Synthesis of the 14α- and 16α-Epimers of Bufotalin Acetate and 16-Deacetylcinobufagin[1]

Yoshiaki Kamano,[a] George R. Pettit,[a,*] Masuo Inoue,[a] Machiko Tozawa,[b] Cecil R. Smith,[a] and David Weisleder[c]

[a] *Cancer Research Institute and Department of Chemistry, Arizona State University, Tempe, Arizona 85287, USA*

[b] *Department of Chemistry, The Jikei University School of Medicine, Kokuryo, Chofu-shi, Tokyo, 182, Japan*

[c] *Northern Regional Research Center, ARS, USDA, Peoria, IL 61604, USA*

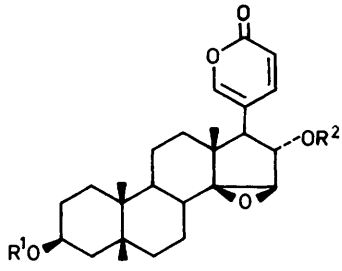
The syntheses of two new bufadienolides with ring-D oxygen substituents in abnormal configurations have been summarised. Sodium borohydride reduction of 3β-acetoxy-16-oxo-14β,15β epoxy-5β-bufa-20,22-dienolide (**3**) provided a readily separable (1 : 1) mixture of 16α-deacetylcinobufagin (**1a**) and its previously known 16β-epimer. Also, 14-*epi*-bufotalin acetate (**2a**) was obtained by a sequence in which 14-dehydrobufotalin acetate (**7**) was epoxidised with *m*-perchlorobenzoic acid to give the diacetate (**5a**), selective alkaline hydrolysis of which gave the monoacetate (**5b**), which was then oxidised with chromium trioxide to give the ketone (**8**); reduction of (**8**) with chromium(II) acetate gave the alcohol (**9**), which was then reduced at the carbonyl to the diol (**2b**) with Urushibara nickel, and finally acetylated to afford the synthetic objective (**2a**).

Bufadienolides as well as their amino acid esters (bufotoxins) and glycoside derivatives are being encountered with increasing frequency as potent animal and plant constituents.[2] Such substances exhibit a far-ranging variety of pharmacological activities, including antineoplastic,[3] cardiotonic,[4] blood pressure stimulating,[5] respiration stimulating,[6] and anaesthetic[7] actions. Hellebritoxin from the Southern European green toad *Bufo viridis* Laur[8] and orbicusides A—C,[9] toxic plant bufadienolide glycosides, provide interesting and recent illustrations.

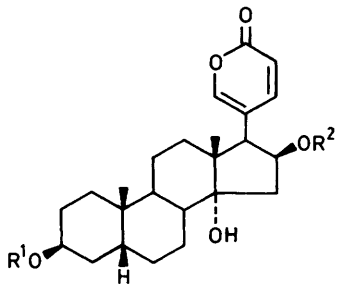
The present study was undertaken to extend knowledge of bufadienolide structure-activity relationships.[7] To this end, we undertook synthesis of the previously unknown 16α-epimer of 16-deacetylcinobufagin (**1a**), and the 14α-epimer of bufotalin (**2a**). In both of these bufadienolides, the hydroxy group was introduced in a configuration opposite to the naturally occurring isomers.

Sodium borohydride reduction[10] of 3β-acetoxy-16-oxo-14β,15β-epoxy-5β-bufa-20,22-dienolide (**3**)[11] in dioxane–water (3:1) has previously been used to obtain the 16β-alcohol (**4**). The key step for obtaining 16α-deacetylcinobufagin was achieved when sodium borohydride reduction of the ketone (**3**) was carried out in ethanol–methanol (5:1). The 16α-alcohols (**1a**) and (**1b**) were obtained by this means and separated by column chromatography.

