where the ring strain may spread the angle between the two carboxyl groups to a point where intramolecular hydrogen transfer is seriously impeded. It is this strain and the greater entropy requirements involved that might be presumed to displace the cyclopropanedicarboxylic acid points from the general linear relationship of ΔH^* *vs.* ΔS^* for the other acids, although the failure of the point for sulfuric acid to fall with the other four solvents is not readily interpretable.

A third steric factor is the bulk of the solvent as it solvates the acid and/or the departing carbon dioxide. Entropy terms ought to show up markedly here, and the consistently negative values of ΔS^* for collidine compared with the other solvents indicates that it is indeed involved in solvation. However, while the entropy requirements are high, the ability to solvate carbon dioxide[1] compensates, and the enthalpy of activation is considerably lower in this solvent. Certainly no real base catalysis is observed, however.

The fact that the points for the three larger rings yield a single ΔH^* *vs.* ΔS^* line indicates a good compensation of these two factors and the probability that there is only one important "interaction mechanism" involved. That this is not ring strain seems clear; thus the best alternative is solvent action on a cyclic transition state. Some reinforcement for this notion comes from the positioning of the cyclobutanedicarboxylic acid points on the upper right portion of the enthalpy–entropy diagram (Figure 6), since the small ring would be expected to pull back the methylene groups to permit easy access of solvent.

The summation of these ideas would suggest that the transition state must lie closer to starting materials than enol in decarboxylation and that in general the entropies and enthalpies of activation compensate each other to produce only a small range of rates through the ring series investigated.

Experimental Section

The 1,1-cycloalkanedicarboxylic acids of the three-, four-, five-, and six-membered rings were prepared by the method of Vogel,[6] as originally worked out for the four-membered ring acid in which a polymethylene dibromide is condensed with sodium malonic ester. The acids were purified by recrystallization.

The products, prepared by using the apporpriate polymethylene dibromide, are described in Table III.

TABLE III

1,1-Cycloalkanedicarboxylic acid	M.p., °C. This work	M.p., °C. Lit.[a]	% yield[b]
Cyclopropane	139–141	140–141	12
Cyclobutane	156–157	157	27
Cyclopentane	183–184	184–186	29
Cyclohexane	174–176	179.5	32

[a] From ref. 6. [b] Calculated on the basis of polymethylene dibromide.

Decarboxylation of the Acids.—Weighed samples of the dicarboxylic acids (0.001 mole) were introduced into a small reaction flask together with 1.5 ml. of solvent and heated in mineral oil under constant temperature conditions. The volume of carbon dioxide generated in the reaction vessel was measured in a gas buret over saturated sodium chloride solution, and the volume observed was corrected to standard conditions. The decarboxylations without solvent were carried out by heating the reaction flask to temperatures in excess of the melting points of the acids until carbon dioxide evolution was complete. The decarboxylations in collidine, concentrated H_2SO_4, 85% H_3PO_4, and diethylene glycol were carried out by heating also, again until gas evolution was complete. In a typical example, 0.1440 g. (0.001 mole) of 1,1-cyclobutanedicarboxylic acid was introduced into a small reaction flask together with 1.50 ml. of solvent and heated in the constant-temperature oil bath. In a series of three such runs, the gas evolution was 21.82, 21.70, and 22.20 cc. at STP (22.40 cc. at STP theoretical). The reaction was carried out at three tempratures between 155 and 195° for this acid, in triplicate for each temperature. The gas volumes, corrected to standard conditions, were used to prepare Arrhenius plots, and from these the rate constants were obtained from the slopes. See Figures 2–5. The rate constants for the four-membered ring acid are collected in Table II.

From absolute reaction rate theory, ΔH^* and ΔS^* were computed by solving the following equation as simultaneous expressions at two different temperatures, and ΔH^* was plotted against ΔS^* in Figure 6.

$$k_1 = \frac{kT}{h} e^{\frac{-\Delta H^*}{RT}} e^{\frac{\Delta S^*}{R}}$$

Acknowledgment.—We are indebted to Dr. Scott MacKenzie and the University of Rhode Island Computer Laboratory for the least-squares treatment of the data.

(6) A. I. Vogel, *J. Chem. Soc.*, 1487 (1929).

