Bufadienolides. Part XXIII.[1] 14,15β-Epoxy-3β,11α-dihydroxy-5β-bufa-20,22-dienolide (11α-Hydroxyresibufogenin)

By **George R. Pettit** * and **Yoshiaki Kamano**, Department of Chemistry, Arizona State University, Tempe, Arizona 85281, U.S.A.

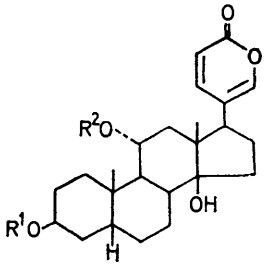
Synthetic procedures have been developed for conversion of gamabufotalin (3β,11α,14-trihydroxy-5β-bufa-20,22-dienolide) (1a) into 11α-hydroxyresibufogenin (5a). Selective dehydration of gamabufotalin with hydrochloric acid in methanol gave the 14-ene (2a). Addition of, for example, hyprobromous acid to this olefin (2a) afforded the bromohydrin (4b) which upon treatment with pyridine easily afforded 11α-hydroxyresibufogenin. As part of this study 11α-hydroxybufalone (11α,14-dihydroxy-3-oxo-5β-bufa-20,22-dienolide) (3a) was also prepared.

THIRTY different bufadienolides have been isolated hitherto from the Chinese toad venom preparation Ch'an Su.[2] One of these, resibufogenin, is used in Japan as a respiratory stimulant. With a view towards extending the medical utility of bufadienolides we undertook preparation of 11α-hydroxyresibufogenin;[3] this material was also required to facilitate its detection in toad venom mixtures.

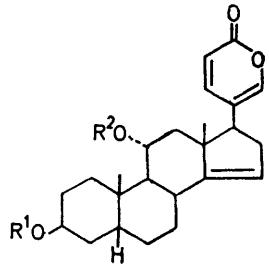
Gamabufotalin (1a) was isolated from commercial Ch'an Su and selectively dehydrated with hydrochloric acid in methanol[4] to 14,15-anhydrogamabufotalin (2a).[5] Also, gamabufotalin diacetate (1b) was easily dehydrated with thionyl chloride in pyridine to yield the olefin (2b). When the hydrochloric acid–methanol dehydration was applied to gamabufotalin diacetate (1b) the major product was gamabufotalin 11-acetate, which was accompanied by gamabufotalin (1a) and 14,15-anhydrogamabufotalin (2a). The structure of the 11α-acetate (1c) was confirmed by mass and n.m.r. spectral data as well as by acetylation, which gave the starting diacetate (1b). The selective hydrolysis of the diacetate (1b) to the monoacetate (1c) illustrates the sterically protected environment of the 11-position.

At this point, the selective oxidation of gamabufotalin to 11α-hydroxybufalone (3a) was studied; the ketone (3a) is also a possible constituent of toad venom. Accordingly, the triol (1a) was selectively oxidized to the ketone (3a) with *N*-bromoacetamide in acetone–water. Acetylation of the 11α-hydroxy-group of the product gave the acetate (3b), also obtained by oxidation of gamabufotalin 11-acetate (1c) with *N*-bromoacetamide.

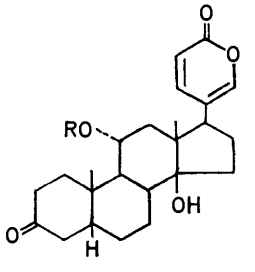
Reaction of 14,15-anhydrogamabufotalin (2a) with *N*-iodosuccinimide or *N*-bromoacetamide in acetone–water led, respectively, to the halogenohydrins (4a and b). Without further purification the halogenohydrins were treated with pyridine to afford 11α-hydroxyresibufogenin (5a) in 32—36% yields. The yields of the β-epoxide (5a) were considerably less than those (*ca.* 80%) experienced when the same reaction sequence was used in our earlier syntheses of resibufogenin[3] and cinobufagin.[6] Apparently the 11α-hydroxy-substituent impedes the halogenohydrin reaction. Application of this sequence to the 11α-acetate (2b) gave higher yields (40—46%) of the β-epoxide (5b).



