cm^{-1}; pmr δ 0.98 and 1.35 (C-18 and -19 methyls), 2.00 (C-3 acetate), 2.6–2.9 (m, C-22 methylene), 4.92 (H-3α), and 5.87 (H-21); mass spectrum m/e 416 (M^+) and 356 ($M^+ - 60$).

Anal. Calcd for $C_{25}H_{36}O_5$: C, 72.08; H, 8.71; O, 19.20. Found: C, 72.12; H, 8.64; O, 19.42.

The last fraction eluted from the column weighed 0.2 g and was virtually pure isodigitoxigenin acetate with only traces of rearrangement product **9** present.

It was later found that the reaction was greatly concentration dependent, and that if the volume of benzene was reduced to *ca.* 35 ml/1 g of isodigitoxigenin acetate, no starting material at all remained after 24 hr at reflux. Reducing the volume still further or prolonging the reflux time lead to increasing amounts of C-norcardenolide **6**. The products were most satisfactorily purified by preparative layer chromatography on large plates (40 × 20 cm), developed up to eight times in chloroform. On silica gel HF_{254} the rearrangement product gave a pale blue fluorescence under ultraviolet light, and the extent of the band owing to unchanged starting material was revealed by spraying the plates with water.

Alcoholysis of 3β-Acetoxy-12(13→14)*abeo*-13α-methyl-13β,-21α-epoxy-5β-cardanolide (9).—A solution prepared from cardanolide **9** (0.11 g), methanol (10 ml), water (0.5 ml), and *p*-toluenesulfonic acid (10 mg) was heated at reflux for 26 hr. The crude product was isolated and acetylated essentially as summarized above for the preparation of acetals **4b** and **4c**. Following acetylation, a thin layer chromatogram ($CHCl_3$ mobile phase) showed two components. Purification by preparative layer chromatography in $CHCl_3$ gave the faster moving acetal **10a** as an oil which crystallized from methanol as large prisms (52 mg): mp 103–105°; $[\alpha]D$ +91.5° (*c* 0.71); RD (*c* 0.71) $[\alpha]_{300}$ +416°, $[\alpha]_{350}$ +289°, $[\alpha]_{400}$ +212°, $[\alpha]_{450}$ +162°, $[\alpha]_{500}$ +130° $[\alpha]_{589}$ +91.5°, and $[\alpha]_{600}$ +91.5°; pmr δ 0.96 and 1.29 (C-18 and -19 methyls), 2.02 (C-3 acetate), 3.25 (C-21 methoxyl), 3.66 (methyl ester), 4.84 (doublet, J = 5 Hz, H-21), and 5.05 (H-3α).

Anal. Calcd for $C_{27}H_{42}O_6$: C, 70.10; H, 9.15. Found: C, 69.69; H, 9.30.

The more polar isomer acetal **10b** (30 mg) was isolated as an oil that resisted all attempts at crystallization. However, a thin layer chromatogram ($CHCl_3$ mobile phase) indicated presence of only one component: pmr δ 0.99 and 1.26 (C-18 and -19 methyls), 2.04 (C-3 acetate), 2.48, (C-22 methylene) 3.28 (C-21 methoxyl), 3.62 (methyl ester), 4.72 (H-21β), and 5.07 (H-3α).

Conversion of Acetals 10a and 10b into C-Norcardanolide 9 and C-Norcardenolide 6.—Preparation of acetals **10a** and **10b** was repeated on a somewhat larger scale. A solution of acetal **10a** (0.24 g) in benzene (60 ml) containing *p*-toluenesulfonic acid (0.05 g) was distilled until 20 ml of solvent was removed. Heating was continued at reflux for 2 hr and the solution was cooled, diluted with diethyl ether, and washed successively with water, dilute sodium bicarbonate solution, and water. Solvent was removed and the residual oil (0.17 g) was purified by preparative layer chromatography with 9:1 chloroform–ethyl acetate. The product separated into three zones with the most polar corresponding to cardanolide **9**. Crystallization from methanol provided 0.069 g, mp 195–196°. The product was identical[20] with an authentic specimen of cardanolide **9**. The next most polar zone corresponded to cardenolide **6**. Crystallization from methanol gave needles (36 mg), mp 165–166°, identical[20] with an authentic sample. The least polar zone provided 0.13 g of oil that resisted crystallization. Repeated purification by preparative layer chromatography failed to yield a crystalline product.

A solution of acetal **10b** (0.112 g) in dry benzene (30 ml) containing *p*-toluenesulfonic acid (20 mg) was heated at reflux for 14.5 hr until tlc showed that no starting material was present. The crude product was isolated and purified by preparative layer chromatography as summarized in the preceding paragraph. The most polar zone again corresponded to cardanolide **9** (25 mg), mp 187–193°. Recrystallization from methanol gave a sample, mp 194–196°, identical[20] with an authentic specimen. Again, cardenolide **6** (10 mg), mp 151–154°, was isolated from the middle zone. Recrystallization from methanol gave a specimen, mp 160–162°, identical[20] with authentic material. The least polar zone corresponded on the basis of thin layer mobility to the analogous zone obtained from acetal **10a** and could not be persuaded to crystallize.

Registry No.—**4b**, 14892-11-6; **4c**, 14892-12-7; **4f**, 17150-44-6; **4g**, 23353-49-3; **5a**, 23353-50-6; **5b**, 17150-43-5; **6**, 23353-51-7; **7**, 23353-52-8; **8**, 23353-53-9; **9**, 23353-54-0; **10a**, 23353-55-1.

