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Bufadienolides. XX. 14β-Chloro-bufadienolides[1]

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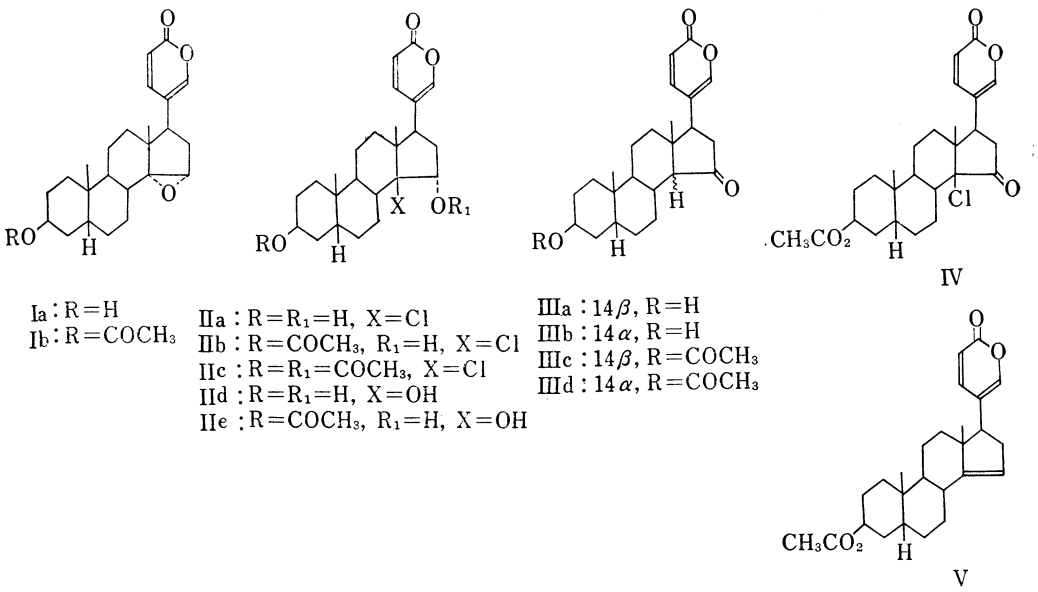
A common structural feature of the naturally occurring bufadienolides and cardenolides is a 14β-hydroxy group which seems important for high biological activity.[3-5] The present study was undertaken to develop a synthetic route to 14β-chloro-bufadienolides and thereby allow an assessment of thiss tructural modification upon biological activity. Previous syntheses of 14β-chloro steroids have been limited to the cardenolide,[6] progesterone,[7] and etianic acid ring systems.[8] In the sequel we have summarized an approach to 14β-chloro-bufadienolides and their further conversion to 14α, 15α-epoxides and 14-olefin derivatives (Chart 1).



Ia : R=H
Ib : R=COCH₃

IIa : R=R₁=H, X=Cl
IIb : R=COCH₃, R₁=H, X=Cl
IIc : R=R₁=COCH₃, X=Cl
IId : R=R₁=H, X=OH
IIe : R=COCH₃, R₁=H, X=OH

IIIa : 14β, R=H
IIIb : 14α, R=H
IIIc : 14β, R=COCH₃
IIId : 14α, R=COCH₃

Chart 1

Reaction of the resibufogenin isomer 14α,15α-epoxide (Ia) with dry hydrogen chloride in chloroform gave 3β,15α-dihydroxy-14β-chloro-5β-bufa-20,22-dienolide (IIa, mp 209—211°).

1) Refer to Y. Kamano, G.R. Pettit, and P. Brown, *J. Org. Chem.*, in Press for part 19 (Steroids and Related Natural Products. 75). The present investigation was supported by Public Health Service Research Grants CA-10612-04 and CA-10115-04 from the National Cancer Institute.