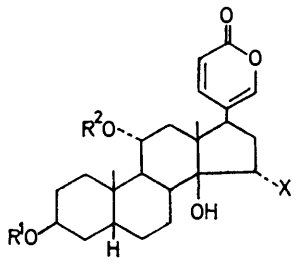
(1) a; $R^1 = R^2 = H$
b; $R^1 = R^2 = Ac$
c; $R^1 = H, R^2 = Ac$



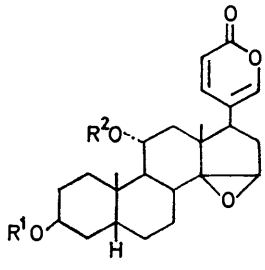
(2) a; $R^1 = R^2 = H$
b; $R^1 = R^2 = Ac$



(3) a; R = H
b; R = Ac



(4) a; $R^1 = R^2 = H, X = I$
b; $R^1 = R^2 = H, X = Br$
c; $R^1 = R^2 = Ac, X = I$



(5) a; $R^1 = R^2 = H$
b; $R^1 = R^2 = Ac$

EXPERIMENTAL

All solvents were redistilled and gamabufotalin was isolated from the Chinese medicinal preparation, Ch'an Su.

[1] Part XXII, G. R. Pettit and Y. Kamano, *Experientia*, 1972, **28**, 768.

[2] For example, see Y. Kamano, K. Hatayama, M. Shinohara, and M. Komatsu, *Chem. and Pharm. Bull. (Japan)*, 1971, **19**, 2478; G. R. Pettit, B. Green, and G. L. Dunn, *J. Org. Chem.*, 1970, **35**, 1367; Y. Kamano, *Kagaku no Ryoiki (Tokyo)*, 1970, **24**, 57; R. Ode, Y. Kamano, and G. R. Pettit, in 'MTP International Review of Science, Organic Chemistry Series One,' ed. by W. D. Johns, Butterworths, London, 1972, vol. 8.

[3] Our prior efforts in this area culminated in a total synthesis of resibufogenin. For leading references see G. R. Pettit, Y. Kamano, F. Brüschweiler, and P. Brown, *J. Org. Chem.*, 1971, **36**, 3736.

[4] G. R. Pettit, P. Hofer, W. J. Bowyer, T. R. Kasturi, R. C. Bansal, R. E. Kadunce, and B. Green, *Tetrahedron*, 1963, **19**, 1143.

[5] H. Wieland and F. Vocke, *Annalen*, 1930, **481**, 215; H. Kondo and S. Ohno, *J. Pharm. Soc. Japan*, 1939, **59**, 186 (*Chem. Zentr.*, 1940, **111**, I, 1997).

[6] G. R. Pettit and Y. Kamano, *J. Org. Chem.*, 1972, **37**, 4040.

Acetylation reactions were generally conducted at room temperature during *ca.* 18 h. The mixture was then poured into ice–water and extracted with chloroform. The chloroform extract and all other solvent extracts employed in the following experiments were dried over anhydrous sodium sulphate and concentrated or evaporated under reduced pressure. Silica gel (0·05—0·20 mm; Merck) was used for column chromatography and silica gel t.l.c. plates were also supplied by Merck. Each analytical sample gave a single spot on t.l.c. [developed with acetone–chloroform–hexane (3 : 3 : 4) and ethyl acetate–hexane (9 : 1)]. Identity of specimens was confirmed by mixed m.p., i.r. spectral, and t.l.c. comparisons. Ligroin refers to light petroleum of boiling range 60—90°.

M.p.s and spectral data (provided by Dr. P. Brown, Miss K. Reimer, and Messrs. R. Scott and E. Kelley) were determined as indicated in Part XXI.[6] Solvents were as follows: u.v. 95% ethanol; n.m.r. deuteriochloroform. I.r. spectra were observed for potassium bromide pellets. Elemental analytical data were determined in the laboratory of Dr. A. Bernhardt, 5251 Elbach Uber Engelskirchen, West Germany.

