

potassium carbonate, and evaporated to give 2.6 g of **12b** as a colorless oil, bp 205–210° (bath temperature) (0.05 mm), after purification by distillation, $\nu_{\text{max}}^{\text{CHCl}_3}$ 2780 cm^{-1} (NCH_3).

Anal. Calcd for $\text{C}_{25}\text{H}_{34}\text{BrNO}_5$: C, 62.59; H, 6.16; N, 2.52. Found: C, 62.33; H, 6.13; N, 2.40.

1-(2-Bromo-3,4,5-trimethoxyphenethyl)-1,2,3,4-tetrahydro-7-hydroxy-6-methoxy-2-methylisoquinoline (13b).—A mixture of 2.6 g of the preceding isoquinoline (**12b**), 30 ml of concentrated hydrochloric acid, and 30 ml of ethanol was refluxed for 4 hr. The solvent was evaporated, and the remaining residue was basified with 10% ammonia and extracted with chloroform. The extract was washed with water and dried over potassium carbonate. Evaporation of the solvent afforded 1.8 g of **13b** as a pale brownish oil, which was difficult to crystallize and therefore used in the following reaction without purification, $\nu_{\text{max}}^{\text{CHCl}_3}$ 3510 (OH) and 2730 cm^{-1} (NCH_3).

Photolysis of 13b.—A stirred mixture of 1.8 g of the phenolic isoquinoline **13b**, 0.5 g of sodium hydroxide, 250 ml of ethanol, and 750 ml of water was irradiated using a 450-W Hanovia mercury lamp with a Pyrex filter under water cooling for 7 hr. The mixture was extracted with chloroform after the addition of 6 g of ammonium chloride. The extract was washed with water, dried over potassium carbonate, and evaporated to leave 1.6 g of a brownish oil which was chromatographed on silica gel (50 g). Removal of the eluate with 1% methanol–chloroform gave a dienone fraction (440 mg), which was further rechromatographed on silica gel (10 g). Evaporation of the eluate with chloroform–methanol (99:1) afforded 210 mg of the dienone fraction, which was again rechromatographed on 10 g of neutral alumina. The elution with benzene–chloroform (19:1) gave 50.5 mg of *O*-methylandrocymbine (**8**). Recrystallization from ether–hexane

afforded colorless prisms, mp 154–156.6°, the spectroscopic data of which were identical with those of an authentic specimen.⁷

Anal. Calcd for $\text{C}_{22}\text{H}_{27}\text{NO}_5$: C, 68.55; H, 7.06. Found: C, 68.68; H, 7.24.

Removal of the subsequent elution after collection of the dienone fraction afforded 40 mg of kreisigine (**17**): mp 187–188° (from ethanol) (lit.¹⁵ mp 187–188°); $\nu_{\text{max}}^{\text{CHCl}_3}$ 3500 cm^{-1} (OH); $\lambda_{\text{max}}^{\text{MeOH}}$ 258 and 291 nm ($\log \epsilon$ 4.02 and 3.82); nmr (CDCl_3) 7.60 (3 H, singlet, NCH_3), 6.38 (3 H, singlet, OCH_3), 6.12 (9 H, singlet, 3OCH_3), 3.41 (1 H, singlet, aromatic proton), 3.38 (1 H, singlet, aromatic proton); mass spectrum m/e 385 (M^+), 368 ($\text{M}^+ - 17$). The spectral data were identical with those of an authentic sample.¹⁵

Anal. Calcd for $\text{C}_{22}\text{H}_{27}\text{NO}_5$: C, 68.55; H, 7.06; N, 3.68. Found: C, 68.35; H, 7.28; N, 3.62.

Registry No.—**8**, 31735-12-3; **9a**, 31735-13-4; **9b**, 31790-84-8; **10a**, 31735-15-6; **10a HCl**, 31735-14-5; **10b**, 31790-85-9; **10b HCl**, 31735-16-7; **11a**, 31790-87-1; **11b**, 31735-17-8; **12a** methiodide, 31735-18-9; **12b**, 31735-19-0; **13a** methiodide, 31790-86-0; **13b**, 31735-20-3; **15**, 31735-21-4; **17**, 31735-22-5.

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(18) In a previous paper,⁷ we reported *O*-methylandrocymbine to be an oil, but, after being allowed to stand for a long time, it crystallized.