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3) Ch. Tamm, "Proceedings of the First International Pharmacological Meeting, "Vol. 3, ed. by W. Wilbrandt and P. Lindgren, Pergamon Press, London, 1963, pp. 11—26.
4) T. Shigei, M. Katori, H. Murase, and S. Imai, *Experientia*, **20**, 572 (1964).
5) T. Shigei and S. Mineshita, *Experientia*, **24**, 466 (1968).
6) H. Ishii, T. Tozyo, and D. Satoh, *Chem. Pharm. Bull.* (Tokyo), **11**, 576 (1963).
7) H. Hasegawa, Y. Sato, and K. Tsuda, *Chem. Pharm. Bull.* (Tokyo), **9**, 409 (1961).
8) A. Lardon, H.P. Sigg, and T. Reichstein, *Helv. Chim. Acta*, **42**, 1457 (1959).

Mild acetylation of diol (IIa) with acetic anhydride-pyridine (10 hr) afforded 3β-acetoxy-15α-hydroxy-14β-chloro-5β-bufa-20,22-dienolide (IIb, mp 197—199°). Monoacetate (IIb) was also prepared from 3β-acetoxy-14α,15α-epoxide (Ib) by reaction with dry hydrogen chloride. Prolonged acetylation of 3-monoacetate (IIb) afforded 3β,15α-diacetoxy-14β-chloro-5β-bufa-20,22-dienolide (IIc, mp 211—213°). Acetylation of diol (IIa) with refluxing (1 hr) acetic anhydride-pyridine gave in one step the 3β,15α-diacetoxy-derivative (IIc).

Reaction for 10 min between 14α,15α-epoxide (Ia) and conc. hydrochloric acid in chloroform-acetone was also found to yield 3β,15α-dihydroxy-14β-chloro-bufadienolide (IIa). The same treatment of 3β-acetoxy-14α,15α-epoxide (Ib) afforded the corresponding 3-monoacetate (IIb). However, in both reactions with conc. hydrochloric acid, small amounts of 14α-artebufogenin (IIIb or IIId), 14β-artebufogenin (IIIa or IIIc), and 15α-hydroxy-bufalin (IId or IIe) were also formed.

Chemical evidence for the 14-chloro substitution pattern was obtained by oxidizing chlorohydrin (IIb) with chromium trioxide to chloro ketone (IV) followed by a zinc-acetic acid reduction step to provide 14α-artebufogenin acetate (IIId). Analogous reduction of chlorohydrin (IIa) afforded a mixture of 14α,15α-epoxide (Id) and 14-dehydrobufalin acetate (V).

Assignment of the 14β-chloro configuration resided with proton magnetic resonance spectral data and by ready elimination of the chloro group with basic alumina or pyridine to yield 14β-artebufogenin (IIIa) and the starting 14α,15α-epoxide (*cf.*, Ia). The latter transformation would also be expected for a 15β-chloro isomer. Formation of 14β-artebufogenins (IIIa and IIIb) from chlorohydrins (IIa and IIb) suggested an enolate intermediate as noted in Chart 2.

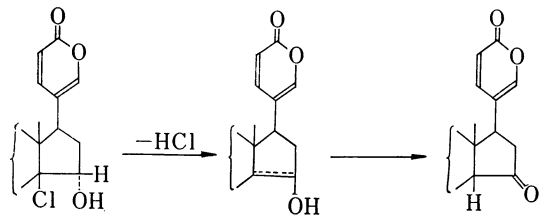

Chart 2

In the proton magnetic resonance (PMR) spectra of 14β-chlorobufadienolides (IIa, IIb, or IIc), signals assignable to the α-pyrone ring C_{22}-proton were shifted further downfield than those of the C_{23}-proton, as usually observed with 14β-hydroxy- and 14β,15β-epoxy-bufadienolides. The deshielding was therefore attributed to a 14β-oriented chloro group. The 14β-chloro orientation was further substantiated by PMR signals corresponding to the 15α-hydroxy or 15α-acetoxybufadienolide (IIb or IIc) C-15β proton which appeared respectively at δ 4.67 and 5.47 (as a doublet, J=5 cps, due to coupling with the C_{16}-protons). Decoupling experiments using alcohol IIc confirmed the C-15 proton assignment.

