$\Delta^{20,23}$-choladien-3α-ol acetate (m.p. 150°; $[\alpha]^{25}D$ +89° (chloroform); ϵ_{3050} 24,900 in ethanol) was oxidized with 12.1 g. (121 millimoles) of chromium trioxide precisely as in the foregoing experiment, and the subsequent saponification was likewise identical. The amorphous product, $E^{1\%}_{1cm.}$ 258 at 3375 Å., was chromatographed on alumina. After several mobile oily fractions, 3.918 g. of yellow product was obtained which on recrystallization from benzene–acetone yielded 1.26 g., $E^{1\%}_{1cm.}$ 547 at 3375 Å. in alcohol. This substance was a solvate which became amorphous when dried at 100° *in vacuo*. It was recrystallized three times from ethyl acetate and formed thin prisms melting 151.5–153°; $[\alpha]^{24}D$ +55.5° (chloroform); ϵ_{2420} 13,900, ϵ_{3375} 31,800.

Anal. Calcd. for $C_{36}H_{44}O_2$: C, 85.19; H, 8.72. Found: C, 84.95; H, 8.86.

1:1 Molecular Compound of Pregnanolone and 3α-Hydroxy-21-(1,1-diphenylacrylal)-pregnan-20-one.—Later fractions from the chromatogram yielded pale yellow crystals from acetone which melted 160–162°; $E^{1\%}_{1cm.}$ 374 at 3375 Å.; calculated for a 1:1 molecular compound of the acrylal and pregnanolone is 373. A mixture of 5.1 mg. (0.01 millimole) of 3α-hydroxy-21-(1,1-diphenylacrylal)-pregnan-20-one (m.p. 151.5–153°) and 3.2 mg. (0.01 millimole) of 3α-hydroxypregnan-20-one was dissolved in acetone and the solvent allowed to evaporate spontaneously. After drying at 100° the crystalline residue melted 163–163.5° and exhibited an infrared spectrum indistinguishable from the product isolated from the chromatogram.

Diacetate of the Enol of 3α-Hydroxy-21-(1,1-diphenylacrylal)-pregnan-20-one.—Three hundred mg. of the 1:1 molecular compound of the acrylal and pregnanolone was acetylated by the procedure of Marshall, *et al.*[4] Crystallization from acetone–methanol and from ethyl acetate yielded 130 mg. of needles, m.p. 132–135°; $[\alpha]^{25}D$ +100° (chloroform); ϵ_{2450} 16,200, ϵ_{3350} 47,600.

Anal. Calcd. for $C_{40}H_{48}O_4$: C, 81.04; H, 8.16. Found: C, 81.43; H, 8.18.

Summary

After oxidation of 24,24-diphenyl-$\Delta^{20,23}$-choladiene with an excess of chromic acid, the 20-ketosteroid and diphenylacrolein condensed in the presence of aqueous alkali with the formation of a 21-diphenylacrylal. The constitution of the product was proved by degradation, and some of the properties of these derivatives were studied.

NEW YORK 21, N. Y. RECEIVED JUNE 21, 1950

[CONTRIBUTION FROM THE SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH]

The Action of Alcoholic Potassium Hydroxide on Δ^{16}-20-Ketosteroids[1]

BY DAVID K. FUKUSHIMA AND T. F. GALLAGHER

An attractive means for the preparation of steroid hormones of the adrenocortical type was advanced by Marker[2] as a result of his finding that a Δ^{16}-20-ketosteroid by addition of the elements of water in the presence of methanolic alkali formed a 17-hydroxy-20-ketosteroid. The reaction was presumed to account for the fact that heating the oxidation product of pseudobotogenin diacetate, 3β-acetoxy-$\Delta^{5,16}$-pregnadiene-12,20-dione, with potassium hydroxide in methanol yielded a compound having an additional atom of oxygen in the molecule. Since the newly introduced oxygen function could not be acetylated, Marker concluded that it was present as a tertiary alcohol group at C-17. Because of our interest in the preparation of 17-hydroxy cortical hormones, we wished to confirm and extend this important reaction, so novel in its simplicity and so readily applicable to the synthesis of adrenal steroids from a widely distributed class of natural products. We therefore investigated the products obtained when a pure sample of 3β-acetoxy-$\Delta^{5,16}$-pregnadien-20-one (I) was heated with a methanolic solution of potassium hydroxide, and found that the reaction resulted in the formation of a quite different compound than that postulated by Marker.