Microbiological Hydroxylation of Alkaloids from *Funtumia latifolia*

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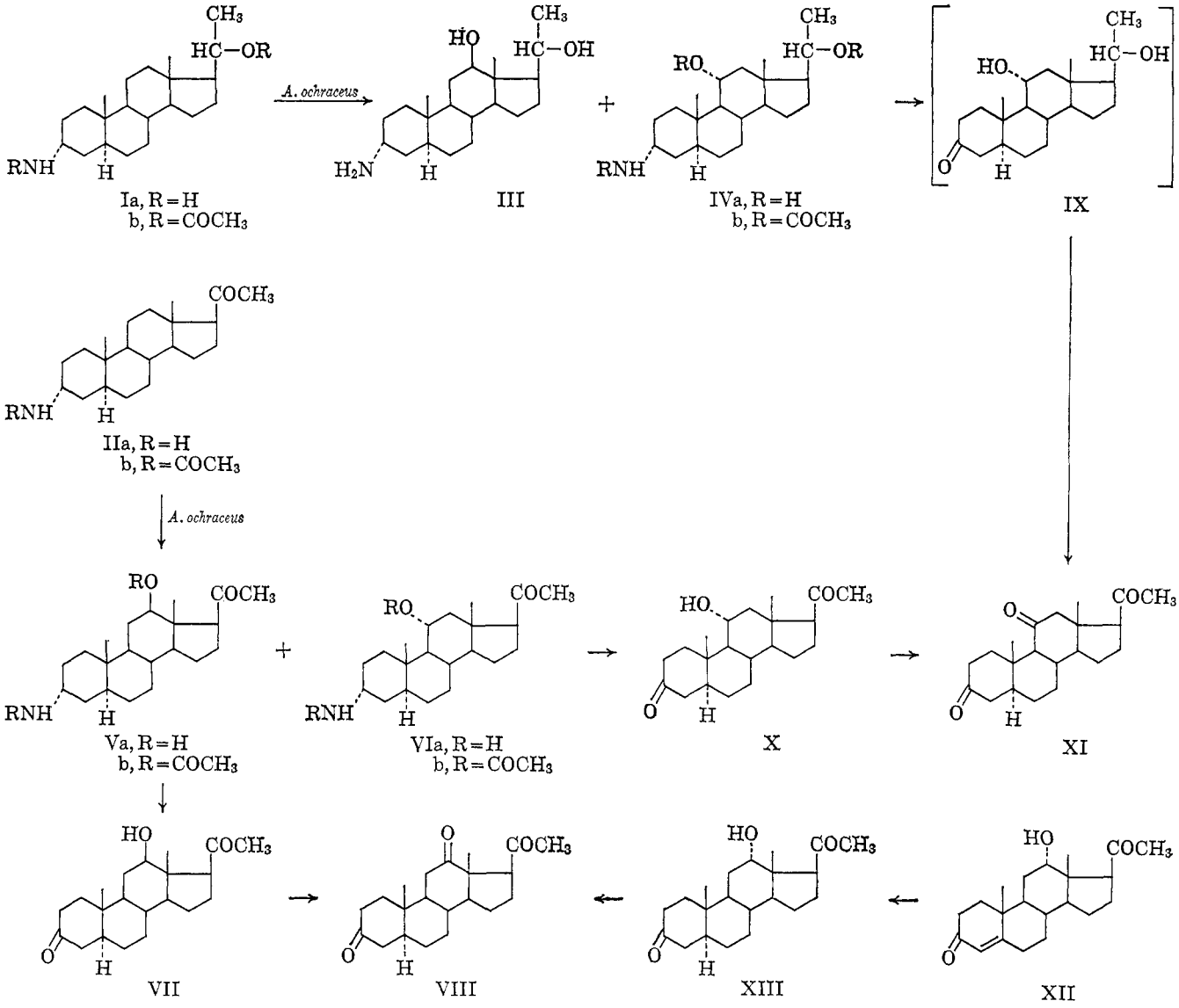
The two steroidal alkaloids funtumine and funtumidine are both monohydroxylated by the fungus *Aspergillus ochraceus* in the 11α and 12β positions. The structures of the hydroxylated products were established by infrared and n.m.r. spectra, optical rotation, and conversion to the respective 5α-pregnane-3,11,20-trione and 5α-pregnane-3,12,20-trione.

Steroidal alkaloids from the leaves of *Funtumia latifolia* have been subjected to chemical modication but not, reportedly, to microbiological transformation. Our findings on the fungal alterations of funtumidine (Ia) and funtumine (IIa)[1] are presented here. Microbiological modification of other steroidal alkaloids (conessine,[2] solasodine,[3] and tomatidine[3a,4]) has recently been reported.

Funtumidine (Ia) and funtumine (IIa) are both

(1) M.-M. Janot, Q. Khuong-Huu, and R. Goutarel, *Compt. rend.*, **246**, 3076 (1958); **248**, 982 (1959).

