(150 g). Continued elution with the same solvent resulted in some (0.45 g) recovery of aldehyde **12e**. Elution with 9:1 hexane–ethyl acetate afforded methyl ester **15** (1.5 g). An analytical sample was obtained by preparative chromatography on ChromAR 1000 with 17:3 hexane–ethyl acetate development (band eluted with ether) followed by crystallization from ethyl acetate–hexane: mp 123–126°; ν_{max} 2960, 1740 (broad), 1390, and 1260 cm^{-1}; pmr δ 0.62 (singlets, 3 H, C-18 methyl of C_{20} epimers), 0.7, 0.8 (3 H, C-19 methyl), 2.05 (3 H, acetate), 3.65 (3 H, methyl ester), and 9.5 (d, J = 4 cps, aldehyde).

Anal. Calcd for $C_{27}H_{42}O_5$: C, 72.61; H, 9.48. Found: C, 72.91; H, 9.26.

In somewhat larger scale experiments, molecular sieve type 4A (3.5 g/3 g of aldehyde **7e**) was employed in place of anhydrous potassium carbonate with comparable results. The crude product in 2:1 pentane–benzene was chromatographed on a column of silica gel (250 g/3 g of starting aldehyde). Fractions eluted by 9:1 pentane–ethyl acetate contained aldehyde **12e** and those elated with 17:3 pentane–ethyl acetate contained methyl ester **15**. Yields of methyl ester **15** ranged from 45 to 49%.

3β-Acetoxy-5α-14α-buf-20(21)-enolide (16).—To a solution of methyl ester **15** (0.55 g) in tetrahydrofuran (15 ml)–methanol (6 ml) was added 10 ml of 5% aqueous sodium carbonate. The mixture was stirred at room temperature for 3 hr, neutralized with 6 *N* hydrochloric acid and concentrated to *ca.* 10 ml using a rotating evaporator. The aqueous phase was acidified with 6 *N* hydrochloric acid and extracted with ethyl acetate. The combined ethyl acetate extract was extracted with 10% aqueous potassium carbonate. Next, the combined aqueous solution was acidified with 6 *N* hydrochloric acid and extracted with ethyl acetate. The ethyl acetate extract was washed with water and evaporated to yield 0.38 g (72%) of colorless, crystalline carboxylic acid exhibiting one spot on a thin layer chromatogram with 4:1:0.2 pentane–ethyl acetate–acetic acid mobile phase. A specimen (0.53 g) prepared in the same manner was dissolved in dry benzene (50 ml) containing *p*-toluenesulfonic acid (0.06 g). The solution was heated at reflux for 25 hr employing a Dean–Starke trap containing molecular sieve type 4-A. The solution was cooled and added to a column of silica gel (7 g). Elution with benzene (400 ml) gave 0.37 g (73%) of colorless crystals, mp 181–184°. The product **16** appeared as a single spot on a thin layer chromatogram with 4:1 pentane–ethyl acetate mobile phase. Recrystallization from ethyl acetate–hexane afforded an analytical sample as needles: ν_{max} 2940, 1760 (enol lactone carbonyl), 1740 (acetate carbonyl), 1670 (olefin), 1260, and 1140 cm^{-1} (doublet); RD (25°, *c* 0.515) $[\alpha]_{420}$ 0° (slightly negative 420–650°), $[\alpha]_{350}$ +27.2°, $[\alpha]_{320}$ +58.3°, and $[\alpha]_{300}$ +166.9°; pmr δ 0.60 (3 H, C-18 methyl), 0.83 (3 H, C-19 methyl), 2.05 (3 H, acetate), and 6.36 (broad, 1 H, H-21).

Anal. Calcd for $C_{26}H_{38}O_4$: C, 75.32; H, 9.24. Found: C, 75.27; H, 8.99.

3β-Acetoxy-5α-14α-bufa-20,22-dienolide (17).—An intimate mixture of enol lactone **16** (0.10 g) and sulfur (0.20 g) was heated at 221–227° under a nitrogen atmosphere for 0.5 hr. After 1 min in the required temperature range, evolution of hydrogen sulfide was detected using moist lead acetate paper and by odor. After cooling, the mixture was dissolved in carbon disulfide. A thin layer chromatogram with 4:1 pentane–ethyl acetate mobile phase indicated a major component accompanied by a lesser quantity of starting material **16** and a more polar side product. The carbon disulfide solution was chromatographed on a column of silica gel (20 g). The oily fraction (**17**) eluted by 2:1 benzene–ether weighed 0.06 g (60%) and was essentially pure by tlc. The analytical sample was further purified by preparative tlc on ChromAR 1000 with 10:1 pentane–ethyl acetate mobile phase and recrystallized twice from methanol to afford needles: mp 194–195°; λ_{max} 300 mμ (ϵ 5500); ν_{max} 1740, 1640, 1540, 1250, 835, and 800 cm^{-1}; pmr δ 0.53 and 0.83 (C-18 and -19 methyls), 4.7 (diffuse, H-3α), 6.25 (d, J = 10.5 cps, H-23), and 7.20–7.41 (complex, 2-pyrone ring protons).[30]

Anal. Calcd for $C_{26}H_{38}O_4$: C, 75.69; H, 8.80; mol wt, 412. Found: C, 75.75; H, 9.03; mol wt, 412 (mass spectrum).

Registry No.—**2a**, 23079-69-8; 2,4-dinitrophenylhydrazone of **2a**, 23330-09-8; **3**, 23079-70-1; **4**, 23330-10-1; **5**, 23079-71-2; **6a**, 2312-10-9; **6b**, 23330-13-4; **7a**, 23330-14-5; **7b**, 23330-15-6; **7d**, 23330-16-7; **7e**, 16934-54-6; **8a**, 23367-52-4; **9**, 23017-35-8; **11**, 23330-19-0; **12a**, 23017-30-3; **12e**, 23017-32-5; **15**, 23017-33-6; **16**, 23017-34-7; **17**, 23017-36-9; 3β-acetoxy-20-oxo-21-diazo-5α-pregnane, 23330-24-7.

(30) Decoupling experiments showed the doublet at δ 6.18 coupled to the δ 7.20–7.41 signals and further supported the structural assignment.

Bufadienolides. 8. 12(13→14)*abeo* Skeletal Rearrangements[1]

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Several methods were developed for converting isodigitoxigenin (**2a**) into methyl acetals **4b** and **4c**. Of these, methanolysis (followed by acetylation) of isodigitoxigenin in the presence of *p*-toluenesulfonic acid proved most useful. Each isomer reached an equilibrium corresponding to *ca.* 3:1 acetal **4c** to **4b** within 15 min in benzene containing *p*-toluenesulfonic acid. Addition of dihydropyran to the equilibrium mixture resulted in excellent conversion into vinyl ether **5a**. Heating either acetal **4b** or **4c** in benzene containing *p*-toluenesulfonic acid led to a skeletal rearrangement culminating in formation of C-norcardenolide **6**. In addition to results of physical measurements, the structure of spiran **6** was confirmed by degradation to methyl ketone **8**. Similar rearrangement of isodigitoxigenin gave spiran **9** accompanied by C-norcardenolide **6**. Treating lactone **9** with *p*-toluenesulfonic acid in methanol–water provided acetals **10a** and **10b**, which on further contact with *p*-toluenesulfonic acid in refluxing benzene gave lactone **9** and cardenolide **6**. Evidence underlying the stereochemical assignments noted for structures **4**, **9**, and **10** was also discussed.

