Experimental

We would like to thank R. T. Dillon and associates for analyses, rotations, and spectra. Melting points are uncorrected. The analytical samples were dried for 2 hr. at 100° under moderate vacuum (about 10 mm.). N.m.r. spectra were obtained on a Varian A-60 spectrometer at 10% concentration in deuteriochloroform using tetramethylsilane as an internal standard. Chemical shifts—figures in parentheses—are reported in cycles per second downfield from the standard.

Column chromatography was carried out by N. Bilek and M. Blaumeiser (direction E. G. Daskalakis). Quantitative hydrogenations were performed by W. M. Selby.

12a-Aza-C-homo-1,4-pregnadiene-3,12,20-trione (II).—*Nocardia sp.* A.T.C.C. 14558 (Searle A20-16) was grown as a submerged culture in a stainless steel fermentor in 35 l. of medium containing 200 g. of Difco Nutrient Broth and 10 g. of silicone emulsion (Dow Corning Antifoam AF Emulsion). The culture was agitated by means of a paddle-type stirrer operating at 200 r.p.m. and was aerated with 10 l.p.m. of sterile air which entered through a sparger located below the agitator. The incubation temperature was 25°. After an initial growth period of 30 hr., 10.0 g. of 12a-aza-3β-hydroxy-C-homo-5α-pregnane-12,20-dione (I)[3] in 200 ml. of acetone and 50 ml. of methanol was added and incubation continued for 14 hr.

The culture was extracted with two 18-l. portions of methylene chloride and the combined extracts distilled to dryness. The residue, 10.26 g., was crystallized from 200 ml. of 1:1 benzene–cyclohexane and the desired product separated as irregular prisms, 6.10 g. (62%), m.p. 180–184°. Recrystallization from benzene raised the m.p. to 183–185°; $[\alpha]^{26}D$ +48° (*c* 1, methanol); λ_{max}^{MeOH} 240 mμ (ε 16,100).

Anal. Calcd. for $C_{21}H_{27}NO_3$: C, 73.87; H, 7.97; N, 4.10. Found: C, 74.03; H, 8.10; N, 4.06.

A sample (19.5 mg.) in 95% ethanol was hydrogenated over 5% palladium on carbon (4.0 mg.) in the apparatus of Clauson-Kaas.[10] Hydrogen uptake ceased at 102% of two double bonds. Similar results were obtained with model $\Delta^{1,4}$-3-ketones.

The n.m.r. spectrum was interpreted as follows: 19-CH_3 (70), 8-CH_3 (75.5), 21-CH_3 (129.5), 4-H (broad singlet, 361), 2-H (doublet of doublets, 367 and 377), NH (422), 1-H (doublet, 422 and 432). $J_{1,2}$ had the usual value of 10 c.p.s. while $J_{2,4}$ was about 2 c.p.s.

In a second run under identical conditions, the product had m.p. 200–203°. The infrared spectrum in chloroform was identical with that of material with m.p. 183–185°.

12a-Aza-17α-hydroxy-C-homo-1,4-pregnadiene-3,12,20-trione (IV).—The fermentation was conducted as described except that the organism employed was *Nocardia sp.* A.T.C.C. 14559 (Searle A20-17). Following an initial growth period of 26 hr., 6.0 g. of 3β-acetoxy-12a-aza-17α-hydroxy-C-homo-5α-pregnane-12,20-dione (III)[3] dissolved in 250 ml. of acetone was added and incubation continued for 21 hr. The crude material obtained by methylene chloride extraction was chromatographed on silica gel. Elution with ethyl acetate gave the desired product, 1.01 g. (17%), m.p. 252–256°. Crystallization from methanol yielded square prisms, m.p. 260–262°; $[\alpha]^{23}D$ +45° (*c* 1, chloroform); λ_{max}^{MeOH} 240 mμ (ε 16,300).

Anal. Calcd. for $C_{21}H_{27}NO_4$: C, 70.56; H, 7.61; N, 3.92. Found: C, 70.46; H, 7.93; N, 4.33.

The n.m.r. spectrum was very similar to that of compound II.

