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BUFADIENOLIDES—19

3β-ACETOXY-15-OXO-5β,14β-BUFA-8,20,22-TRIENOLIDE[1]

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Abstract—Reaction of 3β - hydroxy - 14α,15α - epoxy - 5β - bufa - 20,22 - dienolide (**1a**) with 72% perchloric acid was found to yield 14β - artebufogenin (**2a**), 14α - artebufogenin (**2b**), 15α - hydroxy-bufalin (**3a**), and a new substance 3β,15ξ - dihydroxy - 5β,14ξ - bufa - 8,20,22 - trienolide (**4a**). Similar results were realized by antimony (III) chloride and an iron(III) chloride catalyzed ring opening of epoxide **1a**. Selective acetylation of the new bufadienolide (**4a**) followed by oxidation completed a route to the title substance.

Aqueous acid promoted ring opening of 3β - hydroxy - 14α,15α - epoxy - 5β - bufa - 20,22 - dienolide (**1a**) was shown in a prior study to afford mixtures composed of the 14β - and 14α - artebufogenins (**2a** and **2b**) and 15α - hydroxy-bufalin (**3a**).[2] Each of these experiments also seemed to lead to trace amounts of a new substance which was not further characterized. We have now found that short (30 min) contact of epoxide **1a** with 72% perchloric acid in chloroform-acetone afforded the new substance (**4a**) as major product. Similar results were obtained using antimony(III) chloride and iron(III) chloride. In each case the mixture also contained bufadienolides **2a**, **2b** and **3a**. As the new results of epoxide-ring opening seem to offer promise of entry into still more difficultly accessible bufadienolides, the present investigation concerned with the structure of olefin **4a** was undertaken.

Firstly it was found that similar perchloric acid treatment of acetate **1b** proceeded in analogous fashion to afford alcohol **4b**. Selective acetylation of the original product, diol **4a**, also led to monoacetate **4b**. A prolonged acetylation applied to either diol **4a** or monoacetate **4b** gave the same product, diacetate **4c**. These reactions established that the newly introduced OH group was relatively hindered and about comparable to that expected of a 15α-OH substituent. That the new substance was indeed a diol was shown by chromium trioxide oxidation to diketone **5** and analogous oxidation of monoalcohol **4b** to ketone **6**. No OH absorption appeared in the IR spectrum of diacetate **4c** and the PMR spectrum displayed signals at δ 1·99 and 2·06 corresponding to only the acetate groups at C-15 and C-3 respectively. Also, no OH absorption was detected in the IR spectrum of diketone **5** and the 19-Me chemical shifts of compounds **4**, **5** and **6** were all at lower field than that usually observed with, for example, bufadienolides **1**, **2** or **3**. This suggested deshielding of the 19-Me 1 group by an 8(9) - olefin system.

The position of the isolated olefin was established as follows. The UV spectra of ketones **5** and **6** did not suggest the presence of a conjugated ketone system thereby excluding an 8(14) - position for the olefin. The 7 and 9-positions for the olefin were eliminated by lack of vinyl proton signals in the PMR spectrum of olefin **4a**. The PMR spectra of compounds **4**, **5** and **6** did, in each case, exhibit vinyl proton signals attributable to the 5-substituted 2-pyrone system.

Further evidence[3] for the partial structural assignment of diol **4a** was obtained by results of mass spectral determination employing bufadienolides **4**, **5** and **6**. For example, the characteristic peak [3a] due to loss of the pyrone ring + C-17, C-16, C-15 + any attached substituents was observed at *m/e* 274 (M-152, M-$C_8H_8O_3$) in the mass spectrum of **4b**, followed by expulsion of acetic acid (*m/e* 214) and a Me radical (*m/e* 199). The same peaks obtain with ketone **6**, thus establishing the location of the oxygen substituent in ring D. Were this OH group in **4b** situated at C-14, then the ion at *m/e* 274 would contain one extra O atom and be shifted to *m/e* 290. The presence of ions at *m/e* 153 (comprising the pyrone ring + C-17, C-16, C-15 + substituents) with **4b** and *m/e* 151 with the corresponding ketone **6** ($C_8H_9O_3$ and $C_8H_7O_3$ in 15 - hydroxy - and 15 - ketobufadienolides respectively)[3a] provides compelling evidence for oxygen substitution at C-15 in both compounds.