Bufadienolides. 14. Synthesis of Bufotalien, 15 α -Hydroxybufalin, and Resibufogenin¹

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Conversion of 14-dehydrobufalin (**2a**) to bufotalien (**4a**) was accomplished. Peracid oxidation of 3 β -acetoxy-14-dehydrobufalin (**2b**) was employed to obtain 14 α ,15 α -epoxide **5b**. Sulfuric acid catalyzed opening of epoxide **5b** was used to complete a route to 15 α -hydroxybufalin (**6b**). Treatment of diol **6b** with methanesulfonyl chloride led to a new synthesis of 3 β -acetoxyresibufogenin (**3b**). Conversion of 14-dehydrobufalin to the halohydrins represented by structures **6d–g** followed by treatment with basic alumina or hot pyridine afforded resibufogenin in good yield. The epoxide formation catalyzed by alumina was also shown to yield 14 α -artebufogenin (**8b**).

Interest in the chemistry and physiological action of amphibian venom constituents, for example, from the family Bufonidae, continues to increase.² We recently summarized a total synthesis of bufalin (**1a**) and resibufogenin (**3a**) employing 14-dehydrobufalin (**2a**) as relay.³ The study was subsequently expanded to preparation of bufotalien⁴ and to establish alternative routes from 14-dehydrobufalin to resibufogenin. A summary of these new conversions now follows.

To verify the structure of bufotalien⁴ it became necessary to extend the total synthesis of 14-dehydrobufalin^{3,5} to bufotalien (**4a**). An extensive attempt to convert olefin **2b** to diene **4b** by means of sulfur de-

hydrogenation proved impractical. However, mild treatment of olefin **2b** with *N*-bromosuccinimide followed by pyridine-catalyzed dehydrohalogenation did afford 3 β -acetoxybufotalien (**4b**). Selective saponification of acetate **4b** to bufotalien (**4a**) was achieved using alumina. The synthetic diene (**4a**) was identical with a specimen prepared by acid-catalyzed dehydration of bufotalin (**1d**) essentially as previously reported.⁶

As part of the bufotalin investigation we were led to restudy the *m*-chloroperbenzoic acid oxidation of 14-dehydrobufalin.³ When the oxidation was carried out with more recently purchased samples of *m*-chloroperbenzoic acid, formation of 14 α ,15 α -epoxide **5** was obtained in high yield. The oxidation was repeated several times each with alcohol **2a** and acetate **2b** in chloroform or benzene with the same result (**5**). Unlike the initial study³ no isolatable amounts of β -epoxide **3** were detected. Thus it became important to more firmly establish transformation of 14-dehydrobufalin (**2a**) to resibufogenin (**3a**). Toward this

(1) For paper 13 (Steroids and Related Natural Products. 67), refer to G. R. Pettit and J. Dias, *J. Org. Chem.*, **36**, 3207 (1971).