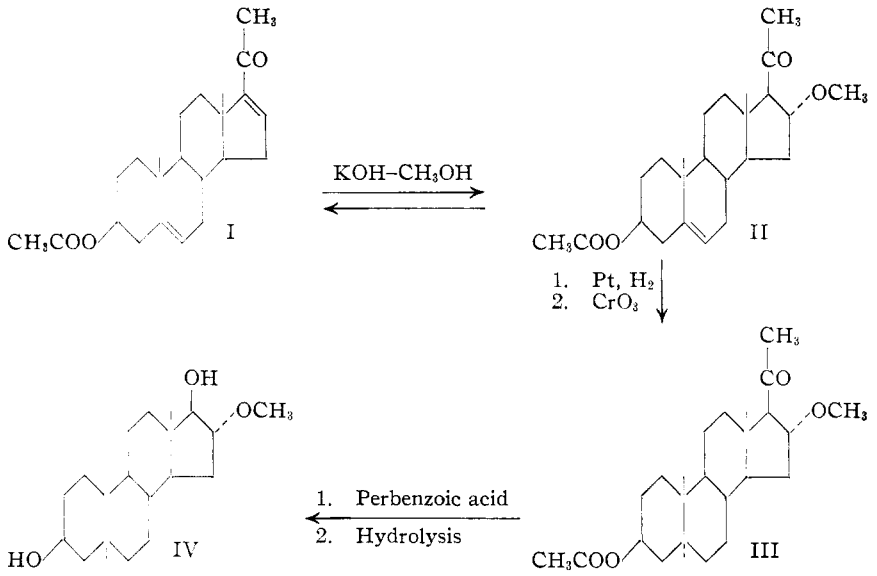
The ultraviolet absorption proved an extremely useful tool in following the reaction. From the decrease in the extinction coefficient at 2390 Å., it was apparent that in the presence of alkali more than half the product had been converted to a substance that no longer had an α,β-unsaturated ketone system. The mixture proved difficult to separate by fractional crystallization, but after acetylation, chromatography yielded two easily distinguished products. One was 3β-acetoxy-$\Delta^{5,16}$-pregnadien-20-one and the other a white crystalline compound, m. p. 158.5–159.5°, $[\alpha]D$ −28°, that had no absorption above 2250 Å. The infrared spectrum in the region 3000 to 4000 cm.$^{-1}$ showed that no free hydroxyl group was present in the new compound. This fact alone would have eliminated either diastereoisomer of 3β-acetoxy-17-hydroxy-Δ^5-pregnen-20-one from consideration but, fortunately, the physical constants of both known epimers[3,4] were sufficiently different from one another and from the unknown product to confirm this conclusion. It was possible that a 17-hydroxy-20-ketopregnane formed initially had been subsequently transformed to a D-homosteroid by alkali; this structure, too, was unlikely from the physical constants[4] as well as from the absence of the hydroxyl band in the infrared spectrum. The elementary analysis and a Zeisel determination showed that there was an additional carbon atom present as a methoxyl group. Therefore, the compound isolated was probably formed by the addition of a molecule of methanol to the unsaturated ketone. This conclusion was confirmed when an *ethanol* solution of potassium hydroxide was used for the reaction, and a different product was isolated, which proved to be the corresponding ethoxy derivative by elementary analysis and ethoxyl determination. A Δ^{16}-20-ketosteroid, therefore, formed an alkoxy derivative with loss of the α,β-unsaturation when treated with base in alcohol solutions. The base-

(1) This investigation was supported by grants from the Jane Coffin Childs Memorial Fund for Medical Research, the Teagle Foundation, Inc., the Lillia Babbit Hyde Foundation, and the National Cancer Institute, United States Public Health Service.

(2) Marker, THIS JOURNAL, **71**, 4149 (1949).

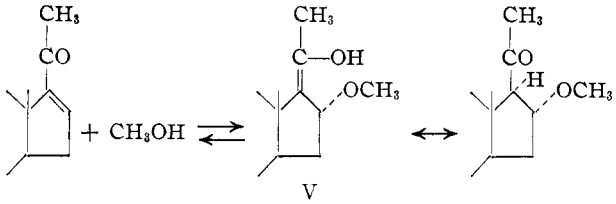
(3) Hegner and Reichstein, *Helv. Chim. Acta*, **24**, 828 (1941).

(4) Shoppee and Prins, *ibid.*, **26**, 201 (1943).



catalyzed addition of alcohols to α,β-unsaturated esters, nitriles[5] and aldehydes[6] with the formation of β-alkoxy derivatives is a well-known reaction similar in all respects to that observed with the Δ^{16}-20-ketosteroid. By analogy with these compounds the methoxyl group in the steroid should be at C-16, and the product obtained, therefore, was 3β-acetoxy-16-methoxy-Δ^5-pregnen-20-one (II). Proof for the structure II was provided by oxidative removal of the acetyl group at C-17. This was effected with perbenzoic acid using the saturated analog III. The product IV obtained from the oxidation still retained the methoxyl group. These results clearly established the constitution of the compound formed from a Δ^{16}-20-ketosteroid in alcoholic base.

The configuration occupied by the side chain and the methoxyl group are of fundamental importance for further investigations. Consideration of the mechanism of the reaction permits the assignment of configuration with a very considerable degree of assurance. It is highly probable that 1,4-addition to the unsaturated ketone leading to the formation of the enol V is the first stage of the reaction. The methoxyl would almost certainly add to the α face



of C-16 by an attack from the rear of the molecule, just as an addition reaction at C-11, C-12 and C-17 results in orientation of the entering group in the α-configuration.[7] With the methoxyl group in the α-configuration, two factors operate to force the side chain to the normal or β-orientation. First, the β side chain orientation is favored over the α in the ratio of 3 to 1 when 20-ketosteroids are equilibrated with acid[8] or base,[9] and it is to be expected that at least this ratio would exist in the equilibrium mixture we have studied. Second, a *trans* relationship of the substituents at C-16 and C-17 would be more favorable sterically and thus result in a thermodynamically more stable compound. While the combination of these factors would almost certainly lead to the exclusion of alternative configurations, additional evidence can be brought to bear on this question.