(2) (a) J. de Flines, A. R. Marx, W. F. van der Waard, and D. van der Sijde, *Tetrahedron Letters*, **No. 26**, 1257 (1962); (b) S. M. Kupchan, C. J. Sih, S. Kubota, and A. M. Rahim, *ibid.*, **No. 26**, 1767 (1963); (c) E. L. Patterson, W. W. Andres, and R. E. Hartman, *Experientia*, **20**, 256 (1964).



attacked by *Aspergillus ochraceus* NRRL 405 in aerobic fermentation, each producing two Dragendorff-positive components more polar on thin layer chromatograms than the parent substrates. From the relative chromatographic mobilities it was presumed that the two funtumidine products, III and IVa, bore the same relationship to the funtumine products, Va and VIa, that funtumidine bore to funtumine, *i.e.*, that III and IVa were 20α-dihydro derivatives of Va and VIa, respectively. This relationship was established by reduction of Va to III with sodium in alcohol.

The proportion of products depended on substrate. Funtumine gave a greater proportion of the more mobile Va (6.7%) than of VIa (0.3%), whereas funtumidine gave a greater proportion of the more polar IVa (8.5%) than of III (6.5%).

O,N-Diacetate derivatives (Vb and VIb) were formed from Va and VIa, respectively, and an O,O,N-triacetate derivative IVb was formed from IVa. From elemental analyses and infrared and proton n.m.r. spectra of III–VI, it was concluded that monohydroxylation had occurred at nuclear secondary positions.

Degradation of IVa by osmium tetroxide[5] gave a partially characterized 11α,20α-dihydroxy-5α-pregnan-3-one (IX), which was oxidized by chromic acid to the known 5α-pregnane-3,11,20-trione (XI). Similarly, osmium tetroxide degradation of VIa led to the known 11α-hydroxy-5α-pregnane-3,20-dione (X), which, *via* the 3,11,20-trione XI, linked the two hydroxylation products IVa and VIa as 11-hydroxy derivatives of the respective funtumidine and funtumine substrates.

The degradation product X clearly established the 11α configuration for the hydroxyl group in VIa. The 11α configuration in VIa is assigned on the basis of molecular rotational data and proton n.m.r. spectra. The $\Delta[M]_D$ values for IVa and IVb are -57 and $-27°$, respectively, which values agree in sign and magnitude with rotational increments associated with the 11α-hydroxyl group (11β-hydroxyl group increments are of opposite sign).

The downfield shift of the 19-methyl group proton resonance in IVa (Table I) in comparison with that of funtumidine, together with a lack of such shift on the 18-methyl group proton resonance, clearly establishes an 11α-hydroxyl group in IVa, as does the chemical shift (3.68 p.p.m.) for the 11-proton geminal to the

(3) (a) Y. Sato and S. Hayakawa, *J. Org. Chem.*, **26**, 4181 (1961); (b) *ibid.*, **28**, 2739 (1963); (c) Y. Sato, J. A. Waters, and H. Kaneko, *ibid.*, **29**, 3732 (1964).

(4) Y. Sato and S. Hayakawa, *ibid.*, **29**, 198 (1964).

(5) R. Pappo, U. S. Patent 2,919,285 (Dec. 29, 1959).

(6) L. L. Smith, *Steroids*, **4**, 395 (1964).

TABLE I

PROTON RESONANCES OF FUNTUMINE DERIVATIVES[a]

Steroid	C-18	C-19	C-21	x-H[b]	3β-H	20β-H	N-H	N-Acetyl	O-Acetyl
Funtumine (IIa)	0.63	0.79	2.12	...	3.21 (m)	...	...	...	...
Funtumine N-acetate (IIb)	0.63	0.80	2.12	...	4.13 (b)	...	5.97	2.00	...
11α-Hydroxyfuntumine (VIa)	0.62	0.92	2.12	3.92 (m)	3.15 (m)	...	...	...	...
11α-Hydroxyfuntumine N,11α-diacetate (VIb)	0.68	0.92	2.12	5.17 (q)	4.17 (m)	...	5.88	2.01	2.09
12β-Hydroxyfuntumine (Va)	0.69	0.78	2.23	3.43 (q)	3.21 (q)	...	...	...	...
12α-Hydroxyfuntumine N,12β-diacetate (Vb)	0.82[c]	0.84[c]	2.08	4.74 (q)	4.13 (b)	...	5.97	2.00	2.00
Funtumidine (Ia)	0.65	0.77	1.18[d]	...	3.18 (b)	3.64 (m)	...	...	...
Funtumidine N,20α-diacetate (Ib)	0.65	0.80	1.20[d]	...	4.08 (m)	4.90 (m)	...	1.95	1.98
11α-Hydroxyfuntumidine (IVa)	0.67	0.94	1.26[d]	3.68 (m)	3.20 (m)	3.68 (m)	...	...	...
11α-Hydroxyfuntumidine N,11α,20α-triacetate (IVb)	0.75	0.93	1.22[d]	5.20 (m)	4.10 (b)	4.95 (m)	5.87	2.01	2.01
12β-Hydroxyfuntumidine (III)[e]	0.69	0.78	1.13[d]	3.39 (q)	3.22 (b)	3.67 (qn)	...	...	...
12β-Hydroxy-5α-pregnane-3,20-dione (VII)[e]	0.73	1.01	2.17	3.42 (q)	...	...	...	...	...
5α-Pregnane-3,12,20-trione (VIII)[e]	0.98	1.08	2.24	...	...	...	...	...	...