Selection of digitoxigenin (**1a**) as a starting point for total synthesis of isobufalin and bufalin required a number of accessory experiments. Protection of the 14-oxygen substituent during reconstruction of the digitoxigenin lactone ring seemed best performed by utilizing isodigitoxigenin (**2a**), which could be converted to hemiacetal **4a**. Model experiments could then be undertaken to determine the direction of cleavage reactions which might be anticipated with acetals such as **2b** and **4c**. Accordingly, digitoxin (**1b**)[3a] was con-

(1) (a) Part 7: G. R. Pettit, D. C. Fessler, K. Paull, P. Hofer, and J. C. Knight, *J. Org. Chem.*, **35**, 1398 (1970). This investigation was supported by Public Health Service Research Grants CA-04074-07, CA-10115-01, and CA-10115-02 from the National Cancer Institute. Summaries, in part, of the present investigation have been presented: (b) T. R. Kasturi, G. R. Pettit, and J. Occolowitz, *Chem. Commun.*, 334 (1967); (c) G. R. Pettit, J. C. Knight, and T. R. Kasturi, *ibid.*, 688 (1967).

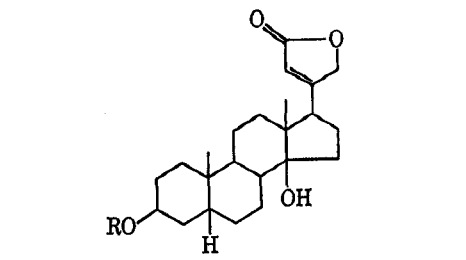
(2) On sabbatical leave from the Indian Institute of Science, Bangalore, India.

(3) (a) M. Kuhn, H. Lichti, and A. von Wartburg, *Helv. Chim. Acta*, **45**, 881 (1962); (b) S. Rangaswami and T. Reichstein, *ibid.*, **32**, 939 (1949); (c) N. Danieli, V. Mazur, and F. Sondheimer, *Tetrahedron*, **22**, 3189 (1966).

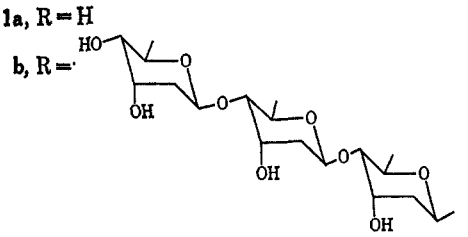
verted[3b] *via* digitoxigenin (**1a**)[3c] into isodigitoxigenin (**2a**).[4a]

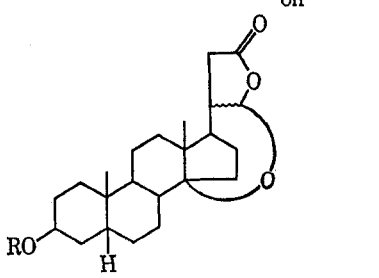
Next, isodigitoxigenin was saponified and methylated with diazomethane to yield the methyl ester of isodigitoxigeninic acid (**4a**) as described by Reichstein.[4b] When hemiacetal **4a** was allowed to react with methanol containing 48% hydrobromic acid, acetals **4b** and **4c** were obtained following acetylation and chromatographic separation. By another route, hemiacetal **4a** was acetylated[4b] to yield acetate **4d** and the latter, upon contact[5] with methanol, gave acetal **4b** with inversion at C-21. Treatment of lactone **2a** with refluxing aqueous methanol[6] containing a catalytic amount of *p*-toluenesulfonic acid and acetylation of the product furnished the most convenient route to acetals **4b** and **4c**. Following separation by column chromatography, acetals **4c**, mp 147–149°, and **4b**, mp 194–199°, were obtained. The acid-catalyzed methanol technique was comparably effective for converting isodigitoxigeninic acid (**4e**), easily isolated from the mother liquors[4a] remaining from preparation of isodigitoxigenin, into the isomeric acetals. Each of the foregoing procedures gave identical specimens of the acetals (**4b** and **4c**), and elemental analyses and pmr and infrared spectra were completely consistent with the assigned structures.

The not unequivocal stereochemical assignments for acetals **4b** and **4c** were based on the following evidence. The H-20–H-21 coupling constant for the 21 proton of acetal **4c** was 8 Hz, while in acetal **4b** a value of J = 5.5 Hz was observed. With at least a quasichair conformation for the pyran ring, the larger coupling constant would be assigned to the *trans* vicinal protons of acetal **4c** and the smaller value to *cis* protons of **4b**.[7] Support[8] for the epimeric nature of acetals **4b** and **4c** was obtained by treating each in benzene with *p*-toluenesulfonic acid. Within 15 min both isomers gave an equilibrium mixture containing *ca.* 3:1 isomer **4c** to **4b**. One hour after adding dihydropyran to either mixture, essentially complete conversion into vinyl ether **5a** occurred. Similarly, when equatorial acetal **4c** was saponified and remethylated and the product was dissolved in benzene and treated with *p*-toluenesulfonic acid and dihydropyran, pyranyl ether **5b** was obtained, accompanied under these conditions by acetal **4f**. For large-scale preparation of pyranyl ether **4f** it was found most convenient to treat the crude epimer mixture obtained by methanolysis of isodigitoxigenin, directly with dihydropyran and *p*-toluenesulfonic acid in benzene. Chromatographic separation on silica gel gave **4f**, accompanied by smaller amounts of axial epimer **4g**. The most significant feature in the pmr spectrum of dihydropyran **5a** was a sharp singlet at δ 5.97 attributable to the 21-vinyl proton.