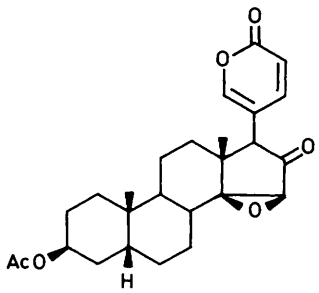
In the ^{1}H n.m.r. spectrum of the 3β-acetate (**1b**) (m.p. 217—219 °C), the 17α-proton doublet at δ 2.33 showed a coupling constant of 5.0 Hz, reasonable for the interaction of *trans* protons at 16β and 17α, but smaller than that observed (J 9.5 Hz) for the 16β-alcohol (**4**), where the 16α and 17α protons are coupled. The 15α-proton appeared as a singlet at δ 3.52 in compound (**1b**). This was consistent with $\Phi \approx 90°$ for 15α- and 16β-H, where J should approach zero according to the Karplus relationship. In the 16β-alcohol (**4**), $\Phi \approx 30°$ for the protons at 15α and 16α suggested a J value of *ca.* 6 Hz. However, the 300 MHz ^{1}H n.m.r. spectrum of the 16β-alcohol (**4**) showed a 15α,16α coupling of only 1 Hz, considerably less than would be expected from inspection of a Dreiding model. Retention of the 3-acetate group in the bufadienolide (**1b**) was verified by a sharp singlet at δ 2.05. Oxidation of the alcohol (**1b**) with chromium trioxide in acetic acid regenerated the ketone (**3**). The result lent support to the conclusion that the alcohol



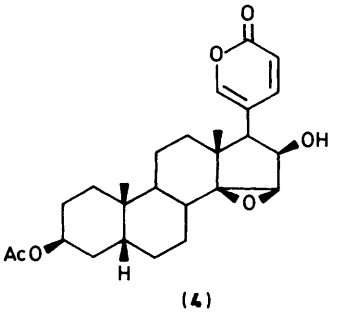
(**1**) **a**; $R^1 = R^2 = H$
b; $R^1 = Ac$, $R^2 = H$
c; $R^1 = R^2 = Ac$



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



(**3**)

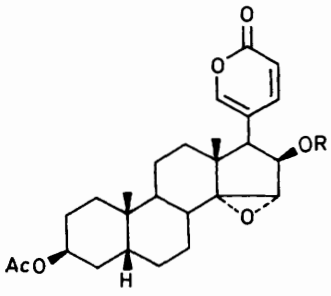


(**4**)

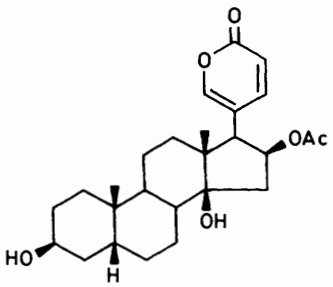
(**1b**) was the 16α-epimer of alcohol (**4**). Acetylation of the 16α-alcohol (**1b**) with acetic anhydride–pyridine afforded the 3,16-diacetate (**1c**).

The other sodium borohydride reduction product of ketone (**3**), *i.e.* the diol (**1a**) (m.p. 204–206 °C), gave an apparent molecular ion at *m/z* 400 and elemental analyses consistent with the molecular formula $C_{24}H_{32}O_5$. Absence of an acetyl group was demonstrated by ^{1}H n.m.r., whereas other signals denoted a close relationship between the alcohols (**1a**) and (**1b**). The structures assigned to the alcohols (**1a**) and (**1b**) were confirmed by interconversion using hydrolysis in methanol under either acidic or basic conditions as well as by acetylation of each to give the diacetate (**1c**).

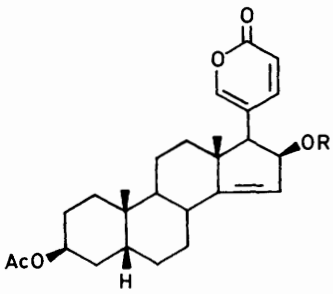
Attention was next turned to the 14α-epimer of bufotalin (**2a**). The synthesis was initiated employing the 14α,15α-epimer (**5a**) of cinobufagin acetate, prepared previously from bufotalin (**6**)



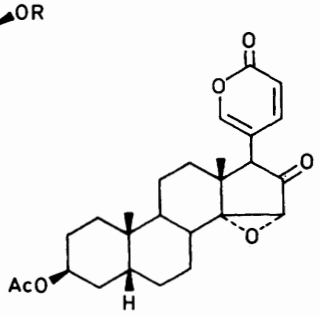
(**5**) a; R = Ac
b; R = H



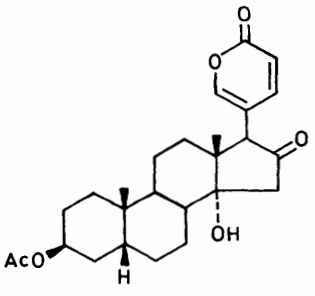
(**6**)