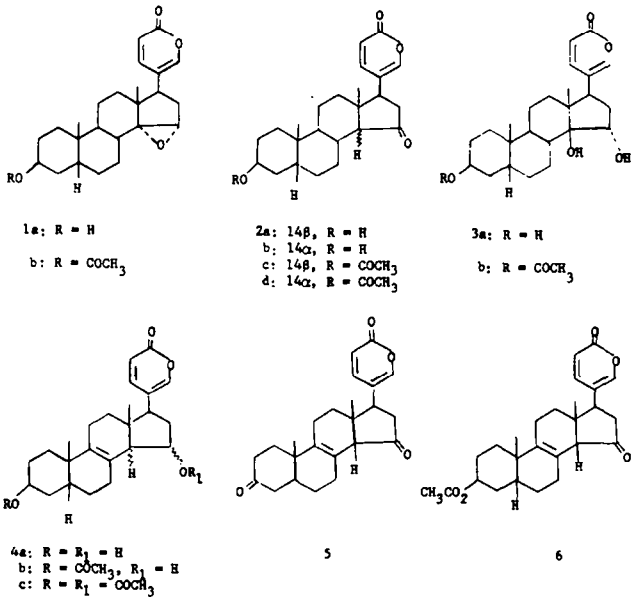
Bufadienolides **4b** and **6** also exhibited peaks at *m/e* 312 and 310 respectively (M-114, M-CH_3CO_2H-C_4H_6), due to initial elimination of acetic acid from ring A followed by a retro-Diels/Alder expulsion of C-1 through C-4 as butadiene.[3a] It has been noted that this fragmentation pathway is only followed in the bufadienolides in the presence of some structural stabilizing influence on the ionized 5(10)-double bond formed in the M-114 ion, such as the 5β-hydroxyl group in telocinobufagin.[3a] In the proposed structures for compounds **4b** and **6** in this series, the 8(9)-olefin moiety nicely accounts for this behavior, since it would be directly conjugated with the ionized 5(10)-double bond in the M-114 ion.

While exact stereochemical assignments at positions 14 and 15 for diol **4a** were not made in the present study, it was possible to make a reasonable assignment at position 14 for ketones **5** and **6** by evaluating their optical rotatory dispersion behavior. The ORD curve of ketone **6** was analogous to that obtained using 14β-artebufogenin acetate (**2c**)[4] and indicated assignment of the 14β-configuration.

Thus, mild treatment of epoxide **1a** with perchloric acid followed by selective acetylation and oxidation completes an experimentally convenient route to the first example of a bufa-8,20,22-trienolide. The ready formation of 8-ene **4a** from epoxide **1a** suggests that such bufadienolides may be present in certain natural products. Also, this conversion opens attractive possibilities for completing synthetic routes to naturally occurring bufadienolides of the gamabufotalin and argentinogenin types.

EXPERIMENTAL

All TLC assessments were performed using commercial (E. Merck Darmstadt) silica gel HF-254 plates with acetone-$CHCl_3$-*n*-



1a: R = H
b: R = $COCH_3$

2a: 14β, R = H
b: 14α, R = H
c: 14β, R = $COCH_3$
d: 14α, R = $COCH_3$

3a: R = H
b: R = $COCH_3$

4a: $R = R_1 = H$
b: R = $COCH_3$, R_1 = H
c: $R = R_1 = COCH_3$

5

6

He (3:3:4) (A) and MeOH–$CHCl_3$ (1:9) (B) as solvent. Thin-layer chromatograms were developed with conc H_2SO_4. M.pts were recorded using a Reichert micro-hot stage apparatus and are uncorrected. Other general experimental and chromatographic techniques including elemental analysis (laboratory of Dr. A. Bernhardt) have been summarized in introductions to the experimental sections of parts 5, 7 and 10 of this series.[5] The UV (95% EtOH soln), IR (in KBr), and NMR [deuterochloroform soln, (TMS)] measurements were recorded by Miss Katie Reimer using the instruments specified in Ref. 4*a*. The low resolution mass spectra were recorded by Mr. E. C. Kelley using an Atlas CH-4B instrument equipped with a molecular beam inlet system under the following conditions: electron energy 70eV, trap current 19μA, source temp. 215–224°, probe temp. 125–180°, accelerating voltage 3 kV. Accurate mass measurements were made by Mr. R. Scott using an Atlas SM-1B double focusing instrument with electron energy 70 eV, trap current 300μA, source temp. 175°, probe temp. 135–215°, accelerating voltage 8 kV, and resolution approx. 10,000.