12a-Aza-C-homo-5α-pregn-1-ene-3,12,20-trione (VI).—The fermentation was carried out as described for compound II except that the organism used was *Arthrobacter sp.* A.T.C.C. 14560 (Searle B22-46). After an initial growth period of 26 hr., 10.0 g. of 3β-acetoxy-12a-aza-C-homo-5α-pregnane-12,20-dione (V)[3] in 200 ml. of acetone was added and incubation continued for 21 hr. The crude product was chromatographed on silica gel. Elution with ethyl acetate yielded compound VI, 2.56 g. (26%), m.p. 194–196°. Crystallization from 1:1 benzene–cyclohexane gave clusters of needles, m.p. 206–207.5°; $[\alpha]^{26}D$ +32° (*c* 1, methanol); λ_{max}^{MeOH} 227.5 mμ (ε 11,100).

Anal. Calcd. for $C_{21}H_{29}NO_3$: C, 73.43; H, 8.51; N, 4.08. Found: C, 73.56; H, 8.37; N, 3.99.

A sample (21.63 mg.) in 95% ethanol was hydrogenated over 6.18 mg. of 5% palladium on carbon. Hydrogen uptake ceased at 98% of one double bond.

(10) N. Clauson-Kaas and F. Limborg, *Acta Chem. Scand.*, **1**, 884 (1947).

The n.m.r. spectrum was interpreted as follows: 19-CH_3 (57), 18-CH_3 (75), 21-CH_3 (130), 2-H (doublet, 345 and 355), NH (417), 1-H (doublet, 425 and 435). $J_{1,2}$ had the value of 10 c.p.s.

Steroid Epoxy Ketones. II. 2,3-Oxygenated Steroids from 1α,2α-Oxidocholestan-3-one

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In the preceding paper of this series,[1] base-catalyzed ring contraction of appropriate α,β-epoxy ketones[2,3] was suggested as a novel approach to norsteroids. However, this mode of reaction was not observed when 4,5-oxidocholestan-3-one was treated with methanolic base, the major product being 4-methoxy-Δ^4-cholesten-3-one (VI).[1,4] We now report the results of a similar study of 1α,2α-oxidocholestan-3-one (I).

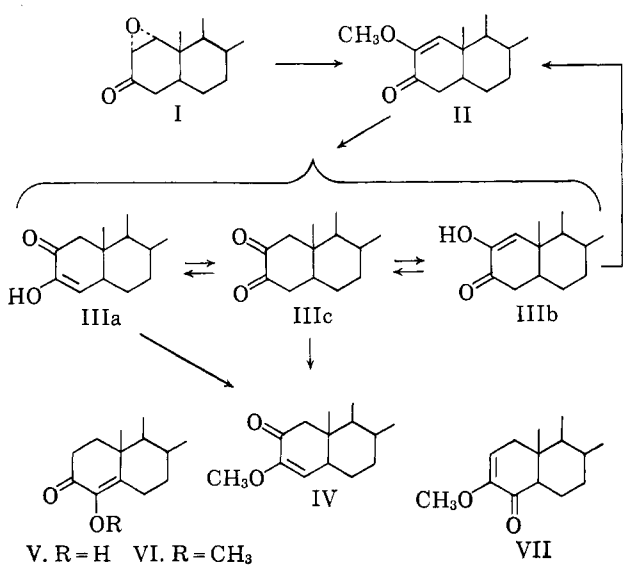
Treatment of I with refluxing methanolic sodium hydroxide yielded 2-methoxy-Δ^1-cholesten-3-one (II) as the major product. This structure assignment was based on the infrared spectrum, $\lambda_{max}^{CCl_4}$ 5.95 and 6.23 μ, the ultraviolet spectrum, $\lambda_{max}^{C_2H_5OH}$ 265 mμ (ε 7800), the n.m.r. spectrum (vinyl hydrogen and methoxyl hydrogen appear as singlets, 4.18 and 6.57 τ, respectively, with an area ratio of 1:3.3), and the elemental analysis. In addition, acid hydrolysis of II to the diosphenol IIIa and an independent synthesis of II from diosphenol IIIb provide chemical evidence for this structure. The reaction probably proceeds by opening of the oxirane ring through methoxide ion attack at C-2 followed by β-elimination of water.

