6,16-Dimethylated Steroids. II. Studies on the Synthesis of 6,16α-Dimethyl-17α-hydroxyprogesterones[1]

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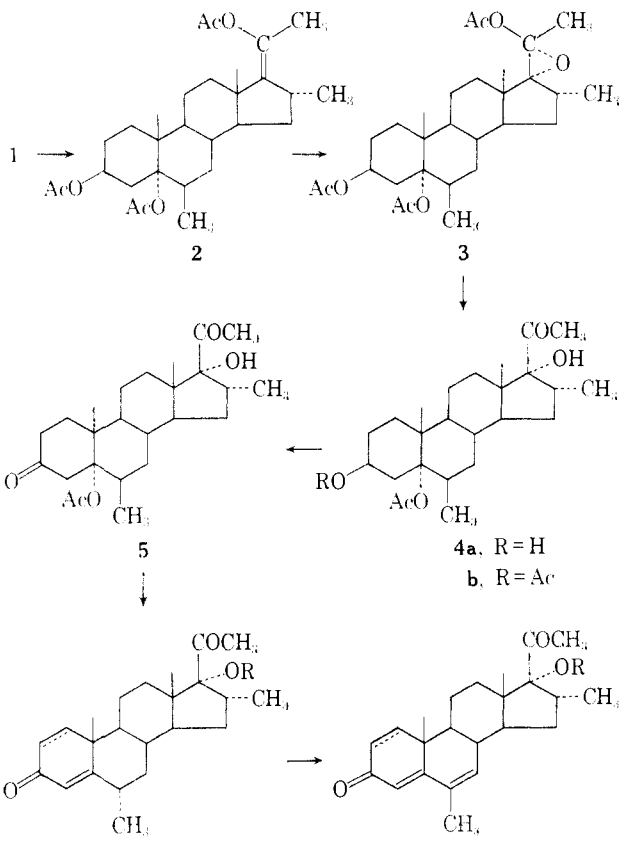
The synthesis of several 6,16α-dimethyl-17-oxygenated progesterones by a variety of routes is described herewith. Of particular interest is the utilization of the readily available 5α,6α-epoxy-16-dehydropregnenolone acetate (**1**) as the starting material for two of the routes. In the first of these, placement of the methyl groups at C_6 and C_{16} as well as the hydroxyl group at C_{17} is accomplished in a straightforward sequence of *only three steps*. Syntheses from 16-dehydropregnenolone acetate (**16**) and from 6-methyl-16-dehydropregnenolone acetate (**19**) are also described. The series of 17-acetates resulting from these sequences comprises one of the most potent groups of orally active progestational compounds known to date. The most active member of this series, 6,16α-dimethyl-6-dehydro-17α-acetoxyprogesterone (**7b**), has an activity at least 130 times that of ethisterone.

The search for synthetic progestogens of high potency and reduced side effects has engaged the attention of numerous groups of investigators during the last several years. A wide variety of compounds have been described both in the area of substituted progesterones and that of substances derived from the C_{19}-steroids. Some of the most active materials, as determined by conventional animal assays, are 17α-acyloxyprogesterones substituted in positions 6 and 16 by halogen, methyl, or methylene groups. Of particular interest, because of their unusually high oral potency in the Clauberg assay, are 6-chloro-6-dehydro-17α-acetoxyprogesterone[2-4] and 6-methyl-16-methylene-6-dehydro-17α-acetoxyprogesterone.[5]

In an earlier paper we described the synthesis of a series of 6,16α-dimethylprogesterones.[6] Although these substances had only a low order of oral progestational activity in the Clauberg assay, they did show that 6,16-dimethyl substitution greatly enhanced the oral activity of the parent progesterones. We now wish to report the details of the synthesis of another series of highly active progestational agents, the 6,16α-dimethyl-17-acyloxyprogesterones. Several members of this series have also been described by other groups.[7]

The initial synthesis of 6α,16α-dimethyl-17α-hydroxyprogesterone (**6a**) (6α,16α-dimethyl-4-pregnen-17α-ol-3,20-dione) and its various esters and dehydro derivatives proceeded from the readily available and highly versatile intermediate 5α,6α-epoxy-16-dehydropregnenolone acetate (**1**) (5α,6α-oxido-16-pregnen-3β-ol-20-one acetate).[8] Treatment of this unsaturated keto epoxide with ethyl Grignard under forcing conditions followed by *in situ* acetylation[9] provided the 6β,16α-dimethyl-17,20-enol triacetate (**2**) as a mixture of its *cis–trans* isomers.[10] The acylation of the intermediate Grignard complex, having –OMgBr groups at C_3, C_{5}, and C_{20}, was not readily accomplished. Substantial excesses of either acetyl chloride or acetic anhydride were required and, in spite of the vigorous conditions, the C_{17}-desoxy compound, 6β,16α-dimethylpregnane-3β,5α-diol-20-one 3-acetate,[6] was subsequently



(1) Preliminary communication: R. P. Graber and M. B. Meyers, *J. Org. Chem.*, **26**, 4774 (1961).

(2) H. J. Ringold, E. Batres, A. Bowers, J. Edwards, and J. Zderic, *J. Am. Chem. Soc.*, **81**, 3485 (1959); R. Sciaky, *Gazz. Chim. Ital.*, **91**, 545 (1961).

(3) K. Brückner, B. Haegel, and U. Johnson, *Chem. Ber.*, **94**, 1225 (1961); K. Brückner, German Patent 1,075,114 (1960).

(4) The following are references to clinical data: (a) J. Martinez Moreno, M. Magnen, R. A. Gilbert, and J. W. Goldzieher, *Fertility Sterility*, **13**, 2:169 (1962); (b) G. I. M. Swyer and V. Little, *J. Endocrinol.*, **24**, xxii (Proc.) (1962); (c) F. Kopel, *Endokrinologie*, **40**, 257 (1961).

(5) D. N. Kirk, V. Petrow, and D. M. Williamson, British Patent 886,619 (1962); for clinical data see ref. 4b.

(6) Paper I of this series: R. P. Graber, M. B. Meyers, and V. A. Landeryou, *J. Org. Chem.*, **27**, 2534 (1962); preliminary communication, R. P. Graber and M. B. Meyers, *Chem. Ind.* (London), 1478 (1960).

(7) (a) J. Iriarte and M. L. Franco, *J. Org. Chem.*, **26**, 2047 (1961); (b) B. Ellis, S. P. Hall, V. Petrow, and H. M. Williamson, *J. Chem. Soc.*, 22 (1962); *cf.* S. P. Barton, B. Ellis, and V. Petrow, British Patent, 884,544 (1961).

(8) G. Slomp, U. S. Patent 2,751,381 (1956); *see* also ref. 6.

(9) *Cf.* K. Heusler, J. Kebrle, C. Meystre, H. Ueberwasser, P. Wieland, G. Anner, and A. Wettstein, *Helv. Chim. Acta*, **42**, 2043 (1959).

(10) For a discussion of the rationale of this synthetic approach to 6,16-dimethylated compounds, see ref. 6.

isolated as a minor by-product together with the desired 3,5-diacetate (**4b**).

Without separation, the *cis–trans* enol acetate mixture (**2**) was oxidized with either perbenzoic acid in benzene or with peracetic acid in buffered acetic acid solution to afford the corresponding dimethyl-17α,20-epoxide triacetate (**3**) again as a mixture of *cis–trans* isomers.[11] No attempts were made to separate the isomeric 17,20-epoxides since in the subsequent step they gave the same 17α-hydroxy-20-ketone (**4a**).

Hydrolysis of the triacetate (**3**) with methanolic potassium carbonate effected saponification of the unhindered 3- and 20-ester functions without attack on the highly hindered 5-acetate group.[12] Isolation of the dimethyltriolone 5-monoacetate (**4a**) proved to be difficult. However, after reacetylation, the 3,5-diacetate (**4b**) was readily obtained by direct crystallization.

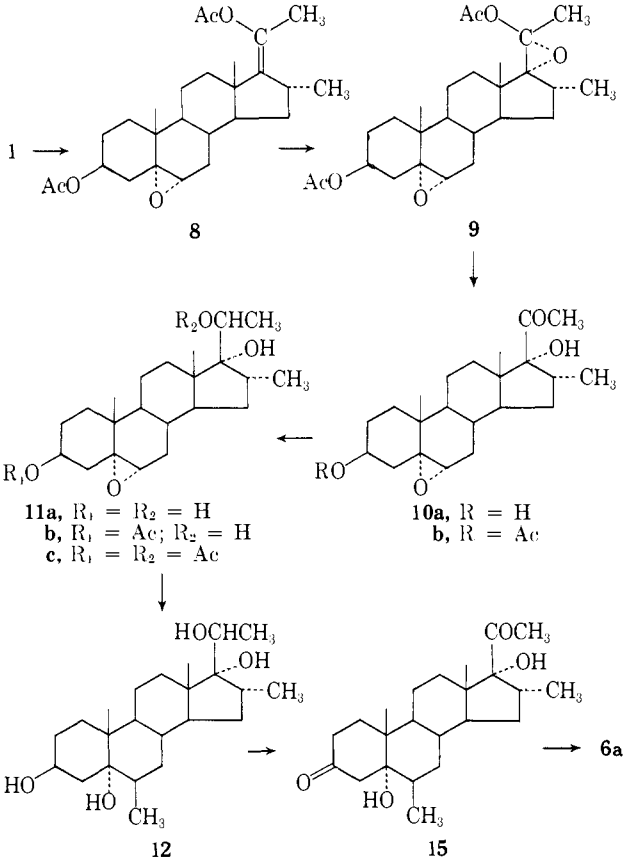
The purified dimethyltriolone 3,5-diacetate (**4b**) was hydrolyzed in excellent yield with potassium bicarbonate in aqueous methanol to the 5-monoacetate (**4a**). Oxidation with 8 *N* chromic acid–sulfuric acid in acetone[13] proceeded smoothly to the dimethyldioldione 5-monoacetate (**5**). Acid-catalyzed elimination[14] of the 5-acetoxy function occurred with concomitant epimerization of the 6β-methyl to the more stable 6α-configuration giving 6α,16α-dimethyl-17α-hydroxyprogesterone (**6a**).

