## 6,16-Dimethylated Steroids. II. Studies on the Synthesis of 6,16α-Dimethyl-17α-hydroxyprogesterones<sup>1</sup>

ROBERT P. GRABER, MARTIN B. MEYERS, LEROY G. HICKMAN, EUGENE H. BOROCHOFF, AND ARTHUR D. ODELL

The Central Research Laboratories, General Mills, Inc., Minneapolis 27, Minnesota

Received August 19, 1963

The synthesis of several  $6,16\alpha$ -dimethyl-17-oxygenated progesterones by a variety of routes is described herewith. Of particular interest is the utilization of the readily available  $5\alpha,6\alpha$ -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<sub>6</sub> and C<sub>16</sub> as well as the hydroxyl group at C<sub>17</sub> 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,  $6,16\alpha$ -dimethyl-6-dehydro-17 $\alpha$ -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  $17\alpha$ 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- $17\alpha$ acetoxyprogesterone<sup>2-4</sup> and 6-methyl-16-methylene-6dehydro- $17\alpha$ -acetoxyprogesterone.<sup>5</sup>

In an earlier paper we described the synthesis of a series of  $6,16\alpha$ -dimethylprogesterones.<sup>6</sup> 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 6,16 $\alpha$ -dimethyl-17-acyloxyprogesterones. Several members of this series have also been described by other groups.<sup>7</sup>

The initial synthesis of  $6\alpha$ ,  $16\alpha$ -dimethyl- $17\alpha$ -hydroxyprogesterone (**6a**) ( $6\alpha$ ,  $16\alpha$ -dimethyl-4-pregnen- $17\alpha$ -ol-3, 20-dione) and its various esters and dehydro derivatives proceeded from the readily available and highly versatile intermediate  $5\alpha$ ,  $6\alpha$ -epoxy-16-dehydropregnenolone acetate (1) ( $5\alpha$ ,  $6\alpha$ -oxido-16-pregnen-3 $\beta$ ol-20-one acetate).<sup>\*</sup> Treatment of this unsaturated keto epoxide withm ethyl Grignard under forcing con-

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

The following are references to clinical data: (a) J. Martinez Moreno,
 M. Maqueo, 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. Kinel, *Endokrinologie*, **40**, 257 (1961).

(1962); (c) F. Kinel, Endokrinologie, 40, 257 (1961).
(5) D. N. Kirk, V. Petrow, and D. M. Williamson, British Patent 886,619 (1962); for clivical 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 D. M. Williamson, J. Chem. Soc., 22 (1962);

ef. 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.

ditions followed by *in situ* acetylation<sup>9</sup> provided the  $6\beta$ ,  $16\alpha$ -dimethyl-17, 20-enol triacetate (2) as a mixture of its *cis-trans* isomers.<sup>10</sup> The acylation of the inter-



mediate Grignard complex, having -OMgBr groups at C<sub>3</sub>, C<sub>5</sub>, and C<sub>20</sub>, was not readily accomplished. Substantial excesses of either acetyl chloride or acetic anhydride were required and, in spite of the vigorous conditions, the C<sub>17</sub>-desoxy compound,  $6\beta$ ,  $16\alpha$ -dimethyl-pregnane- $3\beta$ ,  $5\alpha$ -diol-20-one 3-acetate, <sup>6</sup> was subsequently

(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.

<sup>(1)</sup> Preliminary communication: R. P. Graber and M. B. Meyers, J, Ocg. Chem., 26, 4774 (1961).

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

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- $17\alpha$ ,20-epoxide triacetate (3) again as a mixture of *cis-trans* isomers.<sup>11</sup> No attempts were made to separate the isomeric 17,20-epoxides since in the subsequent step they gave the same  $17\alpha$ -hydroxy-20ketone (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.<sup>12</sup> 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<sup>13</sup> proceeded smoothly to the dimethyldioldione 5-monoacetate (5). Acid-catalyzed elimination<sup>14</sup> of the 5-acetoxy function occurred with concomitant epimerization of the 6 $\beta$ -methyl to the more stable 6 $\alpha$ configuration giving  $6\alpha$ ,  $16\alpha$ -dimethyl- $17\alpha$ -hydroxyprogesterone (6a).

The  $17\alpha$ -acetate (**6b**) was prepared in the usual manner in acetic anhydride-acetic acid solution with *p*-toluenesulfonic acid catalysis.<sup>15</sup> The 3,5-diendiol 3,17-diacetate<sup>7a</sup> 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  $6\alpha$ ,  $16\alpha$ -dimethyl- $17\alpha$ -acetoxyprogesterone. Similar treatment of **6a** with caproic anhydride-caproic acid in the presence of *p*-toluenesulfonic acid gave the  $17\alpha$ -caproate (**6c**).