An isomeric derivative, 3-methoxy-Δ^3-cholesten-2-one (IV), was prepared by methylation of a mixture of diosphenols IIIa and IIIb with alkaline dimethyl sulfate. The infrared and ultraviolet spectra of IV were similar to those from II; however, the n.m.r. spectrum of the former exhibited a doublet at 4.83 τ, having an area ratio to the methoxyl resonance at 6.49 τ of 1:2.9, in contrast to the singlet vinyl resonance observed for II. The exclusive formation of isomer IV in this reaction is interesting in view of the fact that there are no obvious steric factors favoring alkylation of one diosphenol over the other. Since equilibrium between the two diosphenols and their conjugate bases is undoubtedly established in the alkaline medium employed in this reaction, we suggest this selectivity reflects a difference in the stability of the diosphenol conjugate bases and/or the methylation derivatives.[5] An instructive contrast is provided by the preparation[1] of 3-methoxy-Δ^2-cholesten-4-one (VII) from diosphenol V through similar treatment with dimethyl sulfate. In this case, steric

(1) W. Reusch and R. LeMahieu, *J. Am. Chem. Soc.*, **85**, 1669 (1963).
(2) W. Treibs, *Ber.*, **64**, 2178, 2545 (1931).
(3) H. House and W. Gilmore, *J. Am. Chem. Soc.*, **83**, 3972 (1961).
(4) B. Camerino, B. Patelli, and R. Sciaky, *Gazz. chim. ital.*, **92**, 709 (1962).
(5) The diosphenols IIIa and IIIb, the corresponding conjugate bases, and the methyl derivatives II and IV probably have corresponding stability orders. The common factors responsible for the stability differences are discussed in ref. 9.

hindrance of the C-4 oxygen appears to be an important factor.

In a parallel investigation, Camerino, Patelli, and Sciaky reported[4] that the salt prepared by treatment of V with sodium hydride in refluxing xylene reacted with methyl iodide to give VI. We interpret this result as implying rapid, irreversible formation of the conjugate base of V followed by methylation of the C-4 oxygen, which is the site of greatest negative charge. A corresponding study of diosphenols IIIa and IIIb has disclosed a similar relationship. Thus, methylation of the enolate anion prepared by reaction of IIIa with sodium hydride gave a mixture of methyl derivatives consisting of 96% IV and 4% II.[6] Equivalent treatment of the enolate anion derived from IIIb, on the other hand, produced a mixture of 44% II and 56% IV.[6] Apparently, interconversion of the isomeric enolate anions is occurring in the latter case.



The diosphenols IIIa and IIIb and the α-diketone IIIc constitute a remarkable tautomeric system. Several procedures for the preparation and isolation of the diosphenols have been described,[7] and chemical evidence[7b] for the structural assignments has been buttressed by n.m.r. measurements.[8] An inspection of molecular models indicates that isomer IIIa has fewer and less severe nonbonded interactions[9] than IIIb; therefore, the former should be more stable and predominate in an equilibrium mixture of the two tautomers. In view of this, the preparation of IIIb by acid treatment of a diosphenol mixture[7] is unexpected. However, a careful examination discloses that IIIb is actually obtained in relatively low yield and that the mother liquors consist roughly of 80% IIIa and 20% IIIb. Although previous workers have often disregarded the difference between the diosphenols and the α-diketone IIIc, it is clear that the substances described in their reports were primarily the former. Thus, infrared, ultraviolet, and n.m.r. spectra of the isomers melting at 144° (IIIa) and 162° (IIIb) are in complete agreement with the assigned structures; a modification having a melting point of 132–135° was demonstrated[7a] to be an equimolar mixture of the diosphenols.

