Bufadienolides. 2. 20-Hydroxy-21-nor-5α-cholanic Acid γ-Lactones (24→20)[1]

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Sodium borohydride reduction of both methyl 3β,23-diacetoxy-20-oxo-21-nor-5-cholenate (**1a**) and the corresponding 23-methyl ether **1b** was found to produce 3β,20-dihydroxy-21-nor-5-cholenic acid γ-lactone (24 → 20). Concomitant elimination of the 23 substituent (to yield lactone **2a**) again occurred with methyl 3β,23-diacetoxy-20-oxo-21-nor-5α-cholanate (**3**) to give γ-lactone **4a**, which was also obtained by palladium-catalyzed hydrogenation of olefin **2a**. Hydrogenation of methyl 3β-acetoxy-20-oxo-21-nor-5-*trans*-22-choladienate (**5**) gave 5α-cholanate **6a** as a major product accompanied by small quantities of 3-deoxycholanate **6b** and γ-lactone **4b**. Sodium borohydride reduction of ketone **6a** yielded γ-lactone **4b**, thereby confirming structures assigned to lactones **2** and **4**. Platinum-catalyzed hydrogenation of 23-methyl ether **1b** and of 23-acetate **3** led, respectively, to 23-substituted lactones **4c** and **4d**. A potentially useful lactone (3-oxo-4-ene, **8**) for biological evaluation was obtained by Oppenauer oxidation of alcohol **2a**.

Once experimental conditions had been devised for condensing glyoxylic acid with 20-oxopregnanes, a practical route to 23-substituted lactones of the isocardanolide type[1c] became the next objective. To this end, 23-acetoxy- (**1a**) and 23-methoxy- (**1b**) 20-oxo-cholenates[1a] were reduced by sodium borohydride[3] with the expectation of obtaining the corresponding γ-lactones, which are members of one of two possible groups of isocardanolides (*cf.* ref 1c).[4] Spectral data and microanalyses indicated that both ketones **1a** and **1b** had been converted into the same γ-lactone, namely, **2a**. A β-type elimination[5] of the oxygen substituent during reduction would have yielded an α,β-unsaturated lactone, but, if β elimination occurred in the presence of sufficient sodium borohydride, reduction of the olefin or a suitable intermediate might occur.[6] Also a direct deoxygenation reaction would seem plausible, in which the hydride ion displaces the oxygen substituent by a nucleophilic substitution mechanism.

Analogous reduction and saponification of the product from methyl 3β,23-diacetoxy-20-oxo-21-nor-5α-cholanate (**3**) led to γ-lactone **4a**, again involving elimination of the 23-acetoxy group. The structural relationship of lactones **2a** and **4a** was readily established by palladium-catalyzed hydrogenation of the former to γ-lactone **4a**. Specimens of this lactone ranged in melting point from 237–241 to 248–253° with optical rotation values at the sodium D line of −72 to −102°, but all exhibited identical infrared spectral and thin layer chromatographic properties. The undefined melting-point and rotation values for lactone **4a** suggest epimeric mixtures at C-20.[7] Unequivocal support for the gross structure of lactones **2** and **4** was obtained as follows. Hydrogenation with palladium of *trans* 22 olefin **5** gave 20-oxo-5α-cholanate (**6a**) as the major product accompanied by methyl 20-oxo-21-nor-5α-cholanate[8] (**6b**) and lactone **4b** in very low yield. Application of the sodium borohydride procedure to ketone **6a** and extension of the reaction period to 72 hr gave lactone **4b**, which was identical with a sample obtained by acetylating lactone **4a**. Subsequently, by sodium borohydride reduction of ketone **4a** at 0°, a 56% yield of the 20-hydroxy methyl ester intermediate was isolated, which was readily converted into γ-lactone **4b** by absorption on a column of silica gel for 4 days.

For the main purpose of providing chemical evidence for the *trans* configuration assigned olefin **5**, reduction with sodium borohydride followed by saponification gave the *trans* hydroxy acid **7**, which failed to lactonize. As expected, palladium-catalyzed hydrogenation of this product followed by acetylation provided only lactone **4b**. Hydrogenation[9] of the *cis* geometrical isomer of olefin **5** in acetic acid containing platinum again provided lactone **4b**.

