[CONTRIBUTION FROM THE RESEARCH LABORATORY, WALLACE AND TIERNAN PRODUCTS, INC.]

Functional Variants of Diethylstilbestrol

BY MARTIN RUBIN AND HENRY WISHINSKY

The synthetic estrogen diethylstilbestrol[1] (4,4'-dihydroxy-α,α'-diethylstilbene) has achieved a position of importance as a therapeutic agent because of its availability and efficacy on oral administration. That significant therapeutic advantages might be offered by modification of the functional groups in the molecule as in the monomethyl ether,[2,3] led us to investigate other variants of a similar type.

By procedures described in the experimental section, we have prepared variants of diethylstilbestrol in which one or both of the phenolic hydroxyls have been replaced by $-NH_2$, —Br, —COOH, and $-OCH_3$ groups. For comparative purposes we desired to assay diethylstilbene. This compound has been reported previously as a solid melting at 89–90°,[4] and 70–71°,[5] and as an oil.[6,7,8] 3,4-Diphenyl-hexanol-3 was prepared as described by Brownlee, *et al.*,[8] according to the method used by Dodds, *et al.*,[1] for diethylstilbestrol. Dehydration of this carbinol by heating at 180–200° with potassium acid sulfate gave a liquid diethylstilbene from which we were able to separate a crystalline isomer of m. p. 57–58°. From the analogy with diethylstilbestrol we believe this isomer to be *trans*-diethylstilbene.

We are indebted to Drs. Geschickter and Byrnes for the estrogenic assay of the compounds recorded in Table I. The assays of several previously prepared related compounds are included for comparative purposes.

TABLE I

$RC_6H_4C(C_2H_5)=C(C_2H_5)C_6H_4R'$

R	R'	Activity, γ
HO	HO	0.3
MeO	HO	2.5
MeO	NH_2	1000
HO	NH_2	7.5
MeO	COOH	Inactive at 1000
MeO	Br	1000
HO	Br	100
H	H	Inactive at 1000

The figures given in Table I are the weights in γ of the compounds which equal one rat unit as found by the Allan–Doisy method. The rat unit is the minimum amount of the estrogen required to produce cornification of the vaginal smear in 50% or more of a group of ten castrated female rats.

The previously reported bactericidal activity of di-(*p*-hydroxyphenyl)-alkanes[9] and bacteriostatic activity of stilbenes[8,10] led us to examine several of the compounds listed above for bacteriostatic properties. In Table II are recorded the minimum concentrations of these compounds which prevent visible growth (after forty-eight hour incubation at 37°) of the F. D. A. 209 strain of *Staphylococcus* used in these experiments. We are indebted to Mr. Strandskov for these data.

TABLE II

R	R'	Concn.
H	OH	1:640,000[8]
H	H	1:10,000[8]
		<1:10,000
NH_2	OH	1:100,000
Br	OH	1:1,000,000

Experimental

M. p.'s and b. p.'s are uncorrected.

4'-Methoxy-4-nitrodesoxybenzoin.—To a solution of 20.2 g. of *p*-nitrophenylacetyl chloride[11] and 32.4 g. of anisole in 200 cc. of carbon disulfide, 13.4 g. of aluminum chloride was added in small portions at room temperature. After stirring for four hours the reaction mixture was poured into ice and 50 cc. of concentrated hydrochloric acid. The solvent and excess anisole were removed by steam distillation. The non-volatile residue crystallized on cooling. After recrystallization from ethanol 19.4 g. (70.3%) of product melting at 117–118° was obtained.

Anal. Calcd. for $C_{15}H_{13}O_4N$: C, 66.67; H, 4.80. Found: C, 66.65; H, 4.99.

4'-Methoxy-4-nitro-α-ethyldesoxybenzoin.—To a solution of 2.3 g. of sodium in 200 cc. of absolute ethanol was added 27.6 g. of 4'-methoxy-4-nitrodesoxybenzoin and 23.4 g. of ethyl iodide. The reaction mixture was stirred and heated under reflux until the disappearance of the violet color indicated the completion of ethylation (two hours). Two hundred cc. of water was added, the alcohol removed by distillation and the aqueous residue extracted with ether containing 5% of ethanol. The combined extracts were dried over sodium sulfate and concentrated *in vacuo*. Distillation of the residue gave 23 g. (76.6%) of pale yellow oil, b. p. 210–215° at 0.8 mm.