The convenient synthesis of 14β-chloro-bufadienolides described above should allow ready access to the analogous 14β-bromo- and possibly 14β-iodo-bufadienolides for further structure/activity studies.

Experimental[9]

3β,15α-Dihydroxy-14β-chloro-5β-bufa-20,22-dienolide (IIa)——A solution of α-epoxide (Ia) (150 mg) in 5 ml of dry $CHCl_3$ was cooled (ice bath) and dry HCl gas was introduced over a 10 min period. The mixture was allowed to stand for an additional 90 min at room temperature and then consecutively washed with H_2O, dil. $NaHCO_3$ solution and H_2O. Removal of solvent afforded 163 mg of residue, which was crystallized from acetone to give diol (IIa) (115 mg), mp 205—209°. The diol was recrystallized from MeOH–ether to yield an analytical sample as needles, mp 209—211°. Beilstein Test: positive. UV $\lambda_{max}^{95\%\ EtOH}$ mμ (log ε): 300 (3.85). IR ν_{max}^{KBr} cm^{-1}: 3520 and 3400 (OH), 1730—1710 (conjugated CO), 1645, 1545 (conjugated C=C), 1260, 1250 (ester C–O), 955, 900, 750, 738 (C=C), 690 (Cl). NMR (10% solution in C_5D_5N) δ: 7.68 (1H, dd,

9) Anhydrous sodium sulfate was used to dry solvent extracts of aqueous solutions and all melting points are uncorrected. Other general experimental and instrumental methods were performed as described in previous papers of this series (see ref. 1).

J=3 and 10.5 cps, 22-H), 7.52 (1H, d, J=3 cps, 21-H), 6.38 (1H, d, J=10.5 cps, 23-H), 4.19 (1H, broad peak, 3-H), 1.05 (3H, s, 18-CH_3), 9.05 (3H, s, 19-CH_3). Mass Spectrum *m/e*: M+ 420, 402 (M+–H_2O), 484 (M+–2H_2O and M+–HCl), 348 (M+–2H_2O–HCl). *Anal.* Calcd. for $C_{24}H_{33}O_4Cl$: C, 68.47; H, 7.49; Cl, 8.42. Found: C, 68.53; H, 7.41; Cl, 8.55.

3β-Acetoxy-15α-hydroxy-14β-chloro-5β-bufa-20,22-dienolide (IIb)——(i) From Epoxide (Ib): A solution of epoxide (Ib) (100 mg) in 3 ml of abs. $CHCl_3$ was treated with dry HCl gas for 10 min (at 0°) and product isolated as described above. Removal of solvent gave 111 mg of residue. which was crystallized from MeOH–ether to afford alcohol (IIb) (87 mg, mp 192—197°). Recrystallization from same solvent gave an analytical sample as needles, mp 197—199°. UV $\lambda_{max}^{95\%\ EtOH}$ mμ (log ε): 301 (3.82). IR ν_{max}^{KBr} cm^{-1}: 3520 (OH), 1750, 1720 (conjugated C=C), 1260—1240 (ester C–O), 958, 753 (C=C), 689 (Cl). NMR (10% solution in $CDCl_3$) δ: 7.68 (1H, dd, J=3 and 10 cps, 22-H), 7.32 (1H, d, J=3 cps, 21-H), 6.33 (1H, d, J= 10 cps, 23-H), 5.08 (1H, broad singlet, 3-H), 4.77 (1H, broad doublet, J=5 cps, 15-H), 2.37 (*ca.* 2H, broad doublet, J=5 cps, 16-CH_2), 2.07 (3H, s, 3-$OCOCH_3$), 0.94 (3H, s, 18-CH_3), 0.92 (3H, s, 19-CH_3). Mass Spectrum *m/e*: M+ 462, 444 (M+–H_2O), 426 (M+–HCl), 408 (M+–H_2O–HCl), 402 (M+–AcOH), 366 (M+–HCl–AcOH), 348 (M+–H_2O–HCl–AcOH). *Anal.* Calcd. for $C_{26}H_{35}O_5Cl$: C, 67.45; H, 7.63; Cl, 7.66. Found: C, 67.39; H, 7.59; Cl, 7.61.

