The Synthesis and Pharmacological Activity of 5-Ethyl-5-(3-hydroxy-1-methylbutyl)barbituric Acid[1]

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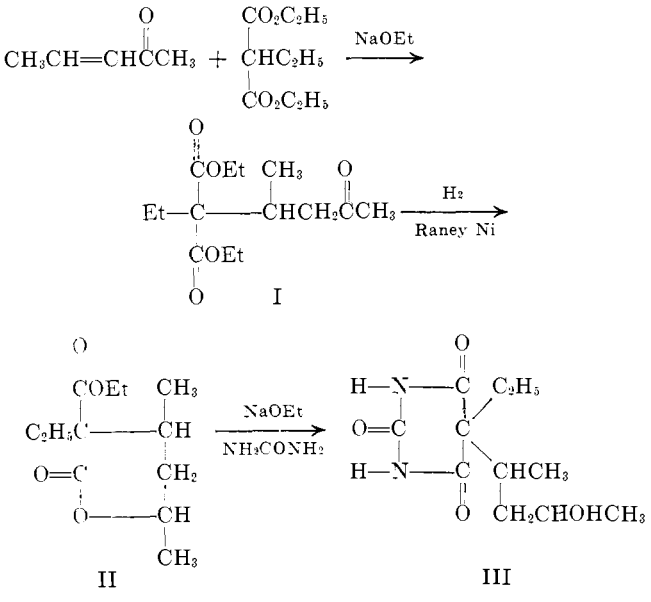
Received August 16, 1965

The anesthetic, pentobarbital, is excreted chiefly as 5-ethyl-5-(3-hydroxy-1-methylbutyl)barbituric acid[2a] (III). Studies using pentobarbital-2-C^{14} show that over 70% of the drug is hydroxylated.[2b] It has been stated that these metabolic products have no pharmacological activity.[3] With the synthesis (Scheme I) of adequate quantities of material, the pharmacological properties of III have been reinvestigated.

SCHEME I



I II III

Pharmacological Data.—Female, Swiss–Webster mice were treated with the test compounds suspended in 1% Methocel and administered orally by stomach tube. Two hours after drug administration, the animals were tested by the maximal electroshock method of Toman, Swinyard, and Goodman.[4] Diphenylhydantoin at a dose of 10 mg/kg was found to be more effective than III at a dose of 500 mg/kg in protecting against the tonic extensor component of convulsions.

By intraperitoneal administration of III as a 1% Methocel suspension, the ED_{38} in the maximal electroshock test was found to be 310 (252–381) mg/kg. No indication of anesthesia or ataxia was noted even in doses of 1 g/kg.

It is concluded from these experiments that III has a very weak anticonvulsant activity and no anesthetic properties. It is doubtful if any of the action of pentobarbital can be ascribed to accumulations of the metabolite.

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Experimental Section

Diethyl Ethyl(1-methyl-3-oxobutyl)malonate (I).—Diethyl ethylmalonate (188 g, 1.0 mole) was added to a stirred solution of sodium (1.3 g, 0.056 g-atom) in 150 ml of dry ethanol at 25°. 3-Penten-2-one (67.2 g, 0.8 mole), prepared by the method of Alexander and Coraor,[5] was added at 10° over a 1-hr period. The mixture was stirred at 10° for 2.5 hr, then neutralized with acetic acid. It was added to 300 ml of water, and the oil layer was separated. The aqueous layer was extracted with two 50-ml portions of ether. The ether extract and the oil layer were combined, and the solvent was removed by distillation. The residue was distilled to yield I (193 g, 88.7%), bp 102–103° (0.5 mm), $n^{25}D$ 1.4440. The product assayed 98% by glc. Infrared and nmr spectra were consistent with the assigned structure.

Anal. Calcd for $C_{14}H_{24}O_5$: C, 61.8; H, 8.85. Found: C, 61.7; H, 8.91.