Anal. Calcd. for $C_{17}H_{17}O_4N$: C, 68.24; H, 5.67. Found: C, 68.44; H, 5.52.

A mixture of 8.4 g. of 4-nitro-α-ethylphenylacetic acid[12] and 10 cc. of thionyl chloride was refluxed for one hour. The excess thionyl chloride was removed by distillation *in vacuo* from a boiling water-bath. To the residue was added 8 g. of anisole in 50 cc. of carbon disulfide. To this mixture, cooled in an ice-bath, was added 5.3 g. of aluminum chloride. When the evolution of hydrogen chloride had ceased, the mixture was warmed for fifteen minutes on a water-bath. The reaction mixture was decomposed with ice and 10 cc. of concentrated hydrochloric acid.

(1) Dodds, Golberg, Lawson and Robinson, *Proc. Roy. Soc.* (London), **B127**, 152 (1939).
(2) Reid and Wilson, THIS JOURNAL, **64**, 1625 (1942).
(3) Geschickter and Byrnes, *J. Clin. Endocrinol.*, **2**, 19 (1942).
(4) Rising and Zee, THIS JOURNAL, **50**, 1706 (1928).
(5) Carlisle and Crowfoot, *J. Chem. Soc.*, 6 (1941).
(6) Ramart-Lucas and Anagnostopoulas, *Bull. soc. chim.*, [4] **43**, 1356 (1941).
(7) Kharasch and Kleiman, THIS JOURNAL, **65**, 11 (1943).
(8) Brownlee, Copp, Duffin and Tonkin, *Biochem. J.*, **37**, 572 (1943).
(9) Heinemann, *J. Lab. Clin. Med.*, **29**, 254 (1944).
(10) Faulkner, *Lancet*, **I**, 38 (1943).
(11) Gabriel, *Ber.*, **14**, 2342 (1881).
(12) Fourneau and Sandulesco, *Bull. soc. chim.*, **41**, 450 (1927).

After removal of solvent and excess anisole by steam distillation the residue was extracted with ether containing 5% of ethanol and purified as described above. The product, 9.6 g. (59.7%), was obtained as a pale yellow oil, b. p. 195–205° at 0.5 mm.

Anal. Calcd. for $C_{17}H_{17}O_4N$: C, 68.24; H, 5.67. Found: C, 68.56; H, 5.87.

4-Amino-4'-methoxy-α-ethyldesoxybenzoin.—To a solution of 30.4 g. of 4'-methoxy-4-nitro-α-ethyldesoxybenzoin in 200 cc. of 95% ethanol was added 47.6 g. of granulated tin. After 130 cc. of concentrated hydrochloric acid had been added in small portions the mixture was heated under reflux until the absence of violet color on the addition of a test sample to an alcoholic sodium hydroxide solution indicated that the reduction was complete. After dilution with 200 cc. of water and removal of the alcohol by distillation, the residue was poured, with vigorous stirring, into excess 10% potassium hydroxide solution. The alkaline solution was extracted with ether, the combined ether extracts filtered and dried over anhydrous sodium sulfate, and evaporated *in vacuo*. The residue, which crystallized on cooling, could be purified either by recrystallization from a mixture of petroleum ether and benzene or by distillation. Sixteen grams (57.5%) of product, b. p. 215–220° at 0.8 mm., m. p. 97–98.5°, was obtained.

Anal. Calcd. for $C_{17}H_{19}O_2N$: C, 75.85; H, 7.06. Found: C, 75.79; H, 7.27.

3-(*p*-Aminophenyl)-4-anisyl-hexanol-3.—To the Grignard reagent from 16.4 g. of ethyl bromide and 3.6 g. of magnesium turnings in 150 cc. of dry ether was added 13.5 g. of 4-amino-4'-methoxy-α-ethyldesoxybenzoin dissolved in anhydrous ether. When the addition was complete the mixture was refluxed with vigorous stirring for one hour. The Grignard addition compound was hydrolyzed with dilute sulfuric acid. The excess acid was neutralized with sodium carbonate solution and the upper ethereal layer separated. The aqueous portion was extracted twice with ether and the combined ethereal extracts washed with water and dried over magnesium sulfate. After concentration *in vacuo* the residue crystallized on cooling. On recrystallization from 60–80° petroleum ether, 9.0 g. of product (60.3%) melting at 113–114° was obtained.