It has been shown[7,10] that conversion of the 20-ketosteroid to a 17-hydroxy C_{19} steroid by means of perbenzoic acid is accomplished without change in configuration. The alteration in molecular rotation accompanying this transformation should provide evidence of configuration at C-17. When an acetyl group at C-17 in the β-configuration is replaced by a β oriented hydroxyl group, the change is accompanied by a marked shift in molecular rotation in a negative direction as evidenced by the data recorded for the molecular rotation differences (ΔM_D) in Table I (Pairs 1–4). Conversely, when a 17α-acetyl group is replaced by a 17α-hydroxyl function, there is a pronounced dextrorotatory shift

TABLE I

THE MOLECULAR ROTATION DIFFERENCES (ΔM_D) IN ALCOHOL FOR THE REPLACEMENT OF A C-17 ACETYL GROUP WITH A C-17 HYDROXYL GROUP

Pair	Compound, C-17β	Ref.	$[\alpha]_D$	M_D	ΔM_D
1	3α-Acetoxypregnan-20-one	11	+123	+442	−369
	Etiocholane-3α,17β-diol	12	+ 25	+ 73	
2	3β-Acetoxyallopregnan-20-one	13	+ 79.8	+288	−276
	Androstane-3β,17β-diol	14	+ 4.2	+ 12	
3	3β-Acetoxy-Δ^5-pregnen-20-one	15	+ 19.9	+ 72	−214
	Δ^5-Androstene-3β,17β-diol	12	− 49	−142	
4	Δ^4-Pregnene-3,20-dione	16	+194	+605	−291
	3-Keto-Δ^4-androsten-17β-ol	17	+109	+314	
5	3β-Acetoxy-16α-methoxyallopregnan-20-one (III)		+ 26.7	+104	−158
	16α-Methoxyandrostane-3β,17β-diol (IV)		− 16.8	− 54	
	C-17α				
6	3α-Acetoxy-17-isopregnan-20-one	9	− 30.6	−110	+110
	Etiocholane-3α,17α-diol	7	0	0	
7	17-Iso-Δ^4-pregnen-3,20-dione	18	0	0	+206
	3-Keto-Δ^4-androsten-17α-ol	19	+ 71.5	+206	

(5) Koelsch, THIS JOURNAL, **65**, 437 (1943).

(6) "Acrolein," Report No. S-13149, Shell Development Company, Emeryville, California, p. 19, 1949.

(7) Gallagher and Kritchevsky, THIS JOURNAL, **72**, 882 (1950).

(8) Moffett and Hoehn, *ibid.*, **66**, 2098 (1944).

(9) Marshall and Gallagher, *J. Biol. Chem.*, **179**, 1265 (1949).

(10) Turner, THIS JOURNAL, **72**, 878 (1950).

(11) Butenandt and Müller, *Ber.*, **71**, 191 (1938).

(12) Ruzicka, Goldberg and Bosshard, *Helv. Chim. Acta*, **20**, 541 (1937).

(13) Fleischer, Whitman and Schwenk, THIS JOURNAL, **60**, 79 (1938).

(14) Butenandt, Tscherning and Hanisch, *Ber.*, **68**, 2097 (1935).

(15) Butenandt and Fleischer, *ibid.*, **70**, 96 (1937).

(16) Butenandt and Westphal, *ibid.*, **67**, 2085 (1934).

(17) Ruzicka and Wettstein, *Helv. Chim. Acta*, **18**, 1264 (1935).

(18) Butenandt, Schmidt-Thomé and Paul, *Ber.*, **72**, 1112 (1939).

(19) Ruzicka and Kägi, *Helv. Chim. Acta*, **19**, 842 (1936).