(**7**) a; R = Ac
b; R = H



(**8**)



(**9**)

via the 14-ene (**7a**).[12] Direct oxidation of the 14-ene (**7a**) with *m*-chloroperbenzoic acid provided the 14α,15α-epoxide (**5a**). Selective hydrolysis of the diacetate (**5a**) with sodium hydrogen carbonate in aqueous methanol–methylene dichloride afforded the 3-monoacetate (**5b**). The n.m.r. spectra of compounds (**5a**), (**5b**), and (**7a**) revealed couplings (*J* values of 5.2, 4.9, and 6.3, respectively) between 16- and 17-H that suggested these protons must have 16β and 17α configurations. The assignments were in accord with those made by Nambara *et al.*[13] in a study of 14β,15β-epoxypregnanes with various groups at C-16.

Oxidation of the 16β-alcohol (**5b**) with chromium trioxide provided the 16-ketone (**8**), whose n.m.r. spectrum appropriately exhibited a sharp singlet for the 17α-proton shifted downfield to δ 3.50. The epoxide (**8**) was reduced with chromium(II) acetate, the same reagent used in our previous syntheses of telocinobufagin[12] and bufotalin.[10] Preparative t.l.c. separation of the resulting product led to a fraction of the crude 14α-alcohol (**9**). Without further purification, this fraction was reduced with Urushibara Nickel A[14] in ethanol.[10] Purification by silica gel column chromatography afforded the 16β-alcohol (**2b**), the 14α-epimer of deacetylbufotalin 3-acetate. Sodium borohydride reduction of the crude 16-ketone (**9**) also gave the 16β-alcohol (**2b**) identical with that obtained from reduction of the ketone (**9**) with Urushibara Nickel A. In the ^{1}H n.m.r. spectrum of the 16β-alcohol (**2b**), the 17α-proton (δ 3.06) showed a coupling of *J* 8 Hz with the 16α-proton. The 15α-proton appeared at δ 2.28, coupled (*J* 14 Hz) with the geminal 15β-proton (δ 1.64) and with the 16α-proton (*J* 8 Hz).

Acetylation of the 16β-alcohol (**2b**) with acetic anhydride–pyridine afforded the diacetate (**2a**). The 16α-proton was found appropriately shifted downfield to δ 5.47. Other ^{1}H n.m.r. shifts for the diacetate (**2b**) were in reasonable agreement with those of the monoacetate (**2a**). The structure of the diacetate (**2a**) was also confirmed by dehydration with thionyl chloride to yield the 14-ene (**7b**). The 60 MHz ^{1}H n.m.r. spectra of the 14α-alcohols (**2a**) and (**2b**) reflected 15α,16α-proton couplings (*J* 7.5 Hz), but not 15β,16α-couplings. However, the 300 MHz ^{1}H n.m.r. spectrum of the structurally related bufotalin (**6**) does show a small 15β,16α-proton coupling (*J* 1.6 Hz). During the chromatographic purification of the 14α,16β-diol (**2b**), small amounts of the 14-ene (**7b**)[8] were isolated from fractions eluted ahead of those containing the diol (**2b**).

The ^{1}H n.m.r. signals of protons on the bufadienolide 2-pyrone ring present a consistent coupling constant pattern. Protons at C-22 and -23 were found coupled with a value of *ca.* 9.5 Hz, whereas 21- and 22-H showed a long-range coupling of 2.5 Hz. In addition, ^{1}H n.m.r. spectra recorded at higher fields (300—400 MHz) showed a small long-range coupling between 21- and 23-H of *ca.* 1.3 Hz which may not have been reported previously. Significant variations in shifts for 22-H occurred with changing stereochemistry and substitution in ring D. Komatsu and co-workers[15] noted earlier that the 22-H signal was shifted markedly downfield by 14β-hydroxy and 14β,15β-epoxy substituents. The C-22 proton of the 14β,15β-epoxides (**4**) and (**6**) exhibit this type of downfield shift.

Initial biological evaluation of 16α-deacetylcinobufagin (**1a**) and 14α-hydroxybufotalin (**2a**) against the P388 lymphocytic leukaemia cell line gave cell growth inhibition at ED_{50} 19 and 11 μg ml^{-1}, respectively.