During the course of our work with these materials, the diosphenol IIIa was found to be unstable in the solid state. Upon standing, either at room temperature or in the refrigerator, this isomer became yellow, the melting point dropped (after thirty days the melting range was 70–75°), strong absorption appeared at 5.78 and 5.89 μ in the infrared, while the 2.94 and 6.02-μ bands which characterized the diosphenol were much diminished, and the vinyl hydrogen and hydroxyl resonances appearing at 4.45 and 4.32 τ for IIIa and 3.82 and 4.32 τ for IIIb were greatly reduced in intensity (the ratio of the combined areas of these low field absorptions to the methyl resonance at 8.37 τ was roughly 0.06). Since over 50% diosphenol IIIa was recovered from this low melting material *via* the potassium salt, it is clear that no deep-seated structural changes occurred. Isomerization of the disophenols to the α-diketone is an attractive rationale for these facts. In contrast to the changes observed in crystalline IIIa, carbon tetrachloride solutions of this isomer do not appear to accumulate significant concentrations of IIIc, although isomerization to IIIb occurs to a small extent. Over 80% of the diosphenols are present as IIIa at equilibrium.

Experimental

Melting points were determined on a Kofler hot stage. The infrared spectra were measured with a Perkin-Elmer, Model 21, spectrophotometer. The ultraviolet spectra were determined with a Beckman DK-2 spectrophotometer. The microanalyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Mich. The n.m.r. spectra were determined in carbon tetrachloride solution using a Varian Associates A-60 high resolution spectrometer.

2-Methoxy-Δ¹-cholesten-3-one (II).—A solution of 1α,2α-oxidocholestan-3-one[10] (I, 1.0 g.) in methanol (90 ml.) was refluxed with 12 *N* sodium hydroxide (3 ml.) for 5 hr. Dilution of the reaction mixture with water followed by extraction with ether gave, upon evaporation of the dried ether extracts, 900 mg. of a neutral oil. This material was chromatographed on neutral alumina (40 g.) and yielded 140 mg. of recovered I and 580 mg. of II, m.p. 69–70°. An analytical sample, m.p. 70–71, was prepared by crystallization from aqueous ethanol.

Anal. Calcd. for $C_{28}H_{46}O_2$: C, 80.54; H, 11.52. Found: C, 80.47; H, 11.55.

The aqueous fraction yielded 70 mg. of acidic material.

Hydrolysis of Enol Ether II.—A solution of 2-methoxy-Δ¹-cholesten-3-one (II, 250 mg.) in 95% ethanol (40 ml.) containing a little concentrated hydrochloric acid (0.5 ml.) was refluxed overnight. Water was added to the reaction mixture which was then extracted with ether. The combined ether extracts were shaken with 20% potassium hydroxide; the potassium salt which formed remained suspended in the aqueous layer. After being dried and evaporated, the ether layer gave 160 mg. of an oil, which proved to be unchanged II on the basis of the infrared spectrum. Acidification and ether extraction of the aqueous fractions containing the suspended potassium salt resulted in the isolation of 90 mg. of a colorless solid, m.p. 137–139°, which exhibited an infrared spectrum identical to that of authentic IIIa. A mixture melting point confirmed the identification.

(6) These analyses were made using the integrated areas of the characteristic vinyl hydrogen resonances in the n.m.r.

(7) (a) E. T. Stiller and O. Rosenkeim, *J. Chem. Soc.*, 353 (1938); (b) L. Ruzicka, Pl. Plattner, and M. Furrer, *Helv. Chim. Acta*, **37**, 524 (1944); (c) J. C. Sheehan and W. F. Erman, *J. Am. Chem. Soc.*, **79**, 6050 (1957); (d) H. Nace and M. Inaba, *J. Org. Chem.*, **27**, 4024 (1962).

(8) C. R. Noller, A. Melera, M. Gut, J. N. Schoolery, and L. F. Johnson, *Tetrahedron Letters*, **15**, 15 (1960). The n.m.r. spectra reported by these workers were measured in deuteriochloroform solution, and the 10-δ values for the vinyl hydrogen resonance are 4.32 for IIIa and 3.63 for IIIb. The former appears as a doublet with $J \sim 3$ c.p.s. We have confirmed the essential features of this study.

(9) In addition to the C-19:C-4 interaction present in IIIb but not in IIIa, the former isomer has serious compressions between C-19:C11 and C-19:C-8.

(10) P. Striebel and Ch. Tamm, *Helv. Chim. Acta*, **37**, 1094 (1954).