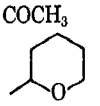
1a, R = H

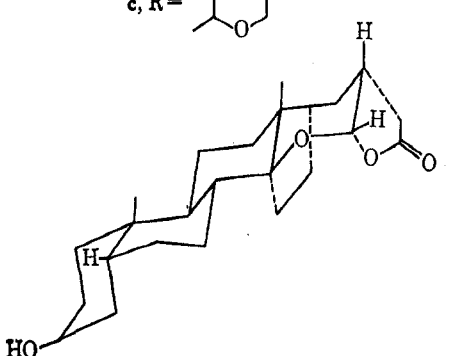
b, R = 



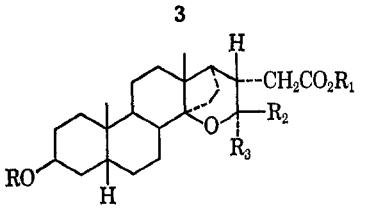
2a, R = H

b, R = $COCH_3$

c, R = 



3



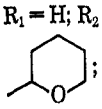
4a, R = H; R_1 = CH_3; R_2 or R_3 = OH or H

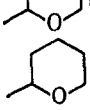
b, R = $COCH_3$; R_1 = CH_3; R_2 = H; R_3 = OCH_3

c, R = $COCH_3$; R_1 = CH_3; R_2 = OCH_3; R_3 = H

d, R = $COCH_3$; R_1 = CH_3; R_2 = $OCOCH_3$; R_3 = H

e, R = R_1 = H; R_2 or R_3 = OH or H

f, R =  ; R_1 = CH_3; R_2 = OCH_3; R_3 = H

g, R =  ; R_1 = CH_3; R_2 = H; R_3 = OCH_3

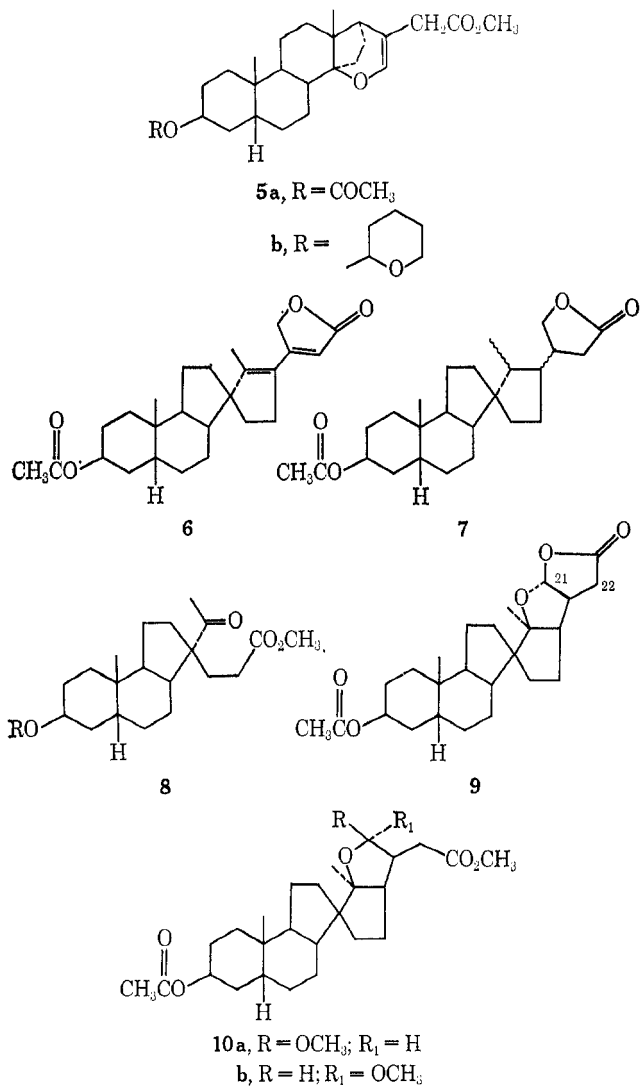
(4) (a) W. A. Jacobs and E. L. Gustus, *J. Biol. Chem.*, **78**, 573 (1928); C. Lindig and K. Repke, *Monatsber. Deut. Akad. Wiss. Berlin*, **4**, 522 (1962); *Chem. Abstr.*, **62**, 4089 (1965). By applying conformational analysis, structure **3** (20*S*,21*S*) was tentatively assigned to isodigitoxigenin by (b) O. Schindler and T. Reichstein, *Helv. Chim. Acta*, **39**, 1876 (1956). On the same conformational basis, possible mechanism of formation, and interpretation of the isodigitoxigenin proton magnetic resonance spectrum, we also support Professor Reichstein's proposal. The protons at positions 20 and 21 in conformer **3** correspond to a dihedral angle of *ca.* 45° and the coupling constant of J = 4 Hz found is consistent with such a relationship.

(5) *Cf.* H. Heymann and L. F. Fieser, *J. Amer. Chem. Soc.*, **73**, 5252 (1951).

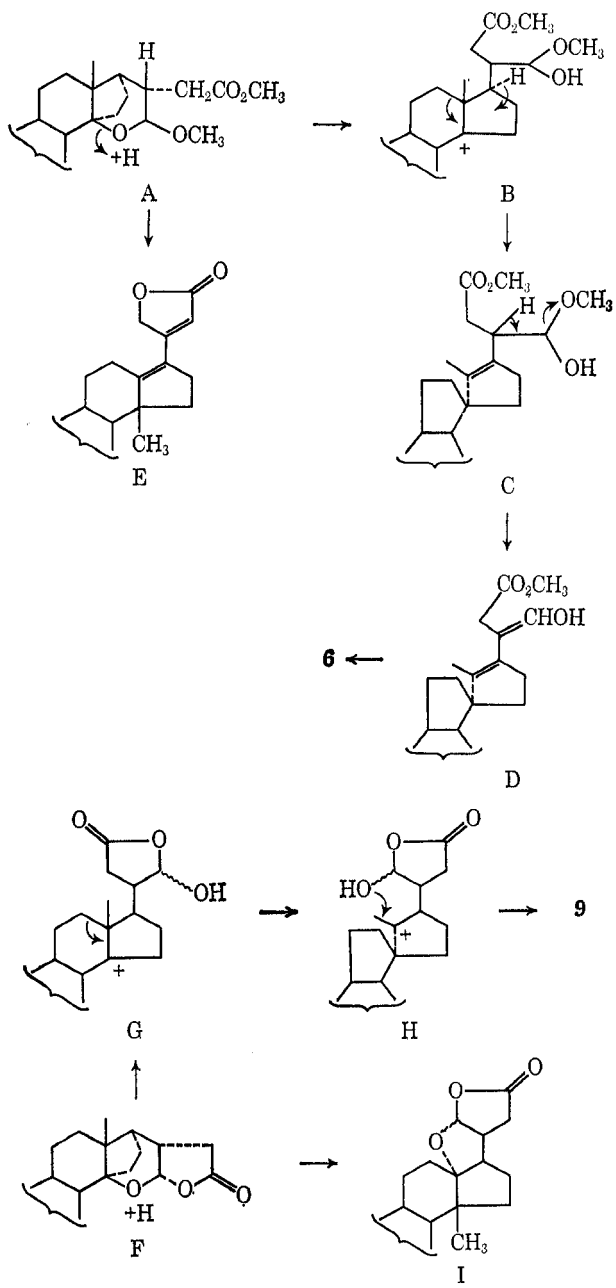
(6) W. S. Johnson, W. A. Vredenburgh, and J. E. Pike, *ibid.*, **82**, 3409 (1960).

