this study of the cyclic sulfides. Similarly, the facile cleavage of cyclic five-membered sulfones under conditions not affecting the six-membered systems was attributed to ring strain in the former.[4]

The isolation of both II and III in this study provides greater evidence that the classical Claisen rearrangement product (Ia) is the initial intermediate formed directly from I (*cf.* ref. 5). However, since neither we nor Kwart have actually isolated this classical product (or Ib) under conditions leading to the formation of II and now also III, this evidence may be circumstantial and an alternate initial path from I cannot yet be definitely precluded.

The Claisen route, with the initial formation of Ia and Ib as *very reactive transients* (*i.e.*, nonisolable)[17] derives further support from related studies. There are indications that benzenesulfenyl anions (ArS^-) are much more reactive nucleophiles than phenoxy anions; *e.g.*, PhS^- attacks benzothiophene 1,1-dioxide forming quantitatively the β-PhS-adduct, while PhO^- is completely unreactive with this substrate.[18] Much greater mesomeric delocalization of the negative charge in the phenoxy systems reasonably accounts for this and similar observations.[19] It follows rationally that while *o*-allylphenols can be isolated from usual Claisen rearrangements, the corresponding thiols may simply cyclize too rapidly to be detected *per se* especially under the high-temperature and alkaline conditions employed.

It has been suggested[20] that I initially might cyclize directly into III-anion which then is reversibly transformed into the anions of Ia, Ib, and II, all in equilibrium, but irreversibly into III by proton abstraction. The experimental facts—sequence of formation of II and III, cleavage of II but not III under these conditions, etc.—together with independent evidence of the greater stability of six- than five-membered cyclic systems containing a sulfur atom[4,14-16] do not support this argument.

A closer examination of these reactions, necessarily under less vigorous conditions, should prove interesting and fruitful.

(17) While this was suggested by Kwart to explain the absence of Ia, no supporting evidence was offered.

(18) F. G. Bordwell and W. H. McKellin, *J. Am. Chem. Soc.*, **72**, 1985 (1950).

(19) See C. C. Price and S. Oae, "Sulfur Bonding," The Ronald Press Co., New York, N. Y., 1962, p. 25.

(20) The authors gratefully acknowledge a referee's suggestion.

Azasteroids. IV. Microbiological Dehydrogenation of C-Ring Azasteroids[1]

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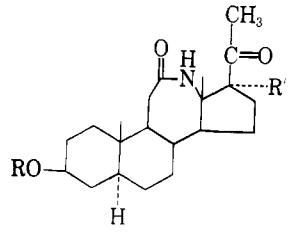
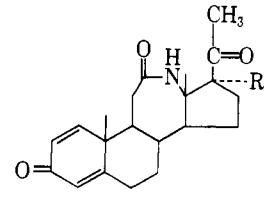
Azasteroids have attracted some attention as a direction in which to search for steroid hormone analogs. Introduction of nitrogen into ring C (with concurrent homoannulation) has been reported by Mazur[2,3] and Zderic.[4,5] All our attempts to convert suitable precursors to A-ring unsaturated compounds by chemical methods have failed—for example, bromination-dehydrobromination,[6] selenium dioxide oxidation,[7] and heating with dichlorodicyanoquinone[8]—and Zderic has mentioned having the same experience.

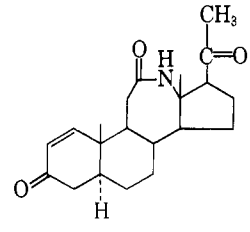
We have now found that fermentation of 12a-aza-3β-hydroxy-C-homo-5α-pregnane-12,20-dione (I) with *Nocardia sp.* A.T.C.C. 14558 gave 12a-aza-C-homo-1,4-pregnadiene-3,12,20-trione (II). Compound II was obtained in two polymorphic forms having identical solution infrared spectra. The structure was confirmed by quantitative hydrogenation and by n.m.r. spectrum. The latter showed the typical complex pattern in the 350–450-c.p.s. region due to interaction of the C-4 proton with the C-2 proton (itself part of an AB system). Similarly, fermentation of 3β-acetoxy-12a-aza-17α-hydroxy-C-homo-5α-pregnane-12,20-dione (III) with *Nocardia sp.* A.T.C.C. 14559 gave 12a-aza-17α-hydroxy-C-homo-1,4-pregnadiene-3,12,20-trione (IV), with typical n.m.r. spectrum in the 350–450-c.p.s. region. A different result was obtained by fermenting 3β-acetoxy-12a-aza-C-homo-5α-pregnane-12,20-dione (V) with *Arthrobacter sp.* A.T.C.C. 14560 which yielded 12a-aza-C-homo-5α-pregn-1-ene-3,12,20-trione (VI); the n.m.r. spectrum showed the AB pattern of a Δ^1-3-ketone.



I, R = R′ = H
III, R = Ac, R′ = OH
V, R = Ac, R′ = H



II, R = H
IV, R′ = OH



VI

It is interesting that the ultraviolet spectra of all products showed a hypsochromic shift of about 4 mμ usually associated with 11-keto steroids. To our knowledge, there is only one previous example of direct conversion of a ring A/B saturated steroid 3-alcohol derivative to a $\Delta^{1,4}$-3-ketone by fermentation.[9]

(1) Part III, R. H. Mazur, *J. Org. Chem.*, **28**, 248 (1963).

(2) R. H. Mazur, *J. Am. Chem. Soc.*, **82**, 3992 (1960).

(3) R. H. Mazur, *ibid.*, **81**, 1454 (1959).

(4) J. A. Zderic, H. Carpio, and D. C. Limon, *J. Org. Chem.*, **27**, 1125 (1962).

(5) J. A. Zderic and J. Iriarte, *ibid.*, **27**, 1756 (1962).

(6) E. S. Rothman and M. E. Wall, *J. Am. Chem. Soc.*, **78**, 1744 (1956).

(7) C. Meystre, H. Frey, W. Voser, and A. Wettstein, *Helv. Chim. Acta*, **39**, 734 (1956).

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

(9) V. E. Origoni, Neil E. Rigler, and J. J. Goodman, U. S. Patent 3,047,468 (July 31, 1962).

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.