The platinum-catalyzed hydrogenation reaction of 23-methyl ether **1b** yielded 23-methoxy lactone **4c**, whose isolation was achieved by preparative layer chromatography; a proton magnetic resonance spectrum displayed a characteristic methyl ether signal at δ 3.46. Similarly, catalytic reduction of diacetate **3** led to 23-acetoxy lactone **4d**, whose pmr spectrum showed two sharp singlets at δ 1.96 and 2.09 from the 3β- and 23-acetate groups. Assuming that the lactone ring may have a preferred spatial orientation,

(1) (a) Part 1: G. R. Pettit, B. Green, and G. L. Dunn, *J. Org. Chem.*, **34**, 1267 (1970). (b) This investigation was supported by Public Health Service Research Grants CY-4074 (C3) to CA-04074-06 and CA-10115-01 from the National Cancer Institute. (c) A preliminary account of the present study was presented in part in Dec 1963 at the VIth Pan-American Congress of Pharmacy and Biochemistry, Mexico City, Mexico; see G. R. Pettit, G. L. Dunn, and B. Green, *Chem. Ind.* (London), 1265 (1964).

(2) To whom correspondence should be addressed.

(3) A variety of δ-lactones have been obtained by sodium borohydride reduction of δ-oxovaleric acids, *e.g.*, R. Lukes, S. Dolezal, and K. Capek, *Collect. Czech. Chem. Commun.*, **27**, 2408 (1962); K. W. Rosenmund and H. Bach, *Chem. Ber.*, **94**, 2401 (1961).

(4) Examples of this type of lactone system have been obtained by synthesis [D. Bertin, French Patent 1,369,319; *Chem. Abstr.*, **62**, 1718 (1965)] and by degradative routes [D. Rosenthal, A. O. Niedermeyer, and J. Fried, *J. Org. Chem.*, **30**, 510 (1965); P. Crabbé, G. Ourisson, and T. Takahashi, *Tetrahedron*, **3**, 279 (1958)].

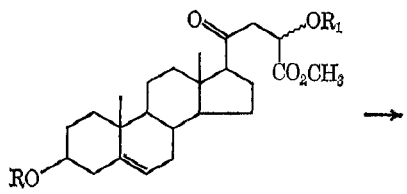
(5) For an example, see G. R. Pettit and T. H. Brown, *J. Chem. Soc., C*, 2024 (1967).

(6) (a) C. Djerassi and W. Rittel, *J. Amer. Chem. Soc.*, **79**, 3528 (1957); (b) G. R. Pettit, B. Green, A. K. Das Gupta, P. A. Whitehouse, and J. P. Yardley, *J. Org. Chem.*, **34**, 1381 (1970).

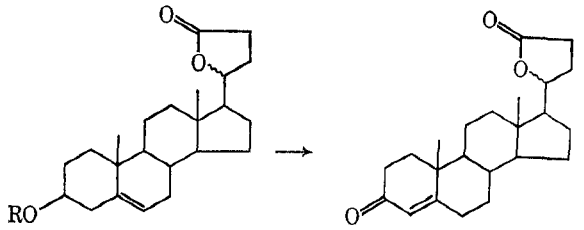
(7) Reduction of steroid 20 ketones using sodium borohydride generally gives 20β alcohols as major products: D. Taub, R. D. Hoffsommer, and N. L. Wendler, *J. Amer. Chem. Soc.*, **81**, 3291 (1959). Assuming that reduction of ketones **1** and **3** follow a similar course, lactones **2** and **4** may consist mainly of the 20β epimer.

(8) Hydrogenolysis of the carbon–oxygen bond at C-3 has been observed with several other 3β-acetoxy-Δ⁵ steroids in our laboratory: G. R. Pettit, A. K. Das Gupta, and R. L. Smith, *Can. J. Chem.*, **44**, 2023 (1966).

(9) *Cf.* P. Kurath, W. Cole, J. Tadanier, M. Freifelder, G. R. Stone, and E. V. Schuber, *J. Org. Chem.*, **28**, 2189 (1963).



