**Bufadienolides. III.¹⁾ Reduction of Resibufogenin
with Sodium Borohydride²⁾**YOSHIAKI KAMANO, HIROSHI YAMAMOTO
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Partial reduction of resibufogenin (III) having 14 β ,15 β -epoxide with sodium borohydride under various conditions was examined. No bufalin (IV) corresponding 14 β -hydroxy compound was obtained in any case, but instead there was produced 14 β ,15 β -epoxy-5 β -chol-20(22)-ene-3 β ,21,24-triol (V) by the cleavage of an α -pyrone ring. Then V was converted into the corresponding triacetate (VI), bromide (VII) and saturated compound (VIII). The result of this reaction differed that of the reaction of Marinobufagin (I), which was reported⁴⁾ to yield Telocinobufagin (II) corresponding 14 β -hydroxy compound. In our case, it is interesting that 14 β ,15 β -epoxy ring is intact by the treatment with sodium borohydride whereas the α -pyrone ring cleavages smoothly to afford $\Delta^{20(22)}$ -compound. This reaction mechanism was considered to be as depicted in Chart 3.

Bufadienolides occur either as 14 β -hydroxy or 14 β ,15 β -epoxy compounds, chemical transformation of the latter to the former compound constitutes not only an important process but also has a practical value. Bharucha, *et al.*⁴⁾ have achieved the transformation of marinobufagin (I) to telocinobufagin (II) by the treatment with sodium borohydride (Chart 1).

Chart 1

Linde and Meyer,⁵⁾ however, failed to detect bufalin (IV) by the treatment of resibufogenin (III) with sodium borohydride, although they observed a few spots due to polar products by paper chromatography. Although the reaction conditions used by these two groups differed slightly, the contradicting results prompted the present authors to reinvestigate the partial reduction of III.

This paper describes the reaction of resibufogenin (III) with sodium borohydride under various conditions, no bufalin was obtained in any case, but instead there was produced 14 β ,15 β -epoxy-5 β -chol-20(22)-ene-3 β ,21,24-triol, V by the cleavage of an α -pyrone ring (Chart 2).

- 1) Part II: Y. Kamano, H. Yamamoto, Y. Tanaka and M. Komatsu, *Tetrahedron Letters*, **1968**, 5673.
- 2) A part of this work was reported at the 85th Annual Meeting of Pharmaceutical Society of Japan, Tokushima, Oct. 1965.
- 3) Location: No. 34-1 Takata 3-chome, Toshima, Tokyo, 170-91, Japan.
- 4) M. Bharucha, H. Jager, K. Meyer, T. Reichstein and O. Schindler, *Helv. Chim. Acta*, **42**, 1395 (1959).
- 5) H. Linde and K. Meyer, *Helv. Chim. Acta*, **42**, 807 (1959).

Chart 2

When resibufogenin (III) was treated with sodium borohydride in 80% ethanol at -15° to -20° at pH 8–9 under the condition of Bharucha, *et al.*,⁴⁾ a few polar products were detected in addition to the starting material by thin-layer chromatography. No bufalin formation was observed in all cases (Fig. 1 (A)).

Fig. 1. Thin-Layer Chromatograms of the Reaction Solutions and Authentic Substances

III: resibufogenin
IV: bufalin
V : 14 β ,15 β -epoxy-5 β -chol-20(22)-en-3 β ,21,24-triol
A⁰: the reaction at -20° at pH 8–9 in 80% EtOH,
(A₁, 30 min; A₂, 60 min; A₃, 120 min)
B⁰: the reaction at 20° for 2 hr in MeOH
C : the reaction was added MgSO₄·2H₂O under the
same way as A
D : the reaction at room temperature for 2 hr in
pyridine
E : the reaction at room temperature in dioxane-
H₂O (4:1); (E₁, 30 min; E₂, 4 hr; E₃, 20 hr; E₄, 48 hr)
solvent system: Me₂CO-CHCl₃-*n*-hexane (3:2:2) (A)

Fig. 2. Infrared Spectra of V (a) and VI (b)

Similar chromatogram was always observed under various reaction conditions (see Fig. 1 (A–E)). As is clear from the chromatogram, there is one major spot. The reaction mixture was chromatographed on silica gel or alumina affording a main product, mp 178 – 180° , as

colorless prisms from acetone, whose analytical values and molecular weight were in good agreement with $C_{24}H_{38}O_4$.