14,15-*Anhydrogamabufotalin* (3β,11α-*Dihydroxy*-5β-*bufa*-14,20,22-*trienolide*) (2a).—A solution of gamabufotalin (1a) (0·20 g), m.p. 258—262°, in methanol (8 ml), containing 35% hydrochloric acid (0·4 ml) was heated at reflux for 2 h, poured into ice–water, and extracted with chloroform. The combined extract was washed with water and concentrated to dryness. The crude product (0·19 g) was column chromatographed and the fraction eluted with ligroin–acetone (5 : 1) was recrystallized from acetone to provide 14,15-anhydrogamabufotalin (2a) as prisms (0·135 g), m.p. 202—204° (lit.,[5] 204°).

14,15-*Anhydrogamabufotalin Diacetate* (2b).[7]—*Method A. From* 14,15-*anhydrogamabufotalin* (2a). When 14,15-anhydrogamabufotalin (0·052 g) was acetylated (room temp.; 20 h) with pyridine (1·1 ml)–acetic anhydride (0·8 ml) and the product isolated by column chromatography [elution with ligroin–acetone (9 : 1)] the diacetate (2b) was obtained as an amorphous solid (0·047 g).

Method B. From gamabufotalin diacetate (1b). The solution prepared from gamabufotalin diacetate (1b) (20 mg), m.p. 265—267°, dry pyridine (2 ml), and freshly distilled thionyl chloride (0·2 ml) was kept for 20 min at *ca.* 5° and then for 5 min at room temp. The mixture was poured into ice–water and extracted with chloroform; the organic phase was washed with water, dilute hydrochloric acid, and water, and evaporated. The crude product was column chromatographed. Elution with ligroin–acetone (9 : 1) provided the diacetate (1b) as an amorphous solid (18 mg).

The diacetate showed $\nu_{max.}$ 3030 (CH), 1740—1720 (conjugated CO and ester CO), 1650, 1550 (conjugated C=C), 1260, 1240 (ester C–O), 953, and 790 (C=C) cm^{-1}, *m/e* (70 eV) 468, 408, 393, 380, 366, 348, 333, 253, 241, 227, 212, 131, 119, 98, 91, and 59, τ 9·19 (10-Me), 8·95 (13-Me), 8·02 (11α-OAc), 7·94 (3β-OAc), 5·06 (11β-H), 4·89 (3α-H), 4·68 (15-H), 3·72 (23-H, d, *J* 10 Hz), 2·77 (22-H, q, *J* 10 and 2·5 Hz), and 2·74 (21-H, d, *J* 2·5 Hz), $\lambda_{max.}$ 298 nm (ε 5250).

Gamabufotalin 11-*Acetate* (11α-*Acetoxy*-3β,14-*dihydroxy*-5β-*bufa*-20,22-*dienolide*) (1c).—Gamabufotalin diacetate (1b) (0·23 g) was treated (1·5 h) in methanol (2·5 ml) with 35% hydrochloric acid (0·05 ml) as described for the preparation of the olefin (2a). The yield of crude product was 0·25 g. The fraction eluted with ligroin–acetone (6 : 1) crystallized as needles from methanol to yield the 11α-*acetate* (1c) (0·15 g), m.p. 143—148°. Further elution with ligroin–acetone (3 : 1) provided gamabufotalin (1a) (0·05 g) and 14,15-anhydrogamabufotalin (2a) (0·02 g). The latter two products were identical with authentic samples.

The 11α-acetate (1c) (Found: C, 70·45; H, 8·0. $C_{26}H_{36}O_6$ requires C, 70·25; H, 8·15%) showed $\nu_{max.}$ 3500, 3350 (OH), 1740, 1720, 1690 (conjugated CO and ester CO), 1630, 1530 (conjugated C=C), 1260, 1240 (ester C–O), 950, and 794 (C=C) cm^{-1}, *m/e* (70 eV) 444, 426, 416, 402, 384, 366, 351, 348, 341, 333, 323, 296, 231, 201, 191, 107, 93, and 55, τ 9·21 (13-Me, s), 8·96 (10-Me, s), 8·03 (11α-OAc, s), 5·86br (3α-H, s), 5·09br (11β-H), 3·75 (23-H, d, *J* 9·5 Hz), 2·79 (21-H, d, *J* 2·5 Hz), and 2·21 (22-H, q, *J* 9·5 and 2·5 Hz), $\lambda_{max.}$ 298 nm (ε 5500).