The 17α-acetate (**6b**) was prepared in the usual manner in acetic anhydride–acetic acid solution with *p*-toluenesulfonic acid catalysis.[15] The 3,5-diendiol 3,17-diacetate[7a] initially formed was not purified but rather treated with methanolic hydrochloric acid for a short period. Selective hydrolysis of the 3-enol ester occurred to afford the desired 6α,16α-dimethyl-17α-acetoxyprogesterone. Similar treatment of **6a** with caproic anhydride–caproic acid in the presence of *p*-toluenesulfonic acid gave the 17α-caproate (**6c**).

6,16α-Dimethyl-6-dehydro-17α-hydroxyprogesterone (**7a**) was prepared by chloranil dehydrogenation[16] of **6a**. The corresponding 17α-acetate (**7b**) was prepared as described above for the parent compound. Finally, the 1-dehydro 17α-acetate (**6d**) and the 1,6-bisdehydro 17α-acetate (**7c**) were prepared readily by the use of 2,3-dichloro-5,6-dicyanobenzoquinone[17] from **6b** and **7b**, respectively.

Initially, the yields of the 3,5-diacetate (**4b**) were rather low and this together with the uneconomic use of large amounts of methyl Grignard at an early stage of the synthesis led us to consider an alternate sequence of reactions proceeding again from 5α,6α-epoxy-16-dehydropregnenolone acetate (**1**). Although this route is somewhat longer, the over-all yields were superior; furthermore, the opening of the 5α,6α-oxide with large excesses of methyl Grignard was reserved for a later stage of the sequence.

Treatment of the epoxypregnenolone acetate (**1**) with methyl Grignard at room temperature followed by *in situ* acetylation with acetyl chloride gave the 5α,6α-epoxy-16α-methyl-17,20-enol diacetates (**8**).[18] Under the mild conditions employed, no attack on the 5,6-oxide was observed.



Epoxidation of the 17,20-enol double bond of **8** was accomplished with peracetic acid in buffered acetic acid solution as in the previous sequence. No attempts were made to separate either the *cis–trans* enol acetates or the *cis–trans* 17,20-epoxides (**9**). Hydrolysis of the ester functions of **9** with methanolic potassium hydroxide under mild conditions provided the 5α,6α-epoxy-16α-methylpregnane-3β,17α-diol-20-one (**10a**).[19]

For the vigorous Grignard opening of the 5α,6α-epoxide, the 20-ketone function required "protection."[11] This was accomplished by sodium borohydride reduction of the epoxydiolone (**10a**) to the epoxytriol (**11a**). The predominant product was assigned the 20β-configuration in agreement with the results of Norymberski and Woods.[20,21] In addition,

(11) Although attack of the 17,20-double bond from the rear or α-side of the molecule predominates, trace formation of the epimeric 17β,20-epoxides cannot be ruled out completely. For an illuminating discussion of the *cis–trans* isomerism of similar pairs of 17,20-enol acetates as well as the derived epoxides, see A. L. Nussbaum and F. E. Carlon, *Tetrahedron*, **8**, 145 (1960).

(12) T. H. Kritchevsky and T. F. Gallagher, *J. Am. Chem. Soc.*, **73**, 184 (1951); B. A. Koechlin, T. H. Kritchevsky, and T. F. Gallagher, *ibid.*, 189 (1951).

(13) K. Bowden, I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, *J. Chem. Soc.*, 39 (1946); A. Bowers, T. G. Halsall, E. R. H. Jones, and A. J. Lemin, *ibid.*, 2548 (1953).

(14) D. Burn, G. Cooley, V. Petrow, and G. O. Weston, *ibid.*, 3808 (1959).

(15) R. B. Turner, *J. Am. Chem. Soc.*, **74**, 4220 (1952); **75**, 3489 (1953); Huang-Minlon, E. Wilson, N. L. Wendler, and M. Tishler, *ibid.*, **74**, 5349 (1952).

(16) E. J. Agnello and G. D. Laubach, *ibid.*, **82**, 4293 (1960).

(17) D. Burn, D. N. Kirk, and V. Petrow, *Proc. Chem. Soc.*, 14 (1960).

(18) See ref. 9; also M. Sletzinger and W. A. Gaines, U. S. Patent 2,940,-968 (1960).

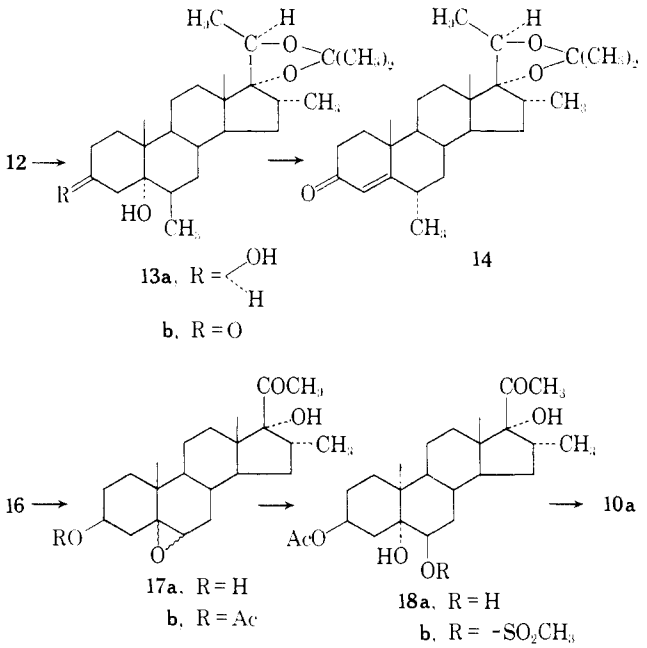
(19) A. Wettstein, G. Anner, and J. Kebrle, U. S. Patent 3,055,887 (1962).

(20) J. K. Norymberski and G. F. Woods, *J. Chem. Soc.*, 3426 (1955).

(21) It is of some interest to note that work-up of the reduction mixture in the usual manner led to a product, m.p. above 300°. Infrared evidence led us to postulate that this product was a 17α,20-cyclic borate ester. Alkaline hydrolysis afforded the desired epoxytriol (**11a**). Revised work-up conditions led directly to the isolation of the epoxytriol. See Experimental section for further details.

comparison of molecular rotation differences served to confirm this assignment.[41] Treatment of **11a** with a *ca.* 20-fold excess of methyl Grignard[22] effected opening of the 5,6-epoxide to give in good yield the 6β,16α-dimethyl-3β,5α,17α,20β-tetrol (**12**).

This tetrol was converted to its 17α,20β-acetonide derivative (**13a**) by acid-catalyzed reaction with acetone[23] and the 3β-hydroxyl oxidized with 8 *N* chromic acid in acetone to the 3-ketone (**13b**). Acid-catalyzed



elimination of the 5α-hydroxyl afforded 17α,20β-isopropylidenedioxy-6α,16α-dimethyl-4-pregnen-3-one (**14**).[24]

Oxidation of the 3- and 20-hydroxyl groups of the dimethyltetrol (**12**) was attempted under a variety of conditions. Not unexpectedly, cleavage of the 17,20-glycol system to the 17-ketone was observed as the predominant reaction under most conditions tried.[25] The desired selective oxidation was achieved successfully, however, by treatment with either N-bromoacetamide or N-bromosuccinimide in acetone solution,[26] or by microbiological oxidation with a strain of *Flavobacterium dehydrogenans*.[27] With NBA or NBS, the 5α,17α-dihydroxy-3,20-diketone (**15**)[79] could be isolated in good yield. Acid-catalyzed elimination and epimerization afforded excellent yields of 6α,16α-dimethyl-17α-hydroxyprogesterone (**6a**).

The product of the microbiological oxidation was a complex mixture of steroidal and nonsteroidal material. Infrared analysis, however, showed clearly the presence of the 3-ketone and the 17α-hydroxy 20-ketone system. Acid treatment of the crude material followed by chromatographic purification afforded the desired progesterone (**6a**), identical in all respects with the materials prepared by the all-chemical routes.

(22) *Cf.* L. F. Fieser and M. Fieser, "Steroids," Reinhold Publishing Co., New York, N. Y. 1959, p. 199; (b) L. E. Miramontes, M. A. Romero, and F. A. Farjat, U. S. Patent 2,878,246 (1959).

(23) G. Cooley, B. Ellis, F. Hartley, and V. Petrow, *J. Chem. Soc.*, 4373 (1955).

(24) Not unexpectedly, oral Clauberg assay of **14** showed it to have no activity when tested at a level at which ethisterone gave a 2.5–3.0+ response.