TABLE II

THE MOLECULAR ROTATION DIFFERENCES (ΔM_D) IN ALCOHOL FOR 16-OXYGENATED FUNCTIONAL GROUP

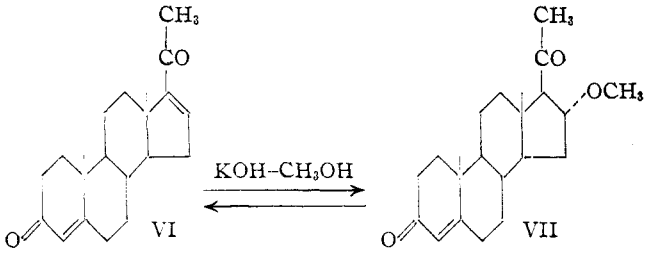
Pair	Compounds	Ref.	$[\alpha]_D$	M_D	
					ΔM_D 16α-OAc
1	3β,20α-Diacetoxyallopregnane	20	+ 2	+ 8	−276
	3β,16α,20α-Triacetoxyallopregnane	20	− 58	−268	
2	3β,20β-Diacetoxyallopregnane	20		+125	−333
	3β,16α,20β-Triacetoxyallopregnane	21	− 25	−208	
3	3β,20α-Diacetoxy-Δ⁵-pregnene	20	− 45	−181	−284
	3β,16α,20α-Triacetoxy-Δ⁵-pregnene	20	−101	−465	
					ΔM_D 16α-OH
4	Estradiol-3,17β	22	+ 81	+220	− 44
	Estriol-3,16α,17β	23	+ 61	+176	
5	Androstane-3α,17β-diol	24	+ 12.6	+ 37	− 55
	Androstane-3α,16α,17β-triol	25	− 5.9	− 18	
6	Etiocholane-3α,17β-diol	12	+ 25	+ 73	− 67
	Etiocholane-3α,16α,17β-triol	25	+ 1.9	+ 6	
7	Androstane-3β,17β-diol	14	+ 4.2	+ 12	− 71
	Androstane-3β,16α,17β-triol	26	− 19	− 59	
					ΔM_D 16α-OCH_3
8	3β-Acetoxy-Δ⁵-pregnen-20-one	15	+ 19.9	+ 72	−182
	3β-Acetoxy-16α-methoxy-Δ⁵-pregnen-20-one (II)		− 28.2	−110	
9	3β-Acetoxyallopregnan-20-one	13	+ 79.8	+288	−184
	3β-Acetoxy-16α-methoxyallopregnan-20-one (III)		+ 26.7	+104	
10	Δ⁴-Pregnene-3,20-dione	16	+194	+605	−240
	16α-Methoxy-Δ⁴-pregnene-3,20-dione (VII)		+106	+365	
11	Androstane-3β,17β-diol	14	+ 4.2	+ 12	− 66
	16α-Methoxyandrostane-3β,17β-diol (IV)		− 16.8	− 54	
					ΔM_D 16β-OAc
12	3β,20α-Diacetoxyallopregnane	20	+ 2	+ 8	+108
	3β,16β,20α-Triacetoxyallopregnane	21	+ 25	+116	
13	3β,20β-Diacetoxyallopregnane	20		+125	+ 97
	3β,16β,20β-Triacetoxyallopregnane	21	+ 48	+222	
					ΔM_D 16β-OH
14	Estradiol-3,17β	22	+ 81	+220	+ 33
	Estriol-3,16β,17β	27	+ 88	+253	
15	Androstane-3β,17β-diol	14	+ 4.2	+ 12	+ 43
	Androstane-3β,16β,17β-triol	28	+ 18	+ 55	

(Pairs 6, 7). 3β-Acetoxy-16α-methoxyallopregnan-20-one (III) was converted to 16α-methoxyandrostane-3β,17β-diol (IV) with perbenzoic acid followed by hydrolysis, and the molecular rotation difference of the two products was found to be −158 (Pair 5). The method of molecular rotation difference, then, confirms the conclusion previously stated, that the acetyl group at C-17 in 3β-acetoxy-16α-methoxy-Δ-⁵pregnen-20-one (II) is β oriented.

That the 16-methoxyl group is α oriented can likewise be deduced from molecular rotation differences. Table II summarizes the rotatory contribution that a 16-oxygenated group makes in a steroid when there is also a functional group in the β configuration at C-17. The contribution of the 16α-methoxy group in the 20-ketosteroids we have investigated was approximately −200 (Pairs 8–10). From the other compounds in the table, it is apparent that the contribution of a 16α-acetoxy group is about −300 (Pairs 1–3). On the other hand, the molecular rotatory contribution of a 16β-acetoxy group is approximately +100 (Pairs 12, 13). Where the substituent at C-17 is a hydroxyl group in the β-configuration, the rotatory contribution of a 16α-methoxy was found to be −66 (Pair 11), and this value is similar in sign and magnitude to that of a 16α-hydroxyl group, *i. e.*, −60 (Pairs 4–7). The 16β-hydroxyl group, on the contrary, contributes approximately +40 to the molecular rotation (Pairs 14, 15). The molecular rotation differences, thus, substantiate the validity of the conclusion drawn from application of the "rule of the rear" to the 16 position in the steroid nucleus. Since all of these considerations are in agreement, the configuration of the substituents at C-16 and C-17 can be considered to be firmly established.

The extremely facile reaction of Δ¹⁶-20-ketosteroids with alcohol immediately raises the question of the extent to which other α,β-unsaturated ketones undergo similar addition. Δ⁵,¹⁶-Pregnadiene-3,20-

(20) Hirschmann and Hirschmann, *J. Biol. Chem.*, **184**, 259 (1950).
(21) Hirschmann, Hirschmann and Daus, *ibid.*, **178**, 751 (1949).
(22) Whitman, Wintersteiner and Schwenk, *ibid.*, **118**, 789 (1937).
(23) Thayer, Levin and Doisy, *ibid.*, **91**, 655 (1931).
(24) Butenandt and Tscherning, *Z. physiol. Chem.*, **234**, 224 (1935).
(25) Lieberman and Dobriner, Abstracts of Papers, Philadelphia Meeting of American Chemical Society, p. 19C, April, 1950.
(26) Ruzicka, Prelog and Wieland, *Helv. Chim. Acta*, **28**, 1609 (1945).
(27) Huffman and Darby, THIS JOURNAL, **66**, 150 (1944).
(28) Huffman and Lott, *ibid.*, **71**, 719 (1949).