[a] Abbreviations used: b, broad; m, multiplet; q, quartet; qn, quintet. All data except as noted were obtained at 60 Mc. [b] The proton geminal to the 11α- or 12β-oxygen function. [c] The C-18 and C-19 methyl proton assignments may be reversed. [d] Doublet signals, J = 6.0 c.p.s. [e] 100-Mc. spectra.

11-hydroxyl in IVa. This chemical shift value is typical of 11α-hydroxy steroids but is outside of the limits found for several 11β-hydroxy steroids.[6]

Osmium tetroxide degradation of the secondary product Va led to a monohydroxy-5α-pregnane-3,20-dione (VII) not readily identified with the derivatives previously described in the literature. Chromic acid oxidation of VII gave the trione VIII, whose eventual identification with authentic 5α-pregnane-3,12,20-trione established that Va and, therefore, III were 12-hydroxylated derivatives of funtumine and funtumidine, respectively.

The trione VIII did not absorb selectively in the ultraviolet region in ethanol solution or in alkaline ethanol solution, thus eliminating 1,3,20-, 2,3,20-, 3,4,20-, 3,6,20-, and 3,16,20-triones as possible structures. Since VIII and XI were not identical, only three reasonable possibilities (the 3,7,20-, 3,12,20-, and 3,15,20-triones) remained. Infrared bands characteristic of a five-membered D-ring 15-ketone were absent from the spectrum of VIII, thus eliminating 5α-pregnane-3,15,20-trione[7] from consideration.

Direct comparison of VIII with the two remaining likely structures, 5α-pregnane-3,7,20-trione[8] and 5α-pregnane-3,12,20-trione,[9,10] established its identity as the latter. The large downfield shift of the proton resonances of the C-18 methyl group ($\Delta\delta$ = 0.33 p.p.m.) in VIII *vs.* the parent 5α-pregnane-3,20-dione[11] is consistent with a 12-ketone but not a 7-ketone formulation.[6] The structures of III, Va, and VII as 12-hydroxypregnanes are thus certain.

The β orientation is assigned to the 12-hydroxy group on the basis of molecular rotational data and n.m.r. spectra. The Δ[M]D values for the newly introduced hydroxyl group in Va and VII are −274 and −321°, respectively, and for the acetoxy derivative Vb, −17°. Δ[M]D values for the 12β-hydroxyl group range between −150 and +50° and those for the 12β-acetoxyl group between −61 and +76°, whereas values for the 12α-hydroxyl group range between +22 and +202° and those for the 12α-acetoxyl group between +165 and +280°.[12]

The 12-proton resonances in III, Va, VII and the diacetate Vb appear as quartets from which coupling constants of 3.7 and 11.0 c.p.s. can be obtained by first-order analysis. From these coupling constants we conclude that the 12-proton is axial in conformation and coupled to one axial and one equatorial proton. The 12-hydroxyl group is thus equatorial and β oriented.[13]

Two special effects were noted in the n.m.r. spectra of the 12β-hydroxy derivatives. The C-21 methyl group protons of Va are found at lower field ($\Delta\delta$ = 0.09 p.p.m.) than are the C-21 protons of other funtumine derivatives. Also, the C-18 methyl group protons of the 12β-acetoxy derivative Vb are deshielded by 0.19 p.p.m. from their resonance frequencies in IIb.[14]

(7) 5α-Pregnane-3,15,20-trione absorbs in Nujol at 5.72, 5.82, and 5.85 μ: see J. Fried, U. S. Patent 2,799,689 (July 16, 1957).

(8) W. Klyne, *J. Chem. Soc.*, 3449 (1951); J. Romo, G. Rosenkranz, and C. Djerassi, *J. Org. Chem.*, **17**, 1413 (1952).

(9) (a) R. B. Wagner, J. A. Moore, and R. F. Forker, *J. Am. Chem. Soc.*, **72**, 1856 (1950); (b) H. A. Walens, S. Serota, and M. E. Wall, *J. Org. Chem.*, **22**, 182 (1957); (c) R. Tschesche, C. Brügmann, H.-W. Marquardt, and H. Machleidt, *Ann.*, **648**, 185 (1961).

(10) We present a new synthesis for 5α-pregnane-3,12,20-trione by reduction of 12α-hydroxyprogesterone (XII) with lithium metal in liquid ammonia followed by oxidation with chromic acid.