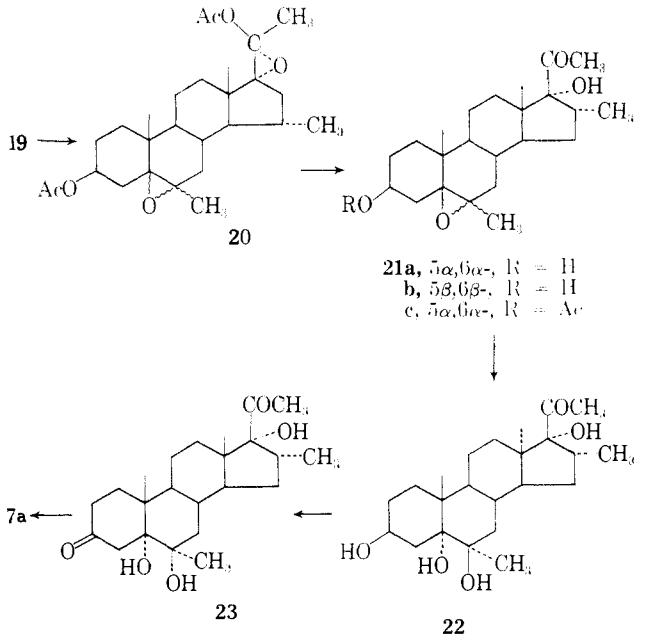
(25) *Cf.* L. H. Sarett, *J. Biol. Chem.*, **162**, 601 (1946).

(26) E. W. Cantrall and S. Bernstein, U. S. Patent 3,019,219 (1962).

(27) A. L. Nussbaum, E. Yuan, E. P. Oliveto, C. Federbush, and W. Charney, *Chem. Ind.* (London), 836 (1960).

An alternate preparation of the 5α,6α-epoxy-16α-methyldiolone (**10a**) was also investigated briefly. 16-Dehydropregnenolone acetate (**16**) was converted by treatment with methyl Grignard and *in situ* acetylation to the mixture of isomeric enol acetates. Epoxidation with perbenzoic acid in benzene gave the mixture of 5,6:17,20-diepoxy isomers which, without separation, was hydrolyzed with methanolic potassium carbonate. The total product, containing both the 5α,6α- and 5β,6β-epoxy isomers (**17a**) was acetylated in the usual manner (**17b**) and then treated with perchloric acid in aqueous acetone. Hydrolytic scission of the oxide linkages in the usual diaxial manner afforded the 16α-methylpregnane-3β,5α,6β,17α-tetrol-20-one 3-monoacetate (**18a**). Reclosure of the 5α,6β-glycol system to the 5α,6α-epoxide (**10a**) was effected by base treatment of the derived 6β-mesylate (**18b**). The product was shown by infrared spectrometry to be identical with that obtained from 5α,6α-epoxy-16-dehydropregnenolone acetate (**1**).[28]

A third sequence of reactions leading directly to 6,16α-dimethyl-6-dehydro-17α-hydroxyprogesterone (**7a**)[29] proceeded from 6-methyl-16-dehydropregnenolone acetate (**19**).[30] Room temperature conjugate Grignard addition followed by *in situ* enol acetylation with acetyl chloride gave the mixture of enol acetates



which on epoxidation with peracetic acid in buffered acetic acid afforded the mixture of isomeric 5,6:17,20-diepoxydimethyldiol diacetates (**20**). Hydrolysis of the ester functions with aqueous methanolic potassium bicarbonate also effected rearrangement of the 17,20-epoxy 20-alcohol to the 17,20-ketol (**21a** and **b**). This mixture of 5,6-epoxides on crystallization gave a small amount of substance, m.p. 235–238°, which was assigned the 5α,6α-epoxy structure (**21a**). Crystallization of the epoxide mixture after acetylation with acetic anhydride and pyridine gave a larger yield of the 3-acetate (**21c**) of the above material.

(28) Wettstein and co-workers[19] have described this synthetic sequence in detail with the isolation and characterization of all the intermediate compounds.

(29) R. P. Graber and M. B. Meyers, U. S. Patent 3,085,090 (1963).

(30) D. Burn, B. Ellis, V. Petrow, I. A. Stewart-Webb, and D. M. Williamson, *J. Chem. Soc.*, 1692 (1957); see also ref. 22b.

Since acid-catalyzed opening of both 5,6-epoxides was expected to lead to the same *trans* 5α,6β-glycol by way of diaxial opening, the mixture was treated with perchloric acid in aqueous acetone. The expected 6α,16α-dimethylpregnane-3β,5α,6β,17α-tetrol-20-one (**22**) was isolated in good yield. Oxidation with chromium trioxide–pyridine complex or with 8 *N* chromic acid in acetone gave the 3-ketone (**23**) which on treatment with hydrochloric acid in absolute ethanol led directly to **7a**.[31]

The oral progestational activities of the various 6,16α-dimethyl-17α-hydroxyprogesterone esters were estimated by the Clauberg method on immature estrogen-primed rabbits.[32] The results are summarized in Table I.

Table I

Clauberg Assays in Rabbits

Compound	Potency[a]
6b	55
7b	130
6d	40
7c	120

[a] Ethinyltestosterone = 1.0.

In the general screening test (subcutaneous injection of 2.0 mg./day for 14 days on intact 21 day old male rats), compound **7b** showed marked adrenal suppresion and thymus involution. This effect, which appears to be specific for the rodent, has been noted earlier by others[33] for 6α-methyl-17α-acetoxyprogesterone.

A granuloma pouch test[34a] demonstrated antiinflammatory activity for **7b** equal to *ca.* 40% of that for hydrocortisone. Sex reversal in the female rat foetus[34b] could not be detected even at challenging dosages of 0.25 mg. of **7b**/day for 6 days (15th through 20th day of pregnancy), either at birth or at sexual maturation of the offspring.

Experimental[35]

6β,16α-Dimethylpregnane-3β,5α,17α-triol-20-one 3β,5α-Diacetate (4b). A. Dialkylation–Enol Acetylation of 5α,6α-Oxido-16-pregnen-3β-ol-20-one Acetate (1).—To a well-stirred mixture of 4.0 g. of cuprous chloride in 410 ml. of ethereal 3 *M* methylmagnesium bromide, was added a solution of 22.4 g. of **1** in 1050 ml. of toluene over a period of 13 min. The mixture was heated to reflux with continued stirring for 140 min. and then cooled and 150 ml. of acetic anhydride[36] added in 15 min. After 30 min. at room temperature, the mixture was heated to 50–55° for 3.5 hr. Saturated aqueous ammonium chloride (700 ml.) was then added slowly with external cooling. The organic layer was separated, washed 3 times with water, and once with saturated sodium chloride solution, and dried,[37] and the solvent evaporated *in vacuo* to give an oil consisting of *cis*- and *trans*-**6β,16α-dimethyl-17(20)-pregnene-3β,5α,20-triol 3β,5α,20-triacetate (2)**, $\lambda_{max}^{CCl_4}$ 5.77, 5.88 (sh), 8.11, 8.18, 8.41, 8.53, and 8.67 μ; $\lambda_{max}^{CCl_4-CS_2}$ 5.70 (sh), 5.75, 5.85, 8.02, and 8.15 μ.

B. Epoxidation of 6β,16α-Dimethyl-17(20)-pregnene-3β,5α,-20-triol 3β,5α,20-Triacetate (2).—The oily triacetate (**2**) above was taken up in 150 ml. of benzene and 170 ml. of a 0.37 *N* solution of perbenzoic acid in benzene was added. After standing for 3 hr., the reaction mixture was diluted with water containing potassium iodide and sodium thiosulfate. The organic layer was separated and washed successively with 5% aqueous sodium bicarbonate solution, water, and saturated sodium chloride solution, and dried, and the solvent evaporated *in vacuo* to give the crude *cis–trans* **epoxide mixture (3)** as an amorphous residue, $\lambda_{max}^{CCl_4}$ 5.77, 5.87 (sh), 8.11, 8.53, 8.67, and 8.88 μ; $\lambda_{max}^{CCl_4+CS_2}$ 5.74, 8.03, and 11.60 μ.

C. Alkaline Hydrolysis of 17α,20-Oxido-6β,16α-dimethylpregnane-3β,5α,20-triol 3β,5α,20-Triacetate (3).—The mixture of *cis* and *trans* epoxides (**3**) was dissolved in 400 ml. of methanol, the solution was heated to boiling, and a solution of 10 g. of potassium carbonate in 100 ml. of water was added. The reaction mixture was heated under reflux for 90 min., then cooled, and treated with 10 ml. of glacial acetic acid. After removing *ca.* 67% of the original volume by evaporation *in vacuo*, water and ethyl acetate were added. The organic layer was separated and washed successively with water, 5% aqueous sodium bicarbonate solution, and saturated sodium chloride solution. After drying, the solvent was removed by evaporation *in vacuo* leaving the crude **dimethyltriolone 5-monoacetate (4a)** as an oily residue which could not be crystallized, $\lambda_{max}^{CCl_4}$ 2.77, 2.86, 5.79, 5.87, and 8.08 μ.

D. Acetylation of 6β,16α-Dimethylpregnane-3β,5α,17α-triol-20-one 5-Acetate (4a).—The crude 5-monoacetate (**4a**) was taken up in 65 ml. of pyridine and treated for 20 hr. with 15 ml. of acetic anhydride. Water was then added and the resulting oil extracted with several portions of ethyl acetate. The organic extracts were washed successively with water, dilute hydrochloric acid, twice with water, and with saturated sodium chloride solution. After drying, the solvent was evaporated *in vacuo* to give a foam which was redissolved in 50 ml. of ether. Skellysolve B (300 ml.) was added and the solution allowed to stand at 7° for 22 hr. The crystalline solid which separated was removed by filtration, washed with Skellysolve B, and dried giving 4.7 g. (17%) of partially purified **3,5-diacetate (4b)**; λ_{max}^{KBr} 5.79, 5.88, 7.89, and 8.0 μ. The solid was dissolved in benzene and the solution introduced onto a column of 200 g. of neutral alumina.[38] Elution with 250-ml. portions of solvent was as follows: 15 portions of 4:1 Skellysolve B–benzene, 3 of 2:1 Skellysolve B–benzene, 3 of 1:1 Skellysolve B–benzene, 4 of benzene, 3 of 19:1 benzene–ether, and 3 of 1:1 benzene–ether.