3β,15ξ-Dihydroxy-5β,14ξ-bufa-8,20,22-*trienolide* (**4a**)

Method A, using 72% perchloric acid. To a soln of **1a**, (0·12 g) in chloroform (2 ml) - acetone (5 ml) was added 0·1 ml of 72% perchloric acid. After 30 min at room temp. the mixture was poured into water and extracted with chloroform. The organic phase was washed with water and concentrated under reduced pressure to dryness. The residue (0·13 g) was chromatographed on a column of silica gel. The fractions eluted by 5:1 and 3:1 ligroin - acetone were found[6] to be 14β - *artebufogenin* (**2a**, 35 mg, m.p. 127–130°), 14α - *artebufogenin* (**2b**, 0·2 mg, m.p. 262–265°), 15α - *hydroxy - bufalin* **3a**, 6 *mg, m.p.* 271–272°), *and* 3β,15ξ - *dihydroxy* - 5β,14ξ - *bufa* - 8,20,22 - *trienolide* (**4a**, 71 mg, m.p. 216–218°). A pure specimen of diol **4a** was obtained by recrystallization from acetone R_f 0·12 (A), 0·15 (B) (color: greenish blue); m.p. 216–218°; λ_{max} 302 (log ε = 3·49 mμ; δ 0·69 (s, 18-methyl), 1·12 (s, 19-methyl), 3·94 (broad, 3α-proton), 4·33 (broad, 15-proton), 6·29 (d, J = 11, 23-proton), 7·27 (d, J = 3, 21-proton), and 7·28 (q, J = 11 and 3, 22-proton); ν_{max} 3420, 1710, 1633, 1537, 958, 910, 755 and 745 cm^{-1}; and mass spectrum *m/e* 384 (M^+), 366, 351, 348, 333, 312, 232, 215, 199, 153, 123 and 95. (Found: C, 74·53; H, 8·41. $C_{24}H_{32}O_4$ requires: C, 74·97; H, 8·39%).

Method B, using antimony(III) *chloride.* Antimony(III) chloride (50 mg) was added to a soln of **1a** (50 mg) in $CHCl_3$ (3 ml). The mixture was stirred 30 min at room temp. and then poured into water. The $CHCl_3$ layer was washed with dil. $NaHCO_3$ soln and water. After removing solvent the residue was chromatographed on a column of silica gel as described above (Method A). By this means the following products were obtained: 12 mg of ketone **2a** (m.p. 127–130°), 3 mg of ketone **2b** (m.p. 263–266°), 7 mg of triol **3** (m.p. 271–273°), and 31 mg of 8-olefin **4a** (m.p. 215–219°).

Method C, using iron(III) *chloride.* The experiment summarized above in Method B was repeated employing α-epoxide **1a** (48 mg) in glacial AcOH (2 ml) with 25 mg of iron(III) chloride hexahydrate. The product comprised 11 mg of ketone **2a** (m.p. 127–129°), 2·5 mg of ketone **2b** (m.p. 262–265°), 4 mg of triol **3** (m.p. 270–273°) and 28 mg of 8-olefin **4a** (m.p. 215–219°).

TLC comparisons were performed using solvents A and B as described above.

3β-Acetoxy-15ξ-hydroxy-5β,14ξ-bufa-8,20,22-*trienolide* (**4b**)