6,16 $\alpha$ -Dimethyl-6-dehydro-17 $\alpha$ -hydroxyprogesterone (7a) was prepared by chloranil dehydrogenation<sup>16</sup> of 6a. The corresponding 17 $\alpha$ -acetate (7b) was prepared as described above for the parent compound. Finally, the 1-dehydro 17 $\alpha$ -acetate (6d) and the 1,6-bisdehydro 17 $\alpha$ -acetate (7c) were prepared readily by the use of 2,3-dichloro-5,6-dicyanobenzoquinone<sup>17</sup> 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  $5\alpha,6\alpha$ -epoxy-16-

(11) Although attack of the 17,20-double bond from the rear or  $\alpha$ -side of the molecule predominates, trace formation of the epimeric 17 $\beta$ ,20-epoxides cannot be ruled out completely. For an illuminating discussion of the *cistrans* 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).

dehydropregnenolone acetate (1). Although this route is somewhat longer, the over-all yields were superior; furthermore, the opening of the  $5\alpha,6\alpha$ -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  $5\alpha, 6\alpha$ -epoxy- $16\alpha$ -methyl-17, 20-enol diacetates (8).<sup>18</sup> 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  $5\alpha,6\alpha$ -epoxy- $16\alpha$ -methylpregnane- $3\beta,17\alpha$ -diol-20-one (10a).<sup>19</sup>

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

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

<sup>(19)</sup> 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).

<sup>(21)</sup> 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  $17\alpha$ ,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 further details.

comparison of molecular rotation differences served to confirm this assignment.<sup>41</sup> Treatment of **11a** with a *ca.* 20-fold excess of methyl Grignard<sup>22</sup> effected opening of the 5,6-epoxide to give in good yield the  $6\beta$ ,  $16\alpha$ -dimethyl- $3\beta$ ,  $5\alpha$ ,  $17\alpha$ ,  $20\beta$ -tetrol (**12**).

This tetrol was converted to its  $17\alpha$ ,  $20\beta$ -acctonide derivative (**13a**) by acid-catalyzed reaction with acctone<sup>23</sup> and the  $3\beta$ -hydroxyl oxidized with 8 N chromic acid in acctone to the 3-ketone (**13b**). Acid-catalyzed



elimination of the  $5\alpha$ -hydroxyl afforded  $17\alpha$ ,  $20\beta$ -isopropylidenedioxy- $6\alpha$ ,  $16\alpha$ -dimethyl-4-pregnen-3-one (14).<sup>24</sup>

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.<sup>25</sup> The desired selective oxidation was achieved successfully, however, by treatment with either N-bromo-acetamide or N-bromosuccinimide in acetone solution.<sup>26</sup> or by microbiological oxidation with a strain of *Flavobacterium dehydrogenans.*<sup>27</sup> With NBA or NBS, the  $5\alpha$ ,  $17\alpha$ -dihydroxy-3, 20-diketone (15)<sup>74</sup> could be isolated in good yield. Acid-catalyzed elimination and epimerization afforded excellent yields of  $6\alpha$ ,  $16\alpha$ -dimethyl- $17\alpha$ -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  $17\alpha$ -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  $\bf 14$  showed it to have no activity when tested at a level at which ethisterone gave a 2.5-3.0+ response.

(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  $5\alpha$ ,  $6\alpha$ -epoxy-16\alphamethyldiolone (10a) was also investigated briefly. 16-Dehvdropregnenolone 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 earbonate. The total product, containing both the  $5\alpha, 6\alpha$ - and  $5\beta, 6\beta$ -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 16 $\alpha$ -methylpregnane-3 $\beta$ , 5 $\alpha$ , 6 $\beta$ , 17 $\alpha$ -tetrol-20-one 3-monoacetate (18a). Reclosure of the  $5\alpha, 6\beta$ glycol system to the  $5\alpha, 6\alpha$ -epoxide (10a) was effected by base treatment of the derived  $6\beta$ -mesylate (18b). The product was shown by infrared spectrometry to be identical with that obtained from  $5\alpha, 6\alpha$ -epoxy-16dehydropregnenolone acetate (1).<sup>28</sup>

A third sequence of reactions leading directly to 6,  $16\alpha$ dimethyl-6-dehydro- $17\alpha$ -hydroxyprogesterone (**7a**)<sup>20</sup> proceeded from 6-methyl-16-dehydropregnenolone acctate (**19**).<sup>30</sup> 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 5,6:17,20diepoxydimethyldiol 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  $5\alpha,6\alpha$ -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.

<sup>(25)</sup> Cf. L. H. Sarett, J. Biol. Chem., 162, 601 (1946).

<sup>(28)</sup> Wettstein and co-workers<sup>19</sup> have described this synthetic sequence in detail with the isolation and characterization of all the intermediate conpounds.