The residues from fractions 3 through 19 were combined and recrystallized from a mixture of methylene chloride and Skellysolve B to give 2.29 g. (8.2%) of **6β,16α-dimethylpregnane-3β,5α,17α-triol-20-one 3β,5α-diacetate (4b)**, m.p. 205–210°. Successive recrystallizations from acetone and Skellysolve B mixtures gave material of m.p. 207–209°, $[\alpha]^{26}D$ −26.5°, λ_{max}^{KBr} 2.89, 5.75, 5.80, 5.83 (sh), 7.83, and 8.00 μ; $\lambda_{max}^{CCl_4+CS_2}$ 2.74, 2.85, 5.74, 5.83, 5.89, 8.01, and 9.62 μ.

Anal. Calcd. for $C_{27}H_{42}O_6$: C, 70.10; H, 9.15. Found: C, 69.74, 69.96; H, 9.26, 9.31.

The residues from fractions 23 through 30 were combined and recrystallized from a mixture of methylene chloride and Skellysolve B producing 0.52 g. of **6β,16α-dimethylpregnane-3β,-5α-diol-20-one 3-acetate**, m.p. 242–247°, $[\alpha]^{35}D$ +21.3°.[6]

6β,16α-Dimethylpregnane-3β,5α,17α-triol-20-one 5-Acetate (4a).—A mixture of 30 ml. of methanol and 300 mg. of the 3,5-diacetate (**4b**) was treated with a solution of 200 mg. of potas-

(31) The approximate over-all yields to **6a** by the various processes described herein may be compared as follows.

	Via		
	1 and **4b**	**1** and **10a**	**16** and **10a**
From diosgenin	1.7%	10–11%	6%
From **16**	3.6%	21–24%	13%

(32) All bioassays by Endocrine Laboratories, Madison 1, Wis.

(33) D. A. Holub, F. H. Katz, and J. W. Jailer, *Endocrinology*, **68**, 173 (1961).

(34) (a) A. Robert and J. E. Nezamis, *Acta Endocrinol.*, **25**, 105 (1957); (b) G. K. Suchowsky and K. Junkmann, *Endocrinology*, **68**, 341 (1961).

(35) Melting points are capillary melting points taken on a Hershberg apparatus unless otherwise specified and are corrected. Rotations were determined in chloroform solution at 1% concentration unless otherwise specified. Ultraviolet spectra were observed in 96% ethanol. Infrared spectra were determined routinely using a Beckman Model IR-5 spectrophotometer. Those indicated as having been run in both CCl_4 and CS_2, however, were determined on a Beckman Model IR-4 spectrophotometer, in which case CCl_4 was used from 2.0–7.8 μ and CS_2 from 7.8 to 15.0 μ.

(36) Acetyl chloride may also be used. Comparative runs, however, indicated that the use of acetic anhydride gave somewhat better results on the basis of a critical comparison of the infrared spectra of the crude products.

(37) In this laboratory, solvent extracts are routinely dried by filtration through a bed of anhydrous magnesium sulfate contained in a sintered glass funnel.

(38) The neutral alumina used in this work was prepared as follows: Merck reagent grade "alumina for chromatography" (alkaline) was suspended in ethyl acetate and allowed to stand at room temperature for 3 days. It was then separated by filtration, washed thoroughly 3 times with fresh ethyl acetate, and dried at 85–90° for 16 hr. at atmospheric pressure. The activity was *ca.* 3.5 on the Brockmann scale.

sium bicarbonate in 3 ml. of water. The mixture was heated under reflux for 1 hr., 1.0 ml. of glacial acetic acid added, and the solution then cooled and evaporated *in vacuo* to *ca.* 17% of the original volume. Water (50 ml.) was added and the resulting solid removed by filtration, washed with water, and dried, m.p. 183–193°. Successive crystallizations from aqueous methanol gave **4a** as a hydrate, m.p. 186–190° (microblock), $[\alpha]^{26}D$ −27.3°, $\lambda_{max}^{CCl_4+CS_2}$ 2.75, 2.87, 5.78, 5.83, 5.90, and 9.55 μ.

Anal. Calcd. for $C_{25}H_{46}O_5 \cdot H_2O$: C, 68.46; H, 9.65. Found: C, 68.60, 68.78; H, 9.64, 9.68.

6β,16α-Dimethylpregnane-5α,17α-diol-3,20-dione 5-Acetate (5).—To a solution of 1.83 g. of the 5-monoacetate (**4a**) in 100 ml. of acetone was added dropwise with stirring in 80 sec., 2 ml. of *ca.* 8 *N* aqueous chromic acid prepared as follows: 2.67 g. of chromium trioxide dissolved in a mixture of 10 ml. of water and 2 ml. of concentrated sulfuric acid. After 4 min. total time, aqueous sodium sulfite was added, the mixture diluted with ethyl acetate, and the organic layer separated and washed successively with 5% aqueous sodium bicarbonate solution and saturated sodium chloride solution. The extracts were dried and the solvent evaporated *in vacuo* to give 1.77 g. (97.3%) of **5** which after two recrystallizations (70% recovery) from acetone and Skellysolve B mixtures melted at 161–163° (microblock), $[\alpha]^{26}D$ −26.9°, $\lambda_{max}^{CS_2}$ 2.76, 2.88, 5.75, 5.80, 5.84, 5.90, 8.06, and 8.21 μ (IR-4).

Anal. Calcd. for $C_{25}H_{38}O_5 \cdot H_2O$: C, 68.77; H, 9.24. Found: C, 68.67, 68.74; H, 9.36, 9.29.

6α,16α-Dimethyl-17α-hydroxyprogesterone (6a). A. From the Dimethyldioldione 5-Monoacetate (5).—A suspension of 1.67 g. of **5** in 150 ml. of absolute ethanol was treated with 0.7 ml. of concentrated hydrochloric acid. The mixture was heated under reflux for 55 min., then cooled and evaporated *in vacuo* to *ca.* 20% of the original volume. After dilution with 250 ml. of water, the resulting solid was removed by filtration, washed with water, and dried giving 1.50 g. of **6a** which on recrystallization from acetone and Skellysolve B mixtures melted at 203–207°, $[\alpha]^{26}D$ +53.5°, λ_{max} 242 mμ (ε 15,400), $\lambda_{max}^{CCl_4-CS_2}$ 2.76, 2.88, 5.84, 5.93, and 6.20 μ (lit.[7a] m.p. 200–202°, $[\alpha]D$ +51.4°, λ_{max}^{EtOH} 240–242 mμ (ε 16,600), λ_{max}^{KBr} 2.73, 5.80, 5.97, and 6.15 μ).

Anal. Calcd. for $C_{23}H_{34}O_3$: C, 77.05; H, 9.56. Found: C, 76.64; H, 9.65.

B. From the Dimethyldioldione (15).—A 1.75-g. portion of **15**, prepared by the NBS oxidation of the tetrol (**14**), was treated with 70 ml. of absolute ethanol containing 0.34 ml. of concentrated hydrochloric acid as described above. Dilution with 650 ml. of water and extraction with ethyl acetate gave 1.77 g. of **6a**, m.p. 195–199°, infrared spectrum identical with that of the material prepared from **5**. One recrystallization from acetone–hexane gave 1.45 g. (87%), m.p. 199–203°. A further recrystallization from the same solvents raised the m.p. to 202–205°.

The samples of **15** prepared by microbiological oxidation (see below) for 5 and 7 days were also converted to **6a**. Portions weighing, respectively, 217.3 and 155.2 mg. were treated with 10 vol. of 0.4% (v./v.) solution of concentrated hydrochloric acid in absolute ethanol under reflux for 30 min. Concentration *in vacuo* to 2–3 ml., dilution with water, and two extractions with ethyl acetate gave 215 and 165 mg., respectively, of crude **6a** identified by infrared spectra and paper chromatographic analyses. These samples were combined and 352 mg. chromatographed on 35.2 g. of neutral alumina.[38] The fractions eluted with 5, 10, 20, and 50% ether in benzene were combined to give 122.1 mg. of **6a** identical by melting point, mixture melting point, infrared spectra, and paper chromatographic comparison with material prepared from **5** or from **15** obtained by chemical oxidation.