(11) Y. Kawazoe, Y. Sato, M. Natsume, H. Hasegawa, T. Okamoto, and K. Tsuda, *Chem. Pharm. Bull.* (Tokyo), **10**, 338 (1962).

(12) Δ[M]D values are from the specific rotations of select 12-substituted 14α-steroids as presented in (a) J.-P. Mathieu and A. Petit, "Pouvoir Rotatoire Naturel. I. Stéroïdes," Masson and Cie., Paris, 1956; (b) L. F. Fieser and M. Fieser, "Steroids," Reinhold Publishing Corp., New York, N. Y., 1959, p. 179; (c) S. W. Pelletier and D. M. Locke, *J. Am. Chem. Soc.*, **79**, 4531 (1957); (d) A. Uffer, *Helv. Chim. Acta*, **39**, 1834 (1956); (e) A. Gubler and C. Tamm, *ibid.*, **41**, 297 (1958); (f) C. Tamm and A. Gubler, *ibid.*, **42**, 473 (1959).

(13) (a) This conclusion is supported by the sluggishness of Va to acetylation with acetic anhydride–pyridine at room temperature (see Experimental Section). (b) Although not fully characterized, the initial product of the lithium metal–liquid ammonia reduction of 12α-hydroxyprogesterone, herein formulated as 12α-hydroxy-5α-pregnane-3,20-dione (XIII), is not identical with and can be resolved from the more polar VII on thin layer chromatograms, thus establishing further the 12β configuration of VII.

(14) Some indication of the deshielding influence of the 12β-acetoxyl group on the C-18 methyl group protons appears in the published spectra of obliquol diacetate.[15] However, the deshielding effect does not occur in 12β-acetoxyl derivatives of the 14β series.[16]

(15) L. B. Kier and W. S. Brey, Jr., *J. Pharm. Sci.*, **52**, 465 (1963).

(16) R. F. Zücher, *Helv. Chim. Acta*, **46**, 2054 (1963).

Experimental Section[17]

Funtumine Fermentation.—Spore suspensions prepared from surface growth on agar slants of *Aspergillus ochraceus* NRRL 405 were used to inoculate 1- and 2-l. flasks containing 200 ml. of yeast extract–glucose medium (10 g. of each/l. of distilled water). The flasks were incubated for 66 hr. at 28° on a rotary shaker (250 r.p.m.), after which mycelial transfers (5%) were made to 42 2-l. flasks containing 400 ml. of medium composed of corn steep liquor (5 g./l.), glucose (50 g./l.), and peptone (20 g./l.) in distilled water. After the flasks had been shaken for 24 hr., 200 mg. of funtumine base in 10 ml. of ethanol was added to each flask, and the incubation was continued.

The course of the transformation was followed by paper chromatography of methyl isobutyl ketone extracts of broth samples using Whatman No. 4 filter paper and a butyl acetate–butanol–acetic acid–water (8:1:1:3) solvent system. The Dragendorff reagent was used for detection of the steroidal alkaloids. Papergrams indicated complete utilization of the substrate at 119 hr., at which time the flasks were harvested.

3α-Amino-12β-hydroxy-5α-pregnan-20-one (Va).—Harvested broth (16.7 l.) obtained by the action of *A. ochraceus* NRRL 405 on 8.34 g. of funtumine (IIa) was filtered, and the mycelial solids were washed repeatedly with ethyl acetate. The aqueous filtrate was adjusted to pH 9–10 with 400–500 ml. of concentrated ammonium hydroxide solution and extracted four times with 10 l. of ethyl acetate. Between extractions the aqueous layer was readjusted to pH 9–10 with ammonium hydroxide. The combined ethyl acetate washes and extracts were concentrated to about 50 ml. under vacuum at 35–45°. The crude crystalline product (3.0 g.) was shown by thin layer chromatography to be an approximate 2:1 mixture of a more mobile product (Va) and a more polar product (VIa), both positive to Dragendorff reagent. Recrystallization four times from chloroform–ethyl acetate yielded needles (550 mg.) of Va, m.p. 202–205°. Further recrystallization gave the analytical sample: m.p. 204–207°; $[\alpha]_D$ +7.9°; λ_{max}^{KBr} 3.00, 3.25, 5.87, 6.30 μ, etc.

Anal. Calcd. for $C_{21}H_{35}NO_2$: C, 75.63; H, 10.58; N, 4.20. Found: C, 75.94; H, 10.69; N, 4.39.

3α-Amino-11α-hydroxy-5α-pregnan-20-one (VIa).—The initial mother liquor from the first recrystallization of Va from chloroform–ethyl acetate was enriched in the more polar component, VIa. Mother liquors from two such fermentations of funtumine gave 10 g. of oil which was chromatographed on 400 g. of silica gel with a mixture of chloroform–methanol (98:2). Only 36 mg. of crystalline material could be recovered, m.p. 144–150°. Recrystallization from ethyl acetate gave 5 mg.: m.p. 150–154°; λ_{max}^{KBr} 3.00, 3.18, 5.87, 6.35 μ, etc.