(7) P. Roffey and M. V. Sargent, *Chem. Commun.*, 913 (1966); K. J. van der Merwe, L. Fourie, and de B. Scott, *Chem. Ind.* (London), 829 (1967); J. A. Knight, J. C. Roberts, P. Roffey, and A. H. Sheppard, *Chem. Commun.*, 706 (1966); G. Buchi, D. M. Foulkes, M. Kurono, and G. F. Mitchell, *J. Amer. Chem. Soc.*, **88**, 4534 (1966).

(8) Further evidence for the isomeric relationship of acetals **4b** and **4c** was obtained by oxidizing both to the same lactone: G. R. Pettit, T. R. Kasturi, J. C. Knight, and K. A. Jaegg, *J. Org. Chem.*, **35**, 1410 (1970).



When either acetal **4b** or **4c** was heated in refluxing benzene with *p*-toluenesulfonic acid, the elimination reaction[9] began to follow a more complex course, and resulted in a new substance, mp 165–166°, which displayed maximal ultraviolet absorption at 288 mμ (ε 22,760) indicative of extended conjugation and split carbonyl absorption in the infrared spectrum at 5.54 and 5.73 μ characteristic of an unsaturated lactone.[10] The molecular ion appeared at 398 and confirmed loss of not 1 but 2 mol of methanol. Signals at δ 1.02 and 1.05 for the tertiary methyl group protons of, for example, acetal **4c** had shifted to δ 0.98 and 1.82 and suggested the presence of a vinyl methyl group. The acetate methyl protons appeared at δ 2.07 as in starting material, but the 21-methoxyl and methyl ester signals were absent. Instead, two methylene protons were apparent at δ 5.1 and one vinyl proton at δ 5.82. These data implicated a Westphalen-type rearrangement[1b,11] involving the C–D ring juncture. Although A–B ring juncture Westphalen rearrangements have invariably been reported to entail methyl migration, the possibility of a methylene shift in the present case could not be readily excluded, and indeed seemed likely.[12] The presence of *p*-toluenesulfonic acid would be expected to give a protonated form of acetal **4c** such as A. The ensuing carbonium ion (B) could undergo Wagner–Meerwein rearrangement with the 12-methylene group to spiran C. Elimination of a second mole of methanol from intermediate C would yield enol D. Lactonization

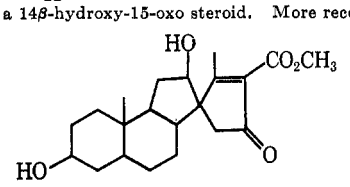


(9) U. Schmidt and P. Grafen, *Justus Liebigs Ann. Chem.*, **656**, 97 (1962).

(10) See, *e.g.*, R. N. Jones, C. L. Angel, T. Ito, and R. J. D. Smith, *Can. J. Chem.*, **37**, 2007 (1959); R. N. Jones and C. Sandorfy, "Chemical Applications of Spectroscopy," Interscience Publishers, New York, N. Y., 1956.

(11) M. M. Janot, P. Devissaquet, M. Pais, Q. K. Huu, F. X. Jarreau, and R. Goutarel, *Bull. Soc. Chim. Fr.*, 4318 (1967).

(12) Later we found that A. Lardon and T. Reichstein [*Helv. Chim. Acta*, **45**, 943 (1962)] had suggested a similar methylene migration to account for rearrangement of a 14β-hydroxy-15-oxo steroid. More recently, structure i



i

has been proposed for an analogous reaction product: C. W. Shoppee, N. W. Hughes, R. E. Lack, and B. C. Newman, *Tetrahedron Lett.*, 3171 (1967); C. W. Shoppee, N. W. Hughes, and R. E. Lack, *J. Chem. Soc., C*, 786 (1968). As indicated in ref 2b, steroids bearing a spiran nuclear ring system are rarely encountered.

and shift of the olefinic double bond into conjugation with the carbonyl group would provide the first example f a C-norcardenolide (**6**). Alternatively, methyl migration by an analogous route would yield 14β-methyl cardenolide E.

To make a definitive choice between alternatives **6** and E, chemical evidence was necessary. Hydrogenation of the rearrangement product **6** resulted in the adsorption of 2 mol of hydrogen and the resulting tetrahydro derivative showed a molecular ion at 402 and no ultraviolet absorption owing to conjugation. The infrared spectrum displayed absorption at 5.65 (γ lactone) and 5.8 μ (acetate) and the pmr spectrum displayed a doublet (J = 7 Hz) at δ 0.78 assigned to a secondary methyl group at position 13. Other aspects of the pmr spectrum were also consistent with spiran structure **7**. Structure E was eliminated conclusively by ozonolysis of olefin **6** at −70°, oxidation of the crude ozonide, methylation with diazomethane, and acetylation. This sequence afforded methyl ketone **8** as major product,[13] and confirmed structure **6**. The pmr spectrum of this ketone exhibited signals at δ 0.94 (19-methyl group), 2.05 (3-acetate), 2.14 (methyl ketone), 3.67 (methyl ester), and 5.08 (3α proton).

Under reaction conditions which led to the rearrangement **4c** → **6**, digitoxigenin gave only 14-dehydrodigitoxigenin,[14] and in a separate experiment the 14-dehydro cardenolide was unaffected.[15] Meanwhile, experiments concerned with cleaving the 14β,21-epoxy bond of isodigitoxigenin were also under way. Treatment with p-toluenesulfonic acid in refluxing benzene gave the only useful results and led to a new substance (**9**) accompanied by C-norcardenolide **6** and starting material. The three-component mixture was separated by preparative layer chromatography. Evidence for assigning C-norcardanolide structure **9** was obtained by considering the empirical formula which was $C_{25}H_{36}O_5$, physical measurements, and possible modes of formation. To accommodate a molecular weight identical with that of starting material **2b** and pmr signals at δ 0.98 (C-19 methyl) and 1.35 (C-18 methyl), skeletal rearrangement of isodigitoxigenin acetate must have occurred. If it is assumed that protonation (F) of isodigitoxigenin results in carbonium ion G, then Wagner–Meerwein rearrangement to H and thence to tetrahydrofuran **9** would seem plausible. The C-norcardanolide (**9**) structure was entirely consistent with the physical data. For example, downfield shift of the C-18 methyl signal to δ 1.35 is quite characteristic of that shown by a methyl group bonded to carbon bearing an electronegative oxygen.[16] Appearance of the 21-proton signal in lactone **9** as a doublet at δ 5.87 with an H-21–H-20 coupling constant of 4 Hz suggested a small dihedral angle. Such information, combined with conformational limitations (as assessed with Dreiding models), suggests structure **9** as a reasonable stereochemical assignment.