Ethyl Ethyl(3-hydroxy-1-methylbutyl)malonate δ-Lactone (II).—A solution of I (29.5 g, 0.11 mole) in 100 ml of absolute ethanol was reduced with Raney Ni at 3 atm of H_2. The solution was filtered and the solvent removed by distillation. Distillation of the residue gave II (20 g, 79%), bp 102° (0.1 mm), $n^{25}D$ 1.4560. Infrared and nmr spectra were consistent with the assigned structure. Purity was 98% by glc.

Anal. Calcd for $C_{12}H_{20}O_4$: C, 63.2; H, 8.77. Found: C, 62.9; H, 8.81.

5-Ethyl-5-(3-hydroxy-1-methylbutyl)barbituric Acid (III).—Urea (15 g, 0.25 mole) was added to a stirred solution of sodium (5.75 g, 0.25 g-atom) in 150 ml of dry ethanol. This was stirred to solution at 40°, and then II (19 g, 0.09 mole) was added over a 30-min period. The solution was then refluxed for 40 hr. The solvent was removed at 20 mm until the pot temperature reached 50°. The residue was dissolved in 200 ml of water at 5–10° and extracted with two 50-ml portions of ether. The aqueous solution was neutralized to a pH of 6.0 with 5 *N* HCl and the dissolved ether was removed under reduced pressure. The solution was cooled and filtered to give III (10.9 g, 51.2%), mp 170–175°. The crude product was recrystallized from water to give 6.7 g of III, mp 187–188° (uncor). Infrared and nmr spectra were consistent with the assigned structure.

Anal. Calcd for $C_{11}H_{16}N_2O_3$: C, 54.5; H, 7.45; N, 11.58. Found: C, 54.7; H, 7.43; N, 11.26.

Samples of III were compared with material extracted from the urine of a cat anesthetized with pentobarbital. The urine was acidified to pH 6.4 and extracted with ethyl acetate. Samples were chromatographed on Whatman No. 1 paper with 1-butanol saturated with 1% ammonia as the descending solvent. The chromatograms were sprayed with a 0.1% saturated solution of cobalt acetate in pyridine. The barbiturate derivatives gave a dull purple color with this reagent. The R_f of the synthetic material in this system was 0.80 which compares favorably with the material (R_f 0.78) extracted from the urine.

Acknowledgment.—The authors wish to acknowledge the technical assistance of Miss Shannon Klug and Mrs. Louise Hargreaves.

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The Synthesis of 3-Fluoroestra-1,3,5(10)-trienes

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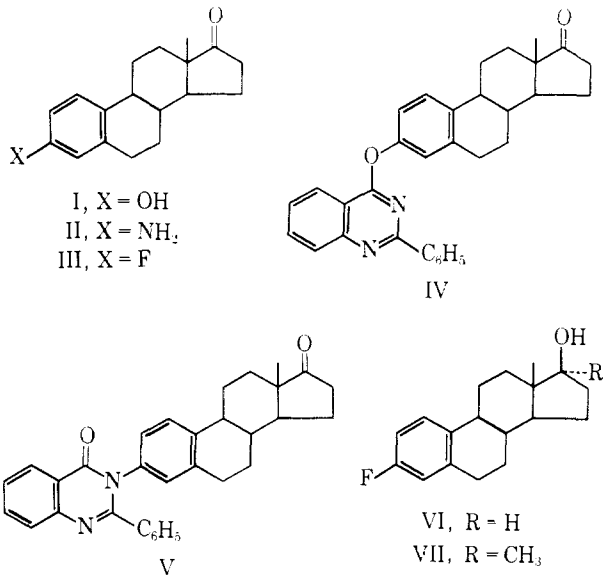
Received October 1, 1965