This compound, which was found to have structure V, exhibited no significant UV-absorption maximum above 300 $m\mu$. The IR-spectrum of compound V exhibited absorption bands corresponding to the double bond of $R_1R_2C=CHR_3$ type at 1670, 845, 835 and 805 cm^{-1} and the methylene band of $-CH_2-C=C-$ type at 1443 cm^{-1} , but had no characteristic absorption bands of α -pyrone ring (Fig. 2 (a)). The results suggested the cleavage of an α -pyrone ring. This was also supported from the NMR spectrum (in C_5D_5N) of V, which did not show low field signals corresponding to α -pyrone ring protons (Fig. 3). There appeared new peaks at 4.29 τ (1H, triplet, $J=7.5$ cps) attributable to an olefinic proton of the type $R_1R_2C=CHR_3$. Peaks at 5.34 and 5.77 τ (2H, AB quartet, $J=12.1$ cps) and 6.14 τ (2H, triplet, $J=6.5$ cps) were assigned to methylene hydrogens of the two different alcoholic groups, respectively. The multiplet at 7.2–7.6 τ suggested the presence of the another methylene hydrogen. Two triplets at 4.29 τ and 6.14 τ were decoupled to two sharp singlets by irradiation at 7.35 τ (Fig. 3). Spin-decoupling results suggested that V had a partial structure of $R_1R_2C=CHCH_2-CH_2-$. Since an AB quartet at 5.34 and 5.77 τ and a triplet at 6.14 τ shifted to a lower field of 4.80 and 5.10 τ and 5.57 τ , respectively, on addition of $(CF_3CO)_2O$ (Fig. 3 (b)), the quartet and the triplet were attributed to be due to the alcoholic methylene signals at C_{21} and C_{23} .

On acetylation with acetic anhydride in pyridine, compound V gave a triacetate VI as a colorless oil (purity checked by TLC). The IR spectrum of VI did not show a hydroxy band and had a C–H stretching vibration band at 3040 cm^{-1} due to a 14 β ,15 β -epoxy grouping (Fig. 2 (b)). The presence of 14 β ,15 β -epoxy grouping was also supported from the NMR spectrum data, which exhibited characteristic signals at 6.48 τ (1H, singlet) (V) and 6.56 τ (1H, singlet) (VI), respectively (Fig. 3).

Furthermore, treatment of V with bromine gave the corresponding bromide (VII), $C_{24}H_{38}O_4Br_2$, mp 217–219.5°. By catalytic hydrogenation over palladium-on-charcoal, compound V afforded the corresponding saturated compound VIII, $C_{24}H_{40}O_4$, mp 155–157°, consuming a molar equivalent amount of hydrogen (Chart 2). Both compounds VII and VIII showed no absorptions due to a double bond in the IR and NMR spectra.

From these data, the structure of V was established as 14 β ,15 β -epoxy-5 β -chol-20(22)-ene-3 β ,21,24-triol.

These results clearly show that 14 β ,15 β -epoxy ring is intact by the treatment with sodium borohydride whereas the α -pyrone ring

Fig. 3. Nuclear Magnetic Resonance Spectra of V (a,b) and VI (c) at 60 Mc

15 β -epoxy ring is intact by the treatment with sodium borohydride whereas the α -pyrone ring

cleavages smoothly. Thus the epoxide ring cleavage observed in marinobufagin is assumed to be an exceptional case possibly ascribable to the structural features. Tamm⁶⁾ reported that the treatment of 3-dehydrobufalin with sodium borohydride afforded 3-epibufalin and a by-product of which partial structure (A) was assumed to have $\Delta^{22,23}$ -double bond, without experimental evidence.