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

<sup>(30)</sup> D. Burn, B. Ellis, V. Petrow, I. A. Stewart-Webb, and D. M. Williamson, J. Chem. Soc., 4092 (1957); see also ref. 22b.

Since acid-catalyzed opening of both 5,6-epoxides was expected to lead to the same trans  $5\alpha,6\beta$ -glycol by way of diaxial opening, the mixture was treated with perchloric acid in aqueous acetone. The expected  $6\alpha, 16\alpha$ -dimethylpregnane- $3\beta, 5\alpha, 6\beta, 17\alpha$ -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.<sup>31</sup>

The oral progestational activities of the various  $6,16\alpha$ -dimethyl-17 $\alpha$ -hydroxyprogesterone esters were estimated by the Clauberg method on immature estrogen-primed rabbits.<sup>32</sup> The results are summarized in Table I.

$T_{\cdot}$	ABLE	: I

	CLAUBERG ASSAYS IN	RABBITS
Compo	und	Potency
6b		55
7b		130
6d		40
7c		120
<sup>a</sup> Ethinylt	estosterone = 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<sup>33</sup> for  $6\alpha$ -methyl-17 $\alpha$ -acetoxyprogesterone.

A granuloma pouch test<sup>34a</sup> demonstrated antiinflammatory activity for **7b** equal to *ca*. 40% of that for hydrocortisone. Sex reversal in the female rat foetus<sup>34b</sup> 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<sup>35</sup>

 $6\beta,16\alpha$ -Dimethylpregnane- $3\beta,5\alpha,17\alpha$ -triol-20-one  $3\beta,5\alpha$ -Diacetate (4b). A. Dialkylation-Enol Acetylation of  $5\alpha,6\alpha$ -Oxido-16-pregnen- $3\beta$ -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<sup>36</sup> added in 15 min. After 30 min. at room temperature, the mixture was heated to 50-55°

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

	<b>1</b> and <b>4b</b>	<b>1</b> and <b>10a</b>	16 and 10a		
From diosgenin	1.7%	10-11%	6%		
From <b>16</b>	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<sub>4</sub> and CS<sub>2</sub>, however, were determined on a Beckman Model IR-4 spectrophotometer, in which case CCl<sub>4</sub> was used from 2.0-7.8  $\mu$  and CS<sub>2</sub> from 7.8 to 15.0  $\mu$ .

(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. 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 drived,<sup>37</sup> and the solvent evaporated *in vacuo* to give an oil consisting of *cis*- and *trans*-6 $\beta$ ,16 $\alpha$ -dimethyl-17(20)-pregnene-3 $\beta$ ,5 $\alpha$ ,20-trial 3 $\beta$ ,5 $\alpha$ ,20-triace-tate (2),  $\lambda_{\max}^{CC14}$  5.77, 5.88 (sh), 8.11, 8.18, 8.41, 8.53, and 8.67  $\mu$ ;  $\lambda_{\max}^{CC14-CS_2}$  5.70 (sh), 5.75, 5.85, 8.02, and 8.15  $\mu$ . B. Epoxidation of  $6\beta$ ,16 $\alpha$ -Dimethyl-17(20)-pregnene-3 $\beta$ ,5 $\alpha$ ,-

B. Epoxidation of  $6\beta$ ,  $16\alpha$ -Dimethyl-17(20)-pregnene- $3\beta$ ,  $5\alpha$ , 20-triol  $3\beta$ ,  $5\alpha$ , 20-triol  $3\beta$ ,  $5\alpha$ , 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 chioide solution, and dried, and the solvent evaporated *in vacuo* to give the crude *cis-trans* epoxide mixture (3) as an amorphous residue,  $\lambda_{\text{max}}^{\text{CCl4}} \leq 5.77$ , 5.87 (sh), 8.11, 8.53, 8.67, and 8.88  $\mu$ ;  $\lambda_{\text{max}}^{\text{CCl4+CS}} = 5.74$ , 8.03, and 11.60  $\mu$ .

C. Alkaline Hydrolysis of  $17\alpha$ ,20-Oxido-6 $\beta$ ,16 $\alpha$ -dimethylpregnane-3 $\beta$ ,5 $\alpha$ ,20-triol 3 $\beta$ ,5 $\alpha$ ,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,  $\lambda_{max}^{CC14}$  2.77, 2.86, 5.79, 5.87, and 8.08  $\mu$ .