Anal. Calcd. for $C_{21}H_{35}NO_2$: C, 75.63; H, 10.58; N, 4.20. Found[21]: C, 76.1, 76.0; H, 10.4, 10.4; N, 4.0, 4.0.

3α-Acetylamino-12β-acetoxy-5α-pregnan-20-one (Vb).—The acetylation of 100 mg. of Va with acetic anhydride and pyridine in the usual manner yielded a mixture of partially acetylated products, as shown by thin layer chromatography. The products were redissolved in acetic anhydride and pyridine and held for 30 hr. at 37°. From the reaction mixture 75 mg. of a single product, Vb, was isolated: m.p. 205–214°; $[\alpha]_D$ +81.2°; λ_{max}^{KBr} 3.10, 5.77, 5.87, 6.00, 6.14, 6.53, 8.03, 8.06 μ, etc.

Anal. Calcd. for $C_{25}H_{39}NO_4$: C, 71.89; H, 9.41. Found: C, 72.24; H, 9.45.

3α-Acetylamino-11α-acetoxy-5α-pregnan-20-one (VIb).—A solution of 400 mg. of VIa in 5 ml. of pyridine and 4.5 ml. of acetic anhydride was kept at room temperature for 18 hr. and the solvents were then removed under vacuum. The residue was chromatographed on 40 g. of silica gel with ethyl acetate, yielding 120 mg. of an amorphous product (VIb), which could not be crystallized even after further chromatography on silica gel thin layer chromatoplates. The product was characterized as a single compound by infrared and n.m.r. spectroscopy and by thin layer chromatography: λ_{max}^{KBr} 2.95, 5.80, 5.86, 6.01, 6.68 μ, etc.

12β-Hydroxy-5α-pregnane-3,20-dione (VII).—A solution of 250 mg. of Va in 25 ml. of benzene was stirred with 250 mg. of osmium tetroxide for 7 hr. Sodium sulfite (800 mg. in 15 ml. of water) and methanol (15 ml.) were added. The mixture was stirred an additional 12 hr. and filtered. The filtrate was extracted with ether, and the ether layer was washed successively with 2 *N* sulfuric acid, 2 *N* sodium carbonate solution, and water. The ether was evaporated under vacuum, leaving 42 mg. of crystalline VII. The product was recrystallized twice from acetone–ether, giving the pure sample: m.p. 154–156°; $[\alpha]_D$ +18.7°; λ_{max}^{KBr} 2.93, 5.84 (shoulder), 5.86 μ, etc. The thin layer chromatographic mobility of VII, with ethyl acetate as solvent, was R_f 0.43, compared with R_f 0.48 for the partially characterized 12α-hydroxy-5α-pregnane-3,20-dione prepared from 12α-hydroxyprogesterone (under method B below).

Insufficient material was available for elemental analyses.

5α-Pregnane-3,12,20-trione (VIII). A. From VII.—To a solution of 20 mg. of VII in 2 ml. of glacial acetic acid was added, over a period of 1 hr., 225 μl. of a 2% solution of chromium trioxide in glacial acetic acid (the calculated amount). After 20 hr. at room temperature, the mixture was worked up in the usual manner, yielding (from ether–hexane) 9 mg. of crystals, m.p. 197–202°, and 4 mg. of a second crop. Thin layer chromatography indicated about 10% of unoxidized VII. Preparative thin layer chromatography (using ethyl acetate) gave homogeneous material which was recrystallized from methanol to afford VIII, m.p. 207–212°, $[\alpha]_D$ +149° (very small sample), λ_{max}^{KBr} 5.87 μ.[22] A mixture melting point (m.p. 205–210°) with 5α-pregnane-3,12,20-trione prepared in B below was undepressed, and the identity of the two samples was established by comparison of infrared spectra and thin layer chromatographic behavior in several solvent systems.

B. From 12α-Hydroxyprogesterone (XII).—A solution of 1.0 g. of XII in 10 ml. of dioxane was poured into 150 ml. of liquid ammonia containing 100 mg. of lithium metal (blue solution). The reaction was terminated immediately by adding 2 g. of solid ammonium chloride, and the ammonia was evaporated. The residue was taken up in ether, and the ether solution was washed with water, dried, and evaporated under vacuum, affording 900 mg. of impure XIII: λ_{max}^{KBr} 2.95, 5.86 μ. Thin layer chromatography with ethyl acetate–methanol (24:1) showed a major component (XIII) more mobile than the starting material, together with two minor, more polar components. The otherwise uncharacterized crude product, 12α-hydroxy-5α-pregnane-3,20-dione (XIII), was dissolved in 30 ml. of glacial acetic acid and treated overnight with 12 ml. of 2% chromium trioxide solution in acetic acid. The product (800 mg.) was isolated in the usual manner. Chromatography on silica gel afforded the trione, VIII, after elution with benzene–ethyl acetate (4:1). The trione was recrystallized from methanol, m.p. 205–209°.