Experimental

Introduction to the Experimental sections of bufadienolides Parts 21[11] and 27[12] provides a general synopsis of techniques employed in the present study. M.p.s were determined on a Reichert micromelting point apparatus and are uncorrected. Plates (Analtech Inc.) of silica gel GHLF (0.25 mm) or GF (2.0

Table. Bufadienolide ^{1}H n.m.r. chemical shifts (δ) expressed in p.p.m. from internal tetramethylsilane. Coupling constants (J), expressed in Hz, are in parentheses. All spectra were recorded in deuteriochloroform

	Bufadienolide [Field (MHz)]												
Position	(**1a**) [100]	(**1b**) [100]	(**1c**) [100]	(**2a**) [100]	(**2b**) [100]	(**3**)[a] [60]	(**4**) [400]	(**5a**) [400]	(**5b**) [400]	(**6**) [300]	(**7a**) [400]	((**8**) [100]	(**9**) [100]
3		5.03, m	5.10, m	5.10, m	5.07, m	5.08, m	4.13, m	5.07, m	5.05, m	4.12, m	5.04, m	5.10, m	5.11, m
15	3.52, s	3.52, s	3.51, s	(α) 2.37, dd (14, 7.5) (β)	(α) 2.28, dd (14, 7.5) (β)	3.52, s	3.57, d (1.0)	3.62, s	3.52, d (1.0)	(α) 2.62, dd (15.6, 9.0) (β) *ca.* 1.9, dd (15.6, 1.6)	5.50, d (2.6)	3.37, s	
16	4.32, d (5.0)	4.32, d (5.0)	5.28, d (5.0)	5.47, dd (8.0, 7.5)	4.64, dd (7.5, 7.5)		4.72, d (9.1)	5.30, dd (5.2, 1.1)	4.38, ddd (5.5, 4.9, 1.1)	5.51, ddd (9.0, 8.9, 1.6)	5.53, dd (6.3, 2.6)		
17	2.35, d (5.0)	2.33, d (5.0)	2.54, d (5.0)	3.28, d (8.0)	3.06, d (7.5)	2.61, s	2.58, d (9.3)	2.60, d (5.3)	2.38 (4.9)	2.85, d (8.9)	2.67, d (6.3)	3.50, s	3.54, s
18	0.99, s	0.98, s	1.01, s	0.97, s	0.99, s	0.97, s	0.79, s	1.01, s	1.01, s	0.77, s	1.05, s	0.85, s	0.80, s
19	1.00, s	1.01, s	1.01, s	1.02, s	1.03, s	1.04, s	0.96, s	1.01, s	1.01, s	0.96, s	1.02, s	1.03, s	1.02, s
21	7.45, d (2.5)	7.45, d (2.5)	7.38, d (2.5)	7.38, d (2.5)	7.48, d (2.5)	7.29, d (3)	7.23, dd (2.5, 1.0)	7.38, dd (2.5, 1.0)	7.54, dd (2.5, 1.0)	7.23, dd (2.6, 0.9)	7.38, dd (2.2, 0.8)	7.37, d (2.5)	7.22, d (2.5)
22	7.72, dd (10.0, 2.5)	7.72, dd (10.0, 2.5)	7.45, dd (10.0, 2.5)	7.44, dd (9.5, 2.5)	7.60, dd (9.5, 2.5)	7.45, dd (10, 3)	7.93, dd (9.5, 2.3)	7.39, dd (9.4, 2.5)	7.55 (9.4, 2.5)	8.02, dd (9.8, 2.6)	7.50, dd (9.9, 2.2)	7.08, dd (9.5, 2.5)	7.10, dd (9.5, 2.5)
23	6.18, d (10.0)	6.18, d (10.0)	6.24, d (10.0)	6.22, d (9.5)	6.20, d (9.5)	6.24, d (10)	6.22, dd (9.5, 1.0)	6.28, dd (9.4, 1.3)	6.27, dd (9.5, 1.0)	6.16, dd (9.8, 0.9)	6.25, dd (9.9, 0.8)	6.30, d (9.5)	6.28, d (9.5)
3-OAc		2.05, s	2.05, s	2.04, s		2.08, s		2.05, s	2.04, s		2.00[b]	2.06, s	2.05, s
16-OAc			2.14, s	2.02, s	2.05, s			2.13, s		1.88, s	2.04[b]		