Bufadienolides. 9. Isobufalin[1]

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Received February 11, 1969

Isobufalin methyl ester (**4a**) was prepared by methanolysis of bufalin (**3**) in the presence of sodium methoxide, and saponification of the 3β-acetoxy derivative **4b** readily afforded isobufalin (**4c**). In each case, the configuration of the side-chain olefin was shown to be *trans* at positions 22 and 23 by proton magnetic resonance measurements. Isodigitoxigenin (**7**), acetal **8e**, and dihydropyran **12a** were prepared from digitoxin by way of digitoxigenin (**6**) as described in part 8. By a four-step reaction sequence *via* intermediates **12b–12d** and **11a**, both methyl esters **8e** and **12a** were converted into methyl 3β-acetoxy-14β,21-epoxy-5β-chol-20(21)-enate (**11b**). Dehydrogenation of methyl ester **11b** employing 2,3-dichloro-5,6-dicyanobenzoquinone completed total synthesis of 3β-acetoxyisobufalin methyl ester and therefore isobufalin.

At an early stage in the extensive and definitive structural investigation of scillaren A by Stoll and colleagues,[3,4] a derivative scillaridin A (**1**) upon contact with potassium hydroxide in methanol was found to yield the methyl ester of an isomeric substance designated isoscillaridin A (**2**).[5] Analogous methanolysis of bufalin[6] (**3**) readily afforded isobufalin methyl ester (**4a**). That a *trans* relationship now existed between the 22 and 23 protons was indicated by proton magnetic resonance signals at δ 5.63 (23 proton) and 7.23 (22 proton) which appeared as a set of doublets with J = 15 Hz. Acetylation of alcohol **4a** gave 3β-acetoxyisobufalin methyl ester (**4b**). Platinum-catalyzed hydrogenation of iso-

(1) (a) This investigation was supported by Public Health Service Research Grants CA-04074-05 to CA-04074-06 and CA-10115-01 to CA-10115-02 from the National Cancer Institute. Part 8: G. R. Pettit, T. R. Kasturi, J. C. Knight, and J. Occolowitz, *J. Org. Chem.*, **35**, 1404 (1970). (b) A preliminary report of the present study was summarized: T. R. Kasturi, G. R. Pettit, and K. A. Jaeggi, *Chem. Commun.*, 644 (1967).

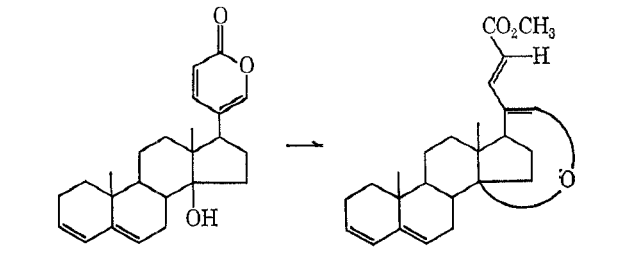
(2) On sabbatical leave from the Indian Institute of Science, Bangalore, India.

(3) A. Stoll, A. Hofmann, and A. Helfenstein, *Helv. Chim. Acta*, **17**, 641 (1934).

(4) Other pertinent references have been summarized: G. R. Pettit, B. Green, and G. Dunn, *J. Org. Chem.*, **35**, 1367 (1970).

(5) The *trans* side-chain geometry presented in structure **2** for isoscillaridin A is based upon results of a proton magnetic resonance study of isobufalin summarized in the sequel. The assignment presumes comparable energy relationships in the olefin systems of isoscillaridin A and isobufalin.

(6) *Cf.* A. von Wartburg and J. Renz, *Helv. Chim. Acta*, **42**, 1620 (1959).



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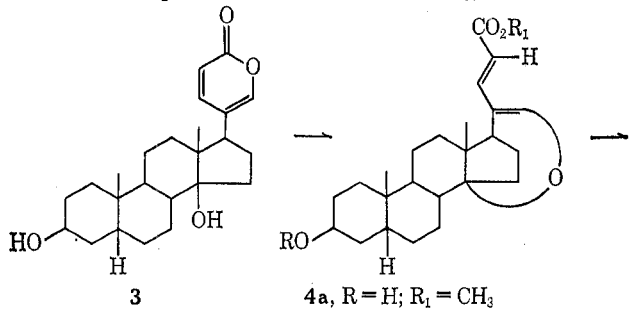
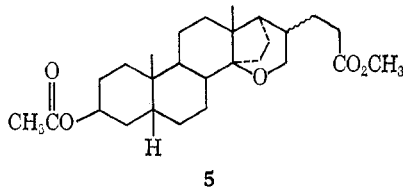


3 4a, R = H; R_1 = CH_3
b, R = $COCH_3$; R_1 = CH_3
c, R = R_1 = H



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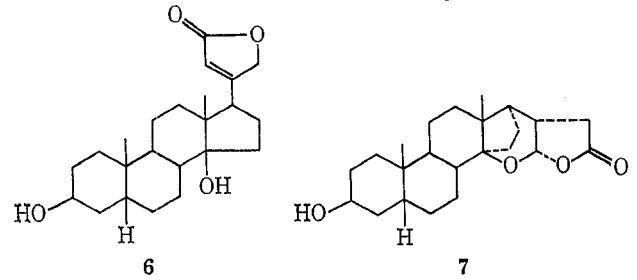

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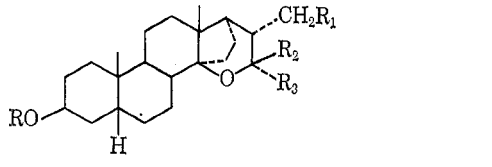
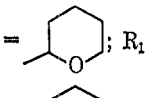
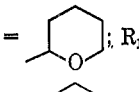
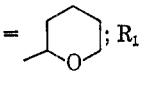


