[CONTRIBUTION FROM THE RESEARCH LABORATORIES, SCHOOL OF PHARMACY, UNIVERSITY OF MARYLAND, AND THE LILLY RESEARCH LABORATORIES]

Synthetic Analogs of the Adrenal Cortical Hormones

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The preparation of compounds which have certain structural features in common with the hormones of the adrenal cortex, has been reported from time to time.[1] The nature of the necessary functional groups and the ring system used as a nucleus, in the absence of objective investigations of either, must be decided on the basis of analogy and empirical information. The successful substitution of a stilbene nucleus for the perhydrocyclopentanophenanthrene nucleus of natural estrogenic hormones, is suggestive of the possibility that the stilbene nucleus or some related nucleus may be substituted for the perhydrocyclopentanophenanthrene nucleus of the adrenal cortical hormones. The use of an α-keto alcohol side chain, α,β-unsaturated ketone grouping, alcoholic hydroxyl group, ketone group or some combination of these, is predicated on the presence of these structural features in natural cortical hormones.

In the simplest case, judging from the synthetic estrogenic hormones, it appears necessary to devise a molecule in which two similar functional groups are separated by an optimum distance.[2]

A series of compounds has been prepared in which two α-keto alcohol groups are separated by distances which can reasonably be expected to bracket the distance found optimum for estrogenic hormones. The possibility that the other functional groups might satisfy the requirements for typical cortical hormone activity will be investigated in a continuation of the work reported here.

4,4'-Bis-(acetoxyacetyl)-biphenyl (I) and 4,4'-bis-(acetoxyacetyl)-bibenzyl (II) have been recently reported to lack any marked activity in survival tests using adrenalectomized rats.[1e] They can be conveniently prepared by acetolysis of the corresponding halogen-substituted ketones. An attempt to prepare 4,4'-bis-(acetoxyacetyl)-stilbene (III) from the halogen substituted ketone resulted in a fluorescent oil.[1e] We likewise failed to prepare it by acetolysis of the halogen-substituted ketone but were able to obtain it by decomposition of the diazomethyl ketone by means of acetic acid. 4,4'-Bis-[(acetoxyacetyl)-methoxy]-α,α'-diethylstilbene (IV) was prepared by way of the diazomethyl ketone.

In a determination of the saponification equivalent of I, an anomalous value was obtained which was indicative of the existence of a form of the alpha-keto alcohol which is alkali-soluble. Investigations now under way are designed to provide information about the generality of the phenomenon and the structure of the alkali-soluble form.

Experimental

4,4'-Bis-(acetoxyacetyl)-biphenyl (I).—The conversion of 4,4'-bis-(chloroacetyl)-biphenyl, prepared by a previously reported procedure,[3] was accomplished by treatment with fused potassium acetate, acetic anhydride and glacial acetic acid mixture, but the yields were poor (15%). The product, purified by recrystallization from acetone (charcoal) and obtained as tan plates, melted at 193–194°; reported m. p. 184–189°.[1e]

Further confirmation of the structure of this compound was obtained by determination of its saponification equivalent. Heating a sample with 1 *N* sodium hydroxide in diethylene glycol produced a solution which remained clear even after dilution with water and titration to a phenolphthalein end-point with standard hydrochloric acid. This was somewhat surprising since the free carbinol was not expected to be very soluble in water. Furthermore the value of the saponification equivalent was one-half of that calculated for the diester, 88.1; theoretical for $C_{20}H_{18}O_6$, 177.1.

Further addition of acid to the solution resulted in the precipitation of a tan solid. Abnormally low values have subsequently been found for the saponification equivalents of other α-keto alcohol esters. As already indicated, it is suspected that alpha-keto alcohols are capable of existing in isomeric forms which have acid hydrogens.

4,4'-Bis-(chloroacetyl)-bibenzyl.—Chloroacetyl chloride (0.75 mole) was caused to react with bibenzyl (0.25 mole) in the presence of anhydrous aluminum chloride (0.75 mole) in carbon disulfide (100 ml.) following the usual Friedel-Crafts procedure. A 50% yield of 4,4'-bis-(chloroacetyl)-bibenzyl was obtained in the form of light yellow needles, m. p. 142–143° by recrystallization from butanol or benzene; reported m. p. 142–143°.[1e]

At the time of its preparation the compound had not yet been described in the literature and its structure was proved by reduction to the known 4,4'-bis-(acetyl)-bibenzyl. A 1-g. sample, dissolved in 50 ml. of dioxane, was refluxed with 10 ml. of concentrated hydriodic acid for three hours. Dilution of the reaction mixture caused the separation of an oil which crystallized on standing. After recrystallization from benzene the product melted at 165–166°; reported m. p. 168°.[4]

4,4'-Bis-(acetoxyacetyl)-bibenzyl (II).—The conversion of 4,4'-bis-(chloroacetyl)-bibenzyl to the diacetate was accomplished by a procedure which was essentially like that already reported,[1e] in which fused potassium acetate, acetic anhydride and glacial acetic acid were employed.