[a] Values quoted from ref. 11. [b] May be interchanged.

mm) were used for analytical and preparative t.l.c., respectively, with the solvent system hexane–chloroform–acetone (4:3:3). The ^{1}H n.m.r. spectra were recorded using deuteriochloroform solutions, and chemical shifts (δ) are expressed in p.p.m. from tetramethylsilane. Solvent extracts of reaction mixtures were routinely washed with water and dried over anhydrous sodium sulphate. Mass spectra refers to low-resolution electron impact data recorded at 70 eV. The mutual identity of specimens prepared by different routes or with natural products was established by mixture melting point determination, thin-layer chromatographic behaviour, and i.r. spectral comparison.

16α-*Deacetylcinobufagin* (**1a**) *and* 3β-*Acetoxy*-16α-*deacetylcinobufagin* (**1b**).—Sodium borohydride (0.70 g) was added to a solution of ketone (**3**) (0.90 g) in ethanol–methanol (5:1; 18 ml). After 20 min at ambient temperature, the reaction mixture was acidified with dilute sulphuric acid to pH 3 and extracted with chloroform. The combined extract was evaporated under reduced pressure to give the crude product (0.85 g) which was found (t.l.c., R_F 0.33 and 0.19) to contain two components. Chromatography on a silica gel column and elution with hexane–acetone (4:1) provided the alcohols (**1b**) (0.31 g) and (**1a**) (0.27 g).

Recrystallisation of the 16α-alcohol (**1b**) from methanol afforded needles: m.p. 217—219 °C (R_F 0.33) (Found: C, 70.29; H, 7.65. $C_{26}H_{34}O_6$ requires C, 70.56; H, 7.74%); $\lambda_{max.}$(MeOH) 299 nm (log ε 3.38); $\nu_{max.}$(KBr) 3 480—3 120 (OH), 3 038 (Hα to epoxy), 1 735 (ester), 1 710 (conjugated C=O), 1 635—1 620, 1 540 (conjugated C=C), and 1 240 cm^{-1} (C–O); ^{1}H n.m.r. (see Table); *m/z* 442 (M^+).

Recrystallisation of the diol (**1a**) from methanol gave needles: m.p. 204—206 °C (R_F 0.19) (Found: C, 72.02; H, 8.14. $C_{24}H_{32}O_5$ requires C, 71.97; H, 8.05%); $\lambda_{max.}$(MeOH) 298 nm (log ε 3.37); $\nu_{max.}$(KBr) 3 500—3 110 (OH), 3 038 (Hα to epoxy), 1 720 (conjugated C=O), 1 705, 1 635, 1 540 (conjugated C=C), 1 220, 1 190 (epoxy C–O), 957 (C=C), 833 (epoxy C–O), 785 and 750 cm^{-1} (C=C); ^{1}H n.m.r. (see Table); *m/z* 400 (M^+).

Hydrolysis of Acetate (**1b**).—*Procedure A* (*base*). Potassium hydrogen carbonate (34 mg) in water (1.0 ml) was added to a solution of acetate (**1b**) (28 mg) in methanol (6 ml). After 5 days at 30 °C, the solution was acidified to pH 3 with dilute sulphuric acid. The mixture was extracted with chloroform, the solvent was evaporated under reduced pressure, and the residue was chromatographed on a preparative t.l.c. plate. The product (**1c**) (16 mg), m.p. 203—206 °C (from methanol), was identical with the diol (**1a**) prepared by sodium borohydride reduction of ketone (**3**).

Procedure B (*acid*). A solution prepared from acetate (**1b**) (25 mg) in methanol (2 ml) and water (0.05 ml) was treated with toluene-*p*-sulphonic acid (40 mg). After 3 days at ambient temperature, the mixture was adjusted to pH 3.0 with dilute sodium hydrogen carbonate, the solvent was evaporated to one-third of the original volume, and the solution was poured into ice–water and extracted with chloroform. The combined extract was concentrated and the residue subjected to preparative t.l.c. as in Procedure A to yield the diol (**1a**) (19.5 mg), identical with the product from base hydrolysis.