The substituent at the 3-position of the steroidal estrogens plays an important role in the pharmacological activities of these compounds. A vast improvement in the ratio of the hypocholesterolemic and geno-

tropic[1] activities to the estrogenic activity has been achieved by the elimination of the 3-hydroxyl group of the natural estrogens[2] or by the preparation of various aminoalkyl ethers of this group,[3] respectively. These changes have greatly lowered the estrogenicity of these compounds, which still retain a relatively large proportion of their antiovulatory and/or hypocholesterolemic activities. The introduction of fluorine atoms onto the nucleus of a variety of steroid hormones had led, in several instances, to additional or greatly enhanced activities for these compounds.[4] The substitution of a fluorine atom for the oxygen function at the 3-position of the estratriene nucleus should lead to an interesting comparison of the effects of these two small, highly electronegative atoms.

3-Fluoroestra-1,3,5(10)-trien-17-one (III) was prepared by a Schiemann reaction of 3-aminoestra-1,3,5-(10)-trien-17-one (II).[5] Other 3-substituted estratrienes have previously been prepared by Hecker[6] by replacement of the corresponding diazonium ion prepared from 17β-acetoxy-3-aminoestra-1,3,5(10)-triene. The starting aniline II was readily prepared from estrone in 58% over-all yield by the general method developed by Scherrer for the conversion of phenols into anilines.[7] This synthesis seems to be superior, from the standpoint of both yield and convenience, to that previously employed for the preparation of this compound.[5] Estrone was condensed with 4-chloro-2-phenylquinazoline[8] to give 3-(2-phenyl-4-quinazolinyloxy)estra-1,3,5(10)-trien-17-one (IV), which was pyrolyzed at 330° to yield 3-(4-oxo-2-phenyl-3(4H)-quinazolinyl)estra-1,3,5(10)-trien-17-one (V). This material was hydrolyzed to give 3-aminoestra-1,3,5(10)-trien-17-one (II), which was converted to III by a standard Schiemann reaction.[9] A similar process was previously used to convert 1-hydroxy-4-methylestra-1,3,5(10)-trien-17-one to the corresponding 1-fluoro derivative.[10] Sodium borohydride reduction of III yielded the estradiol derivative VI, and treatment of III with methylmagnesium bromide afforded the corresponding 17α-methyl analog VII.



These three 3-fluoroestra-1,3,5(10)-trienes (III, VI, and VII) as well as the aromatic amine II were inactive as genotropic agents[1] when assayed orally in mice at 5 mg/kg/day.[11] No oral hypocholesterolemic activity was found for these four compounds in rats at 2.5 mg/kg/day.[12] The estradiol analog VI had no detectable antiestrogenic activity and was a very weak estrogen when compared with estradiol in rats (*ca.* 1/100).[13]

Experimental Section[14]

3-Aminoestra-1,3,5(10)-trien-17-one (II).—A solution of 26.4 g (0.098 mole) of estrone in 1 l. of dried diglyme was treated with 4.89 g (0.104 mole) of a 51% dispersion of NaH in mineral oil. The mixture was stirred under an atmosphere of nitrogen until the evolution of hydrogen ceased. To this solution was then added 25.3 g (0.105 mole) of 4-chloro-2-phenylquinazoline,[8] and the resulting mixture was stirred and refluxed under nitrogen for 1.5 hr. The cooled mixture was poured into ice–water, and the product was collected by filtration, washed well with water, and dried under reduced pressure. The crude **3-(2-phenyl-4-quinazolinyloxy)estra-1,3,5(10)-trien-17-one (IV)**, which weighed 50.45 g and exhibited λ_{max} 256 mμ (ε 31,000) and 286 mμ (ε 16,200), was used directly for the next step.

A suspension of 25.2 g of crude IV in 250 ml of heavy mineral oil was covered with an atmosphere of nitrogen, stirred, and heated at 330–335° for 5 hr. The solution was then cooled to room temperature, diluted with 500 ml of petroleum ether (bp 35–60°), and filtered. The crude **3-(4-oxo-2-phenyl-3(4H)-quinazolinyl)estra-1,3,5(10)-trien-17-one (V)**, which weighed 25.0 g and exhibited λ_{max} 279 mμ (ε 14,100), was used directly for the next step.