1a, R = R_1 = $COCH_3$
b, R = $COCH_3$; R_1 = CH_3



2a, R = H
b, R = $COCH_3$

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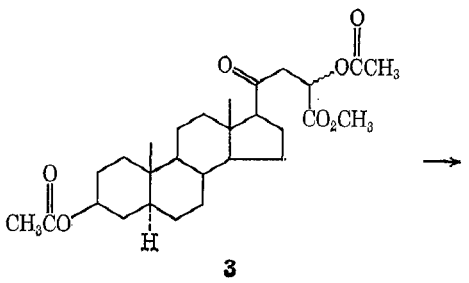

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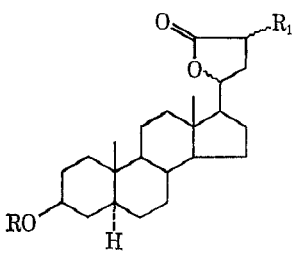
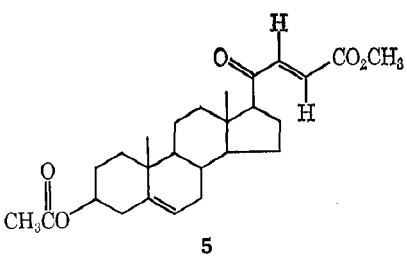


4a, R = R_1 = H
b, R = $COCH_3$; R_1 = H
c, R = $COCH_3$; R_1 = OCH_3
d, R = $COCH_3$; R_1 = $OCOCH_3$



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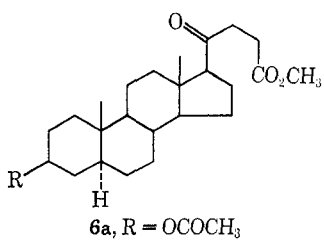
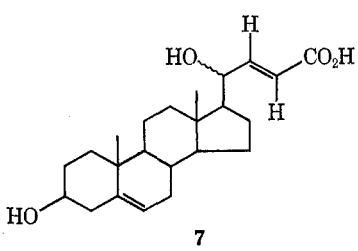


6a, R = $OCOCH_3$
b, R = H



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the sharp signals of the 23 substituents may reflect the presence of essentially one C_{23} epimer.

The terminal objective of the route to isocardanolides was synthesis of A-ring α,β-unsaturated ketone **8** for biological evaluation.[10] Oppenauer oxidation[11] of lactone **2a** yielded lactone **8**, which was isolated by preparative layer chromatography and showed a characteristic C-4 olefinic proton signal (pmr) at δ 5.46.[12]

Experimental Section

All solvents were redistilled. Acetylations were conducted using 1:1 acetic anhydride–pyridine at 25° for 12–15 hr. The combined extracts of aqueous solutions were dried over anhydrous magnesium or sodium sulfate. Acid-washed alumina (Merck, Rahway) and 0.05–0.20-mm-mesh silica gel (E. Merck, Darmstadt) were employed for column chromatography. Thin layer chromatograms were prepared using silica gel G (E. Merck) and visualized with concentrated sulfuric acid. Preparative layer chromatography employed 20 × 20 cm plates coated with a 1-mm layer of silica gel G. All analytical samples were colorless and displayed one spot on a thin layer chromatogram.

Melting points for analytical specimens were determined using a Kofler apparatus and all other melting points were observed in open capillaries (silicone oil bath) and are uncorrected. Physical measurements by Dr. R. Hill, University of Maine, comprised ultraviolet (Perkin-Elmer, Model 400 spectrophotometer), infrared (in potassium bromide unless otherwise indicated, Baird spectrophotometer), and pmr (deuteriochloroform solution with tetramethylsilane as internal standard, Varian A-60) spectra. Elemental microanalytical data was obtained in the laboratory of Dr. A. Bernhardt, Max Planck Institut, Mülheim, Germany, and optical rotations (chloroform solution at 20°) were provided by Dr. P. Demoen, Janssen Pharmaceutica, Beerse, Belgium.

3β,20-Dihydroxy-21-nor-5-cholenic Acid γ-Lactone (24 → 20) (2a). Route A.—To a solution of methyl 3β,23-diacetoxy-20-oxo-21-nor-5-cholenate[1a] (**1a**, 4.0 g, 8.2 mmol) in dimethylformamide (160 ml) was added dropwise during 15 min, with stirring, a solution of sodium borohydride (1.26 g, 32.8 mmol) in water (30 ml) at 15°. The mixture was stirred for 3 hr at room temperature, treated with 25% aqueous acetic acid (40 ml), and poured into water (500 ml). The aqueous mixture was saturated with sodium chloride and extracted with chloroform, and the combined extract was washed with saturated aqueous sodium bicarbonate and water. Evaporation of the dry solvent yielded an oil (3.2 g) which was dissolved in methanol containing 0.5 *N* potassium hydroxide (270 ml)–water (30 ml) and heated at reflux for 2 hr. Dilution with water (300 ml) and concentration to 75 ml *in vacuo* gave a turbid mixture. Following acidification with 3 *N* hydrochloric acid (600 ml), and a 12-hr period at room temperature, the precipitated gel was extracted with chloroform and the solvent was evaporated to a colorless foam (2.6 g), which was dissolved in benzene (0.68 g insoluble) and chromatographed on acid-washed alumina (60 g). Elution with 3:1 benzene–chloroform afforded a solid (1.1 g, 38%), mp 240–244°. Three recrystallizations from acetone furnished rods: mp 244–246°; $[\alpha]_D$ −83° (*c* 0.572); ν_{max} 3400 (hydroxyl) and 1745 cm^{-1} (γ lactone).