8a, R = H; R_1 = CO_2H; R_2 or R_3 = OH
b, R = $COCH_3$; R_1 = CO_2CH_3; R_2 = $OCOCH_3$; R_3 = H
c, R = $COCH_3$; R_1 = CO_2CH_3; R_2 = R_3 = O
d, R = $COCH_3$, R_1 = CO_2CH_3; R_2 = H; R_3 = OCH_3
e, R = $COCH_3$; R_1 = CO_2CH_3; R_2 = OCH_3; R_3 = H
f, R = H; R_1 = CO_2H; R_2 = R_3 = O
g, R = $COCH_3$; R_1 = CO_2H; R_2 = R_3 = O
h, R = $COCH_3$; R_1 = COCl; R_2 = R_3 = O

i, R =  ; R_1 = CH_2OH; R_2 = OCH_3; R_3 = H

j, R =  ; R_1 = $CH_2OSO_2CH_3$; R_2 = OCH_3; R_3 = H

k, R =  ; R_1 = CH_2CN; R_2 = OCH_3; R_3 = H

bufalin methyl ester provided tetrahydropyran **5**. Saponification of methyl ester **4b** with sodium hydroxide in ethanol essentially as described[3] for isoscillaridin A gave isobufalin (**4c**). The H-22–H-23 coupling constant in each case (**4a–4c**) remained at 15 Hz. To further confirm the structure and D-ring stereochemistry of isobufalin and in turn that of bufalin, total[7] synthesis of isobufalin was undertaken.

The initial plan was first to protect hemiacetal acetate **8b**, prepared (**6** → **7** → **8a** → **8b**) from digitoxigenin as already described,[1] by oxidation to lactone **8c** as reported by Schindler and Reichstein.[8] Following extension of the side chain by one methylene group and conversion into acid chloride **9b**, diborane reduction of the δ lactone was expected[9] to result in formation of isodigitoxigenin homolog **10**. The 14β,21-epoxybufanolide **10** was to serve as springboard to both isobufalin and bufalin. In practice, chromium trioxide–glacial acetic acid oxidation of diacetate **8b** gave lactone **8c**, and the same substance was more easily obtained by analogous oxidation of acetal **8d** or **8e**.[1] Ester **8c** was saponified and the product was acetylated to give acid **8g**. This was neutralized with an equivalent amount of sodium methoxide in methanol to give the corresponding sodium salt. After drying, the salt was converted into the acid chloride and treated successively with diazomethane and silver benzoate in dry methanol–triethylamine. Completion of the Arndt–Eistert[10] sequence and purification by column and preparative layer chromatography gave a pure specimen of lactone **9c**. Methyl ester **9c** was transformed into acid chloride **9b** as already noted with acid chloride **8h**. Several attempts to reduce lactone **9b** using diborane in tetrahydrofuran followed by intramolecular cyclization to lactone **10** were unrewarding. In a typical instance, following dilution with water three neutral and two acidic products were obtained. While lactone **10** was not detected, one of the acidic products seemed (by thin layer chromatographic behavior) to be vinyl ether **11a**. Before this route to lactone **10** or acid **11a** could be improved, a more efficient alternative became available.

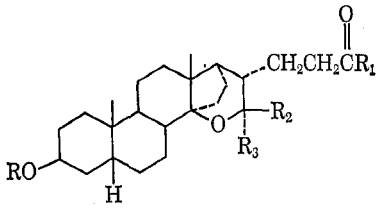
Equatorial acetal **8e**[1] was converted into alcohol **8i** by saponification, methylation, reaction with dihydropyran, and reduction with lithium aluminum hydride in 88% yield. The crystalline alcohol, upon reaction with methanesulfonyl chloride in pyridine, gave oily mesylate **8j**. Nucleophilic displacement of mesylate by reaction with sodium cyanide in dimethylformamide provided crystalline nitrile **8k** in 89% yield. On saponification in ethylene glycol containing potassium hydroxide followed by acidification, nitrile **8k** afforded acid **9d**, which in refluxing acetic acid–water was converted almost completely into vinyl ether **11a**. Elimination of methanol from acetal **9d** was also realized using *p*-toluenesulfonic acid in benzene. However, the acetic acid–water procedure was preferred. An alternative pathway to acid **11a** proceeded from dihydropyran **12a**.[1] The alcohol (**12b**) → mesylate (**12c**) → nitrile (**12d**) →

(7) Total synthesis of digitoxigenin (**6**) from, *e.g.*, 3β-acetoxy-17-oxo-5β-androstane, has been described by Sondheimer and colleagues; for leading references see ref 4.

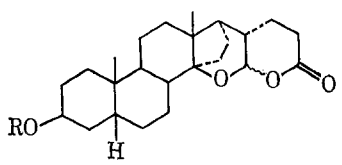
(8) O. Schindler and T. Reichstein, *Helv. Chim. Acta*, **39**, 1876 (1956).

(9) Consult G. R. Pettit, B. Green, G. L. Dunn, P. Hofer, and W. J. Evers, *Can. J. Chem.*, **44**, 1283 (1966), footnote 6, and G. R. Pettit, J. C. Knight, and W. J. Evers, *ibid.*, **44**, 807 (1966), for pertinen treferences to the unreactivity of acid halides toward diborane and reduction of lactones to hemiacetal derivatives by diborane.

(10) See, *e.g.*, M. S. Newman and P. F. Beal, *J. Amer. Chem. Soc.*, **72**, 5163 (1950); J. Klein and E. D. Bergmann, *J. Org. Chem.*, **22**, 1019 (1957).