A solution of 25.0 g of crude V in 1500 ml of absolute ethanol was treated with 350 ml of 40% aqueous NaOH solution and refluxed for 7 hr. This solution was then cooled in ice, treated with 650 ml of 12 *N* HCl, and allowed to stand overnight at room temperature. The mixture then was stirred and refluxed for 1.5 hr, cooled, and filtered. The NaCl precipitate was washed well with ethanol and discarded. The filtrate and washings were concentrated under reduced pressure, poured into water, and filtered. The filtrate was made alkaline with NaOH solution, saturated with K_2CO_3, and filtered. The precipitate was washed well with water, dried under reduced pressure, and sublimed at 165° (0.1 mm). The sublimate was recrystallized from benzene–cyclohexane to yield 7.65 g of II, mp 193–195° (lit.[5] mp 192–192.6°). The yield of product based upon starting estrone was 58%.

3-Fluoroestra-1,3,5(10)-trien-17-one (III).—A solution of 1.00 g (3.72 mmoles) of 3-aminoestra-1,3,5(10)-trien-17-one (II) in 20 ml of ethanol and 10 ml of 48% fluoroboric acid was cooled to 0°, treated with a solution of 0.26 g (3.77 mmoles) of $NaNO_2$ in 1 ml of water, and stirred at 0–5° under an atmosphere of nitrogen for 1.5 hr. The solution was then diluted with 1 l. of ether and filtered. The precipitated diazonium fluoroborate salt was dried

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(14) Melting points were determined on a Fisher-Johns block and are corrected. The ultraviolet spectra were run in methanol. Optical rotations were determined on a 1% solution in $CHCl_3$. All compounds had infrared spectra which agreed with their assigned structures.

under reduced pressure at 70°, at which temperature it decomposed to give the crude 3-fluoro compound. This crude material was dissolved in benzene, filtered, and chromatographed on alumina (Woelm, neutral, activity grade I). The product was eluted with 10% ether in benzene and recrystallized from methanol to give 0.24 g (24%) of III, mp 178–180°, $[\alpha]^{24}D$ +143°.

Anal. Calcd for $C_{18}H_{21}FO$: C, 79.38; H, 7.77; F, 6.98. Found: C, 79.39; H, 8.00; F, 6.83.

3-Fluoroestra-1,3,5(10)-trien-17β-ol (VI).—A solution of 1.00 g (3.69 mmoles) of 3-fluoroestra-1,3,5(10)-trien-17-one (III) in 100 ml of ethanol was treated with 4 ml of 10% NaOH solution and 0.53 g (14.0 mmoles) of $NaBH_4$. After 1 hr at room temperature the solution was poured into water, and the product was extracted with ether. The extract was washed with water, dried, and concentrated to dryness. The residue was recrystallized from hexane to yield 0.65 g (65%) of VI, mp 112–114°, $[\alpha]^{24}D$ +84°.

Anal. Calcd for $C_{18}H_{23}FO$: C, 78.80; H, 8.45; F, 6.92. Found: C, 79.00; H, 8.71; F, 6.67.

3-Fluoro-17α-methylestra-1,3,5(10)-trien-17β-ol (VII).—A solution of 0.84 g (3.09 mmoles) of 3-fluoroestra-1,3,5(10)-trien-17-one (III) in 80 ml of ether was treated with 3.5 ml of 3 *M* methylmagnesium bromide solution. The resulting mixture was stirred and refluxed for 1 hr, cooled, and treated with NH_4Cl solution. The ether layer was separated, washed with water, dried, and concentrated to dryness. The residue was recrystallized from ether–petroleum ether to give 0.40 g (45%) of VII, mp 108–110°, $[\alpha]^{24}D$ +58°.