dione (VI) (16-dehydroprogesterone) provided an excellent material for study of this question, since it has in the same molecule an α,β-unsaturated ketone system in ring A as well as the ring D-side chain system which has been under consideration. In a preparative experiment, 16α-methoxyprogesterone (VII), ϵ_{2410} 16,600 (methanol), was isolated from 16-dehydroprogesterone (VI) after solution in 3% methanolic potassium hydroxide at room temperature for 4 hours. The extinction coefficient clearly proved that the ring A α,β-unsaturated ketone system was unaltered in the product and was directly comparable to progesterone, ϵ_{2410} 16,600 (methanol). Spectrophotometric analysis likewise proved that neither testosterone nor 3-keto-Δ^1-androsten-17-ol had reacted with methanol in the presence of alkali at 23° within a period of 2 hours. Since these two compounds are representative α,β-unsaturated ketones in a six-membered ring, it is highly probable that this system does not react with alcohol in the presence of alkali, or that in these compounds the equilibrium lies far on the side of the unsaturated ketone.

The reversibility of the addition reaction was demonstrated by isolation of 3β-acetoxy-$\Delta^{5,16}$-pregnadien-20-one (I) after heating a pure sample of 3β-acetoxy-16α-methoxy-Δ^5-pregnen-20-one (II) with methanolic base. Spectrophotometric analysis of the reaction showed that with potassium hydroxide in methanol at 23°, equilibrium was rapidly attained, and 30% diene and 70% 16-methoxy compound constituted the equilibrium mixture (Fig. 1). A similar study was made with the corresponding derivatives of progesterone, again approaching the equilibrium from both directions (Fig. 2). When the contribution of the α,β-unsaturated ketone system in ring A was taken into consideration, it was shown that here also an almost identical equilibrium mixture of Δ^{16}-20-ketone and 16-methoxy compound was obtained.

Driving force for reaction of the Δ^{16}-20-ketones must, we believe, be ascribed to the considerable element of strain produced in the five-membered ring D by the presence of an unsaturated bond. Saturation of the olefinic linkage by the addition of alcohol offers a means for the attainment of a more stable ring system, but the tendency to achieve greater stability in this way is opposed by the keto group at 20 which is much more stable in conjugation with the double bond. With the six-membered ring ketones, there is no element of strain to be overcome, and therefore little, if any, reaction takes place.

The equilibrium reaction described in this report provides a simple and very convenient method for the preparation of 16-methoxy-20-ketosteroids, and since these can in turn be readily converted by the excellent peracid oxidation to 17-hydroxy C_{19} steroids, the way is open for the preparation of a wide variety of compounds related to important steroid hormone derivatives. Exploration of the biological activity of these close chemical relatives of hormones may provide very interesting information. The results, in addition, are interesting from a technical standpoint, since preparation of Δ^{16}-20-ketosteroids from sapogenins requires hydrolysis after oxidation of the side chain, and the proper choice of conditions should be dictated by consideration of the addition reaction. The reversibility of the reaction will permit recycling of valuable material which may have been otherwise discarded.

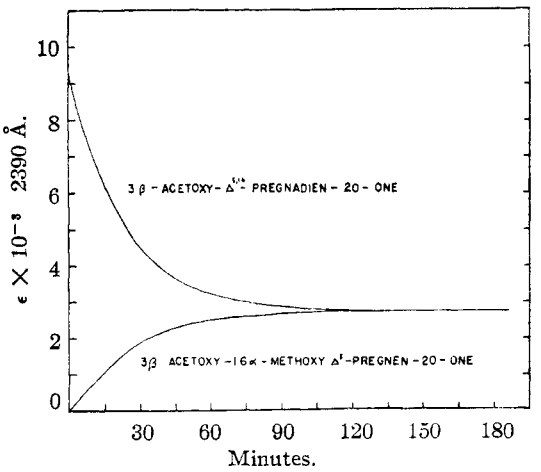


Fig. 1.—Equilibration of 3β-acetoxy-$\Delta^{5,16}$-pregnadien-20-one (c 3.32 × 10^{-4} M) and 3β-acetoxy-16α-methoxy-Δ^5-pregnen-20-one (c 3.16 × 10^{-4} M) in 0.4 N methanolic potassium hydroxide at 23°. With higher concentration (c 1.15 × 10^{-2} M) the same equilibrium was achieved in the same time.

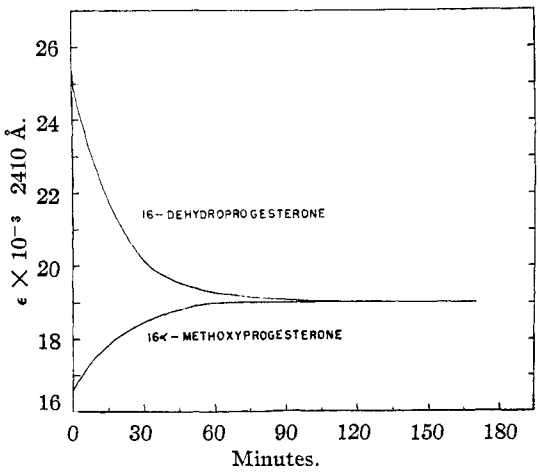


Fig. 2.—Equilibration of 16-dehydroprogesterone (c 4.87 × 10^{-5} M) and 16α-methoxyprogesterone (c 7.26 × 10^{-5} M) in 0.4 N methanolic potassium hydroxide at 23°.

