Pyrolysis of 16-Methylene 17-Oxygenated-20-keto Steroids. IV. Synthesis of 3-Hydroxy-1,3,5(10)-estratrieno[17,16-*c*]-2′-methylfuran

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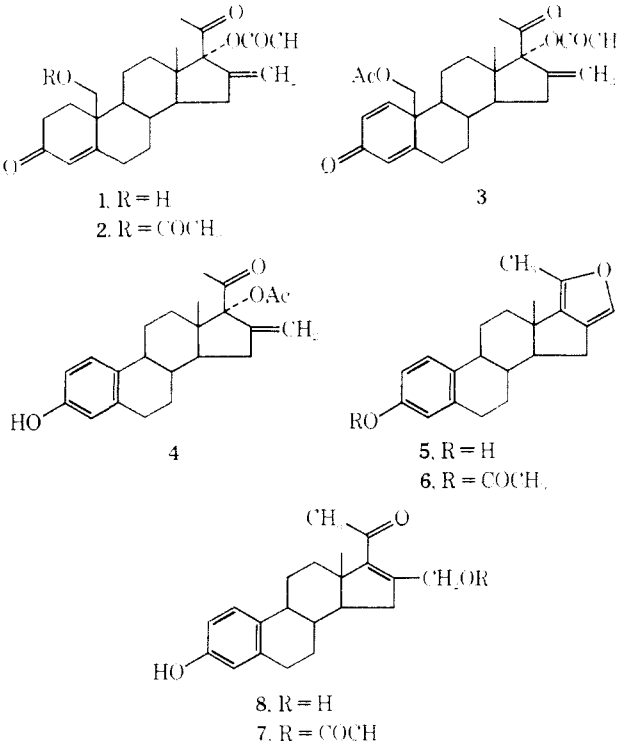
Several groups of workers[1] have reported that estrogens lower serum or plasma cholesterol in humans. The clinical use of estrogens for lowering serum cholesterol in the human male is limited, however, because of the undesirable hormonal side effects of these agents. For some years, attempts have been made to synthesize compounds related to the steroidal estrogens in which the hypocholesterolemic activity is dissociated from the undesirable estrogenic activity.

It has been reported that certain classes of heterocyclic steroids, in which the heterocyclic ring is fused to the C_{16} and C_{17} positions in the steroid skeleton, exhibit increased hypocholesterolemic activity relative to their estrogenicity. Thus, [17,16-*c*]pyrazoles,[2] [17,16-*d*]-2′-methyltriazoles,[3] and [2′,3′-16,17]pyrazines[4] are described as possessing increased hypocholesterolemic activities.

The purpose of this article is to describe the preparation and hypocholesterolemic activity of the 1,3,5(10)-estratrieno-[17,16-*c*]-2′-methylfuran system.

Recently we reported the pyrolytic conversion of 16-methylene-17α-acetoxy-20-keto steroids into the corresponding androstano[17,16-*c*]-2′-methylfurans.[5] In order to prepare **5** a simple representative of this steroidal heterocyclic system, a suitable method for the preparation of the precursor 16-methylene-17α-acetoxy compound **4** had to be devised.

Acetylation of 16-methylene-17α-acetoxy-19-hydroxyprogesterone (**1**)[6] with Ac_2O in pyridine gave **2** which on treatment with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ)[7] afforded the dienone **3**. Short exposure of **3** to ethanolic KOH gave the desired **4**. No hydrolysis of the 17-acetoxy group under these conditions was observed. The overall yield of **4** from **1** was about 55%. Dehydrogenation of **1** with SeO_2 in refluxing *t*-amyl alcohol[8] afforded **4** directly in 12% yield. Pyrolysis of **4** was carried out at 280° under the conditions described earlier.[5] In addition to the desired **5**, a small amount of the acetate **6** was also isolated. Both **5** and **6** had all spectral and analytical properties in agreement with the structure. In addition, direct acetylation of **5** with Ac_2O in pyridine led to **6**. The formation of **6** can be explained by acetylation of **5** with acetic acid, formed during the pyrolysis, under the conditions employed. A third product of pyrolysis **7**, was isolated as a homogeneous oil. Similar thermal allylic rearrangement of 16-methylene-17α-acetoxy-20-keto steroids to 16-acetoxymethyl-16-dehydro-20-keto steroids has been observed earlier.[5] Hydrolysis of **7** with methanolic NaOH gave the crystalline 16-hydroxymethyl **8** which was fully characterized. During the purification of **8** spontaneous cyclization to **5** was observed to a considerable extent, as detected by tlc of the mother liquors.