9a, R = $COCH_3$; $R_1 = OCH_3$; $R_2 = R_3 = O$
b, R = $COCH_3$; R_1 = Cl; $R_2 = R_3 = O$
c, R = $COCH_3$; $R_1 = OCH_3$; $R_2 = R_3 = O$
d, R =  ; R_1 = OH; $R_2 = OCH_3$; R_3 = H
e, R =  ; $R_1 = OCH_3$; $R_2 = OCH_3$; R_3 = H
f, R = $COCH_3$; $R_1 = OCH_3$; $R_2 = OCH_3$; R_3 = H



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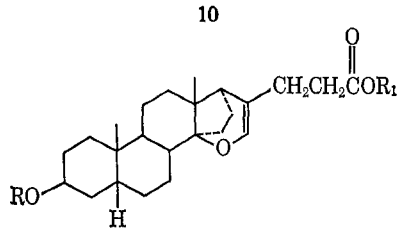
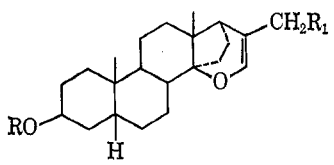


11a, R = R_1 = H
b, R = $COCH_3$; $R_1 = CH_3$
c, R = H; $R_1 = CH_3$
d, R = $COCH_3$; R_1 = H



12a, R =  ; $R_1 = CO_2CH_3$
b, R =  ; $R_1 = CH_2OH$
c, R =  ; $R_1 = CH_2OSO_2CH_3$
d, R =  ; $R_1 = CH_2CN$
e, R = H; $R_1 = CH_2CN$

carboxylic acid (**11a**) procedure again proved effective, and the corresponding 3β-acetoxy methyl ester **11b** was crystallized and characterized.

The final step necessary for interrelating digitoxigenin with bufalin through isobufalin was performed[11] by heating methyl ester **11b** and 2,3-dichloro-5,6-dicyano-benzoquinone in refluxing dioxane. After purification by chromatography, 36 mg of methyl ester **11b** yielded 25 mg of 3β-acetoxyisobufalin methyl ester (**4b**) identical with an authentic specimen prepared from bufalin. The total synthesis of isobufalin (**4c**) was thereby completed.

(11) We wish to thank Dr. A. D. Cross and Dr. J. A. Edwards for kindly providing us, prior to publication, with the experimental details of their procedure for dehydrogenating lactones with DDQ. In this regard refer to A. D. Cross, U. S. Patent 3,296,278 (1967); *Chem. Abstr.*, **66**, 6203 (1967); D. Bevlos, L. Cuellan, R. Grezemkovsky, M. V. Avila, and A. D. Cross, *Proc. Chem. Soc.*, 215 (1964); D. Walker and J. D. Hiebert, *Chem. Rev.*, **67**, 153 (1967).

Experimental Section[12]

3β-Acetoxyisobufalin Methyl Ester (4b).—To a solution of bufalin (**3**, 0.10 g) in dry methanol (5 ml) was added 5% sodium methoxide in methanol (5 ml). The clear solution was allowed to stand at room temperature for 12 hr. Following acidification with 1 *N* hydrochloric acid and dilution with water, the mixture was extracted with chloroform. The combined extract was washed with water. Removal of solvent gave a solid residue (**4a**, 0.10 g) which crystallized as needles, mp 210–213°, from acetone–diethyl ether. An analytical specimen with unchanged melting point displayed the following data: $[\alpha]_D$ −71° (*c* 0.41); RD (*c* 0.20) $[\alpha]_{350}$ −1000°, $[\alpha]_{400}$ −350°, $[\alpha]_{450}$ −210°, $[\alpha]_{500}$ −130°, $[\alpha]_{589}$ −100°, and $[\alpha]_{600}$ −100°; $\lambda_{max}^{cyclohexane}$ 293 mμ (ε 27,520); λ_{max}^{Nujol} 2.82, 5.90, 6.2, 6.26, 11.35, and 11.8 μ; pmr δ 1.0 (C-18 and -19 methyls), 3.73 (methyl ester), 4.13 (H-3α), 5.63 (doublet, *J* = 15 Hz, H-23), 6.58 (H-21), and 7.23 (doublet, *J* = 15 Hz, H-22).

Anal. Calcd for $C_{25}H_{36}O_4$: C, 76.96; H, 9.06; O, 15.98. Found: C, 74.48; H, 8.91; O, 16.42.

Isobufalin methyl ester (**4a**, 0.46 g) was acetylated and the crude product was chromatographed on basic alumina (12 g). Elution with 1:1 hexane–benzene gave **3β-acetoxyisobufalin methyl ester (4b)**. Crystallization from methanol–acetone afforded 0.40 g as small plates: mp 173–175°; RD (*c* 0.48) $[\alpha]_{350}$ −1219°, $[\alpha]_{400}$ −403°, $[\alpha]_{450}$ −252°, $[\alpha]_{500}$ −149°, $[\alpha]_{589}$ −83°, and $[\alpha]_{600}$ −83°; $\lambda_{max}^{cyclohexane}$ 293 mμ (ε 27,120); $\lambda_{max}^{CHCl_3}$ 5.78, 5.81, 6.24, 7.91, and 8.59 μ; pmr δ 1.0 (C-18 and -19 methyls), 2.05 (C-3 acetate), 3.73 (methyl ester), 5.09 (H-3α), 5.63 (d, *J* = 15 Hz, H-23), 6.59 (H-21), and 7.23 (d, *J* = 15 Hz, H-22).

Anal. Calcd for $C_{27}H_{38}O_5$: C, 73.27; H, 8.65; O, 18.07. Found: C, 73.52; H, 8.68; O, 17.19.

Isobufalin (4c).—To isobufalin methyl ester (**4a**, 0.18 g) in warm ethanol (45 ml) was added hot 2 *N* sodium hydroxide solution (45 ml). The mixture was heated on the steam bath for 10 min, water (90 ml) was added, and heating was continued for another 10 min. After cooling, the mixture was acidified to *ca.* pH 6 with 1 *N* sulfuric acid. The crystals, mp 200–210°, which separated were collected and washed with water. Recrystallization from dioxane gave a pure sample of isobufalin as large needles: mp 212–215° (sintering from 205°); $[\alpha]_D$ −63° (*c* 0.32); RD (*c* 0.48) $[\alpha]_{350}$ −1000°, $[\alpha]_{400}$ −438°, $[\alpha]_{450}$ −250°, $[\alpha]_{500}$ −156°, $[\alpha]_{589}$ −125°, and $[\alpha]_{600}$ −125°; λ_{max}^{KBr} 2.94 (broad), 5.86, 6.17, 8.5, 9.57, and 11.76 μ; pmr δ 1.00 (C-18 and -19 methyls), 5.21 (2 H),[13] 5.63 (doublet, *J* = 15 Hz, H-22), 6.63 (H-21), and 7.31 (doublet, *J* = 15 Hz, H-23).