D. Acetylation of  $6\beta$ ,  $16\alpha$ -Dimethylpregnane- $3\beta$ ,  $5\alpha$ ,  $17\alpha$ -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  $7^{\circ}$  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);  $\lambda_{\rm Ms}^{\rm KB}$  5.79, 5.88, 7.89, and The solid was dissolved in benzene and the solution intro-8.0 µ. duced onto a column of 200 g. of neutral alumina.<sup>38</sup> 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 benzeneether, 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 6 $\beta$ ,16 $\alpha$ -dimethylpregnane-3 $\beta$ ,5 $\alpha$ ,17 $\alpha$ -triol-20-one 3 $\beta$ ,5 $\alpha$ -diacetate (4b), m.p. 205-210°. Successive recrystallizations from acetone and Skellysolve B mixtures gave material of m.p. 207-209°,  $[\alpha]^{26}D - 26.5^{\circ}$ ,  $\lambda_{max}^{Kh}$ 2.89, 5.75, 5.80, 5.83 (sh), 7.83, and 8.00  $\mu$ ;  $\lambda_{max}^{CC14+CS2}$  2.74, 2.85, 5.74, 5.83, 5.89, 8.01, and 9.62  $\mu$ .

Anal. Calcd. for  $C_{27}\dot{H}_{42}O_6$ : 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  $6\beta$ ,  $16\alpha$ -dimethylpregnane- $3\beta$ ,- $5\alpha$ -diol-20-one 3-acetate, m.p.  $242-247^{\circ}$ ,  $[\alpha]^{35}D + 21.3^{\circ}$ .<sup>6</sup>

 $6\beta,16\alpha$ -Dimethylpregnane- $3\beta,5\alpha,17\alpha$ -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-

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

<sup>(38)</sup> 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  $85-90^{\circ}$  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°.  $^{14+CS_2}2.75, 2.87, 5.78, 5.83, 5.90, and 9.55 \mu$ .  $\lambda_{m}^{c}$ 

Anal. Caled. for C25H4005 H2O: C, 68.46; H, 9.65. Found: C, 68.60, 68.78; H, 9.64, 9.68.

 $6\beta$ ,  $16\alpha$ -Dimethylpregnane- $5\alpha$ ,  $17\alpha$ -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  $5^{C'}_{C}$  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  $-26.9^{\circ}$ ,  $\lambda_{\max}^{CS_2}(2.76, 2.88, 5.75, 5.80, 5.84, 5.90, 8.06, and 8.21 <math>\mu$ (IR-4).

Anal. Caled. 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.

 $6\alpha$ ,  $16\alpha$ -Dimethyl- $17\alpha$ -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°,  $\begin{array}{l} [\alpha]^{26} \mathrm{D} + 53.5^{\circ}, \ \lambda_{\max} 242 \ \mathrm{m}\mu \ (\epsilon \ 15,400), \ \lambda_{\max}^{\mathrm{CC4}+\mathrm{C8}_2} 2.76, \ 2.88, \\ 5.84, \ 5.93, \ \mathrm{and} \ 6.20 \ \mu \ (\mathrm{lit},^{7a} \ \mathrm{m.p.} \ 200-202^{\circ}, \ [\alpha] \mathrm{D} \ +51.4^{\circ}, \ \lambda_{\max}^{\mathrm{Ec0H}} \end{array}$ 240–242 m $\mu$  ( $\epsilon$  16,600),  $\lambda_{\text{max}}^{\text{KBr}}$  2.73, 5.80, 5.97, and 6.15  $\mu$ ).

Anal. Caled. for C23H34O3: 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 acetonehexane gave 1.45 g. (87%), m.p.  $199-203^{\circ}$ . 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  $0.4C_0^{\prime}$  (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.<sup>38</sup> 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.

 $6\alpha$ ,  $16\alpha$ -Dimethyl- $17\alpha$ -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  $20^{cc}_{c}$  of the original volume and diluted with water. The suspension was extracted

3 times with ethyl acetate. The combined extracts were washed with water,  $5^{C_{e}}_{e}$  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.  $169-171^{\circ}$ :  $[\alpha]^{27} p + 69.0^{\circ}; \lambda_{max} 242 m\mu (\epsilon 15,800); \widetilde{\lambda}_{max}^{CCl_4 \oplus C}$ 5.72, 5.82, 5.94, 6.20, 8.00, and 14.50  $\mu$  (lit<sup>7a</sup> m.p. 170-172°,  $|\alpha|_{\rm b} + 71.1^{\circ}$ ,  $\lambda_{\rm max}^{\rm Etolf}$  240 m $\mu$  ( $\epsilon$  13,800):  $\lambda_{\rm max}^{\rm Re}$  5.75, 5.85, 5.99, and 8.0  $\mu$ : and lit.<sup>7b</sup> m.p. 164–165°:  $|\alpha|_{\rm b} + 74^{\circ}$ ;  $\lambda_{\rm max}^{\rm Etolf}$  240  $m\mu (\epsilon 15, 540)).$ 

Anal. Caled. for C<sub>25</sub>H<sub>36</sub>O<sub>4</sub>: C, 74.96; H, 9.06. Found: C, 74.46; H, 9.13.