Biology.—Compound **5** was suspended in an aqueous medium and administered by gavage once daily for 7 days to 7-week old ovariectomized female rats (Charles River CD strain) in order to determine cholesterol-lowering and estrogenic activities simultaneously. On the eighth day the animals were anesthetized with Et_2O and bled from the abdominal aorta. Serum cholesterol levels were determined by the Technicon Auto-analyzer method. Statistical analysis of the data was performed *via* Dunnett's t statistic. Estrogenicity was determined by (1) examining vaginal smears during the experimental period, and (2) weighing uteri at autopsy. As shown in Table I, **5** was inactive when tested at a daily dose of 1 mg/kg, but was both weakly estrogenic and hypocholesterolemic at 10 mg/kg.

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(4) French Patent, 4741M (Roussel-Uclaf), February 13, 1967; *Chem. Abstr.*, **69**, 96965 (1968).

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TABLE I
ESTROGENIC AND HYPOCHOLESTEROLEMIC ACTIVITY OF 5

Daily dose		Body wt (g)			Uterine		Plasma cholesterol (mg/dl) mean ±
mg/kg	No. of rats	Onset	Final	Δ	wt (mg)	Vaginal smears	standard error
0	6	210	248	38	74.7	6 of 6 diestrus	71.2 ± 5.4
1	6	211	246	35	82.7	6 of 6 diestrus	73.5 ± 4.9
10	6	215	229	14	164.6	6 of 6 proestrous	51.2 ± 3.8 ($p < 0.05$)

Experimental Section[9]

16-Methylene-17α,19-dihydroxy-4-pregnene-3,20-dione Diacetate (2).—A solution of 16-methylene-17α,19-dihydroxy-4-pregnene-3,20-dione 17-acetate (**1**)[10] (1.628 g) in C_5H_5N (8 ml) was allowed to stand with Ac_2O (1.2 ml) for 18 hr. The reaction mixture was added to H_2O, the precipitate collected, dried, and used without any further purification for the preparation of **3**. A 100-mg sample was crystallized from Et_2O–C_6H_{14} affording 56 mg of **2**: mp 216–218°; $[\alpha]_D$ −18°; λ_{max} 239 mμ (ε 16,800). *Anal.* ($C_{26}H_{34}O_6$) C, H.

16-Methylene-17α,19-dihydroxy-1,4-pregnadiene-3,20-dione Diacetate (3).—A solution of **2** (2.11 g) in dioxane (70 ml) was heated at reflux with DDQ (2.45 g) for 17 hr. The solids were removed by filtration and the filtrate passed through a neutral alumina column (Woelm, act. I, 37 × 2.5 cm). Elution with $CHCl_3$ gave 1.488 g (70.7%) of **3**, crystallized from CH_2Cl_2–C_6H_{14}: mp 183–185°; $[\alpha]_D$ −110°; λ_{max} 241.5 mμ (ε 15,200); nmr, δ 4.47, 4.62 (C_{19}-C**H**$_2$O, d, J = 10.5 Hz), 5.49 and 5.62 (C_{16}=C**H**$_2$) ppm. *Anal.* ($C_{26}H_{32}O_6$) C, H.

3,17α-Dihydroxy-16-methylene-19-nor-1,3,5(10)-pregnatrien-20-one 17-Acetate (4). (A) By SeO_2 Dehydrogenation of 1.—A solution of **1** (900 mg) and SeO_2 (414 mg) in t-$C_5H_{11}OH$ (45 ml) was heated at reflux for 4 hr. The solids were removed by filtration. The filtrate was diluted with EtOAc, washed (H_2O), dried, and evaporated to a residue which was chromatographed over silica gel (Baker, 35 × 2.5 cm). Elution with C_6H_{14}–Et_2O (9:1) gave 100 mg of **4**, crystallized from Et_2O–C_6H_{14}: mp 219–223° dec; $[\alpha]_D$ −64°; λ_{max} 281 mμ (ε 2120), 287 (1900, inflexion); nmr, δ 5.09 (C_3-O**H**), 6.59 (C_4-H), 6.63 (C_2-H), and 7.17 (C_1-H) ppm. *Anal.* ($C_{23}H_{28}O_4$) C, H.

(B) By Base Treatment of 3.—A solution of **3** (1.32 g) in EtOH (24 ml), CH_2Cl_2 (24 ml), and H_2O (2.4 ml) was stirred with 1 N ethanolic KOH (12 ml) for 20 min. After neutralization with AcOH and dilution with H_2O, the steroid was extracted with CH_2Cl_2. Evaporation of the solvent gave 1.05 g (95%) of crystalline **4**, which after recrystallization from Et_2O–C_6H_{14} was identical with the product obtained by the SeO_2 dehydrogenation of **1**, as determined by tlc, ir, and nmr.