Gamabufotalin 11-acetate (1c) (20 mg) was acetylated with pyridine (0·4 ml)–acetic anhydride (0·3 ml). By means of column chromatography, and elution with ligroin–acetone (9 : 1), gamabufotalin diacetate (1b), m.p. 263—265°, was obtained, identical with an authentic sample.

11α-*Hydroxybufalone* (11α,14-*Dihydroxy*-3-*oxo*-5β-*bufa*-20,22-*dienolide*) (3a).—To a solution of gambufotalin (60 mg) in acetone (12 ml)–methanol (6 ml), was added *N*-bromoacetamide (60 mg) in acetone (3 ml)–water (3 ml). After stirring for 20 h at room temperature, sodium sulphite (60 mg) in water (6 ml) was added and the mixture was concentrated to about half volume and extracted with chloroform. The combined extract was washed with water and evaporated. The residue (0·065 g) was column chromatographed. Elution with ligroin–acetone (3 : 1) and recrystallization from methanol gave the *ketone* (3a) (41 mg) as needles, m.p. 274—276° (Found: C, 72·05; H, 8·0. $C_{24}H_{32}O_5$ requires C, 71·95; H, 8·05%), $\nu_{max.}$ 3480 (OH), 1720, 1700 (conjugated CO and normal ketone CO), 1630, 1530 (conjugated C=C), 950, and 810 (C=C) cm^{-1}, *m/e* (70 eV) 400, 382, 364, 351, 349, 339, 331, 321, 269, 253, 241, 191, and 123, $\lambda_{max.}$ 298 nm (ε 5630).

11α-*Acetoxy*-14-*hydroxy*-3-*oxo*-5β-*bufa*-20,22-*dienolide* (11α-*Acetoxybufalone*) (3b).—*Method A. From* 11α-*hydroxybufalone* (3a). 11α-Hydroxybufalone (3a) (20 mg) was acetylated with acetic anhydride (0·4 ml)–pyridine (0·6 ml) and the crude product (30 mg) was column chromatographed. Elution with ligroin–acetone (6 : 1) provided the keto-acetate (3b) (26 mg), m.p. 202—203°, as prisms (from ethanol).

Method B. From Gamabufotalin 11-*Acetate* (1c).—Gamabufotalin 11-acetate (1c) (50 mg) in acetone (10 ml–methanol (5 ml) was oxidized with *N*-bromoacetamide (0·05 g) in acetone (2·5 ml)–water (2·5 ml) as described for the preparation of the ketone (3a). The crude product (55 mg) was column chromatographed and the fraction eluted with ligroin–acetone (6 : 1) was recrystallized from methanol to afford the *keto-acetate* (3b) (35 mg) as prisms, m.p. 202—203° (Found: C, 70·6; H, 7·75. $C_{26}H_{34}O_6$ requires C, 70·55; H, 7·75%), $\nu_{max.}$ 3550 (OH), 1760, 1740—1700 (ketone CO, conjugated CO, and ester CO), 1640, 1540 (conjugated C=C), 1240—1220 (ester C–O), 945, 810, and 790 (C=C) cm^{-1}, *m/e* (70 eV) 442, 424, 414, 399, 382, 364, 357, 349, 346, 334, 321, 294, 284, 241, 227, 191, 123, 79, and 55, τ 9·17 (13-Me, s), 8·88 (10-Me, s), 7·98 (11α-OAc, s), 4·96br (11β-H), 3·67 (23-H, d, *J* 10 Hz), 2·66 (21-H, d, *J* 2 Hz), and 2·10 (22-H, q, *J* 10 and 2 Hz), $\lambda_{max.}$ 298 nm (ε 5500).

[7] Preparation of 14,15-anhydrogamabufotalin diacetate (2b) has been reported, but physical constants and spectral data were not presented: M. Komatsu, *Yakugaku Zasshi*, 1963, **84**, 77.

