A Reinvestigation of the Synthesis of 5,16-Pregnadiene-3β,20α-diol. The Nuclear Magnetic Resonance Spectra of Some Epimeric 20-Hydroxypregnane Derivatives

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Received June 13, 1963

In connection with a study of the allylic rearrangement of Δ^{16}-20-hydroxy steroids[1] we had occasion to prepare 5,16-pregnadiene-3β,20α-diol[2] and the epimeric 20β-hydroxy compound[3] by published procedures. Certain apparent anomalies in the physical properties of the 20α epimer induced us to re-examine its synthesis.

Ercoli[2a] reported that zinc and acetic acid reduction of 16-dehydropregnenolone acetate gave predominantly reduction of the 16,17 double bond. Of the nonketonic residue approximately half was obtained in a pure state following acetylation and chromatography. This material was assigned the 20α configuration on the basis of its reduction to a known saturated 3β,20α-diol.

Shapiro and co-workers[2b] investigated the reduction of the same starting material using lithium aluminum hydride. The crude dienediol was purified as its diacetate and hydrolyzed to give what was described as the 3β,20α-diol having physical properties in fair agreement with those reported by Ercoli. Catalytic reduction gave the known 5α-pregnane-3β,20α-diol, however, in only 50% yield.

We have examined the reduction of 16-dehydropregnenolone acetate by several reagent and solvent combinations. Using lithium aluminum hydride in ethyl ether or tetrahydrofuran we isolated a fairly sharp melting diol in about 60% yield, together with considerable 16,17-saturated-20-ketonic material. This diol and its diacetate agreed fairly well in melting point and rotation with the values reported by Shapiro[2b] and by Ercoli[2a] for the 20α epimer and appeared to be homogeneous on the basis of paper chromatographic analysis. The first indication that this compound might not be the pure 20α isomer arose from a consideration of its rotational properties. It is well documented that acetylation of 20α-hydroxy steroids invariably results in a negative shift in molecular rotation.[4] The "20α compounds" prepared by Ercoli and Shapiro as well as our own sample appeared to contradict this rule in that acetylation resulted in a weak positive shift in molecular rotation (see Experimental and Table I).

Marker[3] did not report rotational data for the 5,16-pregnadiene-3β,20β-diol diacetate. However, a sample prepared by his method exhibited the expected strong positive shift in molecular rotation relative to its diol.

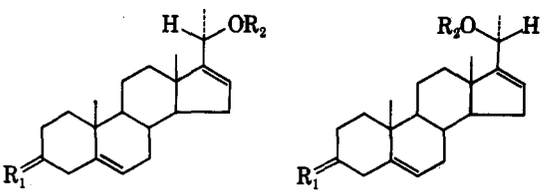
A second indication that our supposedly pure 20α-hydroxy compound was not homogeneous followed from inspection of its n.m.r. spectrum. In the region of the spectrum where one would expect a singlet associated with the absorption produced by the C-18 methyl protons there occured two peaks (52 and 55 c.p.s.), each with one half the intensity of the single C-19 methyl resonance (64 c.p.s.). Furthermore the doublet associated with the C-21 methyl group appeared as a discrete pair of doublets. The epimeric 20β alcohol and its diacetate prepared by the Meerwein–Pondorf reduction of 16-dehydropregnenolone acetate as described by Marker[3] exhibited a single C-18 absorption at 52 c.p.s. and a doublet attributable to the C-21 methyl group. The frequencies of these resonance peaks were identical with one of the sets of apparently anomalous resonances exhibited by the "20α-ol" isolated from the lithium aluminum hydride reduction.