Anal. Calcd. for $C_{19}H_{25}O_2N$: C, 76.26; H, 8.39. Found: C, 76.16; H, 8.72.

4-Amino-4'-methoxy-α,α'-diethylstilbene.—On heating 14 g. of 3-(*p*-aminophenyl)-4-anisyl-hexanol-3 at 180–200° for ten minutes dehydration occurred. Distillation of the residue gave 9.5 g. (68%) of 4-amino-4'-methoxy-α,α'-diethylstilbene as a clear colorless viscous oil, b. p. 180–182° at 1 mm.

Anal. Calcd. for $C_{19}H_{23}ON$: C, 81.14; H, 8.19. Found: C, 81.10; H, 8.30.

The **benzoate,** prepared by interaction of the amine and benzoyl chloride in pyridine, melted at 218–218.5°.

Anal. Calcd. for $C_{26}H_{27}O_2N$: C, 81.04; H, 7.01. Found: C, 80.50; H, 7.01.

4-Amino-4'-hydroxy-α,α'-diethylstilbene.—A mixture of 13 g. of 4-amino-4'-methoxy-α,α'-diethylstilbene, 5 g. of potassium hydroxide and 50 cc. of ethanol was heated in a bomb tube for twelve hours at 180–210°. The reaction mixture was diluted with 50 cc. of water and extracted three times with 50-cc. portions of benzene. The alcoholic alkali layer was neutralized with dilute sulfuric acid and the alcohol removed by distillation. After neutralization with sodium carbonate the amine was extracted with ether. Concentration of the ethereal extracts yielded 8.5 g. of brown viscous oil. On fractionation *in vacuo* 6.0 g. of clear colorless viscous oil, b. p. 180–185° 0.1 mm. was obtained. The product crystallized from methanol and melted at 155–156°. The material was insoluble in cold aqueous alkali, indicating that it was free of diethylstilbestrol.

Anal. Calcd. for $C_{18}H_{21}ON$: C, 80.86; H, 7.92. Found: C, 80.36; H, 7.56.

Demethylation of 4-amino-4'-methoxy-α,α'-diethylstilbene could also be effected by heating the compound for five hours under reflux in diethylene glycol with potassium hydroxide.[13] In this way 7 g. of material, m. p. 155–156° was obtained from 33 g. of starting material.

The **dibenzoate** melted at 247–248° on recrystallization from ethanol.

Anal. Calcd. for $C_{32}H_{29}O_3N$: C, 80.81; H, 6.15. Found: C, 80.56; H, 6.34.

4-Bromo-4'-methoxy-α,α'-diethylstilbene.—To the Grignard solution from 77 g. of *p*-dibromobenzene, 8 g. of magnesium turnings and 250 cc. of anhydrous ether was added 70 g. of anisyl-3-hexanone-4[14] in the course of fifteen minutes. The mixture was refluxed with stirring for one hour, cooled in an ice–salt-bath and decomposed with 10% sulfuric acid. The upper ethereal layer was separated, washed with water, dried over anhydrous calcium chloride and the residue after the removal of the solvent distilled *in vacuo*. Following preliminary fractions consisting of bromobenzene, *p*-dibromobenzene and anisyl-3-hexanone-4, a mixture of 4-bromo-4'-methoxy-α,α'-diethylstilbene and 3-anisyl-4-(*p*-bromophenyl)-hexanol-4 was obtained, b. p. 160–185° at 0.5 mm.

The product was heated at 180–200° for one-half hour with 20 g. of fused potassium acid sulfate. Distillation *in vacuo* gave 115 g. (33%) of 4-bromo-4'-methoxy-α,α'-diethylstilbene, b. p. 170–175° at 0.5 mm.