Based on our results, it is assumed that the partial structure of the compound may be $\Delta^{20,22}$ -structure rather than the Tamm's formulation.

The mechanism of reduction of α -pyrone ring by sodium borohydride is probably the formation of C₂₄-hydroxyl group, followed by the ring-opening, to furnish the reduced $\Delta^{20,22}$ -diol (V) (Chart 3).

Experimental

All melting points are uncorrected. IR spectra were determined in KBr pellets or nujol using a Nihon Bunko Model DS 301 spectrophotometer. UV spectra were recorded on a Hitachi automatic spectrophotometer, Model EPS-2U, in solvents indicated. NMR spectra were measured on a Hitachi Model R-20 spectrometer with tetramethylsilane as an internal standard and are reported in τ values. The solvents used are indicated. Molecular weight were determined using a Hitachi Molecular Weight Apparatus Model 115.

Thin-Layer Chromatography (TLC)—After standing at room temperature for 30–60 min, the silica gel G plates were activated by heating at 140° for 60–90 min. Thin-layer chromatography with silica gel G plates was performed using the following solvent system; (A) acetone-CHCl₃-*n*-hexane (3:2:2) and (B) AcOEt-*n*-hexane (9:1). The spots were detected by spraying conc. H₂SO₄ followed by heating. The reaction of resibufogenin III with NaBH₄ under the following various conditions was followed by thin-layer chromatography as shown in Fig. 1.

The reaction conditions. A⁴⁾ the reaction at -20° at pH 8–9 in 80% EtOH (A₁, 30 min; A₂, 60 min; A₃, 120 min). B⁵⁾ the reaction at 20° for 2 hr in MeOH. C. the reaction was added MgSO₄·2H₂O under the same way as A. D. the reaction at room temperature for 2 hr in pyridine. E. the reaction at room temperature in dioxane-H₂O (4:1) (E₁, 30 min; E₂, 4 hr; E₃, 20 hr; E₄, 48 hr).

Intensity of a spot with an *R_f* value (0.57), corresponding to resibufogenin III, decreased with time, while a spot with an *R_f* value (0.30) corresponding to compound V in Condition A and E was intensified with time. In neither reactions, a spot of bufalin IV (*R_f* 0.43) was detectable.

Sodium Borohydride Reduction of Resibufogenin (III)—a) (Reduction under Condition E.). To a solution of III (1.0 g) dissolved in 120 ml of dioxane-H₂O (4:1), a solution of NaBH₄ (1.0 g) dissolved in 25 ml of the same solvent was gradually added at 0° and the reaction mixture was allowed to stand for 42 hr at room temperature (18–23°). After the excess of NaBH₄ was decomposed and acidified (pH 3) with dil. H₂SO₄ at 0°, the mixture was poured into 240 ml of H₂O and was concentrated *in vacuo* to one-third of the original volume and extracted with CHCl₃. The CHCl₃-extract was washed with dil. NaHCO₃ and H₂O, dried over Na₂SO₄ and evaporated *in vacuo* to give 1.1 g of the crude product, which was chromatographed on 110 g of silica gel (Wakogel C-200). The fraction (0.64 g) eluted with *n*-hexane-acetone (5:1) was collected and recrystallized from Me₂CO to yield 0.58 g of 14 β ,15 β -epoxy-5 β -chol-20(22)-ene-3 β ,21,24-triol, V, mp 178–180°, as colorless prisms, $[\alpha]_D^{25} +0.9^\circ$ (*c*=1.4, CHCl₃). UV λ_{max}^{EtOH} m μ : 205. IR ν_{max}^{KBr} cm⁻¹: 3300 (OH), 3010, 1660, 985, 845, 835, 805, 790 (C=C). NMR (10% solution in C₅D₅N) τ : 4.29 (1H, triplet, *J*=7.5 cps, RRC=CHR), 5.34 and 5.77 (2H, AB quartet, *J*=12.1 cps, 21-CH₂OH), 6.14 (2H, triplet, *J*=6.5 cps, 24-CH₂OH), 6.48 (1H, singlet, 15-CH), 7.2–7.6 (2H, multiplet, -CH₂-CH₂OH), 8.98 (3H, singlet, 19-CH₃),

6) Ch. Tamm, *Helv. Chim. Acta*, **43**, 338 (1960).