Anal. Calcd for $C_{24}H_{34}O_4$: C, 74.58; H, 8.87; O, 16.56. Found: C, 74.10; H, 9.11; O, 16.62.

Methylation of isobufalin using ethereal diazomethane gave exclusively isobufalin methyl ester (**4a**).[14]

Methyl 3β-Acetoxy-14β,21-epoxy-20 ζ-nor-5β-cholanate (5).—A mixture of isobufalin methyl ester (**4a**, 0.15 g) in methanol (15 ml)–tetrahydrofuran (5 ml) containing suspended platinum from platinum oxide (0.075 g) was stirred under a slight positive pressure of hydrogen for *ca.* 8 hr. The solution was filtered and collected catalyst was washed with diethyl ether. Removal of solvent from the filtrate gave an oily residue which was partially purified by filtration in benzene through basic alumina (5 g). Attempts to induce crystallization were unsuccessful and an

(12) Bufalin was used as received from Aldrich Chemical Co., Milwaukee, Wis. Unless otherwise stated, the introduction to the Experimental Section of part 8[1] provides necessary general information for the following experimental summaries.

(13) The δ 5.21 signal disappears upon shaking the deuteriochloroform solution with deuterium oxide. In three different determinations the signal shifted from δ 5.91 to 6.47 and therefore appeared concentration dependent. As no signal corresponding to the carboxyl proton appeared in the spectrum from δ 8 to 15 the signal at δ 5.21 was tentatively assigned to the 3β-hydroxy and carboxyl proton.

(14) Confirmation of identical composition was obtained by results of thin layer chromatographic, proton magnetic resonance, and infrared spectral (in potassium bromide) comparison.

analytical sample was prepared by preparative layer chromatography with 1:1 hexane–ethyl acetate mobile phase and evaporative distillation at 140–150° (bath temperature) and 0.3 mm: $[\alpha]_D$ +25° (*c* 0.52); λ_{max}^{neat} 5.78, 8.0, 8.13, 8.59, and 9.80 μ; pmr δ 0.99 and 1.09 (C-18 and -19 methyls), 2.02 (C-3 acetate), 3.65 (methyl ester), and 5.09 (H-3α).

Anal. Calcd for $C_{27}H_{42}O_5$: C, 72.61; H, 9.48; O, 17.91. Found: C, 72.93; H, 9.16; O, 17.88.

Methyl 3β-Acetoxy-14β,21-epoxy-21-oxonor-5β-(20*S*)-cholanate (8c). Method A.—The digitoxigenin (6) → isodigitoxigenin (7) → isodigitoxigeninic acid (8a) → methyl 3β-(21*S*)-diacetoxy-14β,21-epoxynor-5β-(20*S*)-cholanate (8b) sequence was repeated as previously reported.[1] Diacetate 8b in 0.20-g portions was oxidized with 2% chromium trioxide in glacial acetic acid essentially as summarized by Schindler and Reichstein.[8] A solution of the crude product in chloroform was passed through a column of basic alumina (20 g). Following removal of solvent, the residue was recrystallized from diethyl ether–acetone–hexane to yield lactone 8c (70%), mp 135–138° (lit.[8] mp 145–148°). Several attempts to perform chromium trioxide oxidation of diacetate 8b on a scale larger than 0.20 g afforded lesser yields of lactone 8c. Accordingly, larger quantities of lactone 8c prepared using methods A or B were obtained using a series of 0.20-g scale oxidations.

Method B.—Isodigitoxigenin (7) was transformed to equatorial acetal 8d as previously summarized.[1] A solution of acetal 8d (0.15 g) in glacial acetic acid (2 ml) was treated with 2% chromium trioxide in glacial acetic acid (2 ml) and the mixture was allowed to remain at room temperature for 4 hr. Excess oxidizing agent was destroyed in the violet solution by adding methanol. After a 12-hr period at room temperature, most of the solvent was removed *in vacuo* at 35° and the residue was diluted with 0.1 *N* sulfuric acid (50 ml) and chloroform (30 ml). The mixture was extracted with chloroform and the combined solvent extract was washed successively with water, dilute sodium bicarbonate, and water. Passage of the chloroform solution through a column of basic alumina (10 g) and removal of solvent gave 0.13 g of semisolid. Preparative layer chromatography with 1:4 hexane–ethyl acetate mobile phase gave 0.08 g of lactone 8c. Recrystallization from diethyl ether–acetone–hexane provided needles: mp 137–139° (a mixture melting point with lactone 8c prepared by method A was 138–140°); pmr δ 1.02 and 1.12 (C-18 and -19 methyls), 2.05 (C-3 acetate), 3.72 (methyl ester), and 5.09 (H-3α).