The most reasonable explanation of these data is that the product of lithium aluminum hydride reduction of 16-dehydropregnenolone acetate is in fact a molecular complex of the epimeric 20α- and 20β-diols. In our hands thin layer, paper, gas phase, and conventional column chromatographic techniques were all unsuccessful in effecting a resolution of the two components. A partial separation and isolation of what we feel is the pure 20α epimer was achieved using a gradient elution technique of column chromatography wherein the polarity of the eluent is gradually increased in an exponential manner.[5]

This compound was homogeneous by all conventional criteria including n.m.r. spectroscopy. The positions of the C-18 and C-21 methyl peaks were exactly at those frequencies predicted from a comparison of the n.m.r. spectra of the pure 20β isomer and of what we believe to be the 1:1 complex. The rotation of the pure 20α isomer was found to be in good agreement with the value calculated from the rotations of the 20β isomer and the 1:1 compound. Furthermore, acetylation of this new

TABLE I

MOLECULAR ROTATIONS FOR EPIMERIC 20-HYDROXY-Δ^{16}-PREGNANE DERIVATIVES



			a series = 20α		b series = 20β	
	R_1	R_2	MD	Δ[a]	MD	Δ[a]
I	3β-OH	-H	−272		−210	
II	3β-OAc	-Ac	−369	−63	−116	+128
III		-H	−210		−189	
IV		-Ac	−300	−90	−44	+145

[a] Δ = MD 20-acetate − MD 20-ol and assumes an acetylation increment of −34° for the acetylation of the 3-hydroxy-Δ^5 function of compounds Ia and Ib: L. F. Fieser, "Steroids," Reinhold Publishing Corp., New York, N. Y., 1959, p. 180.

(1) W. R. Benn and R. M. Dodson, to be published.

(2) (a) A. Ercoli and P. de Ruggieri, *Farm. sci. e etc.* (Pavia), **7**, 11, 129, 287 (1952); *Chem. Abstr.*, **46**, 10186 (1952); **47**, 2129, 3325 (1953); (b) E. L. Shapiro, D. Gould, E. B. Hershberg, *J. Am. Chem. Soc.*, **77**, 2912 (1955).

(3) R. E. Marker, D. L. Turner, R. B. Wagner, P. R. Ulshafer, H. M. Crooks, Jr., and E. L. Wittle, *ibid.*, **63**, 779 (1941).

(4) L. F. Fieser and M. Fieser, *Experientia*, **4**, 285 (1948); L. H. Sarett, *J. Am. Chem. Soc.*, **71**, 1165, 1175 (1949).

(5) T. K. Lakshmanan and S. Lieberman, *Arch. Biochem. Biophys.*, **53**, 258 (1954). This technique has been used with a large measure of success by Hirschmann to separate other epimeric 20-hydroxypregnane derivatives. *cf.* D. M. Glick and H. Hirschmann, *J. Org. Chem.*, **27**, 3212 (1962), and earlier papers.

compound resulted in the expected negative shift in molecular rotation.

We have observed a similar tendency toward complex formation in the analogous Δ^{16}-20 alcohols having a 3-ethylene ketal function. Thus, the reduction of the 3-monoethylene ketal of 16-dehydroprogesterone[6] by lithium aluminum hydride in ether or tetrahydrofuran gave a mixture of epimeric 20-hydroxy compounds from which the 20α epimer could be crystallized in fairly pure form. The 20β alcohol, which appeared to be the lesser constituent of the gross reduction product, crystallized as a complex with the 20α epimer. Conventional column chromatography permitted a separation of these two epimeric hydroxy ketals. The acetates formed a sharp melting 1:1 complex which resisted separation.

The positions of the resonance frequencies of the C-18 and C-21 methyl protons in a number of pairs of 20α- and 20β-hydroxy- and acetoxypregnane derivatives are collected in Table II. In each pair of epimeric 20-acetoxy compounds the position of the C-18 absorption in the 20α compound occurs downfield relative to the corresponding absorption of the 20β epimer. The relatively low solubility of the corresponding 20-hydroxy compounds in deuteriochloroform served to limit the number of spectra recorded. With one apparent exception, the configuration of the unesterified 20-hydroxy function had the same influence on the position of the C-18 methyl resonance as did its ester. N.m.r. data must be collected on additional 20-oxygenated epimers before one can establish the reliability of this technique in assigning configuration of this class of compounds.