6α,16α-Dimethyl-17α-hydroxyprogesterone Acetate (6b).—A solution of 3.0 g. of **6a** in 90 ml. of glacial acetic acid was prepared. The reaction flask was flushed several times with nitrogen and then a solution of 2.4 g. of *p*-toluenesulfonic acid monohydrate in 90 ml. of glacial acetic acid and 30 ml. of acetic anhydride was added. The mixture was stirred and kept under a nitrogen atmosphere at room temperature for 16 hr. The resulting solution was poured into 1.5 l. of ice–water and the precipitated solid removed by filtration, washed thoroughly with water, and dried *in vacuo*. The solid was dissolved in 150 ml. of methanol and the solution treated with 1.5 ml. of concentrated hydrochloric acid for 3.5 hr. at room temperature in a nitrogen atmosphere. The solution was then concentrated *in vacuo* at room temperature to about 20% of the original volume and diluted with water. The suspension was extracted 3 times with ethyl acetate. The combined extracts were washed with water, 5% aqueous sodium bicarbonate until neutral, again with water, with saturated sodium chloride solution, and finally dried over magnesium sulfate. After filtration and removal of the solvent *in vacuo*, a crude amorphous product was obtained which on crystallization from acetone–hexane gave **6b**; m.p. 169–171°; $[\alpha]^{25}D$ +69.0°; λ_{max} 242 mμ (ε 15,800); $\lambda_{max}^{CCl_4-CS_2}$ 5.72, 5.82, 5.94, 6.20, 8.00, and 11.50 μ (lit.[7a] m.p. 170–172°, $[\alpha]D$ +71.1°, λ_{max}^{EtOH} 240 mμ (ε 13,800); λ_{max}^{KBr} 5.75, 5.85, 5.99, and 8.0 μ; and lit.[7b] m.p. 164–165°; $[\alpha]D$ +74°; λ_{max}^{EtOH} 240 mμ; ε 15,540).

Anal. Calcd. for $C_{25}H_{36}O_4$: C, 74.96; H, 9.06. Found: C, 74.46; H, 9.15.

6α,16α-Dimethyl-17α-hydroxyprogesterone Caproate (6c).—A stirred mixture of 0.80 g. of **6a**, 50 ml. of caproic acid, and 15 ml. of freshly distilled caproic anhydride was treated under nitrogen with 0.75 g. of *p*-toluenesulfonic acid monohydrate. The mixture was allowed to stir for 90 hr. at room temperature. Pyridine (5 ml.) was then added and the mixture steam distilled until no more oily material appeared in the distillate. The residual mixture was extracted thoroughly with ethyl acetate and the combined extracts were washed successively with dilute hydrochloric acid, water, 5% aqueous sodium bicarbonate solution, and saturated sodium chloride solution. After drying, the solution was concentrated *in vacuo* to an oily residue.

This oily product was dissolved in 63 ml. of methanol and the solution treated with 0.5 ml. of concentrated hydrochloric acid under nitrogen. After stirring for *ca.* 18 hr. at room temperature, the solution was concentrated *in vacuo* to *ca.* 20% of the original volume. Dilution with water precipitated an oil which was extracted with ethyl acetate. The combined extracts were washed with 5% aqueous sodium bicarbonate solution, water, and saturated sodium chloride solution, dried, and concentrated *in vacuo* to an oil.

The oily product was purified by chromatography on 52 g. of neutral alumina.[38] The fractions eluted with 1:3 benzene–hexane were combined and evaporated to dryness *in vacuo*. The purified oily caproate was finally distilled at 198–203° bath temperature (0.055 mm.) to give a waxy solid; m.p. 44–49°; $[\alpha]^{25}D$ +40.6°; λ_{max} 241 mμ (13,200); λ_{max}^{KBr} 5.78, 5.84, 6.00, 6.22, and 11.50 μ[38]; $\lambda_{max}^{CCl_4-CS_2}$ 5.73, 5.81, 5.93, and 6.19 μ.

Anal. Calcd. for $C_{28}H_{42}O_4$: C, 76.27; H, 9.71. Found: C, 76.16, 76.34; H, 9.68, 9.70.

6,16α-Dimethyl-6-dehydro-17α-hydroxyprogesterone (7a). A. By Chloranil Dehydrogenation of 6a.—A mixture of 0.16 g. of 6α,16α-dimethyl-17α-hydroxyprogesterone (**6a**), 0.30 g. of chloranil, and 0.20 ml. of glacial acetic acid in 15 ml. of *t*-butyl alcohol was heated under reflux for 16 hr., then cooled, diluted with methylene chloride, and filtered. The filtrate was washed with water, 5% aqueous sodium hydroxide solution, water, and saturated sodium chloride solution. Evaporation *in vacuo* afforded an oily solid (0.16 g.). Several recrystallizations of this residue from acetone–Skellysolve B mixtures gave **7a**, m.p. 224–229.5° (microblock), identical with material prepared below from **23**, by comparison of the infrared spectra and by mixture melting point.

B. By Acid-Catalyzed Dehydration of 6α,16α-Dimethylpregnane-5α,6β,17α-triol-3,20-dione (23).—To a solution of 0.53 g. of **23** in 50 ml. of absolute ethanol was added 0.2 ml. of concentrated hydrochloric acid. The solution was heated under reflux for 40 min., then cooled, evaporated *in vacuo* to about 10 ml., and diluted with 60 ml. of water. The precipitated solid was removed by filtration and dried giving 0.41 g. (85%) of **7a**, m.p. 204–228°. Several recrystallizations from methylene chloride–Skellysolve B raised the melting point to 220.5–229° (microblock); $[\alpha]^{25}D$ +27.9; λ_{max} 290 mμ (23,200); $\lambda_{max}^{CCl_4}$ 2.74, 2.87, 5.83, 5.90, 5.99, 6.13, 6.29, 11.30, and 11.40 μ (IR-4).

Anal. Calcd. for $C_{23}H_{32}O_3$: C, 77.49; H, 9.05. Found: C, 77.16, 77.26; H, 9.15, 9.33.

6,16α-Dimethyl-6-dehydro-17α-hydroxyprogesterone 17-Acetate (7b).—To a solution of 0.38 g. of **7a** in 20 ml. of glacial acetic acid was added 2.0 ml. of acetic anhydride followed by 0.15 g. of *p*-toluenesulfonic acid monohydrate. After standing for 22 hr. at room temperature, the mixture was poured into 300 ml. of water. The oil which separated was extracted with ethyl acetate. The extracts were washed with 5% aqueous

[38] As in other instances, the 4-en-3-one was contaminated with a small amount of 4,6-dien-3-one. In this instance the sample also showed λ_{max} 280 mμ (3800) corresponding to *ca.* 15% dienone. Small peaks in the infrared (KBr) at 6.11 and 6.33 μ confirmed this finding.

sodium bicarbonate solution and saturated sodium chloride solution, dried, and evaporated *in vacuo* to an oil. This oil was taken up in 20 ml. of methanol and 0.2 ml. of concentrated hydrochloric acid added. After standing for 2 hr. at room temperature, 100 ml. of water was added and the resulting precipitate removed by filtration giving 0.40 g. of crude **7b**, m.p. 163–180°. Successive recrystallizations from Skellysolve B raised the melting point to 189.5–195° (microblock); $[\alpha]^{26}D$ +25.6°; λ_{max} 288 mμ (25,000); $\lambda_{max}^{CCl_4-CS_2}$ 5.73, 5.83, 6.00, 6.14, 6.30, 8.03, and 11.39 μ (lit.[7b] m.p. 202–204°; $[\alpha]D$ +21°; λ_{max}^{EtOH} 286 mμ (22,400); λ_{max}^{Nujol} 5.73, 5.85, 6.05, 6.16, and 6.29 μ).

Anal. Calcd. for $C_{25}H_{34}O_4$: C, 75.34; H, 8.60. Found: C, 75.28, 75.48; H, 8.70, 8.71.

Another sample had m.p. 201–202.5°, $[\alpha]^{26}D$ +20.6°, λ_{max} 290 mμ (24,300).

6α,16α-Dimethyl-1-dehydro-17α-hydroxyprogesterone Acetate (6d).—A mixture of 1.01 g. of **6b** and 0.85 g. of 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) in 50 ml. of benzene was heated under reflux for 4.5 hr. The suspension of solid was diluted with 50 ml. of methylene chloride and filtered. The filtrate was further diluted with 100 ml. of ethyl ether and washed successively with dilute aqueous sodium hydroxide solution, water, and saturated sodium chloride solution. After drying over anhydrous magnesium sulfate and filtering, the solution was evaporated to dryness *in vacuo* to give an oily product. Chromatography on neutral alumina[38] gave the partially purified 1-dehydro compound (**6b**) which on recrystallization from acetone–hexane afforded material of m.p. 163–166°. Infrared analysis indicated that this material was a mixture of **6d** and starting **6b**.

The material was therefore treated again with DDQ as above for 17 hr. Isolation and chromatography as above gave an oil which on 2 crystallizations from ether–hexane afforded the pure material; m.p. 168–173°; $\alpha^{27}D$ +21.7°; λ_{max} 245 mμ (15,800); $\lambda_{max}^{CCl_4+CS_2}$ 5.73, 5.82, 5.99, 6.13, 6.21, 8.02, 11.20, 12.34, and 14.18 μ.

Anal. Calcd. for $C_{25}H_{34}O_4$: C, 75.34; H, 8.60. Found: C, 74.90, 75.09; H, 8.49, 8.56.

6,16α-Dimethyl-1,6-bisdehydro-17α-hydroxyprogesterone Acetate (7c).—A mixture of 0.95 g. of **7b** and 1.0 g. of DDQ in 50 ml. of benzene was heated under reflux for 5 hr. The suspension of solid was diluted with an equal volume of methylene chloride and filtered. The filtrate was washed as above and dried and the solvents were removed *in vacuo* to give an amorphous product. A solution of this product in benzene was placed on a column of neutral alumina[38] and the steroid eluted with mixtures of benzene and Skellysolve B. Mixtures of these solvents in the ratio of 1:1 eluted 0.49 g. of the 1,6-bisdehydro compound (**7d**). One crystallization from methylene chloride–Skellysolve B gave 0.44 g., m.p. 161–166°. Recrystallization from acetone–Skellysolve B afforded the pure material; m.p. 161.5–163°; $[\alpha]^{27}D$ −24.2°; λ_{max} 228 (12,000), 256 (8470), and 302 mμ (11,540); $\lambda_{max}^{CCl_4+CS_2}$ 5.72, 5.82, 6.01, 6.18, 6.28, 8.02, and 11.22 μ.