Anal. Calcd. for $C_{19}H_{21}OBr$: C, 66.09; H, 6.10. Found: C, 66.62; H, 6.28.

4-Bromo-4'-hydroxy-α,α'-diethylstilbene.—Following the procedure of Spaeth,[15] a mixture of 17.5 g. of 4-bromo-4'-methoxy-α,α'-diethylstilbene was heated at 180–220° for fifteen minutes with one mol of methylmagnesium iodide. The gelatinous reaction product was decomposed with dilute sulfuric acid and the oil extracted with benzene. The combined benzene extracts were washed with a solution of 5% potassium hydroxide in 50% alcohol until free of acidic material. The combined alcoholic alkali extracts were acidified, the alcohol removed by distillation and the precipitated oil extracted with ether. Concentration and distillation of the ethereal extracts gave 8.5 g. of viscous colorless oil, b. p. 170–180° at 0.1 mm.

Anal. Calcd. for $C_{18}H_{19}OBr$: C, 65.26; H, 5.79. Found: C, 65.83; H, 5.71.

The ***p*-nitrobenzoate** prepared in the usual way melted at 112–114° on recrystallization from ethyl alcohol.

Anal. Calcd. for $C_{25}H_{22}O_4NBr$: C, 62.50; H, 4.58. Found: C, 62.81; H, 4.87.

4-Carboxy-4'-methoxy-α,α'-diethylstilbene.—A mixture of 18 g. of 4-bromo-4'-methoxy-α,α'-diethylstilbene and 25 g. of ethyl iodide in 75 cc. of anhydrous ethyl ether was added dropwise to 5 g. of magnesium turnings in 100 cc. of anhydrous ether. When the addition was complete the mixture was refluxed with stirring for two hours. The ethereal solution was then poured with vigorous stirring onto finely crushed Dry Ice, and the addition product decomposed with 10% sulfuric acid. The upper ethereal layer was washed with water until neutral to litmus paper, dried over anhydrous sodium sulfate and the ether removed by distillation *in vacuo*. The oily residue was dissolved in 500 cc. of benzene and extracted with two 250-cc. portions of a solution of 5% potassium hydroxide in 50% alcohol. The combined alkali extracts were extracted once with 200 cc. of benzene, filtered through dry filter paper and the clear filtrate acidified with concentrated hydrochloric acid. The oil which separated was extracted with ether, and the combined ethereal extracts washed with three 100-cc. portions of water. After drying over sodium sulfate the ether was removed by distillation and the residue allowed to stand at room temperature. After one week the mixture partially crystallized. The crystals were removed by filtration, washed with petroleum ether

(13) Corse, U. S. Patent 2,325,307 (1943).

(14) Tiffeneau, Levy and Weill, *Bull. soc. chim.*, **49**, 1709 (1931).

(15) Spaeth, *Ber.*, **47**, 766 (1914).

and recrystallized from 50% acetic acid. The product, 3.2 g. (20%) melted at 165.5–167°. Prepared by another procedure[16] this product has been reported as melting at 167°.

Anal. Calcd. for $C_{20}H_{22}O_3$: C, 77.21; H, 7.14. Found: C, 76.92; H, 7.14.

Diethylstilbene.—A mixture of 150 g. of 3,4-diphenylhexanol-3[8] and 50 g. of fused potassium acid sulfate was heated at 180–200° for one-half hour. The product was extracted with ether, and the ethereal extracts concentrated. Distillation of the residue gave 120 g. of clear colorless fairly viscous oil, b. p. 128–131° at 0.8 mm. The oil was diluted with two volumes of methanol and placed in the ice-box overnight. The crystalline precipitate after two recrystallizations from methanol melted at 57–58°.

(16) Jaeger and Robinson, *J. Chem. Soc.*, 744 (1941).

Anal. Calcd. for $C_{18}H_{20}$: C, 91.49; H, 8.51. Found: C, 90.96; H, 8.32.

We wish to thank Mr. A. Kozlowski for technical assistance and Mr. S. Gottlieb for the microanalyses reported here.

Summary

1. The preparation of several variants of diethylstilbestrol in which one or more of the phenolic hydroxyl groups have been replaced by $-NH_2$, —Br, —COOH and $-OCH_3$ groups has been described.