To eliminate further the possibility of the isodigitoxigenin rearrangement product arising by a methyl migration (*cf.* I), additional information was collected. Acetals **10a** and **10b** were obtained by methanolysis of C-norcardanolide **9**. The result was comparable with the reaction leading to acetals **4b** and **4c**, and stereochemical assignments were made in analogous fashion. Heating either acetal **10a** (21R) or **10b** (21S) in benzene containing p-toluenesulfonic acid provided a mixture composed of spirans **6** and **9**, thereby eliminating a 14β-methyl possibility.[17]

The 12(13 → 14)*abeo*[18] skeletal rearrangements discovered during the present investigation may actually be fairly general in scope, and steroids containing the spiro C–D ring juncture may be uncovered in natural products. Since such rearrangement reactions could now be recognized and/or avoided in projected synthetic approaches to bufalin, the objectives of the present study were reached. Future X-ray crystallographic determinations in this area are planned to settle unequivocally the stereochemical assignments for isodigitoxigenin (**2**), tetrahydropyran **4**, and spirans **6**, **9**, and **10**.

Experimental Section

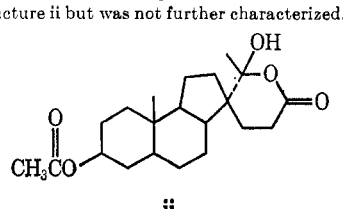
Solvent extracts of aqueous solutions were dried over sodium sulfate and concentrated under reduced pressure using a rotatory evaporator. Acetylation refers to 1:3 acetic anhydride–pyridine at room temperature for 20 hr. Chromatographic solvents were redistilled and ligroin refers to a fraction boiling at 60–80°. Basic alumina (Merck, Rahway, "suitable for chromatography") and silica gel (0.05–0.20 mm, E. Merck, Darmstadt) were used for column chromatography. Thin layer and preparative layer chromatographic plates were prepared using, respectively, silica gel HF_{254} and silica gel G supplied by E. Merck. The introduction to the experimental section of part 7[1] provides other general information necessary here.

Digitoxigenin (**1a**).[19]—In a typical experiment, digitoxin (**1b**, 10 g) in a solution prepared from methanol (500 ml) and 0.1 N sulfuric acid (500 ml) was heated at reflux for 30 min. The hydrolysis and isolation procedure was based on one reported by Rangaswami and Reichstein.[3b] Recrystallization of the crude product from methanol–diethyl ether gave 4.82 g of digitoxigenin, mp 245° (lit.[4a] mp 252°).

Isodigitoxigenin (**2a**).—By essentially the procedure of Jacobs and Gustus,[4a] digitoxigenin (**1a**, 21.0 g) was ground to a fine powder and stirred with methanol (200 ml) containing potassium hydroxide (5.0 g) at 15° for 1 hr. The solid isodigitoxigenin was collected and washed with methanol to yield 13.5 g. One recrystallization from ethanol and one from acetone gave needles, mp 270–273° (at *ca.* 175° the needles become rectangular plates, lit.[4a] mp 271°). Recrystallization from acetone–chloroform was equally effective.

Dilution of the methanol filtrate with water gave a precipitate of digitoxigenin. Acidification of the filtrate and extraction

(13) In one experiment a neutral product was also obtained which might correspond to structure ii but was not further characterized.



ii

(14) E. Hauser, H. Linde, and K. Meyer, *Helv. Chim. Acta*, **49**, 1212 (1966).

(15) Results of these experiments indicted that transformation A → **6** may be a concerted process, such as steroid "backbone"-type rearrangements; see, *e.g.*, J. Bascoul and A. Crastes de Paulet, *Chem. Commun.*, 256 (1908); J. C. Jacquesy, J. Levisalles, and J. Wagnon, *ibid.*, 25 (1967); J. W. Blunt, J. M. Coxon, M. R. Hartshorn, and D. N. Kirk, *Tetrahedron*, **23**, 1811 (1967). Also, the exclusive formation of spiran **6** in preference to a 14β-methyl derivative again points to a concerted reaction.

(16) See, *e.g.*, T. R. Kasturi, E. Raghavan, S. Dev, and D. K. Banerjee, *Tetrahedron*, **22**, 745 (1966).

(17) The results of this study led us to reinvestigate the classical Westphalen rearrangement involving, *e.g.*, 3β-chloro-5α-hydroxy-6β-acetoxycholestane reported by A. Fischer, M. J. Hardman, M. P. Hartshorn, D. N. Kirk, and A. R. Thawley, *ibid.*, **23**, 159 (1967). Evidence so far accumulated completely substantiates methyl migration in the A–B system.

(18) IUPAC–IUB Revised Tentative Rules, *J. Org. Chem.*, **34**, 1517 (1969).

(19) We wish to thank Dr. K. A. Jaeggi for performing several of these experiments.

with chloroform led to isodigitoxigeninic acid (**4e**, 5.7 g).[4a] Acid **4e** was not further purified but used as summarized below in method C for obtaining acetals **4b** and **4c**.

Acetylation of isodigitoxigenin and recrystallization from acetone–chloroform afforded fine needles: mp 258–262° (lit.[4b] mp 250°); pmr δ 1.00 (C-18 and -19 methyls), 2.00 (C-3 acetate), 2.39–2.90 (complex, $COCH_2$–), 4.96 (H-3α), and 5.70 (J = 4 Hz, OCHO).

Methyl 3β-Acetoxy-14β,21-epoxy-21ζ-methoxy-5β-(20*S*)-norcholanate (4b and 4c). Method A.—Isodigitoxigenin (**2a**, 0.5 g) was saponified and the resulting isodigitoxigeninic acid was methylated with diazomethane essentially as reported by Jacobs and Gustus.[4a] The crude methyl ester,[4a] mp 126–127° (lit.[4a] mp 128°), in methanol (25 ml) containing 48% hydrobromic acid (2 drops) was allowed to remain at room temperature for 20 hr. The solution was concentrated to a small volume and then diluted with water. A diethyl ether extract of the aqueous solution was washed with dilute sodium bicarbonate and water. Following removal of solvent, the residue was acetylated and the resulting acetate ester was chromatographed on basic alumina (12 g). Elution with hexane gave a semisolid (0.16 g) which crystallized from hexane or methanol to yield short, thick needles of acetal **4b**, mp 194–199°.

Anal. Calcd for $C_{27}H_{42}O_6$: C, 70.10; H, 9.15; O, 20.75. Found: C, 70.25; H, 9.12; O, 20.54.

Continued elution with 1:1 hexane–benzene gave a viscous oil (0.18 g) which crystallized from methanol to yield large prisms of acetal **4c**, mp 147–149°.

Anal. Found: C, 69.98; H, 9.02; O, 20.85.

Method B.—The methyl ester of isodigitoxigeninic acid (**4a**, see method A or preparation of isodigitoxigenin) was acetylated as described by Schindler and Reichstein.[4b] A specimen of diacetate **4d** was thus obtained: mp 174–176° (lit.[4b] mp 173–175°); pmr δ 1.02 and 1.18 (C-18 and -19 methyls), 2.07 (C-3 acetate), 2.12 (C-21 acetate), 3.7 (methyl ester) and 5.7 (J = 8 Hz, H-21α).