11α-Hydroxy-5α-pregnane-3,20-dione (X).—A solution of 200 mg. of VIa in 6 ml. of benzene was stirred with 250 mg. of osmium tetroxide at room temperature for 10 hr., at which time 6 ml. of methanol and 800 mg. of sodium sulfite in 5 ml. of water were added. After being stirred for 8 hr., the mixture was filtered, and the filtrate was extracted with ether. The ether extract was washed successively with 2 *N* sulfuric acid, 2 *N* sodium carbonate solution, and water, dried, and evaporated. The residue (85 mg.) of dark foam was passed through a column of alumina (15 g.) and crystallized from acetone–ether, giving crystals, m.p. 185–195°. Recrystallization raised the melting point to 190–195°. The melting point of authentic 11α-hydroxy-5α-pregnane-3,20-dione, 198–200°,[23] was not depressed on admixture with X. Identity of the two samples was also established by infrared spectral and thin layer chromatographic comparisons.

Funtumidine Fermentation.—One-liter erlenmeyer flasks with 200 ml. of a medium composed of corn steep liquor (40 g./l.)

(17) Melting points were taken on a Kofler block under microscopic magnification. Optical rotations were obtained on 1% solutions in chloroform. Solvents for chromatography of amino steroids on both columns and chromatoplates were equilibrated with concentrated ammonium hydroxide solution immediately before use.[18] Thin layer chromatography was carried out on silica gel plates prepared with rice starch,[19] using chloroform–methanol mixtures. Amino steroids were detected with a modified Dragendorff reagent,[20] keto steroids with an acidified methanolic solution of 2,4-dinitrophenylhydrazine. N.m.r. spectra were obtained on 10% solutions in deuteriochloroform using a Varian Associates A-60 spectrometer. Chemical shifts were measured downfield from tetramethylsilane.

(18) L. Lábler and V. Černý, *Collection Czech. Chem. Commun.*, **28**, 2932 (1963).

(19) L. L. Smith and T. Foell, *J. Chromatog.*, **9**, 339 (1962).

(20) H. Schriftman, *J. Am. Pharm. Assoc., Sci. Ed.*, **48**, 111 (1959).

(21) Performed by Dr. Orville Hinsvark.

(22) 5α-Pregnane-3,12,20-trione is described in the literature: m.p. 207–209°, $[\alpha]_D$ +189°[9a]; m.p. 206–208°, $[\alpha]_D$ +184°[9a]; m.p. 207–212°[9b]; m.p. 215.5–218°, also 210–214°, $[\alpha]_D$ +186°, ν_{max}^{KBr} 2928, 2841, and 1696 cm.$^{-1}$.[9c]

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and glucose (20 g./l.) in distilled water were inoculated with a spore suspension prepared in distilled water from the growth of *A. ochraceus* NRRL 405 on agar slants. The flasks were incubated for 68 hr. at 28° on a rotary shaker (250 r.p.m.), after which mycelial transfers (5%) were made to 2-l. flasks containing 400 ml. of a medium consisting of corn steep liquor (10 g./l.) and glucose (5 g./l.) in distilled water; incubation was continued for 25 hr. Funtumidine (Ia) (160 mg.) dissolved in ethanol (6.4 ml.) was added to each flask, and the flasks were shaken for an additional 73.5 hr. before harvesting. Paper chromatography disclosed two polar products and complete utilization of the substrate.

3α-Amino-5α-pregnane-12β,20α-diol (IIII). A. From Funtumidine.—Harvested fermentation broth obtained by the action of A. *ochraceus* NRRL 405 on 7.0 g. of funtumidine (Ia) was filtered, adjusted to pH 9–10, and extracted with ethyl acetate in the same manner described for funtumine fermentations. The combined residues from two 7.0-g. fermentations (about 7.0 g. of crude product) were shown by thin layer chromatography to be a mixture of two Dragendorff-positive components. Chromatography on 300 g. of silica gel, with elution by chloroform equilibrated with ammonia, yielded 920 mg. of III, homogeneous on thin layer chromatography but noncrystalline. Repeated chromatography and sublimation failed to give a preparation which could be crystallized. The product was identified as Va, prepared under method B below, as shown by infrared and thin layer comparisons.