Chromium Trioxide Oxidation of the 16α-*Alcohol* (**1b**).—A solution of the 16α-alcohol (**1b**) (22 mg) in acetic acid (0.5 ml) was treated with a solution of chromium trioxide (10 mg) in acetic acid (0.25 ml). The mixture was stirred (1 h) and allowed to stand (16 h) at room temperature. Methanol (0.2 ml) was added, and the mixture was poured into water and extracted with chloroform. After evaporating the solvent, preparative t.l.c. separation of the crude product gave the 16-ketone (**3**) (15 mg), m.p. 226—228 °C (from acetone–methanol), identical with an authentic sample.

3β-*Acetoxy*-16α-*cinobufagin* (**1c**).—3β-Acetate (**1b**) (45 mg) was treated with acetic anhydride (0.7 ml) and pyridine (1.0 ml) for 2 h at ambient temperature. Evaporation of solvent under reduced pressure afforded a crude product (42 mg) which was purified by preparative t.l.c. to give the 3,16-diacetate (**1c**) (38 mg), m.p. 210—212 °C (from acetone) (Found: C, 70.70; H, 7.86. $C_{16}H_{34}O_6$ requires C, 70.56; H, 7.74%); $\lambda_{max.}$(MeOH) 300 nm (log ε 3.66); $\nu_{max.}$(KBr) 3 480—3 120 (OH), 3 040 (Hα to epoxy), 1 740 (ester), 1 710 (conjugated C=O), 1 635, 1 540 (conjugated C=C), 1 260, 1 240, 1 220, 1 190 (C–O), 957, 833, 786, and 748 cm^{-1}; ^{1}H n.m.r. (see Table); *m/z* 442 (M^+).

When the 3β,16α-diol (**1a**) (20 mg) was treated with acetic anhydride–pyridine as described above for acetylation of 16β-ol (**1b**), a sample of diacetate (**1c**) (15 mg) was obtained, identical with the product from 16β-ol (**1b**).

3β-*Acetoxy*-16β-*hydroxy*-14α,15α-*epoxy*-5β-*bufa*-20,22-*dienolide* (**5b**).—Sodium hydrogen carbonate (10% aqueous, 10 ml) was added to a solution of diacetate (**5a**) (1.0 g) in methylene dichloride–methanol (1:4; 200 ml). After 7 days at 30 °C, the mixture was acidified to pH 3 with dilute sulphuric acid, and the solvent concentrated to half-volume under reduced pressure. The residual mixture was poured into ice–water and extracted with chloroform. Following evaporation of solvent, the crude product was chromatographed on a silica gel column with a hexane–acetone gradient (5:1→3:1→2:1) to yield 3β-monoacetate (**5b**) (0.385 g), m.p. 209—211 °C (from acetone–hexane) (Found: C, 70.33; H, 7.71. $C_{26}H_{34}O_6$ requires C, 70.56; H, 7.74%); $\lambda_{max.}$(MeOH) 301 nm (log ε 3.79); $\nu_{max.}$(KBr) 3 450 (OH), 1 730 (ester C=O), 1 720 (conjugated C=O), 1 640, 1 540, 1 260—1 240, 1 230, 956, and 755 cm^{-1}; ^{1}H n.m.r. (see Table); *m/z* 442 (M^+).

3β-*Acetoxy*-16-*oxo*-14α,15α-*epoxy*-5β-*bufa*-20,22-*dienolide* (**8**).—Chromium trioxide (1.5 g) in dry pyridine (9 ml) was added to a solution of 16β-ol (**5b**) (356 mg) in dry pyridine (5 ml). The mixture was stirred (4.5 h; ambient temperature), poured into ice–water, acidified (to pH 3) with dilute sulphuric acid, and extracted with methylene dichloride. Evaporation of solvent afforded a crude product which was separated by dry column chromatography (silica gel column). Elution with a solvent gradient of hexane–acetone (5:1→3:1) gave the 16-ketone (**8**) (0.21 g), m.p. 222—225 °C (needles from acetone) (Found: C, 70.95; H, 7.18. $C_{26}H_{32}O_6$ requires C, 70.89; H, 7.32%); $\lambda_{max.}$(MeOH) 301 nm (log ε 3.82); $\nu_{max.}$(KBr) 1 740 (ester), 1 720 (conjugated C=O), 1 700 (ketone), 1 645, 1 543, 1 265, 1 252, 1 233, 956, 865, and 755 cm^{-1}; ^{1}H n.m.r. (see Table); *m/z* 440 (M^+).