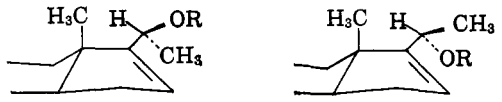
TABLE II[a]

C-18 AND C-21 METHYL PROTON RESONANCE FREQUENCIES IN EPIMERIC 20-HYDROXY- AND ACETOXYPREGNANE DERIVATIVES

Compound	Configuration	Methyl resonance frequencies, CH_3-18, c.p.s.	CH_3-21, c.p.s.	
5,16-Pregnadiene-3β,20-diol (Ia and Ib)	20α	55	75.5	83
	20β	52	78	84
5,16-Pregnadiene-3β,20-diol diacetate (IIa and IIb)	20α	54.5	78	84.5
	20β	51	79	86
20-Hydroxy-5,16-pregnadien-3-one 3-ethylene ketal (IIIa and IIIb)	20α	54	75.5	82
	20β	52	78	84.2
20-Acetoxy-5,16-pregnadien-3-one 3-ethylene ketal (IVa and IVb)	20α	54.5	78	84.5
	20β	51.5	79.8	86.5
5-Pregnene-3β,20-diol diacetate	20α	41.5	71	77
	20β	37	66.6	72
5β-Pregnane-3α,20-diol diacetate	20α	39.5	69.5	76
	20β	37	66	71.5
3β,20-Diacetoxy-16α,17-oxido-5-pregnene	20α	58	76	83
	20β	53	62	69
20-Hydroxy-4-pregnen-3-one	20α	43	72	77
	20β	47.5	65	72
20-Acetoxy-4-pregnen-3-one	20α	44	71	78
	20β	41	66	72

[a] N.m.r. spectra were determined in deuteriochloroform using a Varian Associates A-60 spectrometer operating at 60 Mc./sec. Resonances are expressed in cycles per second downfield relative to an internal standard of tetramethylsilane.

A consideration of molecular models of the epimeric 20-acetoxy compounds offers a possible explanation of the observed relative positions of the C-18 methyl group resonances. The most favorable conformation of the side chain, wherein the bulky C-21 methyl group and the acetoxy function are directed away from the remainder of the steroid nucleus, places the angular C-18 methyl protons closer spatially to the oxygen function in the 20α epimer than in the 20β compound. Deshielding of the angular methyl protons by electrons of the oxygen function is, therefore, stronger in the case of the 20α compound and thus results in a greater downfield shift in the position of methyl resonance of that epimer. In the unesterified compounds, less rigorous steric requirements may permit other conformations accounting for the anomolous spectrum of the epimeric 20-hydroxy-4-pregnen-3-ones.



20α 20β

The relative position of the C-18 methyl group absorption appears to follow a fairly consistent pattern depending primarily upon the configuration at C-20. By contrast, the greater conformational flexibility of the side chain causes the position of the C-21 methyl doublet to be influenced to a greater extent by substituents at C-16 and C-17 than was the C-18 methyl group. Thus, it is seen from Table II that, relative to the 16,17-unsubstituted compounds, introduction of a 16,17 double bond causes deshielding of the C-21 protons of both C-20 epimers, but to a slightly greater extent in the 20β than in the 20α compounds. On the other hand, a 16α,17α-epoxide function deshields the C-21 methyl group of the 20α epimer but not of the 20β.