 $6\alpha$ ,  $16\alpha$ -Dimethyl- $17\alpha$ -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.<sup>38</sup> The fractions eluted with 1:3 benzenehexane 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°;  $\begin{array}{l} |\alpha|^{27} D + 40.6^{\circ} : \lambda_{\max} : 241 \ \mathrm{m}\mu \ (13,200) : \lambda_{\max}^{Kbr} : 5.78, \ 5.84, \ 6.00, \ 6.22, \\ \mathrm{and} : 11.50 \ \mu^{39} : \lambda_{\max}^{CCQ+CS2} : 5.73, \ 5.81, \ 5.93, \ \mathrm{and} \ 6.19 \ \mu. \\ Anal. \ Calcd. \ \mathrm{for} \ C_{29} H_{44} O_4 : \ C_i \ 76.27 ; \ H. \ 9.74. \ Found : \end{array}$ 

C. 76.16, 76.34; H. 9.68, 9.70.

6,16 $\alpha$ -Dimethyl-6-dehydro-17 $\alpha$ -hydroxyprogesterone (7a). A. By Chloranil Dehydrogenation of 6a.—A mixture of 0.16 g. of  $6\alpha$ ,  $16\alpha$ -dimethyl- $17\alpha$ -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  $6\alpha$ ,  $16\alpha$ -Dimethylpregnane- $5\alpha$ ,  $6\beta$ ,  $17\alpha$ -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 racuo 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°  $(microblock); \ \ [\alpha]^{29} p \ \pm 27.9; \ \lambda_{max} \ 290 \ m\mu \ (23,200); \ \ \lambda_{max}^{ccl_4} \ 2.74.$  $2.87, 5.83, 5.90, 5.99, 6.13, 6.29, 11.30, and <math>11.40 \mu$  (IR-4).

Anal. Calcd. for C<sub>23</sub>H<sub>32</sub>O<sub>3</sub>: C, 77.49; H, 9.05. Found: C, 77.16, 77.26; H, 9.15, 9.33.

6,16 $\alpha$ -Dimethyl-6-dehydro-17 $\alpha$ -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

<sup>(39)</sup> 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  $\lambda_{\rm max}\,290$  $m_{\mu}$  (3800) corresponding to ca, 15% dienone. Small peaks in the infrared (KBr) at 6.14 and 6.33 a confirmed this finding.

545

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);  $[\alpha]^{26}D + 25.6^{\circ}$ ;  $\lambda_{max} 288 m\mu (25,000); \lambda_{max}^{Cl4^{-}CS_2} 5.73, 5.83, 6.00, 6.14, 6.30, 8.03,$  $and 11.39 <math>\mu$  (lit.<sup>7b</sup> m.p. 202–204°;  $[\alpha]D + 21^{\circ}; \lambda_{max}^{Eud} 286 m\mu$  $(22,400); \lambda_{max}^{Nuol} 5.73, 5.85, 6.05, 6.16, and 6.29 \mu$ ).

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°,  $\lambda_{max}$  290 mµ (24,300).

 $6\alpha$ ,  $16\alpha$ -Dimethyl-1-dehydro- $17\alpha$ -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<sup>38</sup> 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°;  $\lambda_{max}$  245 m $\mu$  (15,800);  $\lambda_{max}^{\text{CC14+CS2}}$  5.73, 5.82, 5.99, 6.13, 6.21, 8.02, 11.20, 12.34, and 14.18  $\mu$ .

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.

6,16 $\alpha$ -Dimethyl-1,6-bisdehydro-17 $\alpha$ -hydroxyprogesterone Acetate (7c).—A mixture of 0.95 g. of 7b and 1.0 g. of DL Q 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<sup>35</sup> 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°;  $\lambda_{max}$  228 (12,000), 256 (8470), and 302 m $\mu$  (11,540);  $\lambda_{max}^{Cl4+CSg}$  5.72, 5.82, 6.01, 6.18, 6.28, 8.02, and 11.22  $\mu$ .

Anal. Calcd. for C<sub>28</sub>H<sub>32</sub>O<sub>4</sub>: C, 75.72; H, 8.13. Found: C, 75.43, 75.35; H, 8.16, 8.07.

(10a).  $5\alpha$ ,  $6\alpha$ -Oxido-1 $6\alpha$ -methylpregnane- $3\beta$ ,  $17\alpha$ -diol-20-one A. From  $5\alpha$ ,  $6\alpha$ -Oxido-16-pregnen-3 $\beta$ -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;  $\lambda_{max}^{CCl4}$  5.75, 5.92, 8.13, 8.21, and 11.50  $\mu$ . The product was a mixture of cis- and trans- $5\alpha$ ,  $6\alpha$ -oxido- $16\alpha$ -methyl-17(20)-pregnene- $3\beta$ , 20diol diacetate (8).