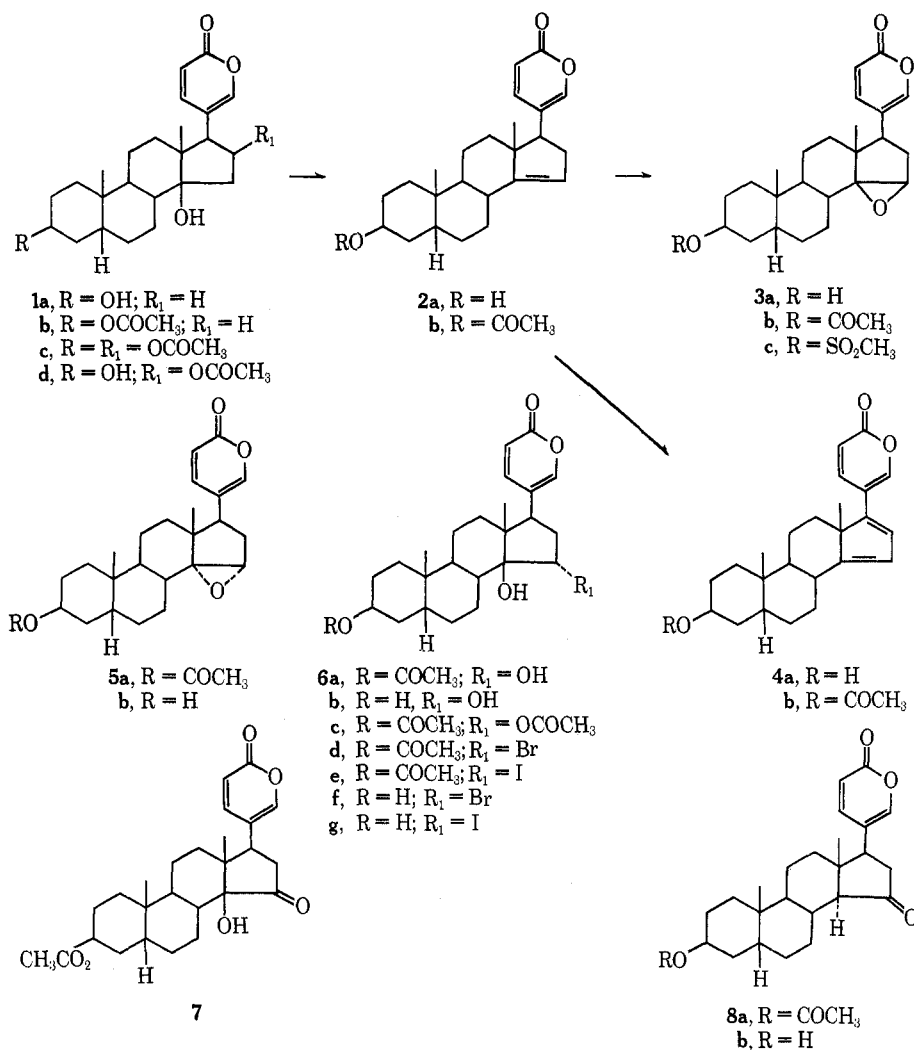
(2) For example, see G. Habermehl, *Naturwissenschaften*, **56**, 615 (1969); Y. Kamano, *Kagaku No Ryoiki*, **24** (4), 57 (1970); Y. Kamano, *ibid.*, **24** (5), 27 (1970); G. R. Pettit, B. Green, and G. L. Dunn, *J. Org. Chem.*, **35**, 1367 (1970); and W. Haede, W. Fritsch, K. Radsch, U. Staehle, and H. Ruschig, *Justus Liebig's Ann. Chem.*, **741**, 92 (1970).

(3) G. R. Pettit, L. E. Houghton, J. C. Knight, and F. Bruschweiler, *J. Org. Chem.*, **35**, 2895 (1970).

(4) The bufotalien synthesis reported herein in detail was summarized in a preliminary communication: G. R. Pettit, P. Brown, F. Bruschweiler, and L. E. Houghton, *Chem. Commun.*, 1566 (1970).

(5) F. Sondheimer, W. McCrae, and W. G. Salmond, *J. Amer. Chem. Soc.*, **91**, 1228 (1969).

(6) K. Meyer, *Helv. Chim. Acta*, **32**, 1993 (1949); H. Wieland, J. Hesse, and R. Huttel, *Justus Liebig's Ann. Chem.*, **524**, 203 (1936); H. Kondo and S. Ikawa, *J. Pharm. Soc. Jap.*, **53**, 23 (1933); *Chem. Abstr.*, **27**, 1887 (1933).



end the mild aqueous sulfuric acid catalyzed opening of epoxide **5** was viewed. Both acetate **5a** and alcohol **5b** led in good conversion to diol **6a** and triol **6b**, respectively. Compelling evidence for the 15 α -hydroxybufalin structure (**6a,b**)⁷ was obtained from mass spectral⁸ proton magnetic resonance and optical rotatory dispersion measurements. Further diol **6a** was easily oxidized by chromium trioxide to ketone **7** and when treated with methanesulfonyl chloride provided a useful route to 3 β -acetoxyresibufogenin (**3b**). The same reaction was applied to triol **6b** to provide 3 β -methanesulfonyloxyresibufogenin (**3c**) which was also easily prepared by reaction between methanesulfonyl chloride and resibufogenin.

In addition to the synthesis of resibufogenin *via* α -epoxide **5**, a variety of bromohydrin approaches were also evaluated and found to be particularly useful.^{5,9} When 3 β -acetoxy-14-dehydrobufalin (**2b**) was treated with *N*-bromoacetamide in dioxane-water containing perchloric acid, bromohydrin **6d** was obtained in high yield. When the crude bromohydrin was chromato-

graphed on basic alumina, 3 β -acetoxyresibufogenin (**3b**) was obtained in 83% yield. When the bromohydrin was heated in pyridine, the same product (**3b**) was isolated in 75% yield. Comparable results were realized with the same bromohydrin from *N*-bromosuccinimide and from iodohydrin **6e** derived from an *N*-iodosuccinimide sequence. An even more direct synthesis of resibufogenin was achieved by applying the NBA, NBS, and NIS halohydrin pathways to 14-dehydrobufalin (**2a**). Here both the basic alumina- and pyridine-catalyzed elimination reactions led to 56–66% overall yields of resibufogenin. Also noteworthy was the isolation of small amounts of 14 α -artebufogenin¹⁰ (**8b**) from the products obtained using basic alumina. Whether the 14 α -artebufogenin arose from resibufogenin or a precursor was not determined.