A 63% yield of long, amber-colored needles, m. p. 163.5–165°, was obtained by recrystallization of the reaction product from acetic anhydride and acetone; reported m. p. 163–165°.[1e]

4,4'-Stilbenedicarboxylic Acid Chloride.—4,4'-Stilbenedicarboxylic acid was prepared by the procedure previously described[5] and by hydrolysis of 4,4'-dicyanostilbene with 10% potassium hydroxide in propylene glycol, using 200 ml. of the solution for each 10 g. of the dinitrile.

The product was generally a mixture of the free acid and potassium salt and difficulty was encountered in efforts to

(1) (a) Linnell and Roushdi, *Quart. J. Pharm. Pharmacol.*, **14**, 270 (1941); (b) Long and Burger, *J. Org. Chem.*, **6**, 852 (1941); (c) Walker, *J. Chem. Soc.*, 347 (1942); (d) The Wellcome Foundation Limited, Browntee and Duffin, British Patent 550,262, May 14, 1942; (e) Ross, *J. Chem. Soc.*, 538 (1945).

(2) Schueler, *Science*, **103**, 221 (1946).

(3) Silver and Lowy, THIS JOURNAL, **56**, 2429 (1934).

(4) I. G. Farbenind. A.-G., German Patent 637,384, Oct. 29, 1936.

(5) Hager, Van Arendonk and Shonle, THIS JOURNAL, **66**, 1982 (1944)

obtain a product that would burn without leaving variable quantities of ash. The mixture could be converted in fair yields to the acid chloride by means of a mixture of thionyl chloride and phosphorus pentachloride although neither reagent alone gave satisfactory results.

4,4'-Stilbenedicarboxylic acid–salt mixture (17 g.) was powdered and refluxed with a mixture of thionyl chloride (100 ml.) and phosphorus pentachloride (40 g.). An additional 15 g. of phosphorus pentachloride was added to the refluxing mixture in portions until at the end of three hours, a red suspension of yellow needles resulted. After volatilization of the solvent at atmospheric pressure, the residue was extracted with 450 ml. of dioxane. On chilling, the dioxane solution deposited yellow needles of 4,4'-stilbenedicarboxylic acid chloride (12.5 g.) (61%), m. p. 227–228°. Successive runs generally produced a yield of 70% based on the dinitrile.

4,4'-Bis-(diazoacetyl)-stilbene.—The product resulting from the reaction of the acid chloride with diazomethane varied considerably in respect to its stability on heating and to its solubility, with evolution of nitrogen, in acetic acid. The conditions for the reaction must be rather carefully defined if consistent results are to be obtained.

4,4'-Stilbenedicarboxylic acid chloride (3 g., 0.01 mole) was powdered and suspended in dioxane (100 ml.). The suspension was added in one portion to an ether solution of diazomethane prepared from 18.5 g. of nitrosomethylurea. The mixture, contained in a 1-liter Erlenmeyer flask closed by a calcium chloride tube, was kept in an icebox for five hours with occasional shaking, and then let stand at room temperature overnight. By filtration of the reaction mixture, 2.8 g. (90%) of 4,4'-bis-(diazoacetyl)-stilbene was obtained as a brilliant yellow solid which appeared to decompose at about 160°.

Lower yields were obtained when lower proportions of diazomethane were employed and when the procedure was varied; *e. g.*, by mechanical stirring in an ice-bath.

4,4'-Bis-(acetoxyacetyl)-stilbene (III).—The diazomethyl ketone was added to glacial acetic acid (25 ml. for each g. of ketone) which had been previously heated to boiling. The compound dissolved with the evolution of nitrogen and the reaction was completed by heating for one-half hour. The product obtained by chilling and filtering the reaction mixture was refluxed for thirty minutes with a mixture of equal parts of acetic anhydride and glacial acetic acid containing 3% fused potassium acetate. The crystals obtained on chilling and filtering were washed with water, alcohol and ether. By this procedure was obtained a 50–60% yield of 4,4'-bis-(acetoxyacetyl)-stilbene as a yellow crystalline solid, m. p. 192–196°.

Anal. Calcd. for $C_{22}H_{20}O_6$: C, 69.5; H, 5.3. Found: C, 69.4; H, 5.2.