2. The estrogenic and bacteriostatic activities of these compounds has been determined.

BELLEVILLE, N. J. RECEIVED JULY 21, 1944

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF WINTHROP CHEMICAL CO., INC.]

Allicin, the Antibacterial Principle of *Allium sativum*. I. Isolation, Physical Properties and Antibacterial Action

BY CHESTER J. CAVALLITO AND JOHN HAYS BAILEY

While investigating plant extracts for antibacterial activity, it was observed that a freshly prepared infusion of ground garlic cloves possessed high antibacterial activity when tested by the cylinder-plate method[1] used for the assay of penicillin.

A literature investigation showed that *Allium sativum*, the common garlic, has been endowed with therapeutic virtues both in legend and in the scientific literature. Most of the claims have been poorly substantiated. Several investigators have observed antibacterial activity of garlic extracts and have attributed this activity to diallyl sulfide,[2,3] unstable sulfur in alkyl polysulfides,[4] a bacteriophage,[5] acrolein or some similar unsaturated aldehyde,[6,7] and recently to a chemically undefined group of substances designated as phytoncides.[8]

A preparation of oil of garlic obtained by steam distillation of the crushed cloves was subjected to fractional distillation and the fractions separated as described by Semmler.[9] When tested by the cylinder-plate method, the natural diallyl disulfide and diallyl polysulfides showed practically no antibacterial action. Synthetic diallyl sulfide was equally ineffective. Aqueous solutions of acrolein and crotonaldehyde showed a diffuse reaction and no sharply defined zones of inhibition. The garlic antibacterial, hereinafter called allicin, showed a sharp zone of inhibition with the periphery accentuated by a line of heavy growth. The phytoncides as described[8] appear to be more volatile and less stable than allicin, which has been obtained thus far only from species of garlic.[10]

It was found convenient during this investigation to use the penicillin[1] assay method and penicillin as a reference standard for the assay of crude garlic preparations.

Having shown allicin to differ from previously claimed garlic antibacterials, tests were conducted on the stability of infusions of garlic. These solutions showed rapid loss of activity when heated, considerable stability when refrigerated and immediate inactivation upon addition of alkalies. Dilute acids had no effect.

The antibacterial agent, allicin, has been isolated in the pure state as a colorless liquid.[11]

The compound contains approximately 40% sulfur, and no nitrogen or halogens. The oil cannot be dry distilled without decomposition. It is soluble in water to the extent of approximately 2.5% at 10°, is miscible with alcohol, benzene and ether, and fairly insoluble in the Skellysolves. It has a d^{20} of 1.112; $n^{20}D$ 1.561, and is optically inactive. The pure product is irritating to the skin and the odor is much more characteristically that of garlic than is that of the various allyl sulfides.

Aqueous solutions of allicin have a *p*H of approximately 6.5 and, upon standing, an oily precipitate forms; the acidity slowly increases from formation of small quantities of sulfur dioxide and

(1) Abraham, Chain, *et al.*, *Lancet*, [2] **241**, 177 (1941).
(2) Uemori, *Chem. Abst.*, **24**, 2191 (1930).
(3) Dittmar, *Z. Krebsforsch.*, **49**, 515 (1939).
(4) Kitagawa and Amano, *Chem. Abst.*, **30**, 3019 (1936).
(5) L. M. Jacobson, *ibid.*, **31**, 6689 (1937).
(6) Vollrath, Walton and Lindegren, *Proc. Soc. Exptl. Biol. Med.*, **36**, 55 (1937).
(7) Carl, McKnight, Scott and Lindegren, *Am. J. Hyg.*, **29**, 32 (1939).
(8) B. Tokin, *Amer. Review Soviet Med.*, **1**, 237 (1944).
(9) Semmler, *Archiv. der Pharmazie*, **230**, 434 (1893).

(10) *Allium vineale* also showed antibacterial activity. Osborn, *Brit. J. Exp. Path.*, **24**, 227 (1943), also reports antibacterial activity from *Allium ursinum* and *Allium triquetrum*. We had tested approximately two hundred species of plants up to the time of appearance of the article by Osborn.

(11) The chemistry of allicin is to be discussed in subsequent papers.