Method A, from epoxide **1b**. A soln of **1b**, (40 mg) in acetone (3 ml) was treated with 0·04 ml of 72% perchloric acid as described aove (see Method A) for obtaining diol **4a**. In this example the fractions eluted from the silica gel column by 9:1 and 5:1 ligroin-acetone were retained to afford 8 mg of ketone **2c** (m.p. 234–236°), 2 mg of ketone **2d** (m.p. 218–221°), 6 mg of monoacetate **3b** (m.p. 279–280°), and 21 mg of 8-olefin **4b** (m.p. 213–216°, prisms from An). The results of physical measurements for olefin **4b** were: R_f 0·34 (A), 0·39 (B) (color: blue); λ_{max} 301 (log ε = 3·98) mμ; δ 0·70 (s, 18-Me), 1·12 (s, 19-Me), 2·04 (s, 3-acetate), 4·32 (broad, 15-proton), 4·97 (broad, 3α-proton), 6·29 (d, J = 11, 23-proton), 7·26 (d, J = 3), and 7·28 (q, J = 11 and 3, 22-proton); ν_{max} 3510, 1740–1720, 1690, 1640, 1540, 1260, 1240, 1210, 955, 910 and 748 cm^{-1}; and mass spectrum *m/e* 426 (M^+), 408, 366, 351, 348, 333, 312, 294, 274, 215, 199, 153, 123 and 95. (Found: C, 72·91; H, 8·04. $C_{26}H_{34}O_5$ requires: C, 73·21; H, 8·04%).

Method B, from diol **4a**. Selective acetylation (Ac_2O-pyridine, room temp, approx. 24 hr) of diol **4a** (20 mg) afforded 17 mg of 3β-acetate **4b**, m.p. 212–215° following recrystallization from acetone. The samples of acetate **4b** prepared by Methods A and B were identical.[6]

3β,15ξ-Diacetoxy-5β,14ξ-bufa-8,20,22-*trienolide* (**4c**)

Method A, from diol **4a**. Extended (48 hr) acetylation of **4a** (20 mg) with Ac_2O (0·3 ml)-pyridine (0·4 ml) at room temp. led to **4c** (14 mg). The product was obtained by chromatography on silica gel and elution with ligroin-acetone (9:1). A pure specimen was obtained as a colorless amorphous solid; R_f 0·49 (A), 0·39 (B) (color: purple); λ_{max}301 (log ε = 3·50) mμ; δ 0·71 (s, 18-Me), 1·11 (s, 19-Me), 1·99 (s, 15-acetate), 2·05 (s, 3-acetate), 4·96 (broad, 3α-proton), 5·47 (broad, 15-proton), 6·29 (d, J = 10·5, 23-proton), 7·25 (d, J = 3, 21-proton), 7·27 (q, J = 10·5 and 3, 22-proton); ν_{max} 1750–1720, 1645, 1540, 950, 900, 740 cm^{-1}; and mass spectrum *m/e* 468 (M^+), 408, 393, 366, 354, 348, 333, 294, 215 and 199. (Found: C, 71·98; H, 7·63. $C_{28}H_{36}O_6$ requires: C, 71·77; H, 7·74%.)

Method B, from monoacetate **4b**. Application of the extended acetylation procedure (see Method A, **4c**) to 80 mg of monoacetate **4b** afforded 66 mg of diacetate **4c**. Both Methods A and B gave mutually identical[6] samples of diacetate **4c**.

3,15-*Dioxo*-5β,14β-*bufa*-8,20,22-*trienolide* (**5**)

A 4% soln (1 ml) of CrO_3 in glacial AcOH was added to **4a** (0·10 g) in 3·5 ml of glacial AcOH. The mixture was stirred 2 hr at room temp. and excess CrO_3 was reduced by adding MeOH. The resulting mixture was diluted with water and extracted with $CHCl_3$. The combined extract was washed with dil $NaHCO_3$ and water. Removal of solvent gave a residue (0·10 g) which recrystallized from MeOH to yield (43 mg) **5** as needles melting at 187–189°: R_f 0·32 (A), 0·37 (B) (color: orange yellow); λ_{max} 300 (log $\epsilon = 3{\cdot}88$) mμ; δ 0·90 (s, 18-Me), 1·20 (s, 19-Me), 6·38 (d, J = 9, 23-proton), 7·27 (q, J = 9 and 3, 22 - proton), and 7·34 (d, J = 3, 21-proton); ν_{max} 1760, 1730, 1705, 1645, 1545, 950, 755 and 740 cm^{-1}; rd (A) $[\alpha]^{25}$ (nm): 0° (570), −10° (500), −60° (422), −290° (360), −500° (347), −700° (340), −850° (335) (trough), −600° (320), −400° (327) (peak), −530° (312), −760° (305) (trough), −620° (301), −500° (298) (peak), −630° (292), −765° (290) (trough), −650° (287), −565° (283), −490° (278) (peak), −630° (274), −750° (270) (trough), −675° (269) (peak), −750° (265) (trough), −600° (263), −300° (260), 0° (258), +300° (256), +600° (254), +900° (252); and mass spectrum *m/e* 380 (M^+), 352, 310 and 173. (Found: C, 75·65; H, 7·33. $C_{24}H_{28}O_4$ requires: C, 75·76; H, 7·40%)