4,4'-Bis-(carboxymethoxy)-α,α'-diethylstilbene.—Stilbestrol (26.8 g., 0.1 mole) was dissolved in a solution of sodium (4.6 g., 0.2 equivalent) in absolute ethanol (130 ml.). Ethyl chloroacetate (24.8 g., 0.2 mole) was added in one portion and the mixture was heated gently on a steam-bath for one hour. To the mixture was added 10% sodium hydroxide solution (130 ml.) and, after warming for ten minutes, the mixture was diluted with water (650 ml.), partly decolorized with charcoal and filtered. Acidification of the filtrate caused the separation of an oil which crystallized almost at once. Recrystallization from acetic acid and washing with *i*-propyl ether produced 19 g. (50%) of 4,4'-bis-(carboxymethoxy)-α,α'-diethylstilbene, m. p. 228–229°, neutral equivalent, 187.5; theoretical for $C_{22}H_{24}O_6$: 192.2.

Anal. Calcd. for $C_{22}H_{24}O_6$: C, 68.7; H, 6.3. Found: C, 67.4; H, 6.2.

The analytical data on this sample are inconclusive. Subsequent reactions, however, and analyses of its derivatives confirm the proposed structure.

The ethyl ester was prepared in a similar manner by dissolving stilbestrol (8 g., 0.03 mole) in a solution of sodium (1.4 g., 0.06 equivalent) in absolute methanol (60 ml.) and treating the solution with ethyl chloroacetate (15 g., 0.12 mole). After refluxing for six hours, the mixture was filtered and concentrated to a thick oil by removal of solvent under reduced pressure. The residue was taken up in ether and washed with cold 10% sodium hydroxide to remove unchanged stilbestrol. From the ether solution 5.2 g. (40%) of white solid was obtained which crystallized from ethanol or *i*-propyl ether in colorless plates, m. p. 131–132°.

Anal. Calcd. for $C_{26}H_{32}O_6$: C, 70.9; H, 7.3. Found: C, 70.0; H, 7.4.

4,4'-Bis-[(chloroformyl)-methoxy]-α,α'-diethylstilbene.—4,4'-Bis-(carboxymethoxy)-α,α'-diethylstilbene (13.5 g.) was refluxed in a mixture of ligroin (135 ml.) and thionyl chloride (100 ml.) on a steam-bath until a practically clear solution resulted. The solvent was distilled from the filtered mixture at atmospheric pressure and the residue taken up in 40 ml. of ligroin. After standing overnight in an icebox, 12 g. (81%) of almost colorless crystalline 4,4'-bis-[(chloroformyl)-methoxy]-α,α'-diethylstilbene was filtered off and washed with petroleum ether, m. p. 117–118°.

4,4'-Bis-[(diazoacetyl)-methoxy]-α,α'-diethylstilbene.—4,4'-Bis-[(chloroformyl)-methoxy]-α,α'-diethylstilbene (12 g., 0.03 mole) was dissolved in dioxane (60 ml.) and added slowly during fifteen minutes to an ether solution of diazomethane prepared from 26.8 g of nitrosomethylurea (approximately 0.18 mole of diazomethane or 1.5 times the theoretical amount), while shaking in an ice-bath. A yellow solid began to separate almost immediately and the reaction was completed by allowing the reaction mixture to stand in a flask closed by a calcium chloride tube in an ice-box overnight. On filtration, 8 g. (65%) of yellow crystalline product was obtained, m. p. 156–159° dec.

4,4'-Bis-[(acetoxyacetyl)-methoxy]-α,α'-diethylstilbene (IV).—The bis-diazomethyl ketone (12 g.) was dissolved in 40 ml. of a mixture of fused potassium acetate, (1) acetic anhydride (10) and glacial acetic acid (10) by heating on a steam-bath for one hour or until nitrogen evolution ceased. After the mixture had been let stand at room temperature overnight, 2.5 g. (18%) of light tan crystalline solid was filtered off and washed with water, ethanol and ether. An amorphous white solid was obtained by chilling a solution of this material in 2-propanol, m.p. 163–164° (after softening at 150°).

Anal. Calcd. for $C_{28}H_{32}O_8$: C, 67.8; H, 6.5. Found: C, 67.0; H, 6.6.

Summary

1. Four compounds have been prepared as synthetic analogs of the adrenal cortical hormones in which an attempt has been made to separate two α-keto alcohol ester groups by distances which might reasonably be expected to bracket the distance found optimum for estrogenic hormones.

2. Anomalous values have been found for the saponification equivalents of this type of compound which seem to indicate that it is capable of existing in an isomeric form which is soluble in alkali.

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