Experimental[7]

1:1 Molecular Complex of 5,16-Pregnadiene-3β,20α- and 3β,-20β-diol (Ia:Ib).—A slurry of 3.56 g. of 3β-acetoxy-5,16-pregnadien-20-one and 2.0 g. of lithium aluminum hydride in 400 ml. of dry ether was stirred at ice-bath temperature for 3 hr. The excess hydride was decomposed with ethyl acetate, 20 ml. of 10% potassium hydroxide solution was added, and the reaction was stirred for 0.25 hr. The ether solution was decanted from the precipitated solids, dried over sodium sulfate, and evaporated to a white solid residue. Recrystallization from acetone gave 1.85 g. (59%) of colorless needles, m.p. 177–179°, $[\alpha]_D$ −72.5°. An analytical sample from acetone had m.p. 180–182°, $[\alpha]_D$ −75°. Pertinent portions of the n.m.r. spectrum are described in the discussion and in Table II. (The reported values for 5,16-pregnadiene-3β,20α-diol agree fairly well with our 20α:20β complex: lit.[2a] m.p. 180–181°, $[\alpha]_D$ −72°; lit.[2b] m.p. 174–176°, $[\alpha]_D$, −74.6°.)

Anal. Calcd. for $C_{21}H_{32}O_2$: C, 79.70; H, 10.19. Found: C, 79.43; H, 10.18.

Subsequent crystalline crops had broader melting ranges higher than 181° and showed considerable absorption in the infrared at 5.82 μ indicating reduction of the 16,17 double bond and no carbonyl reduction.[8,9]

(6) F. Sondheimer; M. Velasco; G. Rosenkranz; *J. Am. Chem. Soc.*, **77**, 192 (1955).

(7) Melting points were taken on a Fisher melting point hot stage. Rotations were taken in chloroform at a concentration of about 1% at 26 ± 2° and the infrared spectra as potassium bromide disks. We are indebted to Dr. R. T. Dillon and his associates for the spectra and analytical data reported, and to Dr. E. G. Daskalakis for chromatographic assistance.

(8) In contrast to the behavior with lithium aluminum hydride, reduction with lithium tri-*t*-butoxyaluminohydride in tetrahydrofuran appeared to result exclusively in reduction of the Δ^{16} double bond rather than the carbonyl group yielding, on the basis of the infrared spectrum and rotation of the crude product, a mixture of pregnenolone acetate and the 17-iso compound (no hydroxyl band; 5.76 μ, acetate; 5.84 μ, saturated carbonyl).

1:1 Molecular Complex of 5,16-Pregnadiene-3β,20α- and 3β,-20β-diol Diacetates (IIa:IIb).—Material from the previous experiment, m.p. 177–179°, was acetylated with acetic anhydride in pyridine to give a product having m.p. 140–141°. Recrystallization from methanol afforded leaflets with m.p. 143–143.5°, $[\alpha]_D$ −57°; for n.m.r. see Table II. This product is the 1:1 mixture of the 20α and 20β epimers. (The reported values for 5,16-pregnadiene-3β,20α-diol diacetate are as follow: lit.[2a] m.p. 137–138°, $[\alpha]_D$ −57°; lit.[2b] m.p. 138–140°, $[\alpha]_D$ −60°.) The M_D increment for conversion of the mixed 20-ols to the acetate complex[10] is as follows: calculated from our data, Δ = +42°; from the data of Ercoli,[2a] Δ = +33°; from the data of Shapiro[2b], Δ = +30°.

Anal. Calcd. for $C_{25}H_{36}O_4$: C, 74.96; H, 9.06. Found: C, 75.22; H, 8.98.

Separation of 5,16-Pregnadiene-3β,20α- and 3β,20β-diols (Ia and Ib).—A pure sample of the 1:1 diacetate mixture, m.p. 143–143.5°, $[\alpha]_D$ −57°, was hydrolyzed with methanolic potassium hydroxide in approximately quantitative yield and the crude product, m.p. 180–182°, $[\alpha]_D$ −72°, was placed on an alumina column (Woelm, neutral, activity III, 50 to 1 ratio) and subjected to gradient elution[5] by gradually enriching the benzene eluant with a solution of 6% ethyl alcohol in benzene. The products were eluted as a plateau by the 0.6 to 0.8% ethyl alcohol–benzene eluants. The early fractions were pooled and evaporated; the product was crystallized from methanol in the form of leaflets, m.p. 186.5–188.5, $[\alpha]_D$ −86°. The yield of pure material was 13%.