3β - *Acetoxy* - 15 - *oxo* - 5β,14β - *bufa* - 8,20,22 - *trienolide* (**6**)

To a soln of acohol **4b** (0·13 g) in glacial AcOH (10 ml) was added to a soln of CrO_3 (0·10 g) in glacial AcOH (5 ml). The soln was stirred at room temp. 30 min and excess oxidizing agent was removed by adding MeOH. The crude product was isolated as summarized in the case of diketone **5** and chromatographed on silica gel. Elution with ligroin-acetone (9:1) gave 15-ketone **6** (84 mg) as needles from acetone: R_f 0·43 (A), 0·48 (B) (color: yellow); m.p. 149–152°; λ_{max} 299 (log $\epsilon = 3{\cdot}44$) mμ; δ 0·88 (s, 18-Me), 1·10 (s, 19-Me), 2·07 (s, 3-acetate), 5·02 (broad peak, 3α-proton), 6·40 (d, J = 9, 23-proton), and 7·45–7·20 (m, overlapping signals corresponding to the 22- and 21-proton); ν_{max} 1740, 1720, 1695, 1645, 1545. 1260–1240, 950 and 745 cm^{-1}; rd (B) $[\alpha]^{25}$ (nm): 0° (550), −10° (510), −30° (450), −110° (380), −290° (350), −500° (338), −600° (335), −650° (331) (trough), −600° (329), −500° (328), −320° (222) (peak), −340° (314), −440° (308), −630° (302) (trough, −525° (299) (peak), −610° (294) (trough), −470° (289) (peak), −600° (286), −735° (285) (trough), −580° (282), −495° (280) (peak), −600° (277) (trough), −570° (275) (peak), −620° (274) (trough), −500° (272), −200° (270), 0° (269), +300° (267), +700° (264), +900° (263); and mass spectrum *m/e* 424 (M^+), 364, 349, 310, 274, 214, 199, 160, 151, 123 and 95. (Found: C, 73·64; H, 7·57. $C_{26}H_{32}O_5$ requires: C, 73·56; H. 7·60%.)

REFERENCES

[1]For Bufadienolides 18 and Steroids and Related Natural Products 74, see P. Brown, Y. Kamano and G. R. Pettit, *Org. Mass Spec.* **6**, 613 (1972). The present investigation was supported by the National Cancer Institute (performed pursuant to Contract No. N01-CM-12308 with the Division of Cancer Treatment, N.C.I., Dept. of Health, Education and Welfare) and by the J. W. Kieckhefer Foundation, The Fannie E. Rippel Foundation, and The Motorola Company. The Atlas Mass Spectrometers were purchased using funds from National Science Foundation Grants GB-4939 and GP-6979.

[2]Y. Kamano and G. R. Pettit, *Can. J. Chem.* **51**, 1973 (1973).

[3]However the possibility of a skeletal rearrangement leading to formation of the new diol was not excluded: see, G. R. Pettit, T. R. Kasturi, J. C. Knight and J. Occolowitz, *J. Org. Chem.* **35**, 1404 (1970). For a detailed interpretation of bufadienolide mass spectra refer to: (a), P. Brown, Y. Kamona and G. R. Pettit, *Org. Mass Spec.* **6**, 47 (1972), and (b), Ref. 1.

[4]For the ORD curve of 14β-artebufogenin acetate (**2c**), see Y. Kamano, S. Kumon, T. Arai and M. Komatsu, *Chem. Pharm. Bull.* **21**, 1960 (1973).

[5]G. R. Pettit, C. .L. Herald and J. P. Yardley, *J. Org. Chem.* **35**, 1389 (1970); G. R. Pettit, D. C. Fessler, K. D. Paull, P. Hofer and J. C. Knight, *Ibid*, **35**, 1398 (1970); and J. C. Knight, G. R. Pettit and P. Brown, *Ibid.* **35**, 1415 (1970).

[6]Comparison of thin layer chromatographic, IR spectral, PMR and mixture melting point determination data was used to establish identiy with authentic specimens.