Anal. Calcd for $C_{23}H_{34}O_3$: C, 77.05; H, 9.56; O, 13.39. Found: C, 77.07; H, 9.52; O, 13.19.

Route B.—Methyl 3β-acetoxy-23-methoxy-20-oxo-21-nor-5-cholenate (**1b**, 1.4 g, 3.05 mmol) was reduced with sodium borohydride (0.45 g, 12.2 mmol) in dimethylformamide–water as described in route A above to give, after saponification and acidification, a semicrystalline mass (0.97 g), which was dis-

(10) Endocrinological studies of the steroids described herein are being performed under the auspices of the National Cancer Institute, National Institutes of Health. Isocardanolide **4b** was found to be devoid of cardiac activity, being completely inactive in an ATPase test system at concentrations as high as 0.1 m*M*. We wish to thank Professor Repke for this information; *cf.* H. J. Portius and K. Repke, *Arzneim.-Forsch.*, **14**, 1073 (1964).

(11) J. A. Cella, E. A. Brown, and R. R. Burtner, *J. Org. Chem.*, **24**, 743 (1959).

(12) N. S. Bhacca and D. H. Williams, "Applications of NMR Spectroscopy in Organic Chemistry," Holden-Day, Inc., San Francisco, Calif., 1964, p 90.

solved in 3:1 benzene–chloroform and chromatographed on acid-washed alumina (30 g) to yield 0.51 g, mp 215–240°. Four recrystallizations from chloroform–ether gave plates: mp 237–241°; $[\alpha]D$ −72° (*c* 0.22); ν_{max}^{Nujol} 3400 (hydroxyl) and 1740 cm^{-1} (γ lactone). This sample was identical[13] with a sample prepared by route A.

Anal. Calcd for $C_{23}H_{34}O_3$: C, 77.05; H, 9.56. Found: C, 76.58; H, 9.34.

A portion (0.075 g) of the 3β,20-dihydroxy-21-nor-5-cholenic acid γ-lactone (24 → 20) (**2a**) was acetylated. Recrystallizing the product from methanol furnished **3β-acetoxy-20-hydroxy-21-nor-5-cholenic acid γ-lactone (24 → 20)** (**2b**, 0.06 g): mp 192–197°; ν_{max} 1770 (γ lactone), 1730, and 1250 cm^{-1} (acetate). Four recrystallizations from methanol gave shiny plates, mp 204–205°, $[\alpha]D$ −64.3° (*c* 0.49).

Anal. Calcd for $C_{25}H_{36}O_4$: C, 74.96; H, 9.06. Found: C, 74.62; H, 9.24.

Route C.[14]—A 0.2-g specimen of 3β acetate **2b** was saponified overnight using 10% aqueous potassium carbonate (3 ml) in methanol (16 ml). An analytical sample of alcohol **2a** was obtained by repeated recrystallization from acetone as prisms: mp 248–253°; $[\alpha]D$ −102° (*c* 0.19); ν_{max} 3400 and 1740 cm^{-1}; pmr δ 0.78 (CH_3-18), 1.02 (CH_3-19), 3.55 (multiplet, 1 proton, H-3α), 4.4 (multiplet, 1 proton, H-20), and 5.37 (multiplet, 1 proton, H-6).

Anal. Calcd for $C_{23}H_{34}O_3$: C, 77.05; H, 9.56. Found: C, 76.50, 76.55; H, 9.58, 9.48.