Acknowledgment.—We wish to express our appreciation to Dr. Konrad Dobriner and Phyllis Humphries of this Institute, who determined and interpreted the infrared spectra for us, and to Jean

Rogers for technical assistance. We are greatly indebted to Dr. George S. Rosenkranz of Syntex S. A., Mexico City, Mexico, who supplied us with a generous sample of 3β-acetoxy-$\Delta^{5,16}$-pregnadien-20-one.

Experimental[29]

3β-Acetoxy-16α-methoxy-Δ^5-pregnen-20-one (II) from 3β-Acetoxy-$\Delta^{5,16}$-pregnadien-20-one (I).—A solution of 2.50 g. of 3β-acetoxy-$\Delta^{5,16}$-pregnadien-20-one (m.p. 172–175°; ϵ_{2390} 9100; $E^{1\%}_{1cm.}$ 257) in 250 cc. of 3% methanolic potassium hydroxide solution was refluxed under nitrogen for 1 hour. The solution was cooled, added to water and extracted with ether. The ether solution was washed with water until neutral, dried over sodium sulfate, and the ether evaporated to give 2.49 g. of crystalline residue, $E^{1\%}_{1cm.}$ 121 at 2390 Å. Recrystallization from ether gave 2.34 g. of white crystalline compound, m.p. 165–180°. Repeated recrystallization yielded materials of wide m.p. range. The product was therefore acetylated with acetic anhydride and pyridine overnight at room temperature. Ice was added to the acetylation mixture which was then extracted with ether; the ether was washed with dilute hydrochloric acid, sodium carbonate solution and water, and dried over sodium sulfate. Evaporation of the solvent gave 2.49 g. of crystalline product. Attempted purification by recrystallization gave materials with wide m.p. ranges. The mixture was chromatographed on 75 g. of acid washed alumina. Elution with benzene–petroleum ether (b.p. 60°) (1:19) yielded 690 mg. of 3β-acetoxy-$\Delta^{5,16}$-pregnadien-20-one (I), m.p. 165–170°. A recrystallization from petroleum ether raised the m.p. to 171.5–173°. Further elution with benzene–petroleum ether (1:19) gave 313 mg. of a mixture of compounds. The benzene–petroleum ether (1:10) eluates yielded 1.220 g. of a compound which showed no absorption between 2200 and 3200 Å. Recrystallizations from methanol and from petroleum ether yielded needles of 3β-acetoxy-16α-methoxy-Δ^5-pregnen-20-one (II), m.p. 158.5–159.5°; $[\alpha]^{25}D$ −28.5° (chloroform); $[\alpha]^{32}D$ −28.2 (95% ethanol).

Anal. Calcd. for $C_{24}H_{36}O_3$: C, 74.29; H, 9.34; OCH_3, 7.99. Found: C, 74.53; H, 9.18; OCH_3, 7.99.

3β-Acetoxy-$\Delta^{5,16}$-pregnadien-20-one was heated in 50% aqueous methanol containing 8% potassium carbonate, and the absorption at 2390 Å. was determined on small portions at intervals: after 0.5 hour, $E^{1\%}_{1cm.}$ 150; after 1 hour, $E^{1\%}_{1cm.}$ 137; after 2 hours, $E^{1\%}_{1cm.}$ 152. The reaction mixture was separated as in the foregoing experiment, and 3β-acetoxy-$\Delta^{5,16}$-pregnadien-20-one and 3β-acetoxy-16α-methoxy-Δ^5-pregnen-20-one were isolated from the mixture in substantially similar amounts to those obtained with potassium hydroxide.

3β-Acetoxy-16α-ethoxy-Δ^5-pregnen-20-one from 3β-Acetoxy-$\Delta^{5,16}$-pregnadien-20-one (I).—A solution of 500 mg. of 3β-acetoxy-$\Delta^{5,16}$-pregnadien-20-one (m.p. 172–175°; ϵ_{2390} 9100; $E^{1\%}_{1cm.}$ 257) in 50 cc. of 3% ethanolic potassium hydroxide solution after standing at room temperature for 2 hours was diluted with water and extracted with ether. The ether solution was washed with water, dried, and the solvent evaporated to give a crystalline residue; $E^{1\%}_{1cm.}$ 118 at 2390 Å. The product was acetylated as before and was chromatographed on 100 g. of silica gel. The separation was followed with the aid of Bradys reagent. The ether–petroleum ether (1:1) eluates, which gave a yellow dinitrophenylhydrazone characteristic of a saturated ketone, yielded 125 mg. of product. This was recrystallized from petroleum ether, and 100 mg. of prisms of 3β-acetoxy-16α-ethoxy-Δ^5-pregnen-20-one, m.p. 144–144.5°, $[\alpha]^{23}D$ −30.6° (chloroform) were obtained. The compound was transparent in the ultraviolet. The melting point of a mixture with 3β-acetoxy-16α-methoxy-Δ^5-pregnen-20-one was 125–140°, and the infrared spectra of the two compounds were markedly different.

Anal. Calcd. for $C_{25}H_{38}O_4$: C, 74.59; H, 9.51; OC_2H_5, 11.19. Found: C, 74.62; H, 9.56; OC_2H_5, 10.66.