Anal. Calcd. for $C_{25}H_{32}O_4$: C, 75.72; H, 8.13. Found: C, 75.43, 75.35; H, 8.16, 8.07.

5α,6α-Oxido-16α-methylpregnane-3β,17α-diol-20-one (10a). A. From 5α,6α-Oxido-16-pregnen-3β-ol-20-one Acetate (1). (a) Alkylation and Enol Acetylation.—A mixture of 150 ml. of dry tetrahydrofuran and 20 ml. of 3 *M* ethereal methylmagnesium bromide solution was placed under nitrogen. Ninety ml. of the mixture was removed by distillation, the remaining mixture cooled to room temperature, and 0.45 g. of powdered cuprous chloride added. A solution of 11.20 g. of **1** in 80 ml. of tetrahydrofuran was added over a 5 min. period with stirring. The mixture was allowed to stir at room temperature for 45 min. and then a solution of 4 ml. of acetyl chloride in 40 ml. of tetrahydrofuran was added quickly and stirring continued for 40 min. Ninety ml. of saturated aqueous ammonium chloride was then added followed by 100 ml. of ether. The organic layer was separated, washed with 5% aqueous sodium bicarbonate followed by saturated sodium chloride solution, dried, and evaporated *in vacuo* to an oil, 13.7 g., which slowly crystallized; $\lambda_{max}^{CCl_4}$ 5.75, 5.92, 8.13, 8.21, and 11.50 μ. The product was a mixture of *cis*- and *trans*-**5α,6α-oxido-16α-methyl-17(20)-pregnene-3β,20-diol diacetate (8).**

(b) Epoxidation of *cis*- and *trans*-5α,6α-Oxido-16α-methyl-17(20)-pregnene-3β,20-diol 3,20-Diacetate (8).—The mixture of enol acetates (**8**) was treated with 75 ml. of a 0.44 *N* solution of perbenzoic acid in benzene at room temperature. After 190 min. had elapsed, an aqueous solution of potassium iodide, sodium thiosulfate, and sodium bicarbonate was added. The organic layer was separated, washed with 5% aqueous sodium bicarbonate solution followed by saturated sodium chloride solution, dried, and evaporated *in vacuo* to a partially crystalline residue, 13.5 g., which consisted principally of a mixture of *cis*- and *trans*-**5α,6α:17α,20-dioxido-16α-methylpregnane-3β,20-diol diacetate (9)**; $\lambda_{max}^{CCl_4}$ 5.74, 8.11, 11.50, and 11.60 μ.

(c) Alkaline Hydrolysis of the 5α,6α:17α,20-Dioxido-16α-methylpregnane-3β,20-diol Diacetates (9).—The mixture of diepoxides (**9**) above was placed in 250 ml. of methanol under nitrogen and a solution of 3 g. of potassium hydroxide in 200 ml. of methanol added. The mixture was heated under reflux for 45 min., then cooled, and 10 ml. of glacial acetic acid added. The solution was reduced *in vacuo* to about 67% of the original volume and diluted with 750 ml. of water. The resulting precipitate was removed by filtration and washed with water to give 9.6 g. of crude **5α,6α-oxido-16α-methylpregnane-3β,17α-diol-20-one (10a)**, m.p. 198–202°. Several recrystallizations from aqueous methanol raised the m.p. to 224–229° (219–225°, microblock); $[\alpha]^{31}D$ −87.1°; λ_{max}^{KBr} 5.88, 12.53, and 13.3 μ (lit.[19] m.p. 220–225°, also m.p. 245°).

Anal. Calcd. for $C_{22}H_{34}O_4$: C, 72.89; H, 9.45. Found: C, 72.73, 72.89; H, 9.48, 9.63.

5α,6α-Oxido-16α-methylpregnane-3β,17α-diol-20-one 3-Acetate (10b).—A 2.46 g. sample of (**10a**) (m.p. 213–221°) was treated with a mixture of 15 ml. of pyridine and 15 ml. of acetic anhydride overnight at room temperature. Dilution with water (external cooling) gave crystals which were removed by filtration, washed thoroughly with water, and dried *in vacuo*, 2.79 g., m.p. 192–193°. Recrystallization from chloroform–methanol gave 2.114 g., m.p. 193–195°, $[\alpha]^{29}D$ −77°.

Anal. Calcd. for $C_{24}H_{36}O_5$: C, 71.25; H, 8.97. Found: C, 70.78, 70.50; H, 8.98, 8.88.

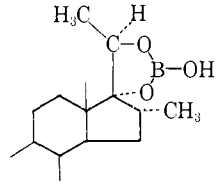
B. From 5,16-Pregnadien-3β-ol-20-one 3-Acetate (16-Dehydropregnenolone Acetate) (16).—A 3.58-g. portion of 16-dehydropregnenolone acetate (**16**) when carried through the sequence of reactions of ref. 19 gave 1.6 g. of crude **10a** which after recrystallization from acetone had m.p. 207–212°, λ_{max}^{KBr} identical with material prepared above from **1**.

5α,6α-Oxido-16α-methylpregnane-3β,17α,20β-triol (11a).—To a solution of 1.09 g. of **10a** in 30 ml. of tetrahydrofuran and 10 ml. of water was added 0.160 g. of sodium borohydride. After heating under reflux for 35 min., the reaction mixture was cooled and 30 ml. of saturated sodium chloride solution added. The tetrahydrofuran layer was separated and washed with 2 portions of saturated salt solution.[40] The organic layer was then diluted slowly with 150 ml. of water. An oil separated which solidified slowly. Filtration gave **5α,6α-oxido-16α-methylpregnane-3β,17α,20(α + β)-triol**, m.p. 207–223°. Several recrystallizations of this material from acetone gave pure **11a**, m.p. 233–236°, $[\alpha]^{31}D$ −91.3°, λ_{max}^{KBr} 12.55 and 13.3 μ.

Anal. Calcd. for $C_{22}H_{36}O_4$: C, 72.49; H, 9.96. Found: C, 72.48; H, 10.05.

5α,6α-Oxido-16α-methylpregnane-3β,17α,20β-triol 3-Acetate (11b).—A solution of 1.924 g. of **10b** (m.p. 193–195°) in 38.5 ml. of tetrahydrofuran and 9.62 ml. of water was treated with 545 mg. of sodium borohydride. The mixture was heated to reflux for 40 min., then cooled to room temperature. Saturated sodium chloride solution (40 ml.) and tetrahydrofuran (35 ml.) were added and the tetrahydrofuran layer sepa-

(40) In earlier runs, the reduction mixture was worked up in the usual manner, as follows. After the reflux period, the excess sodium borohydride was destroyed by the careful addition of excess dilute aqueous acetic acid. The tetrahydrofuran was removed *in vacuo* to give a suspension of crystalline solid which, after further dilution with water, was filtered. The solid was washed thoroughly with water and dried, m.p. above 310°, λ_{max}^{KBr} 6.55, 6.78, and 11.57 μ. This material is believed to be the cyclic borate ester derived from **11a**.



Treatment of this high melting solid (1.1 g.) with 0.5 g. of potassium hydroxide in 40 ml. of methanol under reflux for 15 min., concentration *in vacuo*, and dilution with water afforded crude **11a**, m.p. 202–209°. The infrared spectrum was substantially identical with the material prepared above.

roed, washed 4 times with saturated salt solution to neutrality, dried, and evaporated to dryness *in vacuo* to give 2.04 g. of crystals: m.p. 124–156°; λ_{max}^{KBr} 2.95, 5.80, and 8.45 μ; $\lambda_{max}^{CCl_4}$ 2.8, 5.78, and 8.1 μ. Two recrystallizations from acetone–Skellysolve B afforded 363 mg. of **11b**: m.p. 169–171°; $[\alpha]^{26}D$ −83.4°; λ_{max}^{KBr} 2.82, 2.95, 5.79, 8.05, and 12.5 μ.

Anal. Calcd. for $C_{21}H_{30}O_5$: C, 70.90; H, 9.42. Found: C, 71.04, 71.05; H, 9.40, 9.56.

5α,6α-Oxido-16α-methylpregnane-3β,17α,20β-triol 3,20-Diacetate (11c). A. From 11a.—A 0.700 g. sample of **11a** (m.p. 210–214.5°, obtained in 74% yield from **10a**) was acetylated with a mixture of 10 ml. of pyridine and 10 ml. of acetic anhydride overnight at room temperature. Dilution with water gave a solid which was removed by filtration, washed thoroughly with water, and dried *in vacuo* to give 0.851 g. (99%) of **11c**, m.p. 158–164.8°. Three recrystallizations from acetone–Skellysolve B afforded 0.482 g. (56%) of constant melting material, m.p. 171.1–172.6°; $[\alpha]^{26}D$ −41.4°; λ_{max}^{KBr} 2.85, 5.78, and 8.05 μ.