3β,20-Dihydroxy-21-nor-5α-cholanic Acid γ-Lactone (24 → 20) (4a). Route A.—Methyl 3β,23-diacetoxy-20-oxo-21-nor-5α-cholanate[1a] (**3**, 1.0 g, 2.0 mmol) in dimethylformamide (40 ml) was reduced with sodium borohydride (0.32 g, 8.2 mmol) in water (8 ml) exactly as described above (*cf.* **2a**) for the unsaturated compound. After saponification and acidification, the colorless foam (0.70 g) was treated with boiling benzene (0.25 g, solid residue) and the filtrate was chromatographed on acid-washed alumina (15 g). Elution with 3:1 benzene–chloroform gave a solid (0.30 g), mp 241–244°, which after five recrystallizations from chloroform–hexane afforded an analytical specimen as rods: mp 246–248°; $[\alpha]D$ −18.7° (*c* 0.643); ν_{max} 3400 (hydroxyl) and 1745 cm^{-1} (γ-lactone).

Anal. Calcd for $C_{23}H_{36}O_3$: C, 76.62; H, 10.07. Found: C, 77.07; H, 9.92.

Route B.—A sample (**2a**, 0.10 g) of 3β,20-dihydroxy-21-nor-5-cholenic acid γ-lactone (24 → 20) in tetrahydrofuran (30 ml) containing 1 drop of perchloric acid (70%) and suspended palladium on charcoal (25 mg, 10%) was shaken in an atmosphere of hydrogen for 2 hr. Filtration, dilution with chloroform, washing with saturated aqueous sodium bicarbonate, drying, and evaporation yielded a pale yellow oil (0.07 g). Repeated recrystallization from chloroform–hexane gave rods (0.02 g), mp 238–242°, identical with γ-lactone **4a** prepared by route A.

Hydrogenation of Methyl 3β-Acetoxy-20-oxo-21-nor-5-*trans*-22-choladienate (5).—A solution of methyl 3β-acetoxy-20-oxo-21-nor-5-*trans*-22-choladienate[6b] (**5**, 2.49 g) in ethyl acetate (150 ml) containing suspended palladium-on-charcoal catalyst (0.8 g, 10%) was shaken for 4 hr under hydrogen. Filtration and evaporation furnished a solid, which was chromatographed on acid-washed alumina (75 g) to yield the following products.

(1) A crystalline solid (0.12 g) was eluted by 3:1 hexane–benzene. Recrystallization from methanol gave slender needles (0.07 g), mp 173–177°. Two further recrystallizations from the same solvent gave an analytical specimen of **methyl 20-oxo-21-nor-5α-cholanate (6b)**: mp 181–182°; $\nu_{max}^{CHCl_3}$ 1740 (methyl ester) and 1708 cm^{-1} (ketone), *no* absorption at 1250–1270 cm^{-1} (acetate); pmr δ 2.57 (multiplet, 4 protons, $-COCH_2CH_2CO_2-$) and 3.58 (singlet, 3 protons, methyl ester).

Anal. Calcd for $C_{24}H_{38}O_3$: C, 76.96; H, 10.23. Found: C, 76.81; H, 10.17.

(2) A crystalline solid (1.63 g) was eluted by benzene. Recrystallization from methanol provided **methyl 3β-acetoxy-20-oxo-21-nor-5α-cholanate (6a**, 1.3 g), mp 126.5–128.5°. Further recrystallization gave an analytical specimen as blades: mp 128–129°; $[\alpha]D$ +73.7° (*c* 1.49); pmr δ 1.97 (singlet, 3 acetate methyl protons), 2.56 (multiplet with strong central signal, 4 protons, $COCH_2CH_2CO_2$), and 3.57 (singlet, 3 methyl ester protons).

Anal. Calcd for $C_{26}H_{40}O_5$: C, 72.19; H, 9.32; O, 18.49. Found: C, 72.20; H, 9.56; O, 18.41.

(3) A crystalline solid (0.22 g) was eluted by chloroform. Recrystallization from methanol gave 0.1 g, mp 198–203°, of **3β-acetoxy-20-hydroxy-21-nor-5α-cholanic acid γ-lactone (24 → 20) (4b)**.—Another recrystallization from methanol gave an analytical sample as blades: mp 204–207°; $[\alpha]D$ −25.3° (*c* 0.32); $\nu_{max}^{CHCl_3}$ 1760 (γ lactone) and 1720 cm^{-1} (3-acetate); pmr δ 0.72 (singlet, 3 protons, CH_3-18), 0.80 (singlet, 3 protons, CH_3-19), 1.94 (singlet, 3 acetyl methyl protons), and 4.3 (diffuse area, 2 protons, –COOCH).