The preceding experiments conclusively demonstrated that the halohydrin approach to resibufogenin from 14-dehydrobufalin is convenient and reliable. The simplicity and dependability of this synthesis of resibufogenin-type β -epoxides should eventually facilitate total syntheses of related bufadienolides such as bufotalinin and marinobufagin.²

Experimental Section

All melting points were observed using a micro hot-stage apparatus (Reichert, Austria) and are uncorrected. Proton magnetic resonance (deuteriochloroform solution), ultraviolet

(7) Cf. D. Satoh, M. Horie, and J. Morita, *Chem. Pharm. Bull. (Tokyo)*, **14**, 613 (1966).

(8) A detailed mass spectral study of bufadienolides has been prepared by P. Brown, Y. Kamano, and G. R. Pettit, *Org. Mass Spectrum*, in press.

(9) Addition of hydrobromic acid to a 14-olefin system has been employed a number of times in cardenolide chemistry to form a bromohydrin which on reductive dehalogenation provided a practical synthesis of 14 β -hydroxy-cardenolides. See, e.g., P. D. Meister and H. C. Murray, U. S. Patent 2,930,791 (March 29, 1960); *Chem. Abstr.*, **54**, 17471 (1960); U. Stache, W. Fritsch, W. Haede, K. Radsch, and K. Fachinger, *Justus Liebig's Ann. Chem.*, **726**, 136 (1969); and F. Becke and J. Gnad, *ibid.*, **726**, 110 (1969).

(10) H. Linde and K. Meyer, *Experientia*, **15**, 238 (1958).

(95% ethyl alcohol), infrared (potassium bromide pellets), and mass spectral data (by Messrs. Richard Scott and Gene Kelley) were recorded as indicated in the experimental introductions to parts 5 and 10 of this series.¹¹ The *m*-chloroperbenzoic acid was used as purchased from Aztec Chemicals, Elyria, Ohio. The bufalin and resibufogenin were isolated from the Chinese medicinal preparation *Ch'an Su*. General experimental and chromatographic techniques (acetone-chloroform-*n*-hexane, 3:3:4,¹² were used here as the solvent systems) as well as commercial materials have been noted in the experimental introduction to part 5.¹¹

3 β -Hydroxy-14-dehydrobufalin (2b).—A solution of 3 β -acetoxybufalin (1b, 0.20 g) in methanol (10 ml) containing concentrated hydrochloric acid (0.4 ml) was heated at reflux 2 hr. The mixture was poured into ice-water and the solid was collected and washed with water. Recrystallization of the crude product (0.196 g) from acetone gave 0.16 g of olefin 2b melting at 191–193°. The product was identical¹³ with a specimen obtained by acetylating 14-dehydrobufalin (2a).

Bufotalien (4a).—A solution prepared from carbon tetrachloride (40 ml), 3 β -acetoxy-14-dehydrobufalin (2b, 0.18 g), and *N*-bromosuccinimide (0.10 g) was heated at reflux for 3.5 hr. The solvent was evaporated and the residue treated (3 hr) with pyridine (3 ml)–acetic anhydride (2.4 ml). The mixture was concentrated under reduced pressure, and a solution of the residue in chloroform was washed with 1 *N* hydrochloric acid, 10% aqueous sodium bicarbonate, and water. The solvent was evaporated and the crude product in benzene was chromatographed on a column of silica gel (10 g). Elution with benzene-chloroform (1:1, 5-ml fractions) afforded 0.028 g of 3 β -acetoxybufotalien in the seventh and eighth fractions. Crystallization from chloroform and recrystallization from methanol-ether provided yellow crystals melting at 189–191°: mass spectrum M^+ 408 (base peak), 348, 333, 241, 197, and 107; ν_{\max} 300 μ (log ϵ 4.20); ν_{\max} 1750–1730, 1650, 1620, 1560, 1260–1220, 950, 770; $\text{pmr } \delta$ 1.08 (18-methyl), 1.12 (19-methyl), 2.05 (acetate H), 5.03 (3 α proton), 5.94 (t, $J = 2$ Hz, H-16), 6.34 (q, $J = 1.8$ and 9 Hz, H-23), 6.52 (d, $J = 2$ Hz, H-15), 7.50 (d, $J = 2$ Hz, H-21), 7.60 (q, $J = 2$ and 9 Hz, H-22). The specimen of bufotalien acetate (4b) prepared by this procedure was identical¹³ with a sample by heating (3 hr) 3 β -acetoxybufotalin (1c) in refluxing ethyl alcohol (3 ml) containing 3% concentrated hydrochloric acid followed by reacylation. Selective saponification of 3 β -acetoxybufotalien to bufotalien (4a) was achieved using activated alumina as reported previously for the preparation of resibufogenin.³ The specimens of diene 4a prepared from 14-dehydrobufalin (2a) and bufotalin (1d) were found to be identical.¹³