Anal. Calcd. for $C_{26}H_{40}O_6$: C, 69.61; H, 8.99. Found: C, 70.66, 71.06; H, 9.30, 9.48.[11]

B. From 11b.—A 40.6-mg. sample of **11b** prepared above, m.p. 169–171°, was acetylated in the usual manner with 1.0 ml. each of pyridine and acetic anhydride. The product, isolated by precipitation with water, washing, and drying, weighed 42.3 mg., m.p. 171–172.3°. The mixture melting point with **11c** prepared above from **13a** was 171.2–173.1° and the infrared spectra were superimposable.[12]

6β,16α-Dimethylpregnane-3β,5α,17α,20β-tetraol (12).—A solution of 3.10 g. of **11a** in 70 ml. of tetrahydrofuran was heated to reflux and 55 ml. of a 3 *M* solution of methylmagnesium bromide in ether added over a 5-min. period. Fifty ml. of toluene was added, 60 ml. of the solvent mixture removed by distillation, and the remaining mixture heated under reflux for 130 min. While cooling, 80 ml. of saturated aqueous ammonium chloride solution was added. The organic layer was separated, washed twice with saturated sodium chloride solution, dried, and evaporated *in vacuo* to give crude **12** as a crystalline solid, m.p. 140–194°. Several recrystallizations from ethyl acetate gave pure **12**, m.p. 201–205° (microblock), $[\alpha]^{26}D$ −32.4° (ethanol).

Anal. Calcd. for $C_{23}H_{40}O_4$: C, 72.59; H, 10.60. Found: C, 71.82, 71.96; H, 10.89, 10.77.

17α,20β-Isopropylidenedioxy-6β,16α-dimethylpregnane-3β,5α-diol (13a).—A suspension of 1.0 g. of **12** in 50 ml. of acetone was treated with 0.2 ml. of concentrated hydrochloric acid and the solution heated under reflux for 5 min. The solid dissolved readily and the resulting solution was stored at room temperature for 16 hr. Dilution with water gave a solid which was removed by filtration, washed to neutrality with water, and dried. Successive crystallizations from aqueous methanol and acetone–Skellysolve B gave the sample for analysis: m.p. 117–210°; $[\alpha]^{26}D$ −48.5°; λ_{max}^{KBr} 7.92, 7.99, 8.26, and 8.54 μ.

Anal. Calcd. for $C_{26}H_{44}O_4 \cdot C_3H_6O$: C, 72.76; H, 10.53. Found: C, 72.49, 72.72; H, 10.71, 10.71.

17α,20β-Isopropylidenedioxy-6β,16α-dimethylpregnan-5α-ol-3-one (13b).—A solution of crude **13a** (prepared as above) was oxidized with 8 *N* chromic acid in acetone in the usual manner. After a total elapsed time of 5 min., the reaction was stopped by dilution with aqueous sodium bisulfite solution. Further dilution with water and extraction with ethyl acetate gave a solid, m.p. 165–190°. Recrystallization from acetone–Skellysolve B provided the sample for analysis: m.p. 185–188° (microblock); $[\alpha]^{26}D$ −29.3°; $\lambda_{max}^{CCl_4-CS_2}$ 2.77, 2.88, 5.80, 7.90, 7.97, 8.26, 8.49, and 8.59 μ.

Anal. Calcd. for $C_{26}H_{42}O_4 \cdot C_3H_6O$: C, 73.07; H, 10.15. Found: C, 73.21, 73.27; H, 9.95, 10.19.

17α,20β-Isopropylidenedioxy-6α,16α-dimethyl-4-pregnen-3-one (14).—A solution of 0.94 g. of **13b** in 150 ml. of absolute ethanol was flushed thoroughly with nitrogen and then 7.0 ml. of 0.1 *N* aqueous sodium hydroxide solution added. The mixture was stored at room temperature for 48 hr. The alkali was neutralized by the addition of 1.0 ml. of glacial acetic acid and the solution reduced *in vacuo* to *ca.* one-tenth of the original volume. The product was extracted with ethyl acetate and the combined extracts washed with aqueous sodium bicarbonate solution and with saturated sodium chloride solution. After drying, the solvent was removed *in vacuo* to give an amorphous solid, $\lambda_{max}^{CCl_4}$ 5.97 and 6.22 μ.

The crude material was chromatographed on neutral alumina.[38] The material brought through with 2, 5, and 10% benzene in Skellysolve B was combined and crystallized three times from aqueous acetone to give the sample for analysis: m.p. 163–169° (microblock); $[\alpha]^{26}D$ −1.5°; λ_{max} 242 mμ (16,750); $\lambda_{max}^{CCl_4-CS_2}$ 5.95, 6.21, 7.90, 7.98, 8.25, 8.49 (sh), and 8.55 μ.

Anal. Calcd. for $C_{26}H_{40}O_3$: C, 77.95; H, 10.07. Found: C, 78.16, 78.14; H, 10.23, 10.09.

6β,16α-Dimethylpregnane-5α,17α-diol-3,20-dione (15). A. Oxidation of 12 with NBA or NBS.—A 0.761-g. sample of the tetrol (**12**) was dissolved with warming in a mixture of 85 ml. of acetone and 25 ml. of water. N-Bromoacetamide (1.57 g.) was then added and the solution stored at room temperature for 17 hr. Dilution with 500 ml. of water precipitated a solid which was extracted with 3 portions of ethyl acetate. The combined extracts were washed twice with water, once with saturated sodium chloride solution, dried, and evaporated to dryness *in vacuo* to give 0.803 g. of a partially crystalline residue: λ_{max}^{KBr} 2.83 (sh), 2.92, 5.88, and 5.92 (sh) μ.

Another sample of **12** (1.0 g.) was oxidized as above with 2.66 g. of N-bromosuccinimide in 112 ml. of acetone and 33 ml. of water. Dilution with 850 ml. of water precipitated a crystalline solid which was removed by filtration, washed thoroughly with water, and dried *in vacuo* to give 0.766 g. (77%) of **15**, m.p. 214.5–216°, infrared spectrum identical with that prepared above and showing no characteristic absorption in the ultraviolet. A sample was prepared for analysis by crystallization from aqueous acetone: m.p. 215–216°; $[\alpha]^{30}D$ −23.7°; λ_{max}^{KBr} 2.89 and 5.82 μ (IR-4); (lit.[36] m.p. 250–251°; $[\alpha]D$ −26.7°; λ_{max}^{KBr} 2.72, 2.86, 5.85, and 5.92 μ).

Anal. Calcd. for $C_{23}H_{36}O_4$: C, 73.36; H, 9.64. Found: C, 72.98, 72.97; H, 9.77, 9.81.

B. Oxidation with *Flavobacterium dehydrogenans*.—A culture of *F. dehydrogenans var. hydrolyticum* (ATCC 13930) was carried on slants of "gumbo agar" prepared as follows: agar (15.0 g.), yeast extract (5.0 g.), beef extract (5.0 g.), protease peptone (5.0 g.), sodium chloride (5.0 g.), dextrose (1.0 g.), and distilled water to make 1000 ml. The pH of this mixture was adjusted to 6.8 with aqueous sodium hydroxide before use.

A series of 500-ml. erlenmeyer flasks was prepared, each containing 100 ml. of the basal culture medium prepared as follows: yeast extract (10.0 g.), KH_2PO_4 (4.9 g.), $Na_2HPO_4 \cdot 7H_2O$ (8.83 g.), and tap water to make 1000 ml. Again the pH was adjusted to 6.8 as above.

The flasks containing the basal medium were sterilized and then each flask was inoculated with 1.0-ml. portions of an inoculum prepared by washing a "gumbo agar" slant with 10 ml. of sterile distilled water. The inoculated flasks were then incubated for 72 hr. at room temperature (21.1–26.7°) on a platform-type reciprocal shaker under constant illumination.

A 545.6-mg. sample of the tetrol (**12**) was dissolved in 15 ml. of 95% ethyl alcohol and added to 5 flasks in the following amounts: 116.4, 116.4, 116.4, 116.4, and 80.0 mg. Three cultures containing a total of 312.8 mg. were incubated under the above conditions for 5 days and 2 cultures containing 232.8 mg. for 7 days. The steroidal fermentation products were isolated by extraction with ethyl acetate. The 3 flasks fermented for 5 days were combined and extracted with three 100-ml. portions of ethyl acetate using small amounts of methanol to effect separation of the layers. The combined solvent layers were washed twice with water, with saturated sodium chloride solution, dried, and evaporated to dryness *in vacuo* to give 322.5 mg. of a partly crystalline yellow residue. The 2 flasks fermented for 7 days were combined and extracted as above to give 265.7 mg. of a similar residue.

The two products were partially purified as follows. The 322.5 mg. sample was treated with *ca.* 3.0 ml. of carbon tetrachloride. The yellow gummy material dissolved leaving a granular crystalline residue which was removed by filtration, washed once with carbon tetrachloride, and dried to give 260.1 mg. of product. This product after drying at 78° *in vacuo*

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(11) Considerable difficulty was observed in obtaining satisfactory analytical data for this substance. A sample crystallized one further time as above, m.p. 171.2–172.8°, was submitted to two different microanalytical laboratories. The results were as follows: laboratory A (same as that shown above): C, 68.15, 68.33; H, 8.72, 8.92. laboratory B: C, 70.58, 70.47; H, 9.09, 9.13. The average of all the data shown is as follows: C, 69.88; H, 9.01.