3β-Acetoxy-20-hydroxy-21-nor-5α-cholanic Acid γ-Lactone (24 → 20) (4b). Method A.—To a solution of methyl 3β-acetoxy-20-oxo-21-nor-5α-cholanate (**6a**, 0.25 g, 0.575 mmol) in dimethylformamide (13 ml) was added sodium borohydride (0.20 g, 5.57 mmol) in water (6 ml). After 72 hr at room temperature, isolation was achieved by dilution with water, treatment with 1 *N* hydrochloric acid, and filtration. The solid (0.22 g) was washed well with water, dried, and chromatographed in benzene on acid-washed alumina (10 g). The desired γ-lactone (**4b**, 0.17 g)[13] was eluted by 9:1 benzene–chloroform and recrystallized successively from methanol and isopropyl ether to give analytical specimen as blade clusters: mp 204–207°; $[\alpha]D$ −13.5° (*c* 0.52); ν_{max}^{Nujol} 1770 (γ lactone) and 1735 cm^{-1} (acetate); pmr δ 0.72 (singlet, slight splitting, CH_3-18), 0.80 (singlet, 3 protons, CH_3-19), 1.94 (singlet, 3 protons, acetate), and 4–4.5 (unresolved region, 2 protons, –COOCH).

Anal. Calcd for $C_{25}H_{38}O_4$: C, 74.60; H, 9.51; O, 15.90. Found: C, 74.38; H, 9.36; O, 16.37.

In another experiment[15] employing ketone **6a** (6.1 g, 14 mmol) in dimethylformamide (350 ml) and sodium borohydride (4.8 g, 127 mmol)–water (25 ml) at 0° for 17 hr, careful acidification (at 0° to congo red) and dilution with ice–water gave **methyl 3β-acetoxy-20ζ-hydroxy-21-nor-5α-cholanate**. Recrystallization from methanol–methylene chloride provided 3.4 g (56%) of crystals: mp 171–174°; ν_{max} 3500 (20-hydroxyl), 1725 (acetate and methyl ester), and 1260 cm^{-1}.

Anal. Calcd for $C_{26}H_{42}O_5$: C, 71.85; H, 9.74; O, 18.41. Found: C, 71.99; H, 9.71; O, 18.28.

A sample (0.19 g) of the 20 alcohol in benzene was left on a column of silica gel (6 g) for 4 days. Elution with benzene–ethyl acetate mixtures and recrystallization of the product from methanol–methylene chloride afforded 0.11 g of lactone **4b**,[13] mp 204–207° (sintering from 195°).

Method B.—Methyl 3β-acetoxy-20-oxo-21-nor-5-*trans*-22-choladienate (**5**, 0.50 g, 1.2 mmol) was reduced during 2 hr with sodium borohydride (0.09 g, 2.4 mmol) in dimethylformamide (25 ml)–water (2.5 ml) as described above (method A). The same isolation procedure led to a colorless oil (0.5 g) which was saponified at reflux temperature during 2 hr in 0.5 *N* methanol–potassium hydroxide solution (20 ml). Dilution with water (50 ml), concentration *in vacuo* to 20 ml, acidification with 3 *N* hydrochloric acid, extraction with chloroform, and evaporation of solvent gave colorless acid **7** (0.2 g): mp *ca.* 220° dec; ν_{max}^{Nujol} 3000–3400 and 1726 cm^{-1} (carboxylic acid). A solution of acid **7** in tetrahydrofuran (40 ml) containing perchloric acid (1 drop, 70%) and suspended palladium-on-charcoal catalyst (50 mg, 10%) was hydrogenated at atmospheric pressure and room temperature for 2 hr. Filtration, concentration to 10 ml, dilution with chloroform, washing with sodium bicarbonate solution and water, drying, and evaporation yielded a discolored product (0.12 g). After acetylation a colorless solid (0.10 g), mp 192–197°, was isolated. Three recrystallizations from methanol gave crystals (0.02 g) of lactone **4b**:[13] mp 201–204°; ν_{max} 1770 (γ-lactone), 1730, and 1250 cm^{-1} (acetate).