3 β -Acetoxy-14 α ,15 α -epoxy-5 β -bufa-20,22-dienolide (5b).

Method A. From 14-Dehydrobufalin (2a).—To a solution of 14-dehydrobufalin (2a, 0.81 g) in chloroform (20 ml) was added *m*-chloroperbenzoic acid (0.46 g). After a 2.5-hr period at room temperature the mixture was diluted with chloroform and washed consecutively with aqueous potassium iodide, sodium thiosulfate, sodium bicarbonate, and water. Solvent was removed under reduced pressure and the crystalline residue (0.82 g) was recrystallized from acetone to afford 0.70 g, melting at 235–237°, of 3 β -hydroxy-14 α ,15 α -epoxy-5 β -bufa-20,22-dienolide (5b) identical¹⁴ with a specimen prepared from 14-dehydrobufalin (2a) by perbenzoic acid oxidation.¹⁴

A 0.35-g sample of alcohol 5b was acetylated and the product purified by column chromatography on silica gel. Elution with ligroin-acetone (9:1 and 6:1) afforded 0.31 g of acetate 5a as a colorless amorphous solid identical¹³ with a sample obtained by the perbenzoic acid oxidation route.

Method B. From 3 β -Acetoxy-14-dehydrobufalin (2b).—A 0.10-g amount of 3 β -acetoxy-14-dehydrobufalin (2b) was oxidized with *m*-chloroperbenzoic acid (0.065 g) as summarized in method A. The crude product (0.098 g) was chromatographed in ligroin-acetone (6:1) on a column of silica gel. Elution with the same solvent gave 0.072 g of acetate 5a as an amorphous solid.

The samples of 14 α ,15 α -epoxide obtained by both methods A and B were identical.

3 β -Acetoxy-14 β ,15 α -dihydroxy-5 β -bufa-20,22-dienolide (6a, 3 β -Acetoxy-15 α -hydroxybufalin). **Method A. From α -Epoxide 5b.**—A solution composed of acetone (20 ml), water (1.5 ml), and 1 *N* sulfuric acid (5.0 ml) was added to a solution of α -epoxide 5b (0.15 g) in chloroform (10 ml). After 24 hr at room temperature the mixture was diluted with chloroform and poured into water. The chloroform layer was washed consecutively with water, 1% potassium bicarbonate, and water. Solvent was evaporated and the residue (0.15 g) was chromatographed on a column of silica gel. Elution with ligroin-acetone (3:1) and recrystallization of the product from acetone gave 0.12 g (71%) of 3 β ,14 β ,15 α -trihydroxy-5 β -bufa-20,22-dienolide (6b, 15 α -hydroxybufalin) as colorless needles melting at 272–273°: mass spectrum M^+ 402, 384 ($M^+ - H_2O$), 366 ($M^+ - 2H_2O$); ν_{\max} 301 μ (log ϵ 2.16); ν_{\max} 3580, 3400, 1760, 1740–1720, 1640, 1550, 955, 903, 755, and 745 cm^{-1} ; $\text{pmr } \delta$ (1:3 deuteriochloroform-pyridine), 0.92 (18-methyl), 0.98 (19-methyl), 6.28 (d, $J = 10$ Hz, H-23), 7.39 (d, $J = 3$ Hz, H-21), and 7.94 (q, $J = 10$ and 3 Hz, H-22).

Anal. Calcd for $C_{24}H_{34}O_5$: C, 71.61; H, 8.51. Found: C, 71.44; H, 8.33.