By using the procedure just described, equatorial acetal 8e (0.10 g) was also oxidized to lactone 8c. Purification by preparative layer chromatography afforded 0.02 g, mp 136–138°. Specimens of lactone 8c obtained by methods A and B were mutually identical.[14]

Methyl 3β-Acetoxy-14β,21-epoxy-21-oxo-5β-(20*S*)-cholanate (9a).—In a typical experiment, methyl 3β-acetoxy-14β,21-epoxy-21-oxonor-5β-(20*S*)-cholanic acid was saponified with 5% potassium hydroxide in methanol (5 hr at reflux) and the crude product was acetylated with 1:5 acetic anhydride–pyridine overnight at room temperature. The acetylation mixture was poured onto ice and the pH was adjusted to *ca.* 5 with 2 *N* hydrochloric acid. Before extraction with chloroform, the mixture was allowed to remain at room temperature for 15 min to hydrolize mixed anhydride. By removal of solvent *in vacuo* and recrystallization of the crude product from diethyl ether, a sample of 3β-acetoxy acid 8f, mp 238–240°, was obtained. A 0.40-g specimen of acid 8f in methanol was neutralized with an equivalent quantity of sodium methoxide in methanol. Solvent was removed at room temperature and the residue was dried for 16 hr at 80° (20 mm), powdered, and redried for 3 hr at 100° (0.1 mm). A suspension of the sodium salt in dry benzene was stirred in a nitrogen atmosphere and cooled until part of the solvent crystallized. At this point, oxalyl chloride[15] (10% excess) in benzene was added over a period of 30 min, while the reaction temperature was maintained at 5–10° so that the benzene phase was partially frozen. Before addition of dry collidine (4 μl) and additional oxalyl chloride (0.1 ml), stirring was continued at room temperature for 30 min. Fifteen minutes later, solvent was evaporated at 25°. A solution of acid chloride 8h in benzene was slowly added to excess diazomethane in diethyl ether. The reaction mixture was allowed to remain at *ca.* 0° for 36 hr. Evaporation of the solvent and excess diazomethane gave a residue which was dissolved in superdry methanol (10 ml), and a solution of freshly prepared (and dry) silver benzoate (0.3 g) in dry triethylamine (3 ml) was added. After a lapse of 45 min, 23 ml of nitrogen was evolved. Stirring was continued for a total of 1 hr, at which time evolution of nitrogen appeared complete. Solvent was removed at 30° and the residue in benzene was passed through a column of neutral alumina (20 g, E. Merck, Darmstadt). Elution with either benzene or diethyl ether gave a fraction (0.35 g), which was further purified by preparative layer chromatography with 1:4 hexane–ethyl acetate mobile phase. The least polar zone was eluted with chloroform to yield 0.26 g of semisolid, which crystallized from acetone–hexane. Recrystallization from the same solvent gave 0.19 g, mp 118–121°. Final purification was achieved by chromatography of the ester in diethyl ether on basic alumina (1 g) and recrystallization of a fraction eluted with the same solvent from hexane–diethyl ether. By this means a crystalline, analytical sample of lactone 9a, mp 130–132°, was prepared: pmr δ 0.98 (C-18 methyl), 1.04 (C-19 methyl), 1.98 ($OCOCH_3$), 2.28 (multiplet, C-22 and C-23 methylene), 3.56 (OCH_3), and 4.92 (H-3α).

Anal. Calcd for $C_{27}H_{40}O_6$: C, 70.39; H, 8.75. Found: C, 70.28; H, 9.02.

Methyl 3β-Tetrahydropyranyloxy-14β,21-epoxy(21*S*)-methoxy-23-hydroxy-5β-(20*S*)-norcholane (8i).—A solution of methyl 3β-tetrahydropyranyloxy-14β,21-epoxy-(21*S*)-methoxy-5β-(20*S*)-norcholanate (7.55 g)[1] in dry diethyl ether (100 ml) was added over a 30-min period to a cold (ice bath) mixture of lithium aluminum hydride (3.0 g) and dry diethyl ether (600 ml). Stirring at ice-bath temperature was continued for 2.5 hr. Excess lithium aluminum hydride was removed by cautious addition of ice–water and the ethereal layer was separated. The aqueous phase was extracted with diethyl ether and the combined etheral extract was washed with water. Evaporation of the ether gave a colorless oil which slowly solidified. Recrystallization of the residue from acetone–ligroin afforded alcohol 8i as large prisms (4.34 g). Concentration of mother liquors provided 3.2-g of a pale brown oil. The mother liquor residue in benzene was chromatographed on basic alumina (200 g). Elution with the same solvent gave an additional 1.8 g of alcohol 8i. An analytical specimen recrystallized from acetone–pentane as thick, rectangular plates: mp 149–151°, $[\alpha]_D$ + 187° (*c* 0.24); $\lambda_{max}^{CHCl_3}$ 2.06 μ; pmr δ 0.98 and 1.06 (C-18 and -19 methyls), 3.50 (C-21 methoxy), 4.0 (pyranyl ether acetal proton), 4.30 (doublet, J = 8 Hz, H-21), and 4.66 (H-3α).

Anal. Calcd for $C_{29}H_{48}O_5$: C, 73.07; H, 10.15; O, 16.78. Found: C, 73.34; H, 10.13; O, 16.27.

Methyl 3β-Tetrahydropyranyloxy-14β,21-epoxy-(21*S*)-methoxy-23-cyano-5β-(20*S*)-norcholane (8k).—To a solution of alcohol 8i (6.0 g) in pyridine (20 ml) was added at 0° with stirring methanesulfonyl chloride (3.0 g) in pyridine (5 ml). Before dilution with diethyl ether, stirring was continued for 3 hr at ice-bath temperature. The ethereal solution was repeatedly washed with water and concentrated to a pale yellow oil with no appreciable infrared hydroxyl absorption. A solution of the oily residue in 1:1 ligroin–benzene was chromatographed on basic alumina. Elution with the same solvent gave 5.85 g of mesylate 8j as a colorless oil that crystallized on standing. Without further purification the mesylate (5.85 g) was dissolved in dimethylformamide (100 ml). The solution was stirred at room temperature and sodium cyanide (2.4 g) was added. Stirring was continued for 22 hr and the pale yellow solution was diluted with water, cooled, and filtered. The white solid was crystallized from acetone–water to give nitrile 8k as colorless needles (4.6 g): mp 175–177° after three recrystallizations from the same solvent; $[\alpha]_D$ +35° (*c* 1.05); RD (*c* 1.40) $[\alpha]_{400}$ +54°, $[\alpha]_{450}$ +43°, $[\alpha]_{500}$ +36°, $[\alpha]_{589}$ +22°, $[\alpha]_{600}$ +22°; $\lambda_{max}^{CHCl_3}$ 4.42 μ; pmr δ 0.98 and 1.08 (C-18 and -19 methyls), 3.48 (C-21 methoxyl), 4.0 (tetrahydropyranyl acetal proton), 4.26 (doublet, J = 8 Hz, H-21), and 4.68 (H-3α).