(b) Epoxidation of cis- and trans- $5\alpha$ , $6\alpha$ -Oxido- $16\alpha$ -methyl-17(20)-pregnene- $3\beta$ ,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*-5 $\alpha$ ,6 $\alpha$ :17 $\alpha$ ,20-dioxido-16 $\alpha$ -methylpregnane-3 $\beta$ ,20diol diacetate (9);  $\lambda_{max}^{max}$  5.74, 8.11, 11.50, and 11.60  $\mu$ .

(c) Alkaline Hydrolysis of the  $5\alpha, 6\alpha; 17\alpha, 20$ -Dioxido-16 $\alpha$ methylpregnane-3 $\beta, 20$ -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  $5\alpha, 6\alpha$ -oxido-16 $\alpha$ -methylpregnane-3 $\beta, 17\alpha$ 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°;  $\lambda_{max}^{RBT} 5.88$ , 12.53, and 13.3  $\mu$  (lit.<sup>19</sup> 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.

 $5\alpha, 6\alpha$ -Oxido-16 $\alpha$ -methylpregnane-3 $\beta$ , 17 $\alpha$ -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 chloroformmethanol 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-3 $\beta$ -ol-20-one 3-Acetate (16-Dehydropregnenolone Acetate) (16).—A 3.58-g, portion of 16dehydropregnenolone 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°,  $\lambda_{max}^{\rm KBr}$ identical with material prepared above from 1.

 $5_{\alpha,6\alpha}$ -Oxido-16 $\alpha$ -methylpregnane-3 $\beta$ ,17 $\alpha$ ,20 $\beta$ -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 solution.<sup>40</sup> The organic layer was then diluted slowly with 150 ml. of water. An oil separated which solidified slowly. Filtration gave  $5\alpha,6\alpha$ -oxido-16 $\alpha$ -methylpregnane-3 $\beta$ ,-17 $\alpha,20(\alpha + \beta)$ -triol, m.p. 207-223°. Several recrystalizations of this material from acetone gave pure 11a, m.p. 233-236°,  $[\alpha]^{31}$ D -91.3°,  $\lambda_{max}^{Km}$ 12.55 and 13.3  $\mu$ .

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

 $5\alpha,6\alpha$ -Oxido-16 $\alpha$ -methylpregnane- $3\beta,17\alpha,20\beta$ -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 heat d 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-

<sup>(40)</sup> 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°,  $\lambda_{\rm max}^{\rm Kir}$  6.55, 6.78, and 11.57  $\mu$ . 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.

rated, 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°;  $\lambda_{\max}^{\text{KBr}}$  2.95, 5.80, and 8.05  $\mu$ ;  $\lambda_{\max}^{\text{CG4}}$ 2.8, 5.78, and 8.1 µ. Two recrystallizations from acetone-Skellysolve B afforded 363 mg, of **11b**: m.p. 169-171°;  $|\alpha|^{26} p = 83.4^{\circ}$ ;  $\lambda_{\max}^{\text{KBr}}$  2.82, 2.95, 5.79, 8.05, and 12.5  $\mu$ .

Anal. Caled. for  $C_{24}H_{38}O_5$ : C. 70.90; H, 9.42. Found: C, 71.04, 71.05; H, 9.40, 9.56.

 $5\alpha$ ,  $6\alpha$ -Oxido-16 $\alpha$ -methylpregnane- $3\beta$ ,  $17\alpha$ ,  $20\beta$ -triol 3, 20-Diacetate (11c). A. From 11a.-A 0.700 g. sample of 11a (m.p. 210-214.5°, obtained in  $74^{\circ}_{6}$  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.  $(56^{\circ})^{\circ}$  of constant melting material, m.p. 171.1–172.6°:  $|\alpha|^{26}$ D =41.4°:  $\lambda_{\text{max}}^{\text{KB}}$ 2.85, 5.78, and 8.05  $\mu$ .

Anal. Caled. for  $C_{26}H_{40}O_{8}$ : C, 69.61; H, 8.99. Found: C, 70.66, 71.06; H, 9.30, 9.48.<sup>31</sup>

**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.<sup>12</sup>

 $6\beta$ ,  $16\alpha$ -Dimethylpregnane- $3\beta$ ,  $5\alpha$ ,  $17\alpha$ ,  $20\beta$ -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 evapocated 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),  $[\alpha]^{36}$ D –32.4° (ethanol).

Anal. Caled. for C23H40O4; C, 72.59; H, 10.60. Found: C. 71.82, 71.96; H, 10.89, 10.77.