B. From 12β-Hydroxyfuntumine (Va).—A solution of 200 mg. of Va in 50 ml. of absolute ethanol was stirred at reflux under nitrogen, while 5 g. of sodium metal was added (over a period of 1 hr.). The mixture was poured onto ice and extracted with ethyl acetate. The extracts were washed with water and evaporated under vacuum, leaving a yellow foam (190 mg.). Crystallization from ethyl acetate afforded 35 mg. of III, m.p. 194–204°. Recrystallization from ethyl acetate yielded 12 mg. of pure III: m.p. 203–209°; λ_{max}^{KBr} 3.10, 6.24 μ, etc.

Anal. Calcd. for $C_{21}H_{37}NO_2$: C, 75.17; H, 11.12; N, 4.18. Found[21]: C, 74.8; H, 11.0; N, 4.0.

3α-Amino-5α-pregnane-11α,20α-diol (IVa).—Continued elution with chloroform–methanol (95:5) of the column from which III was obtained afforded 1.46 g. of mixed III–IVa fractions. Still further elution with the same solvent afforded 1.2 g. of crystalline IVa, m.p. 146–155°. Recrystallization from ethyl acetate raised the melting point to 163–164°, and sublimation and recrystallization from ethyl acetate gave the analytical sample: m.p. 158–160°; $[\alpha]_D$ −7.5°; λ_{max}^{KBr} 3.03, 3.14, 6.27, 6.33 μ, etc.

Anal. Calcd. for $C_{21}H_{37}NO_2$: C, 75.17; H, 11.12; N, 4.18. Found: C, 75.10; H, 10.91; N, 4.01.

5α-Pregnane-3,11,20-trione (XI).—A solution of 507 mg. of IVa in 18 ml. of benzene was stirred with 500 mg. of osmium tetroxide for 15 hr. Sodium sulfite (1.5 g. in 10 ml. of water) and methanol (12 ml.) were added and the mixture was stirred for 5 hr. The dark precipitate that formed was filtered off, and the filtrate was extracted several times with ether. The ethereal extract was washed successively with 2 *N* sodium carbonate solution, 2 *N* hydrochloric acid, and water, dried, and evaporated under vacuum. An oil (180 mg.) was recovered which could not be crystallized even after chromatography on silica gel. Thin layer chromatographic behavior of the oil indicated a single ketonic component, which had a mobility consistent with the assigned 11α,20α-dihydroxy-5α-pregnan-3-one (IX) structure. To 160 mg. of the oil dissolved in 6.0 ml. of acetic acid was added, over a period of 1 hr., 3.3 ml. of a 2% solution of chromium trioxide in glacial acetic acid. After standing overnight, the mixture was worked up in the usual manner, yielding 30 mg. of crystals. Recrystallization from methanol afforded pure XI, m.p. 210–215°, $[\alpha]_D$ +125°, identified as 5α-pregnane-3,11,-20-trione by infrared spectra and thin layer chromatography, and by an undepressed melting point on admixture with an authentic sample prepared by chromic acid oxidation of 11α-hydroxy-5α-pregnane-3,20-dione.

3α-Acetylamino-5α-pregnane-11α,20α-diol, 11α,20α-Diacetate (IVb).—A solution of 75 mg. of IVa in 0.7 ml. of pyridine and 0.6 ml. of acetic anhydride was held at room temperature for 18 hr., after which time the solvents were removed under vacuum. The crystalline product, 61 mg., m.p. 152–156°, was recrystallized from ether–hexane to give the analytical sample: m.p. 160–161°; $[\alpha]_D$ +7.9°; λ_{max}^{KBr} 3.05, 5.75, 6.10, 6.48, 8.03 μ, etc.

Anal. Calcd. for $C_{27}H_{43}NO_5$: C, 70.25; H, 9.39; N, 3.03. Found: C, 70.19, 70.12; H, 9.26, 9.49; N, 3.65.

3α-Acetylamino-5α-pregnan-20α-ol, 20α-Acetate (Ib).—A solution of 1.0 g. of funtumidine (Ia) in 3.0 ml. of pyridine and 2.8 ml. of acetic anhydride was maintained at room temperature for 18 hr., after which time the solvents were removed under vacuum. The residue was dissolved in chloroform, and the chloroform solution was washed successively with aqueous sodium carbonate, dilute hydrochloric acid, and water, dried, and evaporated under vacuum. The residue was recrystallized from chloroform–acetone, yielding 700 mg. of Ib: m.p. 265–270° (recrystallization from acetone raised the melting point to 273–275°); $[\alpha]_D$ +15.7° (2% methanol in chloroform); λ_{max}^{KBr} 3.09, 5.78, 6.00, 6.11, 6.50, 8.03, 8.10 μ, etc.

Anal. Calcd. for $C_{25}H_{41}NO_3$: C, 74.40; H, 10.24; N, 3.47. Found: C, 74.30; H, 10.22; N, 3.68.

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