Method C.—A solution of methyl 3β-acetoxy-20-oxo-21-nor-5-*cis*-22-choladienate (0.11 g)[6b] in acetic acid (20 ml) containing perchloric acid (2 drops) and suspended platinum from platinum oxide (0.05 g) was shaken in an atmosphere of hydrogen for 1 hr. Hydrogen adsorption was fast and essentially complete after 30 min. The solution was filtered, diluted with diethyl ether, and washed successively with saturated aqueous sodium bicarbonate and water. Removal of solvent furnished a viscous oil which crystallized slowly on standing. The product (0.12 g) was dissolved in 1:1 hexane–benzene and chromatographed on silica gel (3.5 g). Benzene eluted a colorless solid (0.045 g, essentially pure by thin layer chromatography). Recrystallization from

(13) The structure was confirmed by mixture melting point, thin layer chromatography, and ir spectral comparison with an authentic specimen.

(14) This experiment was performed by Dr. J. P. Yardley.

(15) By Dr. P. Sunder-Plassmann.

isopropyl ether gave lactone **4b**[13] as elongated prisms, mp 198–202°.

Method D.—A 7-mg sample of 3β,20-dihydroxy-21-nor-5-cholenic acid γ-lactone (**2a**) prepared by sodium borohydride reduction of methyl 3β-acetoxy-23-methoxy-20-oxo-21-nor-5-cholenate (**1b**) was acetylated. A solution of the crystalline acetate in ethyl acetate (10 ml) was hydrogenated with platinum oxide (10 mg) during 2 hr. Filtration and evaporation gave a solid residue. Following dissolution in methanol, filtration to remove flocculent material, and evaporation to small volume, lactone **4b**[13] crystallized as colorless needles (5 mg), mp 196–202°.

Method E.—Acetylation of 0.05 g of 3β,20-dihydroxy-21-nor-5α-cholanic acid γ-lactone (24 → 20) (**4a**) led to 0.04 g of solid acetate **4b**, mp 204–207°. Three recrystallizations from methanol gave plates: mp 208–209°; $[\alpha]D$ 0°; ν_{max} 1770 (γ lactone) and 1730 and 1250 cm^{-1} (acetate).

Anal. Calcd for $C_{25}H_{38}O_4$: C, 74.59; H, 9.52. Found: C, 74.20; H, 9.63.

Lactone **4b** obtained by this means was identical[13] in all respects with samples prepared by methods A–D.

3β-Acetoxy-23-methoxy-20-hydroxy-21-nor-5α-cholanic Acid γ-Lactone (24 → 20) (4c).—Methyl 3β-acetoxy-23-methoxy-20-oxo-21-nor-5-cholenate (**1b**, 0.16 g) in acetic acid (30 ml) containing perchloric acid (3 drops, 70%) was shaken in a slight positive pressure atmosphere of hydrogen with a catalyst from platinum oxide (0.075 g) for 2 hr. Filtration and addition to water gave a white precipitate (0.14 g), which was collected and water washed. Additional product (0.02 g) was obtained by extracting the filtrate with diethyl ether. The extract was successively washed with sodium bicarbonate solution and water. A solution of the crude material in benzene was chromatographed on silica gel (6 g). Two major fractions (0.12 g) were eluted by mixtures of 13:3 and 3:1 benzene and chloroform, but thin layer chromatograhy examination showed only partial separation. The total amount (0.12 g) was subjected to preparative thin layer chromatography on four plates each containing silica gel G (25 g). The solvent system was 1:1 hexane–ethyl acetate and bands were detected by water spraying. Components were isolated by chloroform extraction: lower band, 48 mg; center band, 26 mg; and upper band, 7 mg. The lower band crystallized from methanol as elongated prisms (25 mg): mp 228–232°; mixture melting point with 3β-acetoxy-20-hydroxy-5α-cholanic acid γ-lactone (**4b**) was depressed to 191–200°; $[\alpha]D$ 0°; $\nu_{max}^{CHCl_3}$ 1770 (γ lactone), 1720, 1255 (acetate), and 1135 cm^{-1} (methoxyl); pmr δ 0.71 (singlet, 3 protons, CH_3-18), 0.81 (singlet, 3 protons, CH_3-19), 1.96 (singlet, 3 protons, acetate), and 3.46 (singlet, 3 protons, methyl ether).

Anal. Calcd for $C_{26}H_{40}O_5$: C, 72.20; H, 9.32. Found: C, 72.05; H, 8.91.