 $17\alpha$ ,  $20\beta$ -Isopropylidenedioxy- $6\beta$ ,  $16\alpha$ -dimethylpregnane- $3\beta$ ,  $5\alpha$ 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-

Found: C, 72.49, 72.72; H, 10.71, 10.71.

 $17\alpha$ , 20 $\beta$ -Isopropylidenedioxy- $6\beta$ ,  $16\alpha$ -dimethylpregnan- $5\alpha$ 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-Skelly solve B provided the sample for analysis: m.p.  $185\text{--}188^\circ$ (microblock):  $[\alpha]^{29}$ D = -29.3°:  $\lambda_{\text{tex}}^{\text{cCH}-\text{CSe}}$  2.77, 2.88, 5.80, 7.90, 7.97, 8.26, 8.49, and 8.59 µ.

Anal. Caled. for C<sub>26</sub>H<sub>42</sub>O<sub>4</sub> C<sub>5</sub>H<sub>6</sub>O; C. 73.07; H, 10.15. Found: C, 73.21, 73.27; H, 9.95, 10.19.

 $17\alpha$ , 20 $\beta$ -Isopropylidenedioxy- $6\alpha$ ,  $16\alpha$ -dimethyl-4-pregnen-3one (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,  $\lambda_1^{i}$ 5.97 and 6.22 μ.

The crude material was chromatographed on neutral alumina.<sup>38</sup> 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  $-1.5^{\circ}$ ;  $\lambda_{max}$  242 m $\mu$  (16,750);  $\lambda_{teax}^{CC14+CS2}$ 5.95, 6.21, 7.90, 7.98, 8.25, 8.49 (sh), and  $8.55 \mu$ .

Anal. Caled. for C<sub>26</sub>H<sub>40</sub>O<sub>3</sub>: C, 77.95; H, 10.07. Found: C. 78.16, 78.14; H, 10.23, 10.09.

 $6\beta$ ,  $16\alpha$ -Dimethylpregnane- $5\alpha$ ,  $17\alpha$ -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  $m_{racuo}$  to give 0.803 g, of a partially crystalline residue:  $\lambda_{max}^{\rm Mic}$ 2.83 (sh), 2.92, 5.88, and 5.92 (sh)  $\mu$ .

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.  $215-216^{\circ}$ :  $[\alpha]^{31}\text{D} = -23.7^{\circ}$ :  $\lambda_{\text{max}}^{\text{Kir}}$ 2.89 and 5.82  $\mu$  (IR-4) (lit.<sup>7a</sup> m.p. 250-251°;  $[\alpha]\text{D} = -26.7^{\circ}$ :  $\lambda_{\max}^{\text{KBr}}$  2.72, 2.86, 5.85, and 5.92  $\mu$ ).

Anal. Caled. for C<sub>23</sub>H<sub>36</sub>O<sub>4</sub>: 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.), proteose 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: veast extract (10.0 g.), KH<sub>2</sub>PO<sub>4</sub> (4.9 g.), Na<sub>2</sub>HPO<sub>4</sub> · 7H<sub>2</sub>O (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  $(21.1-26.7^{\circ})$  on a platform-type reciprocal shaker under constant illumination.

A 545.6-mg, sample of the tetrol  $(\mathbf{12})$  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

<sup>(41)</sup> 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; II, 9.09, 9.13. The average of all the data shown is as follows: C, 69.88; H. 9.11

<sup>(42)</sup> The  $\Delta$ Mb value (OAC(OH) for **11c** and **11b** is  $\pm 153^{\circ}$ , that for **11c** and 11a is  $\pm 147^{\circ}$ , both values in good agreement with the  $\Delta M p$  for substance J and its diacetate. See ref. 22a, pages 612-622. This establishes the configuration of 11a, 11b, and 11c as 20b 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<sub>4</sub> solvates showed these samples to be substantially identical  $[\lambda_{\max}^{\text{KBr}} 5.85-5.86, 12.7, \text{ and } 13.2 \,\mu (\text{CCl}_4)], \text{ and to contain substantial}$ quantities of  $6\beta$ ,  $16\alpha$ -dimethylpregnane- $5\alpha$ ,  $17\alpha$ -diol-3, 20-dione (15).