A solution of acetate **4d** (0.15 g) in methanol (15 ml) containing a trace of 48% hydrobromic acid was heated at reflux for 2 hr. Upon cooling, the solid which separated was collected and recrystallized from methanol. A pure sample of acetal **4b** was obtained as long, thin plates, mp 195–196°.

Method C.—The following procedure for obtaining acetals **4b** and **4c** proved routinely effective and was considerably more efficient than proceeding by way of methods A and B. Further, isodigitoxigeninic acid could be substituted for isodigitoxigenin with comparable results. For small-scale conversion, isodigitoxigenin (0.5 g) in methanol (50 ml) containing *p*-toluenesulfonic acid (0.05 g) and water (2.5 ml) was heated at reflux for 20 hr. After removal of methanol and dilution with water, the mixture was extracted with diethyl ether. The ethereal extract was washed with dilute sodium bicarbonate and water. Solvent was evaporated and the residue (0.4 g) was acetylated. The resulting acetate was chromatographed on basic alumina (9 g). Elution with hexane provided a solid (0.22 g), mp 187–189°, which recrystallized from hexane as long, thin plates. Recrystallization from methanol led to an analytical sample of acetal **4b**: mp 194–196°; $[\alpha]_D$ −75° (c 1.0); RD (c 1.0) $[\alpha]_{300}$ −370°, $[\alpha]_{350}$ −242°, $[\alpha]_{400}$ −178°, $[\alpha]_{450}$ −140°, $[\alpha]_{500}$ −105°, $[\alpha]_{589}$ −74°, and $[\alpha]_{600}$ −70°; ν_{max}^{KBr} 1728, 1745, and 1235 cm^{-1}; pmr δ 1.02 and 1.05 (C-18 and -19 methyls), 2.07 (C-3 acetate), 3.32 (C-21 acetate), 3.68 (methyl ester), and 4.73 (J = 5.5 Hz, H-21β).

Further elution with 1:1 hexane–benzene provided 0.15 g of solid acetal **4c**, mp 136–139°. Recrystallization from methanol gave a pure specimen as thick plates: mp 142–144°; $[\alpha]_D$ +9.0° (c 1.33); RD (c 0.97) $[\alpha]_{300}$ +72°, $[\alpha]_{350}$ +51.5°, $[\alpha]_{400}$ +41°, $[\alpha]_{450}$ +33°, $[\alpha]_{500}$ +29°, $[\alpha]_{589}$ +21°, and $[\alpha]_{600}$ +20°; ν_{max}^{KBr} 1735, 1240, and 1256 cm^{-1}; pmr δ 1.08 and 1.12 (C-18 and -19 methyls), 2.07 (C-3-acetate), 3.45 (C-21 methoxy), 3.68 (methyl ester), and 4.23 (doublet, J = 8 Hz, H-21α).

A quite useful larger scale method was based on the digitoxigenin–isodigitoxigeninic acid mixture obtained from digitoxigenin. For example, a mixture composed of isodigitoxigenin (8.0 g) and isodigitoxigeninic acid (3.5 g) in methanol (850 ml)–water (40 ml) containing *p*-toluenesulfonic acid (0.85 g) was heated at reflux for 24 hr (reaction was complete as evidenced by thin layer chromatography). Isomers **4b** and **4c** were isolated, acetylated, and separated as noted directly above. Comparable yields of acetal **4b**, mp 194–199°, and acetal **4c**, mp 147–149°, were obtained. Later, experiments showed that the isomers could be readily separated by column chromatography on silica gel and elution with 19:1 ligroin–ethyl acetate.

Samples of isomeric acetals **4b** and **4c** obtained by methods A–C were compared and found identical.[20]

Equilibration of Methyl 3β-Acetoxy-14β,21-epoxy-21-methoxy-5β-(20*S*)-norcholanate (4b and 4c).—To a solution of acetal **4b** (0.30 g) in benzene (10 ml) was added *p*-toluenesulfonic acid (0.05 g). Aliquots were removed at intervals of 15 min and evaluated by thin layer chromatography with 19:1 chloroform–ethyl acetate mobile phase. Under these conditions, acetal **4b** reached equilibrium with epimer **4c** within 15 min and remained constant for the 3-hr period studied. The equilibrium mixture contained a ratio of *ca.* 3:1 acetal **4c** to acetal **4b**. Repeating the experiment with acetal **4c** gave identical results.

Methyl 3β-Acetoxy-14β,21-epoxy-5β-norchol-20(21)-enate (5a).—To each of the equilibrium mixtures of acetals **4b** and **4c** described in the preceding experiment was added dihydropyran (0.5 ml). In each case, within 15 min nearly all the acetal was transformed into vinyl ether **5a**, and with a lapse of 1 hr, olefin **5a** was the only substance present as evidenced by thin layer chromatography with 19:1 ligroin–ethyl acetate mobile phase. The pale yellow reaction mixture was diluted with diethyl ether and washed with dilute sodium bicarbonate and water. Removal of solvent gave a residue which was chromatographed on silica gel. Elution with 19:1 ligroin–ethyl acetate led to olefin **5a**. Recrystallization from methanol gave an analytical specimen (0.38 g combined yield): mp 103–105°; $[\alpha]_D$ −25° (c 0.59); ν_{max}^{KBr} 1742, 1670, and 1255 cm^{-1}; pmr δ 1.10 and 1.06 (C-18 and -19 methyls), 2.04 (C-3 acetate), 2.90 (singlet, C-22 methylene), 3.68 (methyl ester), 5.14 (H-3α), and 6.0 (H-21 vinyl).

Anal. Calcd for $C_{26}H_{38}O_5$: C, 72.52; H, 8.90. Found: C, 72.82; H, 9.02.

Methyl 3β-Pyranyloxy-14β,21-epoxy-5β-norchol-20(21)-enate (5b).—A solution of acetal **4c** [(21*S*)-methoxy, 4.64 g) in methanol (200 ml) containing water (20 ml) and potassium hydroxide (10 g) was heated at reflux for 3 hr. A major portion of the methanol was removed under reduced pressure and the concentrated solution was diluted with water and acidified with 2*N* hydrochloric acid. The mixture was extracted with diethyl ether and the ethereal extract was washed with water. Following removal of solvent, the viscous residue was methylated with diazomethane. The resulting oily ester slowly solidified on standing to yield 4.1 g. To a solution of the ester in dry benzene (30 ml) was added dihydropyran (3.75 ml) and *p*-toluenesulfonic acid (0.10 g). The mixture was stirred at room temperature overnight for 19.5 hr and then diluted with diethyl ether and washed with dilute sodium bicarbonate and water. Solvent was removed *in vacuo* and the resulting yellow oil was chromatographed on silica gel (200 g). Elution with 19:1 ligroin–ethyl acetate and recrystallization of that fraction from methanol provided olefin **5b** as long needles (2.72 g), mp 122–123°. Another specimen purified by chromatography on basic alumina, elution with 1:1 hexane–benzene, and recrystallization from pentane melted at 124–125°. Another recrystallization from pentane gave an analytical sample as needles: mp 125–126°; $[\alpha]_D$ −29° (c 0.80); RD (c 0.75) $[\alpha]_{300}$ −260°, $[\alpha]_{350}$ −137°, $[\alpha]_{400}$ −87°, $[\alpha]_{450}$ −53°, $[\alpha]_{500}$ −37°, $[\alpha]_{589}$ −23°, and $[\alpha]_{600}$ −23°; ν_{max}^{KBr} 1736 and 1662 cm^{-1}; pmr δ 0.97 and 1.05 (C-18 and -19 methyls), 2.88 (C-22 methylene), 3.65 (methyl ester), and 5.97 (H-21 vinyl).