14,15β-*Epoxy*-3β,11α-*dihydroxy*-5β-*bufa*-20,22-*dienolide* (11α-*Hydroxyresibufogenin*) (5a).—*Method A*. To a solution of 14,15-anhydrogamabufotalin (2a) (0·1 g) in acetone (16 ml), was added *N*-iodosuccinimide (0·1 g) in acetone (3 ml)–water (3 ml) and stirring was continued for 20 h at room temperature. Sodium sulphite solution (1%; 10 ml) was then added and the mixture was concentrated to about half the original volume and extracted with chloroform. The crude iodohydrin (4a) (0·12 g) obtained by washing the chloroform extract with water and concentrating, was added to pyridine (4 ml) and the solution was stirred for 4 h at room temperature. After evaporation of solvent the crude product was column chromatographed. Elution with ligroin–acetone (3 : 1) provided 11α-hydroxyresibufogenin (5a) as an amorphous solid (0·036, 36%).

Method B. The procedure of method A was employed with 14,15-anhydrogamabufotalin (2a) (62 mg) and *N*-bromoacetamide (60 mg) in acetone–water. The crude bromohydrin (0·065 g) was stirred in pyridine (2·5 ml) at room temperature. After 4 h the solvent was evaporated off and 11α-*hydroxyresibufogenin* (5a) (20 mg, 32%) was isolated as an amorphous solid, identical with the product of method A (Found: C, 71·95; H, 8·1. $C_{24}H_{32}O_5$ requires C, 71·95; H, 8·05%), $\nu_{max.}$ 3500 (OH), 3040 (CH), 1720—1700 (conjugated CO), 1630, 1530 (conjugated C=C), 955 (C=C), 833 (epoxy C–O), and 790 cm^{-1}, *m/e* (70 eV) 400, 382, 364, 353, 349, 336, 321, 312, 310, 278, 249, 231, 213, 207, 191, 123, 105, 79, and 55, τ 9·18 (13-Me, s), 8·87 (10-Me, s), 6·42 (15α-H, s), 6·11br (11β-H), 5·82br (3α-H, s), 3·69 (23-H, d, *J* 10 Hz), 2·65 (21-H, d, *J* 3 Hz), and 2·14 (22-H, d, *J* 10 and 3 Hz), $\lambda_{max.}$ 298 nm (ε 5500).

3β,11α-*Diacetoxy*-14,15β-*epoxy*-5β-*bufa*-20,22-*dienolide* (5b).—*Method A*. 14,15β-Epoxy-3β,11α-dihydroxy-5β-bufa-20,22-dienolide (5a) (24 mg) was acetylated with acetic anhydride (0·4 ml)–pyridine (0·5 ml). Chromatography of the crude product (30 mg) with ligroin–acetone (7 : 1) and recrystallization from acetone gave the diacetate (5b) (21 mg) as prisms, m.p. 241—243°.

Method B. The mixture prepared from the olefin (2b) (0·102 g), *N*-iodosuccinimide (NIS) (0·110 g), acetone (19 ml), and water (3 ml) was stirred for 18 h at room temperature. The crude iodohydrin (4c) was isolated and treated with pyridine as for the synthesis of the epoxide (5a). Chromatographic purification and recrystallization from acetone gave prisms, m.p. 241—243° (47 mg, 46%) of the diacetate (5b).

Method C. The NIS procedure of method B was repeated with the olefin (2b) (50 mg) and *N*-bromoacetamide (50 mg) in acetone–water to give *prisms* (5b) (20 mg, 40%), m.p. 241—243° (Found: C, 69·25; H, 7·5. $C_{28}H_{36}O_7$ requires C, 69·4; H, 7·5%), $\nu_{max.}$ 3030 (CH), 1740, 1720—1700 (conjugated CO and ester CO), 1635, 1535 (conjugated C=C), 1260, 1250, 1230 (ester C–O), 954 (C=C), 840 (epoxy C–O), and 798 (C=C) cm^{-1}, *m/e* (70 eV) 484, 466, 424, 406, 395, 382, 364, 349, 336, 294, 273, 256, 241, 227, 213, 131, 91, and 55, τ 9·12 (13-Me, s), 8·89 (10-Me, s), 7·98 (11α-OAc, s), 7·90 (3β-OAc, s), 6·37 (15-H, s), 4·99br (11β-H), 4·83br (3α-H, s), 3·68 (23-H, d, *J* 10 Hz), 2·65 (21-H, d, *J* 3 Hz), and 2·18 (22-H, q, *J* 10 and 3 Hz), $\lambda_{max.}$ 298 nm (ε 5250).

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