(ii) From Alcohol (IIa): Acetylation of alcohol (IIa) (100 mg) with pyridine (2 ml)–Ac_2O (1.4 ml) for 10 hr at room temperature gave, after chromatography [silica gel column and elution with ligroin–acetone (5: 1)], acetate (IIb) (62 mg, mp 195—198° from MeOH–ether) and recovered IIa (mp 205—208°, 27 mg). The product (IIb) was found to be identical with the sample obtained in (i).

3β,15α-Diacetoxy-14β-chloro-5β-bufa-20,22-dienolide (IIc)——(i) From Alcohol (IIb): Acetylation of IIb (50 mg) with pyridine (1.0 ml)–Ac_2O (0.7 ml) for 48 hr at room temperature gave, after chromatographic [silica gel column and elution with ligroin–acetone (7: 1)] separation, 38 mg of diacetate (IIc), mp 211—213° as colorless needles from acetone. UV $\lambda_{max}^{methanol}$ mμ (log ε): 295 (3.64). IR ν_{max}^{KBr} cm^{-1}: 1740, 1720 (conjugated CO and ester CO), 1630, 1535 (conjugated C=C), 1250, 1245, 1235, 1210 (C–O), 950, 760 (C=C), 729 (Cl). NMR (10% solution in $CDCl_3$) δ: 7.59 (1H, dd, J=3 and 9 cps, 22-H), 7.25 (1H, d, J=3 cps, 21-H), 6.29 (1H, d, J=9 cps, 23-H), 5.47 (1H, d, J=5 cps, 15-H), 5.02 (1H, broad peak, 3-H), 2.07 (3H, s, 3-$OCOCH_3$), 2.00 (3H, s, 15-$OCOCH_3$), 0.97 (3H, s, 18-CH_3), 0.91 (3H, s, 19-CH_3). Mass Spectrum *m/e*: M+ 505, 469 (M+-HCl), 445 (M+-AcOH), 409 (M+-HCl–AcOH), 385 (M+–2AcOH), 349 (M+-HCl–2AcOH). *Anal.* Calcd. for $C_{28}H_{37}O_6Cl$: C, 66.58; H, 7.38; Cl, 7.02. Found: C, 66.56; H, 7.33; Cl, 7.18.

(ii) From Diol (IIa): A solution of diol (IIa) (50 mg) in pyridine (2.0 ml)–Ac_2O (1.4 ml) was heated at reflux for 60 min. The mixture was poured into ice–H_2O, and extracted with $CHCl_3$. The extract was washed with dil. HCl solution and H_2O, and evaporated (*in vacuo*) to dryness. Silica gel preparative layer chromatography of the residue (52 mg) gave diacetate (IIc) (35 mg), mp 210—213° as needles from acetone, identical with the sample prepared above (i).

Reaction of 3β-Hydroxy-14α,15α-epoxy-5β-bufa-20,22-dienolide Ia with conc. HCl——To a solution of α-epoxide (Ia) (100 mg) in $CHCl_3$ (3 ml)–acetone (8 ml), 0.25 ml of conc. HCl (36.5%) was added. The mixture was stirred at room temperature. After 20 min, a crystalline material separated to yield the crude 14β-chloro-bufadienolide (IIa) (45 mg). Recrystallization from MeOH–ether gave a pure sample of IIa (32 mg, mp 205—209°, colorless needles).

After dilution of the reaction mixture filtrate with $CHCl_3$, the solution was poured into H_2O. The $CHCl_3$ layer was washed with H_2O and evaporated (*in vacuo*) to dryness. The residue (50 mg) was chromatographed on silica gel and elution with ligroin–acetone (3: 1) led to 8.3 mg of 14β-artebufogenin (IIIa, mp 128—130°, prisms from MeOH), 2.6 mg of 14α-artebufogenin (IIIb, mp 267—269°, prisms from acetone), in addition to 22 mg of 3β,14β,15α-trihydroxy-5β-bufo-20,22-dienolide (15α-hydroxy bufalin, IId, mp 203—208°). Compound (IIa) was found to be identical with the sample prepared using dry HCl gas.