Chromium(II) *Acetate Reduction of* 3β-*Acetoxy*-16-*oxo*-14α,15α-*epoxy*-5β-*bufa*-20,22-*dienolide* (**8**).—Chromium(II) acetate (800 mg) was added to a solution of epoxy ketone (**8**) (0.11 g) in ethanol (6 ml). The mixture was stirred (30 min) at room temperature, poured into ice–water, and extracted with methylene dichloride. Evaporation of solvent gave a residue which was separated by preparative t.l.c. to give the crude 14α-ol (**9**) (80 mg), amorphous; the ^{1}H n.m.r. appears in the Table. The product was used in the following preparation without further purification.

3β-*Acetoxy*-14α,16β-*dihydroxy*-5β-*bufa*-20,22-*dienolide* (**2b**).—*Method A. Using Urushibara Nickel A.* A large excess of freshly prepared Urushibara Nickel A was added[10,14] to a solution of ketone (**9**) (80 mg prepared as summmarized in the preceding experiment) in ethanol (8 ml). The solution was

heated under reflux (1 h), filtered, and the solvent evaporated under reduced pressure. The residue was chromatographed on a column of silica gel (12 g) with a solvent gradient of hexane–acetone (5:1→2:1) to give the diol (**2b**) (25 mg) and 14-ene (**7b**) (8 mg) which was eluted before 16β-ol (**2b**) (*vide infra*): m.p. 231—233 °C (Found: C, 70.37; H, 8.25. $C_{26}H_{36}O_6$ requires C, 70.24; H, 8.16%); $\lambda_{max.}$(MeOH) 299 nm (log ε 4.12); $\nu_{max.}$(KBr) 3 580, 3 500, 1 735, 1 720, 1 634, 1 535, 1 265, 1 235, 1 135, 1 020, 950, 830, and 755 cm^{-1}; ^1H n.m.r. (see Table); *m/z* 444 (M^+), 426 ($M^+ - H_2O$), and 408 ($M^+ - 2H_2O$).

The 14-ene (**7b**) crystallised as prisms, m.p. 232—235 °C (from acetone), and was identical with an authentic sample of 3β-acetoxy-16β-hydroxy-5β-bufa-14,20,22-trienolide.[10]

Method B. Using sodium borohydride. Sodium borohydride (7 mg) was added to ketone (**9**) (20 mg) in dioxane–water (6:1; 3.5 ml) at room temperature. After 5 h the mixture was poured into ice–water and acidified with dilute sulphuric acid. After extraction with methylene dichloride, the solvent was removed, and the crude product was purified as described under Method A to yield the diol (**2b**) (18 mg), m.p. 231—233 °C, identical with the product of Method A.

The diol (**2b**) was treated with acetic anhydride (0.8 ml) and pyridine (1.0 ml) for 17 h at ambient temperature. Preparative t.l.c. gave the *diacetate* (**2a**) (15 mg) as needles (from acetone–hexane), m.p. 226—228 °C (Found: C, 69.24; H, 7.92. $C_{28}H_{38}O_7$ requires C, 69.11; H, 7.87%); $\lambda_{max.}$(MeOH) 299 nm (log ε 4.12); $\nu_{max.}$(KBr) 3 450, 1 735, 1 720, 1 710, 1 635, 1 538, 1 260, 1 245, 1 220, 960, 850, 790, and 754 cm^{-1}; ^1H n.m.r. (see Table); *m/z* 486 (M^+), 468 ($M^+ - H_2O$), 426 ($M^+ - MeCO_2H$), 408 ($M^+ - MeCO_2H - H_2O$), and 348 ($M^+ - 2MeCO_2H$).

14-*Dehydrobufotalin Acetate* (**7a**).—Thionyl chloride (0.1 ml) was added to a solution of 14α-ol (**2a**) (14 mg) in pyridine (1 ml). After 25 min at 0 °C, the mixture was poured into ice–water and extracted with methylene dichloride. The combined extracts were washed with water followed by 2% hydrochloric acid, and the solvent was evaporated. Preparative t.l.c. yielded 14-ene (**7a**) (11 mg), m.p. 205—207 °C (from acetone), identical with an authentic sample.[11]

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