Anal. Calcd for $C_{30}H_{47}NO_4$: C, 74.19; H, 9.75; N, 2.88; O, 13.18. Found: C, 74.41; H, 9.81; N, 3.02; O, 12.77.

3β-Tetrahydropyranyloxy-14β,21-epoxy-(21*S*)-methoxy-5β-(20*S*)-cholanic Acid (9d).—A solution of nitrile 8k (4.56 g) and potassium hydroxide (14 g) in ethylene glycol (140 ml) was heated at reflux and stirred in a nitrogen atmosphere for 3 hr. Upon cooling, the clear, pale yellow solution was diluted with water and acidified with concentrated hydrochloric acid. The aqueous mixture was extracted with diethyl ether and the combined extract was concentrated to an oil. Trituration with acetone caused slow crystallization to yield 4.25 g of acid 9d: pmr δ 0.98

(15) Commercial oxalyl chloride was heated at reflux for 10 min and then distilled from freshly fused and powdered potassium carbonate. The redistilled oxalyl chloride was stored over anhydrous potassium carbonate.

and 1.06 (C-18 and -19 methyls), 3.48 (C-21 methoxyl), 4.0 (tetrahydropyranyl acetal proton), 4.27 (doublet, J = 8 Hz, H-21), 4.70 (H-3α), and 9.33 (carboxylate proton). The acid (0.15 g) was characterized as the methyl ester, prepared using diazomethane. The resulting ester **9e** was purified by chromatography in hexane on basic alumina (4 g). Elution with 1:3 hexane–benzene gave a solid fraction (0.1 g). Recrystallization from acetone–hexane afforded methyl ester **9e** as needles: mp 123–125°; $[\alpha]_D$ +88° (c 0.50); $\lambda_{max}^{CHCl_3}$ 5.78 μ; pmr δ 0.97 and 1.03 (C-18 and -19 methyls), 3.44 (C-21 methoxyl), 3.66 (methyl ester), 3.96 (tetrahydropyranyl acetal proton), 4.26 (doublet, J = 8 Hz, H-21), and 4.67 (H-3α).

Anal. Calcd for $C_{31}H_{50}O_6$: C, 71.78; H, 9.72; O, 18.51. Found: C, 71.66; H, 9.42; O, 19.02.

Tetrahydropyranyloxy methyl ester **9e** was converted into the 3β-acetate **9f** as follows. To a solution of ester **9e** (1.1 g) in methanol (50 ml) was added water (1 ml) and p-toluenesulfonic acid (0.10 g). After having been stirred at room temperature for 3.25 hr, the solution was diluted with water and extracted with diethyl ether. Concentration of the ether layer gave an oil which was held *in vacuo* for 2 hr at 60° and then dissolved in a mixture of acetic anhydride (5 ml)–pyridine (5 ml). The solution was allowed to stand at room temperature overnight, diluted with ice–water, and extracted with ether. The ethereal layer was washed with 2 N hydrochloric acid and saturated sodium bicarbonate solution and evaporated. Crystallization of the residue from aqueous methanol gave **3β-acetoxy methyl ester 9f** as fine needles (first crop 0.32 g), mp 108–110°, $[\alpha]_D$ +20.7° (c 1.11).

Anal. Calcd for $C_{28}H_{44}O_6$: C, 70.55; H, 9.31. Found: C, 70.58; H, 9.34.

3β-Tetrahydropyranyloxy-14β,21-epoxy-23-cyano-5β-norchol-20(21)-ene (12d).—A sample of methyl 3β-tetrahydropyranyloxy-14β,21-epoxy-5β-norchol-20(21)-enate (**12a**, 3.5 g) prepared as noted in part 8[1] was reduced in diethyl ether (600 ml) solution with lithium aluminum hydride (1.5 g) as summarized above for obtaining alcohol **8i**. The colorless, oily sample of alcohol **12b** weighed 3.3 g and exhibited a single spot upon thin layer chromatography with 1:4 ethyl acetate–chloroform mobile phase: pmr 0.98 (C-18 methyl), 1.04 (C-19 methyl), 3.98 (THP-yl acetal H), 5.16 (H-3), and 5.94 (H-21). Allowing the oily alcohol (**12b**, 3.3 g) in pyridine (20 ml) to react with methanesulfonyl chloride (1.6 g) in benzene (10 ml) as summarized in the case of sulfonate **8j** afforded mesylate **12c** as a pale yellow, viscous oil (3.4 g) displaying no hydroxyl absorption in the infrared spectrum. As with alcohol **12d**, further purification of mesylate **12c** by column chromatography on basic alumina again gave a product resistant to crystallization. However, the now colorless oily mesylate was sufficiently pure for conversion into nitrile **12d**. Mesylate **12c** (3.4 g) in dimethylformamide (50 ml) was treated with sodium cyanide (1.5 g) as summarized above for the preparation of nitrile **8k**. In this experiment the crude product in ligroin was chromatographed on silica gel. A 1.0-g fraction eluted by 19:1 ligroin–ethyl acetate corresponded to nitrile **12d** and displayed one spot on a thin layer chromatogram with 1:39 ethyl acetate–chloroform mobile phase. A pure sample recrystallized from acetone–water or from pentane as platelets: mp 136–138°; $[\alpha]_D$ −46° (c 0.30); λ_{max}^{Nujol} 4.44 and 6.24 μ; pmr δ 1.0 and 1.04 (C-18 and -19 methyls), 2.32 (multiplet, C-22 and C-23 methylenes), 4.0 (tetrahydropyranyl acetal proton), 4.14 (H-3α), and 6.0 (H-21).