3β,23-Diacetoxy-20-hydroxy-21-nor-5α-cholanic Acid γ-Lactone (24 → 20) (4d).—Methyl 3β,23-diacetoxy-20-oxo-21-nor-5α-cholanate (**3**, 0.20 g) in acetic acid (40 ml) was hydrogenated for 1 hr using perchloric acid (4 drops, 70%) and platinum oxide (100 mg) essentially as noted above (*cf.* **4c**). The crude product (0.195 g) in benzene was chromatographed on silica gel (8 g) to yield in 9:1 → 3:1 benzene–chloroform mixtures a colorless solid (0.13 g, almost homogeneous by thin layer chromatography). Recrystallization from methanol gave needle clusters in two crops (0.06 g total), mp 197–202° and 204–208°. Recrystallization from the same solvent gave the analytical specimen as tiny crystals: mp 204–208°; $[\alpha]D$ −15.5° (*c* 0.32); $\nu_{max}^{CHCl_3}$ 1780 (γ lactone), 1740, and 1718 cm^{-1} (acetates); pmr δ 0.72, 0.74 (doublet, 3 protons, CH_3-18), 0.81 (singlet, 3 protons, CH_3-19), 1.96 (singlet, 3 protons, 3 acetate), and 2.09 (singlet, 3 protons, 23 acetate).

Anal. Calcd for $C_{27}H_{40}O_6$: C, 70.40; H, 8.75; O, 20.84. Found: C, 70.59; H, 8.44; O, 20.83.

3-Oxo-20-hydroxy-21-nor-4-cholenic Acid γ-Lactone (24 → 20) (8). Procedure A.[16]—A solution of lactone **2a** in toluene (15 ml)–cyclohexanone (5 ml) was heated to reflux and 3 ml of toluene was removed by slow distillation to ensure dryness. To the hot solution was added aluminum isopropoxide (0.34 g, Matheson Coleman and Bell) in toluene (4 ml). The reaction mixture was heated at reflux with stirring for 25 min. Upon cooling, water (4 ml) was added. Following acidification with 2 *N* hydrochloric acid and extraction with diethyl ether, the ethereal extract was washed well with water. The residue obtained by removal of solvent was subjected to exhaustive steam distillation and the resulting crystalline residue was dissolved in chloroform and washed with water. Solvent was removed and the residue (0.22 g) was purified by preparative thin layer chromatography. Three plates were used and developed with 1:1 benzene–ethyl acetate. The product was recovered from the silica gel by repeated extraction with 19:1 chloroform–methanol. The 0.14-g sample of lactone **8** obtained in this manner was recrystallized from acetone–hexane to yield 0.12 g. The analytical sample was recrystallized from acetone: mp 220–223°; $[\alpha]D$ +78° (*c* 0.28); ν_{max} 1760 (γ lactone), 1673 (3 ketone), and 1612 cm^{-1} (4 olefin); pmr δ 0.79 (CH_3-18), 1.12 (CH_3-19), 4.25 (multiplet, 1 proton, H-20), and 5.48 (singlet, 1 proton, H-4).

Anal. Calcd for $C_{23}H_{32}O_3$: C, 77.48; H, 9.05. Found: C, 77.52; H, 8.85.

Procedure B.—To a solution of 3β,20-dihydroxy-21-nor-5-cholenic acid γ-lactone **2a** (0.37 g) in toluene (15 ml)–cyclohexanone (2 ml) was added aluminum *t*-butoxide (0.40 g). The mixture was heated at reflux for 4 hr, and the resulting yellow suspension was cooled, diluted with diethyl ether (50 ml), and washed successively with hydrochloric acid (20%), saturated sodium bicarbonate, and water. Evaporation of solvent gave a colorless solid (0.40 g) which was chromatographed on acid-washed alumina (15 g). Elution with 5:1 benzene–chloroform gave a solid (0.23 g, 62%), mp 207–211°. Five crystallizations from chloroform–diethyl ether afforded microneedles: mp 210–211°; ν_{max}^{Nujol} 1740 (γ lactone), 1690 (3 ketone), and 1618 cm^{-1} (4 olefin); λ_{max}^{EtOH} 241 mμ (log ε 4.21).

Anal. Found: C, 77.93; H, 9.09.

Further elution with 5:1 benzene–chloroform returned starting material (0.06 g).

Registry No.—**2a**, 23330-63-4; **2b**, 23330-64-5; **4a**, 23330-65-6; **4b**, 23330-66-7; **4c**, 23367-50-2; **4d**, 23330-67-8; **6a**, 23330-48-5; **6b**, 23330-69-0; **8**, 23330-70-3; methyl 3β-acetoxy-20ζ-hydroxy-21-nor-5α-cholanate, 23330-71-4.

(16) Performed by Dr. J. P. Yardley.