(12) The ΔMD value (OAC–OH) for **11c** and **11b** is +153°, that for **11c** and **11a** is +147°, both values in good agreement with the ΔMD for substance J and its diacetate. See *ref.* 22a, pages 612–622. This establishes the configuration of **11a**, **11b**, and **11c** as 20β but since the yields overall were less than 50% from the 20-ketone, this data *in itself* does not establish with certainty that this is the configuration of the major reduction product.

weighed 230.2 mg., indicating solvation with carbon tetrachloride in the original sample. The 265.7-mg. sample on similar treatment gave 185.6 mg. of partially purified product which on drying weighed 168.0 mg. Infrared comparisons on the CCl_4 solvates showed these samples to be substantially identical [λ_{max}^{KBr} 5.85–5.86, 12.7, and 13.2 μ (CCl_4)], and to contain substantial quantities of 6β,16α-dimethylpregnane-5α,17α-diol-3,20-dione (**15**).

6α,16α-Dimethylpregnane-3β,5α,6β,17α-tetrol-20-one (22). A. Alkylation–Enol Acetylation of 6-Methyl-5,16-pregnadien-3β-ol-20-one 3-Acetate (19).—To 50 ml. of dry tetrahydrofuran under nitrogen, there was added 8 ml. of a 3 *M* solution of methylmagnesium bromide in ether. After removing 28 ml. of the mixture by distillation and cooling to room temperature, 200 mg. of cuprous chloride was added. A solution of 3.70 g. of **19** in 30 ml. of tetrahydrofuran was introduced in a 2 min. period with stirring. The green mixture was stirred for 45 min. at room temperature and then a solution of 1.5 ml. of acetyl chloride in 10 ml. of tetrahydrofuran was introduced. Stirring was continued for 45 min. The reaction was quenched by the addition of 60 ml. of saturated aqueous ammonium chloride solution. The organic layer was diluted with ethyl acetate, separated, and washed twice with saturated sodium chloride solution, dried, and evaporated *in vacuo* to an oily mixture of *cis*- and *trans*-**6,16α-dimethyl-5,17(20)-pregnadiene-3β,20-diol 3,20-diacetate**; $\lambda_{max}^{CCl_4}$ 5.74, 5.90, 8.1–8.2, 8.41, and 8.66 μ.

B. Epoxidation of 6,16α-Dimethyl-5,17(20)-pregnadiene-3β,20-diol 3,20-Diacetate.—The crude *cis–trans* 3,20-diacetate was dissolved in 30 ml. of chloroform and added to a stirred mixture of 10 ml. of 40% peracetic acid and 1.0 g. of anhydrous sodium acetate. The reaction mixture was stirred for 5 hr. at room temperature, poured into water, and diluted with methylene chloride. The separated organic layer was washed with 5% aqueous sodium bicarbonate solution, with water, and finally with saturated sodium chloride solution, dried, and evaporated *in vacuo* to an amorphous solid ($\lambda_{max}^{CCl_4}$ 5.76, 8.13, 8.62, and 8.90 μ) which was a mixture consisting of *cis*- and *trans*-**5α,6α:17α,20-dioxido-6β,16α-dimethylpregnane-3β,20-diol 3,20-diacetate** and *cis*- and *trans*-**5β,6β:17α,20-dioxido-6α,16α-dimethylpregnane-3β,20-diol 3,20-diacetate (20).**

C. Alkaline Hydrolysis of the 5,6:17α,20-Dioxido-6,16α-dimethylpregnane-3β,20-diol 3,20-Diacetates (20).—The mixture of 5,6:17,20-diepoxides (**20**) above was taken up in 100 ml. of methanol to which was added a solution of 2.0 g. of potassium carbonate in 25 ml. of water. The mixture was heated under reflux for 45 min., then cooled, and 2 ml. of glacial acetic acid added. After concentrating the mixture *in vacuo* to *ca.* 20% of the original volume, 200 ml. of water was added and the resulting precipitate removed by filtration, washed, and dried to give a mixture of **5α,6α-oxido-6β,16α-dimethylpregnane-3β,17α-diol-20-one (21a)** and **5β,6β-oxido-6α,16α-dimethylpregnane-3β,17α-diol-20-one (21b)**; λ_{max}^{KBr} 2.90, 5.90, and 11.60 μ.

Crystallization from acetone–methanol–Skellysolve B mixtures gave colorless plates, m.p. 240.5–247° (melted at 235–238° and resolidified), $[\alpha]^{29}D$ −66.2°, λ_{max}^{KBr} 5.93 μ. This material was assigned the **5α,6α-oxide** structure (**21a**).

Anal. Calcd. for $C_{23}H_{36}O_4$: C, 73.36; H, 9.64. Found: C, 72.93; H, 9.68.

Acetylation of a portion of the crude mixture (**21a,b**) with acetic anhydride and pyridine in the usual manner provided the mixture of the 3-acetates. Several crystallizations from acetone–Skellysolve B gave flat needles; m.p. 156.5–159°; $[\alpha]^{29}D$ −59.1°; λ_{max}^{KBr} 5.78 (sh), 5.83, 7.91, and 8.03 μ. This substance was assigned the **5α,6α-oxido 3-acetate** structure (**21c**).

Anal. Calcd. for $C_{25}H_{38}O_5$: C, 71.74; H, 9.15. Found: C, 71.39, 71.58; H, 9.12, 9.35.

D. Hydrolytic Cleavage of the 5,6-Oxido-6,16α-dimethylpregnane-3β,17α-diol-20-ones (21a,b).—The sample of the mixed 5α,6α- and 5β,6β-oxides (**21a,b**) was dissolved in 80 ml. of acetone. Thirty ml. of a 1 *N* aqueous perchloric acid solution was added with stirring. After 45 min. 1 l. of water was added and the resulting suspension extracted with two 100-ml. portions of ethyl acetate. The extracts were washed with water, once with 5% aqueous sodium bicarbonate solution, and finally with saturated sodium chloride solution, dried, and evaporated *in vacuo* to give **6α,16α-dimethylpregnane-3β,5α,6β,17α-tetrol-20-one (22)**, m.p. 130–135°. Recrystallization from acetone–Skellysolve B gave apparently amorphous material changing to a microcrystalline form at 131–139°, finally melting at 209–215°; $[\alpha]^{29}D$ −10.7° (dioxane); λ_{max}^{KBr} 2.90, 5.86, and 9.48 μ. Another preparation obtained substantially as described above and crystallized from aqueous acetone followed by acetone–Skellysolve B had m.p. 235–242° (after melting at 206–210° and resolidification), $[\alpha]^{25}D$ −12.0° (dioxane).

Anal. Calcd. for $C_{23}H_{38}O_5 \cdot H_2O$: C, 66.96; H, 9.77. Found: C, 66.89, 66.90; H, 9.62, 9.62.

6α,16α-Dimethylpregnane-5α,6β,17α-triol-3,20-dione (23).—To a solution of 2.4 g. of **22** in 25 ml. of pyridine was added a solution of 1.8 g. of chromium trioxide in 1.8 ml. of water and 6 ml. of pyridine. The mixture was stored at room temperature for 7.5 hr. and then diluted with water and ethyl acetate. The two phase mixture was filtered through Celite and the residue washed with water and ethyl acetate. The filtrate was separated and the solvent layer was washed once with dilute hydrochloric acid, with water, and with saturated sodium chloride solution. After drying, the solvents were removed *in vacuo* to give a yellow solid, 2.0 g. Recrystallization from acetone–Skellysolve B gave 0.68 g. of needles, m.p. 225–232.5° (microblock). A further crystallization from the same solvents raised the m.p. to 231.5–234.5°; $[\alpha]^{29}D$ −2.9° (dioxane); λ_{max}^{KBr} 2.90, 5.85, and 5.87 (sh) μ.

Anal. Calcd. for $C_{23}H_{36}O_5$: C, 70.37; H, 9.24. Found: C, 69.99, 70.01; H, 9.41, 9.42.

Oxidation of the tetrolone (**22**) with 8 *N* chromic acid in acetone gave the trioldione (**23**) in 43.5% yield.

Mass Spectrographic Analyses.—A number of the materials described above were characterized further by means of their mass spectrometric fragmentation patterns. The spectra of compounds **1**, **6a**, **7a**, and **16** have been described in detail previously.[43] Both compound **10a** and its 3-acetate (**10b**) showed strong parent peaks at m/e = 362 and 404, respectively. Peaks in the spectrum of **10a** at m/e = 344 and 326 indicate the loss of 1 and 2 molecules of water. In the spectrum of **10b**, strong peaks at m/e = 344 and 326 indicate the loss of acetic acid and of acetic acid plus water, respectively.

The 17α-acetate esters **6b**, **6d**, **7b**, and **7d** all showed small but distinct parent peaks at m/e = 400, 398, 398, and 396, respectively. Other high mass peaks of interest are given in Table II.

Table II

Mass Peaks

Fragment identity	Compound 6b	6d	7b	7d
Parent (M)	400 (3.9)[a]	398 (5.8)	398 (2.4)	396 (3.3)
M − H_2O	382 (4.5)	380 (3.0)	380 (3.7)	378 (2.0)
M − HOAc	340 (10.4)	388 (15.4)	338 (8.7)	336 (6.2)
M − [HOAc + CH_3]			323 (4.6)	321 (3.8)
M − [HOAc + CH_3CO]	297 (100.0)	295 (85.0)	295 (100.0)	293 (100.0)
"A-ring fragment"[b]		135 (100.0)		

[a] The figures in parentheses are relative intensities as per cent of the strongest peak. [b] Fragment arising from cleavage of the B-ring at C_9–C_{10} and C_6–C_7 with hydrogen transfer.

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(43) L. Peterson, *Anal. Chem.*, **34**, 1781 (1962). Compound XXVII of this reference is 6α,16α-dimethyl-17α-hydroxyprogesterone (**6a**), not the 6β-methyl epimer as shown.