Triol 6b (40 mg) was acetylated (18 hr at room temperature) and the product was chromatographed on a column of silica gel. Elution with ligroin-acetone (5:1) and recrystallization of the acetate from acetone provided 34 mg (85%) of needles melting at 281–283°. The specimen of acetate 6a was identical¹³ with the product obtained by method B directly below.

Method B. From Acetate 5a.—A 0.10-g amount of acetate 5a was treated with 1 *N* sulfuric acid (2.5 ml) and the product isolated as described above in method A (cf. 6a). Recrystallization from acetone led to 0.065 g (65%) of needles melting at 280–283°: mass spectrum; M^+ 444, 426 ($M^+ - H_2O$), 408 ($M^+ - 2H_2O$), 384 ($M^+ - CH_3CO_2H$), 366, 351, 348, 232, 217, 123, 109, 95, and 67; ν_{\max} 301 μ (log ϵ 2.56); ν_{\max} 3350, 1740, 1700, 1630, 1540, 1260, 1230, 955, 900, 755, 743 cm^{-1} ; $\text{pmr } \delta$ 0.69 (18-methyl), 0.91 (19-methyl), 2.03 (3-acetate), 5.09 (3 α proton), 6.31 (d, $J = 10$ Hz, H-23), 7.34 (d, $J = 3$ Hz, H-21), and 7.73 (q, $J = 10$ and 3 Hz, H-22).

Anal. Calcd for $C_{25}H_{36}O_6$: C, 70.24; H, 8.16. Found: C, 69.70; H, 8.04.

3 β ,15 α -Diacetoxy-14 β -hydroxy-5 β -bufa-20,22-dienolide (6c, 3 β ,15 α -Diacetoxybufalin).—A 38-mg sample of triol 6b was acetylated (60 hr at room temperature) and the crude product was chromatographed on a column of silica gel. A pure sample of diacetate 6c (30 mg, 80% yield) was obtained as a colorless solid by the fraction eluted by ligroin-acetone (6:1) from acetone-*n*-hexane. A later chromatography fraction led to 4 mg of monoacetate 6a, mp 279–281°. The diacetate exhibited in the mass spectrum M^+ 486, 468 ($M^+ - H_2O$), 426 ($M^+ - CH_3CO_2H$), and 408 ($M^+ - CH_3CO_2H - H_2O$); ν_{\max} 299 μ (log ϵ 2.87); ν_{\max} 3600, 1760–1720, 1650, 1550, 1270, 1260, 1230, 953, 905, 754–745 cm^{-1} ; $\text{pmr } \delta$ 0.74 (18-methyl), 0.92 (19-methyl), 2.05 (3-acetate), 2.09 (15-acetate), 5.20–5.10 (3 α ,15 β protons), 6.30 (d, $J = 10$ Hz, H-23), 7.27 (d, $J = 3$ Hz, H-21), and 7.67 (q, $J = 10$ and 3 Hz, H-22).

Anal. Calcd for $C_{25}H_{38}O_7$: C, 69.11; H, 7.87. Found: C, 68.80; H, 7.78.

Acetylation (30 hr, room temperature) of monoacetate 6a and purification of the product as described directly above gave 19 mg (92%) of diacetate 6c.

3 β -Acetoxy-14 β -hydroxy-15-oxo-5 β -bufa-20,22-dienolide (7, 3 β -Acetoxy-15-oxobufalin). **Method A. From α -Epoxide 5b.**—To a solution of α -epoxide 5a (0.15 g) in acetic acid (3 ml) was added a solution composed of acetic acid 0.3 ml, water (0.04 ml), and chromium trioxide (0.04 g). The mixture was stirred at room temperature for 1.5 hr. Excess chromium trioxide was reduced by adding methanol. The mixture was diluted with water and extracted with chloroform. The combined extract was washed with aqueous sodium bicarbonate and water. Solvent was removed and the product (0.13 g) was chromatographed on a column of silica gel. Recrystallization of the fraction eluted by ligroin-acetone (6:1) from acetone led to 0.09 g (60%) of ketone 7 melting at 260–261°: mass spectrum M^+ 442, 424 ($M^+ - H_2O$), 414 ($M^+ - CO$), 399, 396, 382 ($M^+ - CH_3CO_2H$), 364, 292, 232, 151, 123, 109, and 95; ν_{\max} 300 μ (log ϵ 2.88); ν_{\max} 3530, 1740, 1720, 1640, 1540, 1250, 1230, 960, 905, 755, and 765 cm^{-1} ; $\text{pmr } \delta$ 0.86 (18-methyl), 0.94 (19-methyl),

(11) G. R. Pettit, C. L. Herald, and J. P. Yardley, *J. Org. Chem.*, **35**, 1389 (1970); J. C. Knight, G. R. Pettit, and P. Brown, *ibid.*, **35**, 1415 (1970).