3-Hydroxy-1,3,5(10)-estratrieno[17,16-*c*]-2′-methylfuran (5) and 16-Acetoxymethyl-19-nor-1,3,5(10),16-pregnatetraen-3-ol-20-one (7).—A fine suspension of **4** (2.42 g) in mineral oil (50 ml) was passed through a heated column of glass beads[5] at 280°. The resulting pyrolysate was extracted with MeOH. After evaporation of the solvent, the residue was chromatographed over Florisil (33 × 3 cm). Elution with C_6H_{14}–Et_2O (19:1) yielded a small amount (less than 20 mg) of crystalline **6**: mp 152–153°; $[\alpha]_D$ +54°; λ_{max} 267 mμ (ε 1350), 274 (1010, inflexion); ν_{max} 1765, 1213 cm^{-1}. *Anal.* ($C_{23}H_{26}O_3$) C, H.

Further elution with the same solvent mixture gave 441.5 mg (22%) of **5**, crystallized from Et_2O–C_6H_{14}: mp 212–214°; $[\alpha]_D$ +59°; λ_{max} 281 mμ (ε 2070), 286 (1840, inflexion); ν_{max} 3545, 1621, 1587, 1087 cm^{-1}; nmr, δ 2.20 (C_{20}-C**H**$_3$), 6.59 (C_4-H), 6.62 (C_2-H), 6.93 (furanyl-H), 7.18 (C_1-H) ppm. *Anal.* ($C_{21}H_{24}O_2$) C, H.

Elution with CH_2Cl_2–MeOH (9:1) gave an oil which was rechromatographed over deactivated silica gel (Baker, 15% H_2O, 30 × 2.7 cm). Elution with C_6H_6 gave 481 mg (20%) of **7** as a homogeneous oil: λ_{max} 249 mμ (ε 6300), 287 (1750, inflexion); nmr, δ 2.09 (C_{16}-CH_2OCOC**H**$_3$), 2.30 (C_{20}-C**H**$_3$), 4.93 (s, C_{16}-C**H**$_2$O) ppm.

3-Hydroxy-16-hydroxymethyl-19-nor-1,3,5(10),16-pregnatetraen-3-one (8).—A solution of **7** (450 mg) in MeOH (10 ml) and 2 N NaOH–H_2O (1.5 ml) was stirred under N_2 for 45 min. After neutralization with AcOH, the reaction mixture was added to H_2O. The precipitate was collected, dried, and crystallized several times from Me_2CO–C_6H_{14} affording 56 mg of **8** as a Me_2CO solvate: mp 128° (transition), 203–206° dec; $[\alpha]_D$ +29°; λ_{max} 252 mμ (ε 7300), 287 (2120, inflexion); nmr (DMSO-d_6), δ 2.29 (C_{20}-C**H**$_3$), 2.17 (acetone), 4.27 (C_{16}-C**H**$_2$OH) ppm. *Anal.* $C_{21}H_{26}O_3 \cdot 0.5C_3H_6O$) C, H. The combined mother liquor contained large amounts of **5** as indicated by tlc.

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(9) Melting points were determined on a Kofler hot stage microscope and are uncorrected. Rotations are in dioxane at 25° at about 1% concentration, uv spectra are of MeOH solutions and ir spectra were measured in Nujol. The nmr spectra were measured on a Varian A60-A spectrometer in $CDCl_3$ (Me_4Si) unless otherwise stated. Solutions were dried over anhyd Na_2SO_4. Analyses were determined by the Physical Organic Chemistry Department of Schering Corporation. Where analyses are indicated only by symbols of the elements or functions, analytical results obtained for those elements or functions were within ±0.4% of the theoretical values.

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N-Substituted 2,2′-Diphenamic Acids and Diphenimides. I[1]

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Some time ago we submitted a large number of compounds to the Walter Reed Army Medical Center for antimalarial screening, compounds which had been screened earlier for antitumor activity by the Cancer Chemotherapy National Service Center. We were recently notified that one of these substances (**19**) showed significant antimalarial activity in preliminary testing in mice (T-C, 6.9 days, or more than doubled survival time at a dose of 640 mg/kg; 4.9 days at 160 mg/kg; 3.7 days at 40 mg/kg).[2]

There appeared to be few, if any, N-substituted diphenimides of this type in the literature, and certainly none screened for biological activity; therefore, we synthesized the 32 new compounds shown in Table I. Unfortunately, none of these have shown significant activity against malaria in mice, chickens, or mosquitoes and **19** did not give evidence of activity in further testing in Rhesus monkeys against two *Plasmodium* species. All of the compounds were screened by the CCNSC in BDF_1 mice with L1210 lymphoid leukemia and were inactive.[3] *N*-2-(7-Flurofluorenyl)-2′,2″-diphenamic acid was toxic at a dosage of 150 mg/kg.

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(3) These data were kindly supplied by Dr. Harry B. Wood, Jr.