Anal. Calcd for $C_{29}H_{44}O_5$: C, 73.69; H, 9.38; O, 16.92. Found: C, 73.64; H, 9.34; O, 17.08.

A more detailed study of olefin **5b** homogeneity by thin layer chromatography using the solvent system 1:99 ethyl acetate–chloroform saturated with water gave resolution into two components. The two closely related substances were presumed to represent epimers resulting from the newly introduced 3β-tetrahydropyran ring asymmetric center and were not further characterized.

Methyl 3β-Pyranyloxy-14β,21-epoxy-(21*S*)-methoxy-5β-(20*S*)-norcholanate (4f). Method A.—A sample of 3β acetate **4c** (2.8 g) was saponified and remethylated as described in the preceding experiment (see **5b**). A solution of the crude methyl ester in dry benzene (20 ml) containing dihydropyran (2.5 ml) and *p*-toluenesulfonic acid (0.07 g) was stirred at room temperature for 1 hr. The solution was washed with water, dilute sodium bicarbonate, and water. Following removal of solvent, the residue was chro-

(20) The identical composition was established by thin layer chromatographic, proton magnetic resonance, and infrared spectral (in potassium bromide) comparisons.

matographed on basic alumina (100 g). Elution with 1:1 hexane–benzene gave an oily fraction (2.3 g). Crystallization and recrystallization from pentane gave a pure sample as needles: mp 128–130°; $[\alpha]_D$ +31° (*c* 0.59); RD (*c* 1.18) $[\alpha]_{300}$ +101°, $[\alpha]_{350}$ +87°, $[\alpha]_{400}$ +64°, $[\alpha]_{450}$ +49°; $[\alpha]_{500}$ +40°, $[\alpha]_{589}$ +22°, and $[\alpha]_{600}$ +21°; ν_{max}^{KBr} 1735 cm^{-1}; pmr δ 0.96 and 1.08 (C-18 and -19 methyls), 3.44 (acetal methoxy), 3.66 (methyl ester), 3.96 (pyranyl ether acetal proton), 4.25 (*J* = 8 Hz, H-21), and 4.64 (H-3α).

Anal. Calcd for $C_{30}H_{48}O_6$: C, 71.39; H, 9.59; O, 19.02. Found: C, 71.50; H, 9.54; O, 19.16.

Method B.—For routine and larger scale preparation of acetal **4f** and vinyl ether **5b**, the following procedure was preferred. The crude methanolysis product (21.6 g, mixture of acetals **4b** and **4c**) from isodigitoxigenin and isodigitoxigeninic acid was dissolved in dry benzene (110 ml). Dihydropyran (22.5 ml) and *p*-toluenesulfonic acid (0.55 g) were then added. After stirring for 10 min at room temperature, the solution was washed with dilute sodium bicarbonate and water. Solvent was removed and the crude product was chromatographed in 19:1 ligroin–ethyl acetate on silica gel (400 g). Before 9:1 petroleum ether–ethyl acetate was used, a number of impure fractions (12.5 g total) were collected. Elution with 9:1 ligroin–ethyl acetate gave methyl 3β-pyranyloxy-14β,21-epoxy-(21*S*)-methoxy-5β-(20*S*)-norcholanate (**4f**, 10.1 g) as needles, mp 132–134°. Further elution with ethyl acetate gave 3β-pyranyloxy isodigitoxigenin (**2c**, 0.43 g), characterized by the infrared spectrum and by cleavage of the pyranyloxy group with *p*-toluenesulfonic acid in aqueous methanol to give isodigitoxigenin.

Careful rechromatography of the initially eluted fractions provided the epimeric methyl 3β-pyranyloxy-14β,21-epoxy-(21*R*)-methoxy-5β-(20*S*)-norcholanate (**4g**), crystallized from methanol as plates: mp 159–161°; $[\alpha]_D$ −62.4° (*c* 0.75); pmr δ 0.98 (C-18 methyl), 1.04 (C-19 methyl), 3.28 (acetal methoxyl), 3.68 (ester methoxyl), 3.96 (pyranyl acetal proton), 4.68 (H-3), 4.74 (doublet, *J* = 5.5 Hz, acetal OCHO–).

Anal. Calcd for $C_{30}H_{48}O_6$: C, 71.39; H, 9.59; mol wt, 504. Found: C, 71.07; H, 9.30; mol wt 504 (mass spectrum).

Since isomer **4g** was not desired for further work, the initial fractions were usually treated with dihydropyran and *p*-toluenesulfonic acid as described below to convert the axial epimer into the more useful vinyl ether **5b**.

The mixture (12.5 g) eluted prior to acetal **4f** was dissolved in benzene (100 ml), and both tetrahydropyran (20 ml) and *p*-toluenesulfonic acid (0.50 g) were added. The solution was stirred at room temperature for 7.5 hr and the product was isolated and purified as described directly above. The fraction eluted by 19:1 ligroin–ethyl acetate was a colorless, mobile oil which crystallized from methanol as needles corresponding to methyl 3β-pyranyloxy-14β,21-epoxynor-5β-chol-20(21)-enate (**5b**), mp 122–123°. Continued elution gave a mixture of acetal-containing fractions (2.10 g). The crude material was again pooled and combined with the mother liquors from recrystallization of vinyl ether **5b**. Retreatment with dihydropyran and *p*-toluenesulfonic acid in benzene led to an additional quantity (2.32 g) of vinyl ether **5b**.

3β-Acetoxy-12(13→14)*abeo*-$\Delta^{13(17)}$-5β-cardenolide (6).—A solution composed of equatorial acetal isomer **4c** (0.20 g), dry benzene (30 ml), and *p*-toluenesulfonic acid (0.04 g) was heated at reflux for 10 hr. Water was removed during this period by a Dean–Stark trap. After cooling, the clear solution was washed with water, dilute sodium bicarbonate, and water. Following removal of benzene, the residue was chromatographed on basic alumina (6 g). Elution with 1:1 hexane–benzene gave a viscous oil (0.14 g). Trituration with hexane caused crystallization, mp 155–160°. Recrystallization from acetone–hexane provided pale yellow plates: mp 165–166°; λ_{max}^{EtOH} 288 mμ (ϵ 22,760); $[\alpha]_D$ +36° (*c* 0.5); RD (*c* 1.10) $[\alpha]_{350}$ +145°, $[\alpha]_{400}$ +97°, $[\alpha]_{450}$ +68°, $[\alpha]_{500}$ +55°, $[\alpha]_{589}$ +36°, and $[\alpha]_{600}$ +36°; λ_{max}^{KBr} 5.54, 5.73, 6.13, 6.30, 7.92, and 8.0 μ; $\lambda_{max}^{CHCl_3}$ 5.58, 5.73, and 6.16 μ; pmr δ 0.98 and 1.82 (C-18 and -19 methyls), 2.07 (C-3 acetate), 5.1 (C-21 methylene), and 5.82 (H-22 vinyl).