Anal. Calcd for $C_{19}H_{25}FO$: C, 79.13; H, 8.74; F, 6.59. Found: C, 78.90; H, 8.75; F, 6.24.

Acknowledgment.—The authors wish to thank Mr. C. E. Childs and his staff of our Microanalytical Laboratory and Dr. J. M. Vandenbelt and his staff of our Physical Chemistry Laboratory for their valuable technical assistance. The authors are indebted to Dr. O. D. Bird, Dr. G. Rodney, and Dr. M. R. Callantine for the pharmacological activities presented in this paper.

Synthesis of Some Substituted Tryptophols of Possible Physiological Importance and a Study with 3-(2-Acetoxyethyl)-5-methoxyindole (5-Methoxytryptophol O-Acetate) on Sexual Maturation

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Received July 14, 1965

The unique psychopharmacological effects of ethanol, easily formed by biological processes, have been appreciated since antiquity. Recent studies have indicated that hydroxyl derivatives of histamine,[2a] the catecholamines,[2b,c] γ-aminobutyric acid,[3] tryptamine,[4] and serotonin[5] can be metabolites of these biogenic amines. Furthermore, the resultant biogenic alcohols may possess physiological properties. An example is γ-hydroxybutyric acid, a metabolite of γ-aminobutyric acid[3] which has effective sleep-producing properties.[6]

Tryptophol was identified as a metabolite of tryptamine in rats pretreated by the aldehyde dehydrogenase blocking agent, disulfiram.[4] Kveder, *et al.*,[5] presented good evidence that 5-hydroxytryptophol may be the second most important metabolite of serotonin in the rat, rather than N-acetylserotonin as first thought.[7] Bartholini, Pletscher, and Bruderer[8] found a neutral metabolite of serotonin released from isolated blood platelets by reserpine and have presented chromatographic evidence that it is 5-hydroxytryptophol. Evidence has also been presented for the presence of 5-methoxytryptophol and 5-hydroxytryptophol in pineal tissue[9] rather than β-carbolines, once thought to be constituents.[10]

It was necessary for us to prepare a collection of substituted tryptophols, the acetate esters, and related acids for our studies of metabolism, isolation, and pharmacology of these substances. Acetylation alters markedly the hormonal properties of 5-methoxytryptamine. The latter is without effect on frog-skin lightening while the acetylated analog (melatonin) is a potent frog-skin-lightening hormone[11,12] and displays marked inhibitory effects on the incidence of estrus in rats.[13] Therefore, it was of importance to see if chain acetylation of 5-methoxytryptophol might also produce a compound with special biological properties not seen in the precursor.

5-Methoxytryptophol was prepared by the lithium aluminum hydride reduction of either 5-methoxyindole-3-glyoxyloyl chloride or 5-methoxyindole-3-acetic acid. The reduction of the former produced a considerable amount of by-product which could not be removed by distillation or fractional crystallization. However, the 5-methoxytryptophol could be purified by conversion to a solid picrate derivative. Reduction of the acid gave a better product which was easily purified without recourse to the picrate. It was a yellow oil which was acetylated with acetic anhydride to give an oil that did not solidify, but that could be converted into a useful picrate. The metabolic fate and effects on sexual development of 3-(2-acetoxyethyl)-5-methoxyindole in the female rat were investigated; the latter study is reported in this paper, and the former will be reported elsewhere.

Other substituted tryptophols prepared were compounds which could be potential metabolites of 5-methoxytryptophol O-acetate and serve as chromatographic standards. For the selection of authentic compounds to be synthesized for chromatography, three possible metabolic transformations were anticipated. These are described together with synthetic routes (Chart I) being: (1) 6-hydroxylation only to give VII; (2) 6-hydroxylation and hydrolysis to give V; (3) 6-hydroxylation, ester hydrolysis, and oxidation to give III. Usually, indole-3-acetonitriles can

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