(12) K. Manki, Y. Kamano, and M. Suzuki, *Bunseki Kagaku*, **14**, 1049 (1965).

(13) The results of thin layer chromatographic, infrared spectral, and proton magnetic resonance comparisons served to confirm the identical composition of both specimens.

(14) Y. Kamano, *Chem. Pharm. Bull.*, **17**, 1711 (1969).

2.07 (3-acetate), 2.64 (broad singlet, 16-methylene), 5.10 (3 α proton), 6.29 (d, $J = 10$ Hz, H-23), 7.41 (d, $J = 2.5$ Hz, H-21), and 7.86 (q, $J = 10$ and 2.5 Hz, H-22).

Anal. Calcd for C₂₈H₃₄O₆: C, 70.56; H, 7.74. Found: C, 70.46; H, 7.74.

Method B. From Alcohol 6a.—Oxidation of alcohol 6a (26 mg) was conducted as summarized in method A with epoxide 5a. After chromatographic purification and recrystallization from acetone, the ketone (16 mg) was obtained as needles melting at 259–262°. The specimens of ketone 7 prepared by methods A and B were mutually identical.¹⁴

3 β -Acetoxyresibufogenin (3b). **Method A. From Diol 6a.**—Methanesulfonyl chloride (0.05 ml) was added to a cold (ice bath) solution of diol 6a (40 mg) in pyridine (0.4 ml). The mixture was maintained at approximately 10° for 24 hr and then poured into ice–water (50 ml). The mixture was extracted with chloroform, and the combined extract was washed with water, dilute hydrochloric acid, and water. Removal of solvent led to 45 mg of residue which was chromatographed on a column of silica gel. The fraction eluted by ligroin–acetone (6:1) was recrystallized from acetone to yield (19 mg, 47%) of 3 β -acetoxyresibufogenin as needles melting at 235–239°.

Method B. From Olefin 2b Using *N*-Bromoacetamide.—In a typical experiment a solution of *N*-bromoacetamide (0.35 g) in dioxane (3 ml) was added to a mixture prepared from 3 β -acetoxy-14-dehydrobufalin (2b, 0.36 g) in dioxane (15 ml)–water (2.6 ml)–70% perchloric acid (0.45 ml). Before adding a solution prepared from sodium sulfite (0.35 g) and water (7 ml), the mixture was stirred for 20 min at room temperature. The solution was concentrated under reduced pressure to approximately one-third of the original volume and poured into ice–water with stirring. Solid was collected and washed with water to yield 0.37 g of crude bromohydrin 6d. The bromohydrin was used without further purification as follows. A solution of bromohydrin 6d (0.20 g) in benzene was chromatographed on basic alumina. The fraction (0.18 g) eluted by benzene–ethyl acetate (9:1) was crystallized from acetone to afford 0.17 g (83%) of 3 β -acetoxyresibufogenin as needles melting at 234–239°.

Alternatively the crude bromohydrin (95 mg) was heated 30 min in refluxing dry pyridine (10 ml). Concentration to dryness *in vacuo* gave 98 mg of a residue which was dissolved in chloroform and washed with dilute hydrochloric acid and water. Recrystallization of the crude product (76 mg) from acetone gave 71 mg (75%) of 3 β -acetoxyresibufogenin as needles melting at 228–232°.

Method C. Using *N*-Bromosuccinimide.—The preceding reaction (method B with NBA) was repeated using 0.20 g of olefin 2b and 0.20 g of *N*-bromosuccinimide. In this example the reaction time was 15 min at room temperature and the yield of bromohydrin 6d was 0.22 g. The basic alumina- (5 g) catalyzed elimination applied to bromohydrin 6d (0.11 g) provided 0.073 g (66%) of 3 β -acetoxyresibufogenin (3b), mp 230–235°. Application of the pyridine (5 ml) method to 0.11 g of bromohydrin 6d led to a 75% yield (81 mg) of product 3b melting at 229–233°.