9.01 (3H, singlet, 18-CH₃); (in C₆D₅N-CDCl₃ (1:1) added (CF₃CO)₂O, allowed to stand at room temperature for 24 hr) τ : 4.27 (1H, triplet, $J=7.5$ cps, RRC=CHR), 4.80 and 5.10 (2H, AB quartet, $J=12.0$ cps, 21-CH₂OH), 5.57 (2H, triplet, $J=6.2$ cps, 24-CH₂OH), 6.50 (1H, singlet, 15-CH), 7.2—7.6 (2H, multiplet, -CH₂-CH₂OH), 8.98 (3H, singlet, 19-CH₃), 9.10 (3H, singlet, 18-CH₃). Mol. wt. 392. *Anal.* Calcd. for C₂₄H₃₈O₄: C, 73.80; H, 9.81. Found: C, 73.53; H, 9.94. TLC: R_f 0.30 (solvent A); 0.20 (solvent B), Color: reddish brown (conc. H₂SO₄).

b)⁴⁾ (Reduction under Condition A). To a solution of III (250 mg) dissolved in 20 ml of 80% EtOH, a solution of NaBH₄ (75 mg) dissolved in 5 ml of 80% EtOH, was gradually added over a period of 30 min at -15°—-20° at pH 8—9 (the pH was controlled by addition of AcOH) and the mixture was allowed to stand for 2 hr. After acidification (pH 3) with 2N H₂SO₄ at 0°, the reaction mixture was poured into 120 ml of H₂O and was concentrated *in vacuo* to a half of the original volume and extracted with CHCl₃. The extract was successively washed with 2N Na₂CO₃ and H₂O, dried over Na₂SO₄. The crude product (246 mg) was dissolved in a mixture of C₆H₆-CHCl₃ (19:1), chromatographed on a column of Al₂O₃ (5g) eluting successively with C₆H₆-CHCl₃ (19:1), C₆H₆-CHCl₃ (5:1), C₆H₆-CHCl₃ (1:1), CHCl₃ and CHCl₃-MeOH (98:2). The fraction eluted with C₆H₆-CHCl₃ (19:1) and C₆H₆-CHCl₃ (5:1) gave 120 mg of the starting material. The fraction eluted with CHCl₃ was crystallized from acetone to give 13.6 mg of V, mp 177—179°, as colorless prisms. Mixed mp and IR spectrum established the identity with an authentic sample of V, which was obtained by the method a).

c)⁵⁾ (Reduction under Condition B). To a solution of III (100 mg) dissolved in 5 ml of MeOH, a solution of NaBH₄ (35 mg) dissolved in 5 ml of MeOH was added and the mixture was allowed to stand for 2 hr at 20°. The reaction mixture was worked up as described in b). The product was isolated in the way described above. There was obtained 25 mg of prisms, mp 177—179.5°, which was found to be identical with the above V.

d) (Reduction under Condition C). To a solution of III (100 mg) and MgSO₄·7H₂O (20 mg) dissolved in 15 ml of the same solvent was added. The mixture was allowed to stand for 2 hr at -15°. Working up as described in b) gave 14.6 mg of V, mp 178—179°, as colorless prisms, which was found to be identical with the above V.