Anal. Calcd for $C_{25}H_{34}O_4$: C, 75.34; H, 8.60; O, 16.06; mol wt, 398. Found: C, 75.18; H, 8.44; O, 16.80; mol wt, 398 (mass spectrum).

When the preceding experiment was continued over a longer period or when toluene or *p*-cymene was substituted for benzene, the reaction began to follow (as evidenced by thin layer chromatography) a more complex path. Monitoring by thin layer chromatography indicated that the 10-hr reaction period in benzene was most desirable for obtaining C-norcardenolide **6**.

Dehydration of Digitoxigenin (1a).—A solution prepared from benzene (25 ml), digitoxigenin (**1a**, 0.25 g), and *p*-toluenesulfonic acid (0.04 g) was heated at reflux for 20 hr. After cooling, the product was isolated as summarized in the preceding experiment (see **6**) and the residue (0.22 g) was chromatographed on basic alumina (7 g). Elution with 1:1 hexane–benzene gave a solid (0.2 g). Recrystallization from acetone gave 14-dehydrodigitoxigenin,[14] mp 198-200°.[21]

Hydrogenation of 3β-Acetoxy-12(13→14)*abeo*-$\Delta^{13(17)}$-5β-cardenolide (7).—A mixture composed of cardenolide **6** (0.1 g), 5% palladium on barium sulfate (0.1 g), and glacial acetic acid (15 ml) was stirred in a slightly positive pressure of hydrogen for 10 hr. At this point, hydrogenation appeared complete and the catalyst was removed by filtration and washed with diethyl ether. The filtrate was concentrated to a solid residue. A solution of the crude product in diethyl ether (50 ml) was washed with water, dilute sodium bicarbonate, and water. Ether was removed and the residue was chromatographed on neutral alumina (3 g). Elution with 1:1 hexane–benzene led to a solid (0.06 g) which recrystallized from acetone as crystals, mp 201–216°. The isomeric mixture corresponding to structure **7** was not further separated. At this stage, the isomeric mixture exhibited the following data: $[\alpha]_D$ +19° (*c* 1.0); $\lambda_{max}^{CHCl_3}$ 5.65 and 5.80 μ; pmr δ 0.78 (doublet, *J* = 7 Hz, C-18 methyl), 0.95 (C-19 methyl), 2.07 (C-3 acetate), and 5.08 (H-3α).

Anal. Calcd for $C_{25}H_{38}O_4$: C, 74.62; H, 9.45; O, 15.91; mol wt, 402. Found: C, 74.66; H, 9.37; O, 15.90; mol wt, $M^+ - 60$ peak at 342 (mass spectrum).

Ozonolysis of 3β-Acetoxy-12(13→14)*abeo*-5β-$\Delta^{13(17)}$-cardenolide (6).—Ozone (Welsbach ozonator) was passed for 15 min through a solution of diene **6** (0.15 g) in dry ethyl acetate (30 ml) at −70°. The bluish solution was evaporated in a current of dry nitrogen. A solution of the oily residue in glacial acetic acid (30 ml)–water (1 ml) was treated with concentrated hydrochloric acid (1 drop) and 30% hydrogen peroxide (2 ml) at room temperature; *ca.* 2 days later, the solution was diluted with water and extracted with diethyl ether. The ethereal extract was washed with 5% sodium bicarbonate solution and water. Removal of solvent gave 0.05 g of neutral oil, while acidification of the sodium bicarbonate extract followed by extraction with diethyl ether led to 0.1 g of oily carboxylic acid. Methylation of the acid with ethereal diazomethane and acetylation gave oily ketone **8**. Purification by preparative thin layer chromatography gave an oily, analytical sample: bp 120–125° (bath temperature of evaporative distillation at 0.1 mm); $[\alpha]_D$ +34° (*c* 0.52); RD (*c* 1.09) $[\alpha]_{350}$ +225°, $[\alpha]_{400}$ +119°, $[\alpha]_{450}$ +81°, $[\alpha]_{500}$ +60°, $[\alpha]_{589}$ +38°, and $[\alpha]_{600}$ +37°; λ_{max}^{neat} 5.75 and 5.80 μ; pmr δ 0.94 (C-19 methyl), 2.05 (C-3 acetate), 2.14 (methyl ketone), 3.67 (methyl ester), and 5.08 (H-3α); mass spectrum *m/e* 378 (M^+), 360 (M − 18), 347 (M − 31), 335 (M − 43), 318 (M − 60), 291 (M − 87), 243, 201 and 157.

Anal. Calcd for $C_{22}H_{34}O_5$: C, 69.81; H, 9.05; O, 21.13. Found: C, 69.12; H, 8.66; O, 22.40.

Rearrangement of Isodigitoxigenin Acetate (2b).—By distillation, solvent (30 ml) was removed from a solution of isodigitoxigenin acetate (**2b**, 1.87 g) in benzene (250 ml). *p*-Toluenesulfonic acid (0.24 g) was added and the mixture was heated at reflux for 3 days. After cooling and washing with dilute sodium bicarbonate solution and water, solvent was removed. The solid residue was chromatographed on silica gel (200 g) in 4:1 benzene–ethyl acetate, which removed most of the unchanged starting material (1.0 g) and gave a fraction enriched in the less polar rearrangement product **9** (0.75 g).

The enriched fraction was rechromatographed on silica gel in 19:1 benzene–ethyl acetate and separated into three fractions. The first weighed 60 mg and consisted of two nonpolar components arising from loss of the acetate at C-3. Continuing elution with the same solvent gave a second fraction which was further separated by preparative layer chromatography (multiple development, with chloroform as mobile phase) into recovered isodigitoxigenin acetate (200 mg) and C-norcardanolide **9** (120 mg). Recrystallization from methanol gave a pure sample as colorless rosettes: mp 195–196°; $[\alpha]_D$ −9° (*c* 0.53); RD (*c* 0.53) $[\alpha]_{300}$ −132°, $[\alpha]_{350}$ −66°, $[\alpha]_{400}$ −40°, $[\alpha]_{450}$ −23°, $[\alpha]_{500}$ −19°, $[\alpha]_{589}$ −9°, and $[\alpha]_{600}$ −9°; $\nu_{max}^{CHCl_3}$ 1776, 1733, and 1241

(21) Identical (see ref 20) with an authentic specimen prepared as described in reference 14.

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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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).