Reaction of 3β-Acetoxy-14α,15α-epoxy-5β-bufa-20,22-dienolide Ib with conc. HCl——To a solution of α-epoxide (Ib) (50 mg) in $CHCl_3$ (2.5 ml), 0.13 ml of conc. HCl (36.5%) was added and the mixture stirred for 10 min at room temperature. After dilution with $CHCl_3$, the mixture was poured into H_2O. The $CHCl_3$ layer was washed with dilute $NaHCO_3$ solution, and H_2O ,and evaporated (*in vacuo*) to dryness. Recrystallization of the product (47 mg) from acetone gave 14β-chloro-bufadienolide (IIb) (mp 195—198°, prisms, 24 mg), which was found to be identical with the sample prepared by treatment with dry HCl gas. The mother liquor residue (22 mg) from the recrystallization was chromatographed on a column of silica gel. Elution with ligroin–acetone (9: 1) and (5: 1) gave 14β-artebufogenin acetate (IIIc, mp 232—235°, 9 mg), 14α-artebufogenin acetate (IIId, mp 221—223°, 3 mg), and 3β-acetoxy-14β,15α-dihydroxy-5β-bufa-20,22-dienolide (15α-hydroxy bufalin 3-monoacetate, IIe, mp 280—283°, 2 mg), and chlorohydrin (IIb) (6 mg).

Conversion of 3β-Acetoxy-15α-hydroxy-14β-chloro-5β-bufa-20,22-dienolide IIb to 3β-Acetoxy-14α,15α-epoxy-5β-bufa-20,22-dienolide (Ib)——(i) With Al_2O_3: To 75 mg of chlorohydrin (IIb) in 9 ml of $CHCl_3$, 1.5 g of Al_2O_3 (basic, Camag) was added and the mixture stirred at room temperature. After 2 hr, 5 ml of MeOH was added and stirring was continued an additional 30 min. The solution was filtered and removal of solvent gave a residue (71 mg), which was chromatographed on a column of silica gel. Elution with ligroin–acetone (9: 1 and 5: 1) provided 3β-acetoxy-14α,15α-epoxy-5β-bufa-20,22-dienolide (Ib, 40 mg) as

a colorless amorphous solid and 14β-artebufogenin acetate (IIIc) (16 mg), mp 233—235° as prisms from acetone.

(ii) With Pyridine: Chlorohydrin (IIb) (25 mg) in pyridine (3 ml) was stirred for 45 min at room temperature. Removal of solvent, *in vacuo*, gave a residue (27 mg), which was chromatographed on a column of silica gel. Elution with ligroin–acetone (9: 1 and 5: 1) gave α-epoxide (Ib) as a colorless amorphous solid, and 14β-artebufogenin acetate (IIIc) (12 mg), mp 233—236° as prisms from acetone.

Conversion of 3β,15α-Dihydroxy-14β-chloro-5β-bufa-20,22-dienolide (IIa) to 3β-Hydroxy-14α,15α-epoxy-5β-bufa-20,22-dienolide (Ia)——(i) With Al_2O_3: Chlorohydrin (IIa) (48 mg) was dissolved in MeOH (0.2 ml)–$CHCl_3$ (3.8 ml), adsorbed on a column of basic Al_2O_3 (5 g) and eluted with 1: 19 MeOH–$CHCl_3$. The eluate was evaporated (*in vacuo*) to give 11 mg of Ia (3β-hydroxy-14α,15α-epoxy-5β-bufa-20,22-dienolide, mp 226—229°, needles from acetone). Further elution led to 14β-artebufogenin (IIIa) (2 mg, mp 125—129°) and starting material, IIa (25 mg, mp 204—209°).