3-Hydroxy-Δ^3-cholesten-2-one (IIIa).—A mixture of diosphenols IIIa and IIIb was prepared according to the procedure of Ruzicka, *et al.*[7b] Upon shaking an ether solution of this mixture with cold 20% potassium hydroxide, an insoluble potassium salt formed and was filtered and washed with ether. A suspension of this salt in dilute hydrochloric acid was extracted with ether. Evaporation of the dried ether extracts and crystallization of the residue from petroleum ether gave IIIa, m.p. 143–145° (reported m.p. 144–145°); $\lambda_{max}^{CCl_4}$ 2.92, 5.98 and 8.25 μ; n.m.r. absorption at 4.32 and 4.45 τ (doublet J = 2.5 c.p.s.) in carbon tetrachloride solution.

2-Hydroxy-Δ^1-cholesten-3-one (IIIb).—A solution of diosphenol IIIa (400 mg.) in glacial acetic acid (3 ml.) containing a little concentrated hydrochloric acid (0.1 ml.) was heated on a steam bath for 10 min. Upon cooling, a solid precipitated and was crystallized from ethyl acetate to give IIIb (60 mg.), m.p. 161–162° (reported m.p. 160–162°); $\lambda_{max}^{CCl_4}$ 2.93, 6.00 and 11.65 μ; n.m.r. absorptions at 3.82 and 4.32 τ have equal areas.

The mother liquor exhibited three low field resonances at 3.78, 4.12 and 4.45 τ, the areas of which indicate a mixture of 18% IIIb and 82% IIIa. The downfield shift of the hydroxyl absorption (4.32 τ in pure IIIb to 4.12 in the mixture) is characteristic of mixtures of these diosphenols and implies intermolecular hydrogen bonding. A shift of the carbonyl stretching frequency in the infrared to 6.02 μ agrees with this suggestion.

Evidence for Cholestane-2,3-dione (IIIc).—Diosphenol IIIa is not stable in the crystalline state and yellows upon standing at room temperature or in the refrigerator. After 2 weeks it melted at 105–125°, and after a month the yellow solid melted at 70–75°. The infrared spectrum of this low-melting substance showed weak absorption at 2.94 and 6.02 μ and strong absorption at 5.78 and 5.89 μ. Very weak peaks at 3.86, 4.19 and 4.44 τ were observed in the n.m.r. spectrum; when compared with the area of the methyl resonance at 8.37 τ, these combined low field absorptions represent about 10% diosphenol.

A 50-mg. sample of the low melting yellow substance was converted to a potassium salt by shaking with 20% potassium hydroxide. Diosphenols were isolated by treating the salt with dilute hydrochloric acid followed by ether extraction. The crude solid thus obtained (35 mg.) was crystallized from petroleum ether to yield IIIa (20 mg.), m.p. 140–142°, identified by mixture melting point and infrared spectrum. The mother liquors showed strong absorption just above 6.00 μ in the infrared.

3-Methoxy-Δ^3-cholesten-2-one (IV).—A mixture of diosphenols IIIa and IIIb (560 mg.) was dissolved in methanol (40 ml.) containing sodium hydroxide (200 mg.) and then refluxed with dimethyl sulfate (2.5 g.) for 20 hr. The reaction mixture was diluted with water and then worked up by ether extraction in the usual manner. Evaporation of the dried ether portions yielded a yellow oil (550 mg.), which was chromatographed on 30 g. of neutral alumina. The first materials to be eluted were oils (150 mg.) exhibiting saturated carbonyl absorption in the infrared. Elution with benzene gave IV (230 mg.), m.p. 153–155°; $\lambda_{max}^{CCl_4}$ 5.96, 6.18 and 8.40 μ; $\lambda_{max}^{C_2H_5OH}$ 266 mμ (log ϵ 3.9); n.m.r., 4.83 τ (doublet) and 6.49 τ (area ratio 1:2.9). An analytical sample, m.p. 157–158°, was prepared by crystallization from aqueous ethanol.

Anal. Calcd. for $C_{28}H_{46}O_2$: C, 80.54; H, 11.52. Found: C, 80.71; H, 11.33.

Elution with ether gave 60 mg. of recovered diosphenols.