14 β ,15 β -Epoxy-5 β -chol-20(22)-ene-3 β ,21,24-triyl Triacetate (VI)—Acetylation of V with Ac₂O-pyridine in a usual way gave a triacetate (VI) as colorless oil, which exhibited single spot on thin-layer chromatogram developed with solvents A and B. IR $\nu_{\text{max}}^{\text{Nujol}}$ cm⁻¹: 3040 (15-CH), 1740 (ester CO), 1600, 983, 850, 755 (C=C). NMR (10% solution in CDCl₃) τ : 4.39 (1H, triplet, $J=7.5$ cps, RRC=CH-CH₂-), 4.92 (1H, broad, 3-CH), 5.23 and 5.48 (2H, AB quartet, $J=12.0$ cps, -CH₂OR), 5.92 (2H, triplet, $J=6.5$ cps, -CH₂-CH₂OR), 6.56 (1H, singlet, 15-CH), 7.3—7.7 (2H, multiplet, -CH₂-CH₂OR), 7.91 (6H, singlet, 2×-COCH₃), 7.96 (3H, singlet, -COCH₃), 9.00 (3H, s, 19-CH₃), 9.13 (3H, s, 18-CH₃). TLC: R_f 0.56 (solvent A); 0.61 (solvent B), Color: reddish brown (conc. H₂SO₄).

20 ξ ,22 ξ -Dibromo-14 β ,15 β -epoxy-5 β -cholane-3 β ,21,24-triol (VII)—To a stirred solution of 150 ml of V in 45 ml of CHCl₃, a solution of 180 mg of bromine in 3 ml of the same solvent was added over a period of 5 min at 0°, the mixture was then allowed to stand for 5 min and added a solution of Na₂S₂O₃ (150 mg) dissolved in H₂O. The CHCl₃-layer washed fully with H₂O, dried over Na₂SO₄ and evaporated *in vacuo*. The crude product (150 mg) was purified by column chromatography over silica gel (Wakogel C-200, 6.0 g) in *n*-hexane-EtOAc (1:1) to give 45 mg of VII, mp 217—219.5°. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3360 (OH), 1390, 1145, 1080, 1050, 800, 795. NMR (10% solution in CDCl₃) τ : 5.5—6.5 (*ca.* 5H, multiplet, >CH and 2×CH₂OH), 7.19 (1H, singlet, >CH), 8.65 (3H, singlet, >CH₃), 9.03 (3H, singlet, >CH₃). *Anal.* Calcd. for C₂₄H₃₈O₄Br₂: C, 52.37; H, 6.96; Br, 29.03. Found: C, 52.33; H, 6.98; Br, 29.21. TLC: R_f 0.45 (solvent A and B), Color: purple (conc. H₂SO₄).

14 β ,15 β -Epoxy-5 β -cholane-3 β ,21,24-triol (VIII)—A solution of 100 mg of V in 50 ml of EtOH was hydrogenated with H₂ in the presence of 100 mg of 5% Pd-C at 16—18°. When hydrogen was no longer absorbed, after the uptake of 1.2 molar equivalents of H₂, the catalyst and solvent were removed and 98 mg of colorless residue was purified by column chromatography over silica gel (Wakogel C-200, 1 g) in *n*-hexane-acetone (4:1) to give 80 mg of VIII, mp 155—157°, as colorless prisms. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3460—3420 (OH), 3040 (CH), 1445, 1380, 1070, 1050, 1035, 878. NMR (10% solution in CDCl₃) τ : 4.7—5.1 (1H, multiplet, CH), 5.90 (1H, broad peak, 3-CH), 6.37 (2H, triplet, $J=6$ cps, 21-CH₂OH), 6.58 (1H, singlet, 15-CH), 9.03 (3H, singlet, 19-CH₃), 9.10 (3H, singlet, 18-CH₃). *Anal.* Calcd. for C₂₄H₄₀O₄: C, 73.43; H, 10.27. Found: C, 73.48; H, 10.29. TLC: R_f 0.50 (solvent A); 0.49 (solvent B), Color: brown (conc. H₂SO₄).

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