(ii) With Pyridine: A solution of chlorohydrin (IIa) (35 mg) in pyridine (2 ml) was stirred for 4 hr at room temperature. Removal of solvent gave a 34 mg residue, which was chromatographed on a column of silica gel. Elution with ligroin–acetone 9: 1 and 5: 1 gave 9 mg of epoxide (Ia) (mp 227—229°, needles from acetone, 6 mg of IIIa (mp 125—129°), and 12 mg of the starting material, IIa.

3β-Acetoxy-14β-chloro-15-oxo-5β-bufa-20,22-dienolide (IV)——To a solution of chlorohydrin (IIb) (38.5 mg) dissolved in 1.2 ml of AcOH, was added 0.45 ml of a 2% solution of CrO_3 in AcOH, and the mixture was stirred for 2 hr at room temperature. Excess CrO_3 was reduced with MeOH and the mixture was diluted with H_2O to yield a precipitate which was collected by filtration, washed with H_2O, dried, and crystallized from acetone to give 36 mg of ketone (IV), mp 209—213°, as colorless needles. Recrystallization from the same solvent gave an analytical sample, mp 212—214°. Positive Beilstein Test: UV $\lambda_{max}^{CHCl_3}$ mμ (log ε): 298 (3.88). IR ν_{max}^{KBr} cm^{-1}: 1755—1740, 1730—1720 (ester CO and conjugated CO), 1640, 1530 (conjugated C=C), 1250, 1230 (C–O), 955, 745 (C=C), 730 (Cl). NMR (10% solution in $CDCl_3$) δ: 7.58 (1H, q, J=10.5 and 2.5 cps, 22–H), 7.43 (1H, d, J=2.5 cps, 21–H), 6.25 (1H, d, J=10.5 cps, 23–H), 5.06 (1H, broad singlet, 3α–H), 2.78 (2H, s, 16–CH_2), 2.05 (3H, s, 3–OAc), 1.07 (3H, s, 18–CH_3), 0.94 (3H, s, 19–CH_3). Mass Spectrum *m/e*: M^+ 460, 424 (M^+–HCl), 400 (M^+–AcOH), 364 (M^+–HCl–AcOH). *Anal.* Calcd. for $C_{26}H_{33}O_5Cl$: C, 67.72; H, 7.21; Cl, 7.69. Found: C, 67.68; H, 7.22; Cl, 7.71.

Reduction of 3β-Acetoxy-14β-chloro-15-oxo-5β-bufa-20,22-dienolide (IV)——A mixture prepared from chloroketone (IV) (45 mg), sodium acetate (48 mg), zinc powder (90 mg), and acetic acid (2 ml)–methanol (2 ml) was heated at reflux for 2.5 hr. The solution was filtered and solvent removed under reduced pressure to provide the crude product (47 mg), which was submitted to preparative thin-layer chromatography using 3: 3: 4 acetone–chloroform–ligroin. Elution of the absorbent corresponding to a spot at *Rf* 0.57 and recrystallization from acetone gave 14α-artebufogenin acetate (IIId) (29 mg), mp 220—223°, as colorless prisms, which was identical with an authentic specimen.

Reduction of 3β-Acetoxy-14β-chloro-15α-hydroxy-5β-bufa-20,22-dienolide (IIb)——A mixture prepared from chlorohydrin (IIb) (50 mg), sodium acetate (50 mg), zinc powder (125 mg), and acetic acid (3 ml)–methanol (1 ml) was heated at reflux for 6 hr. After filtration, the solution was extracted with chloroform, and the extract was washed with H_2O. Removal of solvent led to 48 mg of residue, which was submitted to preparative thin-layer chromatography using acetone–chloroform–ligroin (3: 3: 4). Elution of the absorbent corresponding to a spot at *Rf* 0.73 and recrystallization from acetone gave 14-dehydrobufalin acetate (V, 8 mg), mp 193°, as colorless needles. Next elution of the absorbent corresponding to another spot at *Rf* 0.66 provided 14α,15α-epoxide (Ib) (14 mg) as a colorless amorphous solide. Both products were found to be identical with authentic samples.[10]

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