Rate-Controlled Methylation of Diosphenols IIIa and IIIb. A.—A freshly prepared and crystallized sample of IIIa (200 mg.) was dissolved in dry benzene (20 ml.) and treated with sodium hydride (50 mg. of a 52.8% dispersion in mineral oil) at reflux for 45 min. When the hydride was added, a flocculent solid formed concurrently with gas evolution. Methyl iodide (2 ml.) was added and the mixture was refluxed an additional 20 hr. Excess sodium hydride was decomposed by a few drops of ethanol and the reaction mixture was diluted with water and extracted with ether. Unchanged diosphenol (70 mg.) was removed as the potassium salt, formed by shaking the ether extracts with 20% potassium hydroxide. The residue obtained by evaporation of the ether extracts was chromatographed on neutral alumina (15 g.) yielding 88 mg. of a solid, upon elution with ether. Crystallization of this material from aqueous ethanol gave 60 mg. of needles, m.p. 121–131°, having an infrared spectrum identical to that of IV. The n.m.r. spectrum showed a very weak resonance at 4.20 τ and stronger absorptions at 4.90 τ (doublet) and 6.48 τ (OCH_3). The ratio of the areas of the vinyl hydrogen doublet to the methoxyl singlet indicated this material to be 94% IV. Additional attempts to remove the small amount of II apparently present by further chromatography or crystallization failed.

B.—A freshly prepared and crystallized sample of IIIb (54 mg.) dissolved in 15 ml. of dry benzene was refluxed with sodium hydride (7 mg. of the oil dispersion) for 50 min. After the addition of methyl iodide (1 ml.) to the reaction mixture reflux was continued for 20 hr. The work-up paralleled the previous procedure and, after chromatography, two crystalline fractions were isolated. Although the melting point ranges of these fractions were different (105–140° and 50–105°), the infrared spectra were very similar to each other and to authentic II + IV mixtures. The combined fractions totaled 52 mg. and exhibited absorption at 4.14, 4.81 (doublet) and 6.52 τ (OCH_3) in the n.m.r. spectrum. A comparison of the areas of the vinyl hydrogen resonances suggested that this material was a mixture of 44% II and 56% IV.

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The Synthesis of Δ^5-Cholestene-3β,19-diol *via* the 3,5-Cyclosterol Rearrangement. A Novel Route to 19-Nor Sterols

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The synthesis of the Δ^5-3β,19-dihydroxy steroid system is of interest because of its use as an intermediate in the preparation of physiologically active 19-nor steroids[1] and as a precursor in the synthesis of certain C-19 oxygenated naturally occurring steroids.[2] We have achieved a synthesis of this system by utilizing the 3,5-cyclosterol[3a] rearrangement of 3α,5α-cyclo-6β,19-oxidocholestane (II).[3b]

The hydrolytic rearrangement of 3α,5α-cyclo-6β-ol steroids to the corresponding 3β-Δ^5-sterols is well known.[3,4] The high solvolytic rate and stereospecificity observed in this reaction are accommodated by postulation of an intermediary nonclassical homoallylic cation. By analogy, it was anticipated that application of this rearrangement to a 3α,5α-cyclo-6β,19-oxido steroid (A) would lead to intramolecular transfer of the 6β oxygen to C-19 resulting in the homoallylic ion (B). Subsequent attack by solvent at C-3 would yield the corresponding 3β-substituted 19-hydroxy-Δ^5-steroid (C).

Such a reaction sequence was realized experimentally and represents a simple and potentially general route to Δ^5-3β,19-dihydroxy steroids.

Treatment of 3α,5α-cyclocholestan-6β-ol (I) in dry benzene with lead tetraacetate and dry calcium carbonate led to a 25% yield, based upon recovered starting

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(3) (a) E. S. Wallis, E. Fernholz, and T. Gephardt, *J. Am. Chem. Soc.*, **59**, 137 (1937); (b) K. Tanabe, *et al.*, have published the preparation and rearrangement of 6β,19-oxido-3α,5α-cycloandrostan-17-one [K. Tanabe, R. Takasaki, K. Sakai, R. Hayashi, and Y. Morisawa, *Chem. Pharm. Bull.* (Tokyo), **10**, 1126 (1962)].

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