Anal. Calcd. for $C_{21}H_{32}O_2$: C, 79.70; H, 10.19. Found: C, 79.68; H, 9.84.

From the n.m.r. spectrum (Table II) and the change in rotation upon acetylation, this compound was assigned the 5,16-pregnadiene-3β,20α-diol structure (Ia).

The 3β,20α-diacetate IIa was prepared from the diol by treatment with acetic anhydride–pyridine. An analytical sample was crystallized from ethyl alcohol, m.p. 139.5–140°, $[\alpha]_D$ −92°.

Anal. Calcd. for $C_{25}H_{36}O_4$: C, 74.96; H, 9.06. Found: C, 75.25; H, 9.06.

From the later fractions of the broad band of material eluted from the chromatographic column, there was obtained in about 20% yield material having identical melting point and infrared spectral properties as an authentic sample of the 3β,20β-diol, prepared by aluminum isopropoxide reduction of 16-dehydropregnenolone acetate followed by alkaline hydrolysis as described by Marker.[3] A sample was crystallized from ethyl acetate–methylcyclohexane, m.p. 169.5–171.5°, $[\alpha]_D$ −66.5° (lit.[3] m.p. 169–171°).

The 3β,20β-diacetate IIb prepared by acetic anhydride–pyridine treatment of the diol, crystallized as leaflets from dilute methanol, m.p. 123.5–125°, $[\alpha]_D$ −29° (lit.[3] m.p. 121°).

20α- and 20β-Hydroxy-5,16-pregnadien-3-one Ethylene Ketal (IIIa and IIIb).—A slurry of 65 g. of 5,16-pregnadiene-3,20-dione 3-ethylene ketal[6] in 7.5 l. of dry ether and 500 ml. of tetrahydrofuran together with 70 g. of lithium aluminum hydride was refluxed for 6 hr. The excess reagent was destroyed with ethyl acetate, and dilute sodium hydroxide solution was added to precipitate the aluminum salts. The ether solution was decanted, dried, and evaporated to dryness. Crystallization from acetone containing a trace of pyridine afforded a 66% yield of material, m.p. 184–188°, $[\alpha]_D$ −55.5°. The melting range could not be narrowed by further recrystallization. The n.m.r. spectrum indicated (see discussion) that the material consisted of 56% of the 20α epimer and 44% of the 20β compound.

The pure epimers were isolated by chromatography of the crude reduction product on Florisil.[11] From the fractions eluted by mixtures of 1 to 2% ether–benzene there was obtained by crystallization from ethyl acetate–methylcyclohexane (pyridine) the pure 20α-hydroxy compound IIIa, m.p. 192.5–195°, $[\alpha]_D$ −58.5°; for n.m.r., see Table II.

Anal. Calcd. for $C_{23}H_{34}O_3$: C, 77.05; H, 9.56. Found: C, 76.94; H, 9.56.

From the fractions eluted by 10% ether–benzene 20β epimer IIIb was obtained as needles from ethyl acetate–methylcyclohexane (pyridine), m.p. 180–182, $[\alpha]_D$ −52.7°.

Anal. Calcd. for $C_{23}H_{34}O_3$: C, 77.05; H, 9.56. Found: C, 77.44; H, 9.65.

20α-Acetoxy-5,16-pregnadien-3-one Ethylene Ketal (IVa).—The 20α-hydroxy compound IIIa was acetylated using acetic anhydride in pyridine to afford the acetate as needles from methylcyclohexane (pyridine), m.p. 170–171°, $[\alpha]_D$ −75°.

Anal. Calcd. for $C_{25}H_{36}O_4$: C, 74.96; H, 9.06. Found: C, 74.76; H, 8.66.

20β-Acetoxy-5,16-pregnadien-3-one Ethylene Ketal (IVb).—The 20β alcohol IIIb was acetylated in the same manner to afford the title compound as blades from methylcyclohexane (pyridine), m.p. 148.5–150°, $[\alpha]_D$ −11°.