Method D. Using *N*-Iodosuccinimide.—When *N*-iodosuccinimide (0.16 g) was substituted for NBA as described in method B above, olefin 2b (0.20 g) led to 0.22 g of crude iodohydrin 6e. Conversion of the iodohydrin (0.10 g) to 3 β -acetoxyresibufogenin by the basic alumina technique resulted in a 68% yield (68 mg) of product, mp 233–236°. The pyridine (5 ml) route with iodohydrin 6e (98 mg) provided a 73% yield (72 mg) of product (3b) melting at 230–235°.

The sample of 3 β -acetoxyresibufogenin (3b) prepared by methods A–D were found identical¹⁴ with material prepared from natural resibufogenin.

Resibufogenin (3a). **Method A. From 14-Dehydrobufalin (2a) Using *N*-Bromoacetamide.**—The procedure summarized

from preparation of 3 β -acetoxyresibufogenin (3b using *N*-bromoacetamide) was repeated employing 14-dehydrobufalin (2a, 0.20 g). The resulting crude bromohydrin (6f, 0.23 g) led, by the basic alumina (10 g) route, to 0.13 g (65%) of resibufogenin (3a). Recrystallization from acetone–hexane afforded a pure sample melting at 108–120 and 162–166°. Continued elution of the alumina column led to 16 mg of 14 α -artebufogenin¹⁴ (8b) as prisms, mp 263–265°.

Method B. By *N*-Bromosuccinimide.—Preparation of bromohydrin 6d from 14-dehydrobufalin (0.10 g) was repeated using *N*-bromosuccinimide (0.10 g); after chromatography of the product from acetone–hexane, 56 mg (56%) of resibufogenin melting at 115–130 and 164–172° was obtained. In addition 12 mg of 14 α -artebufogenin (8b), mp 262–264°, was obtained following recrystallization from acetone.

Method C. By *N*-Iodosuccinimide.—The general procedure (*cf.* 3b, method D) was applied to 95 mg of 14-dehydrobufalin using 90 mg of *N*-iodosuccinimide. The crude iodohydrin (6g, 99 mg) was heated in refluxing dry pyridine (4 ml) for 40 min. Following column chromatography on silica gel (3.5 g), elution by ligroin–acetone (5:1), and recrystallization from acetone–hexane, pure resibufogenin (66% yield, 63 mg) was obtained with the characteristic double melting point at 117–122 and 157–166°.

Each of the resibufogenin samples obtained by methods A–C were identical¹³ with natural resibufogenin, and the specimens of 14 α -artebufogenin were identical¹³ with material prepared from resibufogenin.¹⁴

3 β -Methanesulfonyloxyresibufogenin (3c). **Method A. From Triol 6b.**—A solution prepared from pyridine (0.4 ml), triol 6b (36 mg), and methanesulfonyl chloride (0.05 ml) was allowed to remain at approximately 10° for 20 hr. The mixture was poured into ice–water and extracted with chloroform. The combined extracts were washed with 2% hydrochloric acid and water. Removal of solvent and recrystallization of the residue (31 mg) from methanol provided 26 mg (71%) of mesylate 3c as prisms melting at 160–162°. The product was identical¹³ with the corresponding sample prepared from resibufogenin as described below.

Method B. From Resibufogenin (3a).—Extension of the procedure just described (*cf.* 3c, method A) to resibufogenin (0.10 g) led to 90 mg (90%) of mesylate 3c melting at 161–162°: mass spectrum 366 ($M^+ - CH_3OSO_2H$), 348, 333, 312, 294, 216: $uv \lambda_{max}$ 301 m μ ($\log \epsilon$ 3.09); $ir \nu_{max}$ 3040, 1760–1720, 1640, 1540, 1320, 1300, 1255, 1180, 1170, 1155, 950, 750, and 745 cm^{-1} ; $pmr \delta$ 0.80 (18-methyl), 1.03 (19-methyl), 3.05 (3-methanesulfonyl), 3.55 (s, 15 α proton), 5.10 (s, 3 α proton), 6.27 (d, $J = 10$ Hz, H-23), 7.29 (d, $J = 2.5$ Hz, H-21), and 7.91 (q, $J = 10$ and 2.5 Hz, H-22).

Anal. Calcd for C₂₈H₃₄O₈S: C, 64.90; H, 7.40; S, 6.93. Found: C, 64.92; H, 7.27; S, 7.15.

Registry No.—3a, 465-39-4; 3b, 4029-64-5; 3c, 31444-07-2; 4a, 474-53-3; 4b, 31444-09-4; 6a, 4534-19-4; 6b, 31444-11-8; 6c, 31489-85-7; 7, 31444-12-9; 8b, 468-86-0.

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