Anal. Calcd for $C_{29}H_{43}NO_3$: C, 76.78; H, 9.55; N, 3.09; O, 10.58. Found: C, 76.94; H, 9.71; N, 3.23; O, 10.42.

Further elution of the silica gel column with ethyl acetate provided the corresponding 3β-hydroxy derivative **12e** (0.50 g): λ_{max}^{neat} 2.90–2.98, 4.42, and 6.02 μ; pmr δ 1.02 and 1.06 (C-18 and -19 methyls), 4.15 (H-3α), and 5.99 (H-21). Removal of the pyranyloxy group from nitrile **12d** (1.0 g) was achieved by dissolution in methanol (80 ml)–water (1 ml) containing p-toluenesulfonic acid (0.10 g). After the solution had been stirred for 3 hr at room temperature, essentially quantitative conversion into alcohol **12e** was realized. The glassy alcohol **12e** was combined with the 0.5-g quantity and hydrolyzed to hydroxy acid **11a** as outlined in the following experiment.

Methyl 3β-Acetoxy-14β,21-epoxy-5β-chol-20(21)-enate (11b).—A solution of hydroxy nitrile **12e** (1.55 g) was hydrolyzed with potassium hydroxide (4.5 g) in ethylene glycol (50 ml, redistilled from potassium hydroxide) as summarized above with nitrile **8k** (see **9c**). A colorless, viscous, oily specimen of acid **11a** (1.38 g) was obtained: pmr δ 0.98 and 1.02 (C-18 and -19 methyls), 2.28 (multiplet, C-22 and C-23 methylene), 4.16 (H-3α), 5.90 (H-21), and 5.90 (broad) (carboxylate disappeared on addition of D_2O). No signals appeared further downfield.

Hydroxy acid **11a** was methylated with ethereal diazomethane and acetylated. The product was chromatographed on basic alumina (5 g). Elution with hexane–benzene (3:1) led to oily methyl ester **11b** (0.1 g), which crystallized from methanol as prisms: mp 104–106°; $[\alpha]_D$ −17.4° (c 0.86); RD (c 1.05) $[\alpha]_{300}$ −119°, $[\alpha]_{350}$ −76°, $[\alpha]_{400}$ −52°, $[\alpha]_{450}$ −38°, $[\alpha]_{500}$ −26°, $[\alpha]_{589}$ −21°, and $[\alpha]_{600}$ −21°; γ_{max}^{KBr} 1742, 1662, and 1255 cm^{-1}; pmr δ 1.07 (C-18 and -19 methyls), 2.07 (C-3 acetate), 3.67 (methyl ester), 5.1 (H-3α), and 5.89 (H-21).

Anal. Calcd for $C_{27}H_{40}O_5$: C, 72.94; H, 9.07; O, 17.99. Found: C, 72.54; H, 9.18; O, 18.06.

Conversion of Methyl 3β-Tetrahydropyranyloxy-14β,21-epoxy-(21S)-methoxy-5β-(20S)-cholanate (9d) into Derivatives of 3β-Hydroxy-14β,21-epoxy-5β-chol-20(21)-enic Acid (11a).—A solution of acetal **9d** (0.10 g) in benzene (10 ml) containing p-toluenesulfonic acid (0.02 g) was heated at reflux for 1.5 hr. After cooling, the solution was diluted with diethyl ether and washed with water, dilute sodium bicarbonate, and water. Following removal of solvent the brown oily residue was purified by preparative layer chromatography with 1:9 ethyl acetate–chloroform mobile phase. Several bands were detected, three of which appeared dark under ultraviolet light. The largest zone did not absorb ultraviolet light, and upon elution with diethyl ether gave 0.016 g of oily dihydropyran **11c**. Vinyl ether **11a** could be conveniently prepared by heating for 30 min at reflux a solution prepared from acid **9d** (3.09 g) and acetic acid (100 ml)–water (50 ml). Acid **11a** was isolated by ether extraction as an oil, which was acetylated using 1:1 acetic anhydride–pyridine (20 ml) at steam-bath temperature for 15 min to give acetoxy acid **11d** (2.78 g). Methylation with diazomethane gave acetoxy methyl ester **11b** which was in every way identical[14] with the product prepared from the nitrile **12e** as described above.

Conversion of Methyl 3β-Acetoxy-14β,21-epoxy-5β-chol-20(21)-enate to 3β-Acetoxyisobufalin Methyl Ester (4b).—A solution of ester **11b** (0.036 g) and 2,3-dichloro-5,6-dicyanobenzoquinone (0.030 g) in dry dioxane (5 ml) was heated at reflux for 20 hr. Following cooling the mixture was diluted with methylene chloride and the solid phase was collected and washed with additional methylene chloride. The combined filtrate was passed through a column of neutral alumina (3 g). Removal of solvent from the methylene chloride eluate provided an 0.025-g residue which crystallized as needles, mp 172–174°, from methanol–acetone. A mixture melting point with an authentic sample prepared from bufalin (see **4b**) of 3β-acetoxyisobufalin methyl ester was undepressed. The mutual identity of both specimens was confirmed by comparing ultraviolet, infrared, optical rotatory dispersion, and proton magnetic resonance spectra. In each case, spectra of the methyl ester **11b** dehydrogenation product were superimposable upon those of 3β-acetoxyisobufalin methyl ester prepared from bufalin.

Registry No.—**4a**, 23337-64-6; **4b**, 23337-65-7; **4c**, 23337-66-8; **5**, 23337-67-9; **8c**, 23337-68-0; **8i**, 23337-69-1; **8k**, 23337-70-4; **9a**, 23337-71-5; **9e**, 23337-72-6; **9f**, 23359-80-0; **11b**, 17150-46-8; **12d**, 23337-73-7; **12e**, 23337-74-8.