3β-Acetoxy-$\Delta^{5,16}$-pregnadien-20-one (I) from 3β-Acetoxy-16α-methoxy-Δ^5-pregnen-20-one (II).—A solution of 100 mg. of 3β-acetoxy-16α-methoxy-Δ^5-pregnen-20-one in 25 cc. of 3% methanolic potassium hydroxide solution was refluxed under nitrogen for 1 hour. The hydrolysis mixture was cooled, added to water and extracted with ether. The ether solution was washed with water, dried and the solvent evaporated to give a product with $E^{1\%}_{1cm.}$ 134 at 2390 Å. Acetylation followed by chromatography resulted in the separation of 3β-acetoxy-$\Delta^{5,16}$-pregnadien-20-one (I), m.p. 173.5–175°, and 3β-acetoxy-16α-methoxy-Δ^5-pregnen-20-one (II), m.p. 157.5–159°, in approximately the same yields as those obtained starting with the $\Delta^{5,16}$-20-ketone. Hydrolysis of 3β-acetoxy-16α-methoxy-Δ^5-pregnen-20-one with 0.05 *N* 50% aqueous methanolic sodium hydroxide solution under reflux for 2 hours gave similar results.

3β-Acetoxy-16α-methoxyallopregnan-20-one (III).—A solution of 685 mg. of 3β-acetoxy-16α-methoxy-Δ^5-pregnen-20-one (II) in 20 cc. of acetic acid was hydrogenated with 100 mg. of Adams catalyst at room temperature and atmospheric pressure. The uptake was very slow after 1.4 moles of hydrogen had been absorbed and the reaction was interrupted. The catalyst was filtered off, and the reduction mixture was oxidized with 6 cc. of 2% chromic acid in acetic acid solution at room temperature for 2 hours. The excess chromic acid was reduced with methanol, and after dilution with water the product was extracted with ether. The ether solution was washed with sulfuric acid solution, sodium carbonate solution and water, dried, and the solvent evaporated to give 700 mg. of yellow oil. Recrystallization from petroleum ether (b.p. 60°) gave 252 mg. of white needles of 3β-acetoxy-16α-methoxyallopregnan-20-one (III), m.p. 120.5–122.5°. The analytical sample melted at 122–123.5°; $[\alpha]^{28}D$ +29.3° (chloroform), $[\alpha]^{28}D$ +26.7° (95% ethanol).

Anal. Calcd. for $C_{24}H_{38}O_4$: C, 73.80; H, 9.81; OCH_3, 7.94. Found: C, 74.02; H, 9.92; OCH_3, 8.00.

16α-Methoxyandrostane-3β,17β-diol (IV).—To 200 mg. of 3β-acetoxy-16α-methoxyallopregnan-20-one (III) was added 0.7 cc. of a benzene solution containing 88.2 mg. of perbenzoic acid. The solution was stored at room temperature for 3 days, and then an additional 88.2 mg. of perbenzoic acid was added. The mixture was stored 4 days longer at room temperature and was then diluted with ether. The ether solution was washed with sodium carbonate solution and water, dried, and the solvent evaporated to give 217 mg. of yellow oil. Upon separation with Girard T reagent, 109 mg. was obtained in the ketonic fraction and 90 mg. was found in the non-ketonic fraction. Hydrolysis of the non-ketonic fraction with 5 cc. of 3% methanolic potassium hydroxide solution under reflux for 45 minutes gave 67 mg. of crystalline product. Recrystallization from acetone gave short needles of 16α-methoxyandrostane-3β,17β-diol (IV), m.p. 198.5–199.5°, $[\alpha]^{28}D$ −16.8° (95% ethanol).

Anal. Calcd. for $C_{20}H_{34}O_3$: C, 74.49; H, 10.63. Found: C, 74.24; H, 10.63.

16α-Methoxyprogesterone (VII) from 16-Dehydroprogesterone (VI).—A solution of 5.8 g. of crude 16-dehydroprogesterone in 200 cc. of 3% methanolic potassium hydroxide was allowed to stand at room temperature for 4 hours. Ether was added and the ether solution washed repeatedly with water and dried over sodium sulfate. Evaporation of the solvent yielded a yellow oil, $E^{1\%}_{1cm.}$ 520 at 2410 Å. The product was chromatographed on silica gel and two distinct fractions were obtained. From the ether–petroleum ether (3:1) eluates was obtained 1.34 g. of 16-dehydroprogesterone (VI) which on recrystallization from acetone–petroleum ether gave needles, m.p. 190–192°; $[\alpha]^{32}D$ +133° (95% ethanol); ϵ_{2410} 25,200 (methanol); $E^{1\%}_{1cm.}$ 808. From the ether–petroleum ether (17:3) eluates was obtained 2.07 g. of 16α-methoxyprogesterone (VII) which on recrystallization from acetone–petroleum ether gave needles, m.p. 134–135.5°; $[\alpha]^{32}D$ +106° (95% ethanol); ϵ_{2410} 16,600 (methanol); $E^{1\%}_{1cm.}$ 482.

Anal. Calcd. for $C_{22}H_{32}O_3$: C, 76.70; H, 9.37; OCH_3, 9.01. Found: C, 77.00; H, 9.25; OCH_3, 9.11.