Anal. Calcd. for $C_{25}H_{36}O_4$: C, 74.96; H, 9.06. Found: C, 74.99; H, 9.04.

In the early attempts at purification, the crude lithium aluminum hydride reduction product from 5,16-pregnadiene-3,20-dione 3-ethylene ketal was acetylated and crystallized from aqueous methanol. The material had m.p. 136–137°, $[\alpha]_D$ −40°, and had an analysis consistent with an acetoxy ketal. The n.m.r. spectrum showed resonances at 51.3 and 54.3 c.p.s. of equal amplitude but one-half of the amplitude of the single C-19 methyl resonance peak at 63.8 c.p.s. These data and the rotational value are consistent with a 1:1 molecular complex of the 20α and 20β epimers.

(9) For a recent discussion of the configuration of the reduction products of various 20-keto steroids, see S. Rakhit and C. R. Engel, *Can. J. Chem.*, **40**, 2163 (1962); also K. Heusler and A. Wettstein, *Helv. Chim. Acta*, **45**, 347 (1962).

(10) See footnote *a* of Table I.

(11) A synthetic magnesium silicate product of the Floridin Co., Warren, Pa.

Oxidation of 6β-Hydroxy-3α,5α-cycloandrostan-17-one with Lead Tetraacetate. A Route to 19-Norsteroids from *i*-Steroids

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Received August 12, 1963

This report describes a route to 19-norsteroids starting with *i*-steroids (3α,5α-cyclosteroids).[1] Intramolecular substitution of the steroidal 19-methyl group initiated by abstraction of hydrogen by a 6β-oxygen radical or cation has had many applications, particularly in the formation of 6β,19-oxides by the action of lead tetraacetate on 6β-hydroxy steroids.[2] Lead tetraacetate oxidation of 3β-acetoxy-5α-bromo-6β-hydroxy steroids afforded an excellent route to 19-norsteroids, as the resulting oxides could be converted to 19-oxygenated steroids from which the C-19 could be eliminated.[3]

It was conceived that the use of *i*-steroids would easily provide the 6β-hydroxyl, would protect the potential 3β-hydroxyl-Δ[5] functions while oxygen was introduced at C-19, and would enable these functions readily to be regenerated prior to elimination of the angular C-19. Dreiding models of 3α,5α-cyclosteroids indicate that the shape of ring B is only slightly changed from its shape in 5α-steroids.[4] Ring B is in the chair

(1) After the present work was completed, three reports of independent investigations similar to this were reported: (a) K. Tanabe, R. Takasaki, K. Sakai, R. Hayashi, and Y. Morisawa, *Chem. Pharm. Bull.* (Tokyo), **10**, 1126 (1962), isolated *ca.* 25% yield of 6β,19-oxido-3α,5α-cycloandrostan-17-one (II); (b) J. Tadanier, *J. Org. Chem.*, **28**, 1744 (1963), obtained the 17-ethylenedioxy derivative of II in 22% yield and studied its solvolysis; (c) R. M. Moriarty and T. D. D'Silva, *J. Org. Chem.*, **28**, 2445 (1963), synthesized and hydrolized 6β,19-oxido-3α,5α-cyclocholestane.

(2) (a) C. Meystre, K. Heusler, J. Kalvoda, P. Wieland, G. Anner, and A. Wettstein, *Experientia*, **17**, 475 (1961); (b) A. Bowers, E. Denot, L. C. Ibanez, M. E. Cabezas, and H. J. Ringold, *J. Org. Chem.*, **27**, 1962 (1962).

(3) A. Bowers, R. Villotti, J. A. Edwards, E. Denot, and O. Halpern, *J. Am. Chem. Soc.*, **84**, 3204 (1962).

(4) J. Tadanier and W. Cole, *J. Org. Chem.*, **27**, 4610 (1962).