 $6\alpha$ ,  $16\alpha$ -Dimethylpregnane- $3\beta$ ,  $5\alpha$ ,  $6\beta$ ,  $17\alpha$ -tetrol-20-one (22). 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-6,16 $\alpha$ -dimethyl-5,17(20)-pregnadiene-3 $\beta$ ,20-diol 3,20-diacetate;  $^{314}_{1x}$  5.74, 5.90, 8.1–8.2, 8.41, and 8.66  $\mu$ .  $\lambda_m^{\cup}$ 

B. Epoxidation of  $6,16\alpha$ -Dimethyl-5,17(20)-pregnadiene- $3\beta$ , 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  $(\lambda_{\max}^{CC14} 5.76, 8.13, 8.62, \text{ and } 8.90 \ \mu)$ which was a mixture consisting of cis- and trans- $5\alpha$ ,  $6\alpha$ :  $17\alpha$ , 20dioxido-6 $\beta$ ,16 $\alpha$ -dimethylpregnane-3 $\beta$ ,20-diol 3,20-diacetate and cis- and trans- $5\beta$ ,  $6\beta$ :  $17\alpha$ , 20-dioxido- $6\alpha$ ,  $16\alpha$ -dimethylpregnane-33,20-diol 3,20-diacetate (20).

C. Alkaline Hydrolysis of the 5,6:17 $\alpha$ ,20-Dioxido-6,16 $\alpha$ dimethylpregnane-3,3,20-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  $5\alpha$ ,  $6\alpha$ -oxido- $6\beta$ ,  $16\alpha$ -dimethylpregnane- $3\beta$ ,  $17\alpha$ diol-20-one (21a) and  $5\beta$ ,  $6\beta$ -oxido- $6\alpha$ ,  $16\alpha$ -dimethylpregnane-**3** $\beta$ ,17 $\alpha$ -diol-20-one (21b);  $\lambda_{\max}^{\text{KBr}}$  2.90, 5.90, and 11.60  $\mu$ .

Crystallization from acetone-methanol-Skellysolve B mixtures gave colorless plates, m.p. 240.5–247° (melted at 235–238° and resolidified),  $[\alpha]^{29}D = 66.2^\circ$ ,  $\lambda_{\rm KBr}^{\rm KBr} 5.93 \mu$ . This material was assigned the  $5\alpha$ ,  $6\alpha$ -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°;  $[\alpha]^{29}D = 59.1^{\circ}$ ;  $\lambda_{\max}^{KBr} 5.78$  (sh), 5.83, 7.91, and 8.03  $\mu$ . This substance was assigned the  $5\alpha$ ,  $6\alpha$ -oxido 3-acetate structure (21c).

Anal. Caled. for C25H38O5: 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-6,16 $\alpha$ -dimethylpregnane- $3\beta$ ,  $17\alpha$ -diol-20-ones (21a, b).—The sample of the mixed  $5\alpha, 6\alpha$ - and  $5\beta, 6\beta$ -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  $6\alpha$ ,  $16\alpha$ -dimethylpregnane- $3\beta$ ,  $5\alpha$ ,  $6\beta$ ,  $17\alpha$ -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);  $\lambda_{\text{max}}^{\text{KBr}} 2.90, 5.86, \text{ and } 9.48 \mu$ . Another preparation obtained substantially as described above and crystallized from aqueous acetone followed by acetone-Skellysolve B had m.p.  $235-242^{\circ}$  (after melting at 206-210° and resolidifica-tion),  $[\alpha]^{25}p - 12.0^{\circ}$  (dioxane).

Anal. Caled. for C23H38O3 H2O: C, 66.96; H, 9.77. Found: C, 66.89, 66.90; H, 9.62, 9.62.

 $6\alpha$ ,  $16\alpha$ -Dimethylpregnane- $5\alpha$ ,  $6\beta$ ,  $17\alpha$ -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 vellow 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°;  $[\alpha]^{29}D = -2.9^{\circ}$  (dioxane);  $\lambda_{max}^{\text{KBT}} 2.90, 5.85, \text{and } 5.87 \text{ (sh) } \mu$ . 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 (  ${\bf 23})$  in  ${\bf 43.5\,\%}$  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.<sup>43</sup> 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  $17\alpha$ -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
111/10/0	1 LANG

Fragment	Compound			
identity	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 - [HOAe +				
CH <sub>3</sub> CO]	297 (100.0)	295 (85.0)	295 (100.0)	293 (100.0)
"A-ring fragment" <sup>b</sup>		135 (100.0)		

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

Acknowledgments .--- The authors wish to thank Mr. Robert D. Fisher and Miss Dolores J. Bell for determination of the ultraviolet and the IR-4 infrared spectra, Mr. Harold M. Boyd for determination of the rotational data, Dr. Raphael O. Wagenaar and Mr. Joseph L. Mayou for carrying out the fermentation studies, and Dr. Lowell E. Peterson for the mass spectrographic analyses.

<sup>(43)</sup> L. Peterson, Anal. Chem., 34, 1781 (1962). Compound XXVII of this reference is  $6\alpha$ ,  $16\alpha$ -dimethyl- $17\alpha$ -hydroxyprogesterone (**6a**), not the  $6\beta$ methyl epimer as shown.