(29) The ultraviolet spectra were determined with the Cary recording ultraviolet spectrometer. The solvent employed was 95% ethanol unless otherwise specified. We are indebted to Mr. J. Alicino, Metuchen, New Jersey, the Schwarzkopf Microanalytical Laboratory, Middle Village, L. I., New York, and Mr. R. Funk of the Sloan-Kettering Institute for the microanalyses. All m. p. are corrected.

Summary

The addition of alcohols to Δ^{16}-20-ketosteroids in alcoholic alkali to give 16-alkoxy-20-ketosteroids has been described. The configurations of the 16-alkoxy group as α and the C-17 acetyl group as β have been assigned. The reaction has been shown to be reversible, and the same equilibrium mixture has been obtained from both directions. 16α-Methoxyandrostane-3β,17β-diol was prepared in the course of the proof of structure.

NEW YORK 21, N. Y. RECEIVED JULY 5, 1950

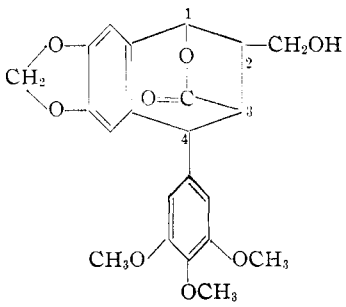
[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF MARYLAND]

Podophyllotoxin and Picropodophyllin.[1] I. Their Reduction by Lithium Aluminum Hydride

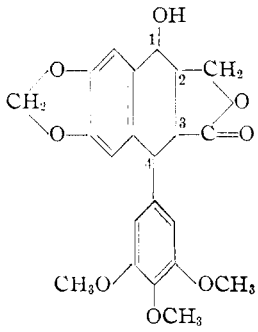
BY NATHAN L. DRAKE AND EDWARD H. PRICE[2]

Podophyllotoxin, one of the constituents of podophyllin resin, and its isomer, picropodophyllin, are of current interest because of their effect upon mitosis, an effect which is apparently shared by other constituents of the resin.[3]

The structures, I and II, for podophyllotoxin and picropodophyllin, respectively, are based upon work of Borsche and Niemann,[4] Späth[5] and Haworth.[6] Neither compound has been synthesized.



I



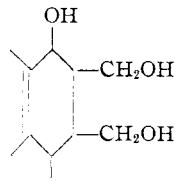
II

The carbon skeleton of the isomers is well established, but the evidence upon which the different lactone structures are based is neither sufficiently extensive nor sufficiently clear-cut to rule out other possibilities. Furthermore, no proof of the presence of a primary hydroxyl in podophyllotoxin and a secondary hydroxyl in picropodophyllin has been advanced, and the stereochemistry of the molecules has been largely neglected.

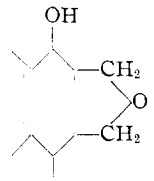
The present work was undertaken to prove whether the lactone ring of each of the isomers was essential to biological activity.

As work progressed, it became evident that podophyllotoxin and picropodophyllin must differ in configuration at carbon number 3 (see I and II). Whether there are other differences between the isomers, differences which are not expressed in the Borsche–Späth formulas, is not proven by our work, but our evidence can be satisfactorily interpreted in terms of the Borsche–Späth formulas.[7]

When podophyllotoxin is reduced by lithium aluminum hydride, a trihydroxy compound, IIIa is produced. This substance is very sensitive



IIIa,b



IVa,b

to acidic reagents and cannot be recrystallized due to the ease with which it undergoes loss of water. The dehydration product is formulated as IVa, although it is quite possible that the tetrahydrofuran ring might be formed through the hydroxyl group on carbon-1. IIIa is characterized by the formation of a tri-*p*-nitrobenzoate formed by the treatment of the alcohol with *p*-nitrobenzoyl chloride in pyridine. The presence of only one hydroxyl group in IVa follows from its conversion into (1) a methyl ether containing four methoxyl groups, (2) a monobenzoate and (3) a mono-*p*-nitrobenzoate. The molecular weight of IVa was also in accord with that calculated assuming internal loss of water from the molecule.

We have been unable to obtain any direct chemical proof of the presence of a *secondary*

(1) From a thesis submitted to the Graduate School of the University of Maryland by Edward H. Price in partial fulfillment of the requirements for the degree of Doctor of Philosophy, June, 1949.

(2) American Chemical Society predoctoral fellow 1946–1949. The junior author wishes to express his thanks to the American Chemical Society for the fellowship which made this work possible. The podophyllotoxin used was made available through a grant-in-aid (C 430 C) from the National Institutes of Health.

(3) Hartwell and Detty, THIS JOURNAL, **72**, 246 (1950).

(4) Borsche and Niemann, *Ann.*, **494**, 126 (1932); *Ber.*, **65**, 1633 (1932).

(5) Späth, *et al.*, *Ber.*, **65**, 1536, 1773 (1932); **66**, 125 (1933).

(6) Haworth and Richardson, *J. Chem. Soc.*, **149**, 348 (1935).

(7) In a paper presented before the Medicinal Chemistry Division at Philadelphia, April 10, 1950, Hartwell and Shrecker presented evidence to show that the sole difference between podophyllotoxin and picropodophyllin is the configuration around carbon-3.