ethyl-6-hydroxy-indene (V).—1.8 g. of 2-(*p*-methoxy-phenyl)-3-ethyl-6-methoxy-indene (VI) dissolved in 24 cc. of glacial acetic acid was treated with 6 cc. of hydrobromic acid (48%). On heating, the solution became clear and was refluxed under carbon dioxide for seventy minutes. The reaction mixture was diluted with water and made alkaline under cooling. A dark oil precipitated and was removed by extraction with ether. The aqueous alkaline layer was filtered, acidified with hydrochloric acid, and three times extracted with ether. The residue from the evaporated ether solution was dissolved in benzene and passed through a tube filled with aluminum oxide (Merck, ignited) and the chromatogram developed with a mixture of 1 part acetone and 3 parts benzene. A dark brown, followed by a yellow zone on top of the column presumably contained decomposition products. The lowest main zone with uniform blue fluorescence under the ultraviolet lamp was separated and

the adsorbed material eluted with dilute aqueous sodium hydroxide. The alkaline solution was filtered from the aluminum oxide and upon acidification with hydrochloric acid a light brown crystalline precipitate was obtained. After recrystallization from dilute ethanol, the substance was obtained in slightly colored crystals, m. p. 136°, which tend to decompose on exposure to air.

Anal. The substance contained no methoxyl. Calcd. for $C_{17}H_{16}O_2$: C, 80.9; H, 6.4. Found: C, 79.8, 79.5; H, 6.5, 6.5.

After saponification of the di-propionic ester described below, the di-hydroxy derivative was again obtained as the same unstable and slightly colored, though crystalline, product, m. p. 136°, giving the same analysis low in carbon.

2-(*p*-Acetoxyphenyl)-3-ethyl-6-acetoxy-indene (VII).—200 mg. of 2-(*p*-hydroxy-phenyl)-3-ethyl-6-hydroxy-indene (V) was refluxed for three hours with 15 cc. of acetic anhydride and 1 g. of anhydrous sodium acetate. After cooling, the reaction mixture was diluted with water and an oil separated which soon crystallized. After recrystallization once from dilute ethanol and twice from ligroin (b. p. 70–90°), the substance was obtained in almost colorless crystals, m. p. 118–120°. Contrary to the free hydroxyl compound, the acetylated derivative is perfectly stable.

Anal. Calcd. for $C_{21}H_{20}O_4$: C, 75.0; H, 6.0. Found: C, 75.3; H, 6.3.

2-(*p*-Propoxyphenyl)-3-ethyl-6-propoxy-indene (VIII).—650 mg. of 2-(*p*-hydroxy-phenyl)-3-ethyl-6-hydroxy-indene (V) was dissolved in 5 cc. of dried pyridine, 3 g. of propionic anhydride (redist.) was added, and the mixture refluxed under nitrogen for ninety minutes at 105° bath temperature. After cooling and diluting with water, an oil separated which crystallized slowly on standing in the cold. After repeated recrystallization from methanol, the dipropionic ester crystallized in colorless leaflets, m. p. 88–89°.

Anal. Calcd. for $C_{23}H_{24}O_4$: C, 75.8; H, 6.6. Found: C, 75.8; H, 6.8.

2-(*p*-Hydroxyphenyl)-3-ethyl-6-hydroxy-indane (X).—Two grams of 2-(*p*-methoxy-phenyl)-3-ethyl-6-methoxy-indene (VI), m. p. 87–88°, was dissolved in 30 cc. of hot absolute methanol and hydrogenated in the presence of palladium. One mole of hydrogen was taken up within two and one-half hours, the catalyst was filtered off, the filtrate evaporated *in vacuo* and the remaining oil dissolved in 25 cc. of glacial acetic acid and 6 cc. of hydrobromic acid (48%). After refluxing under nitrogen for two hours, the reaction mixture was diluted with water and made strongly alkaline with sodium hydroxide. Some tarry material separated but went almost completely into solution when warming the mixture on the steam-bath. The alkaline solution was shaken three times with ether, the extract being discarded. The aqueous layer was filtered, acidified with hydrochloric acid, extracted three times with ether, and the combined ether extracts washed repeatedly with water until the washings were no longer acid to congo. The ether solution was dried over sodium sulfate, evaporated *in vacuo* and the residue recrystallized first from benzene, then repeatedly from ethanol; yield 1.25 g., m. p. 162–163°.

Anal. Calcd. for $C_{17}H_{18}O_2$: C, 80.2; H, 7.2. Found: C, 80.3; H, 7.0.

Summary

1. 2-(*p*-Hydroxy-phenyl)-3-ethyl-6-hydroxy-indene and some of its derivatives have been synthesized and were found to possess considerable estrogenic activity. The subcutaneous activity of the most active indene derivative is one-twelfth of that of stilbestrol, though the ratio of oral to subcutaneous activity is less favorable than for stilbestrol.

2. The absorption spectra of phenyl-indene derivatives and of *cis*- and *trans*-stilbene have been compared with those of stilbestrol and its diacetate. Contrary to expectation the latter were found not to correspond to those of 2-phenyl-indene derivatives and *trans*-stilbene.

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Unsymmetrical Diacyl Derivatives of 4,4'-Diaminodiphenyl Sulfone

BY H. A. SHONLE AND A. M. VANARENDONK

In the search for new chemotherapeutic agents, it was early observed that 4,4'-diaminodiphenyl sulfone was more effective, on a weight basis, than sulfanilamide in combatting streptococcic and certain other infections in white mice. Bauer and Rosenthal[1] reported a therapeutic index of 6 for 4,4'-diaminodiphenylsulfone and a therapeutic index of 3.3 for sulfanilamide in white mice infected with hemolytic streptococcus. Because of the relatively high toxicity of 4,4'-diaminodiphenyl sulfone, many derivatives have been prepared and tested in the hope that in some of these a more favorable therapeutic index might be found.

Nitti, Bovet and Hamon[2] investigated some of the lower aliphatic diacyl derivatives of 4,4'-diaminodiphenyl sulfone and determined the amount of free 4,4'-diaminodiphenyl sulfone in the blood after oral administration. They found that the blood concentration of the free amino compound varied inversely with the length of the carbon chain with the exception of the diacetyl derivative which produced a low concentration of the amino compound in the blood stream. They believed that these varying concentrations represented the relative rates of hydrolysis. In the work here reported, we have prepared and tested a series of unsymmetrical 4,4'-diacylaminodiphenyl sulfones in order to ascertain whether any of these new derivatives would be sufficiently effective to possess clinical usefulness.

Pharmacological Part.—These unsymmetrical 4,4'-diacylaminodiphenyl sulfones were used in the treatment of mice infected with hemolytic streptococcus (C 203) and pneumococcus Type I (from Park; original Neufeld I). The experi-

(1) H. Bauer and S. M. Rosenthal, *U. S. Pub. Health Rep.*, **53**, 40 (1938).

(2) F. Nitti, D. Bovet and V. Hamon, *Compt. rend. soc. biol.*, **128**, 26 (1938).