[Chem. Pharm. Bull. 17(6)1246—1250(1969)]

UDC 615.324 : 547.918.02 : 615.357.011.4

Bufadienolides. III.[1] Reduction of Resibufogenin with Sodium Borohydride[2]

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(Received December 25, 1968)

Partial reduction of resibufogenin (III) having 14β,15β-epoxide with sodium borohydride under various conditions was examined. No bufalin (IV) corresponding 14β-hydroxy compound was obtained in any case, but instead there was produced 14β,15β-epoxy-5β-chol-20(22)-ene-3β,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 reported[4] to yield Telocinobufagin (II) corresponding 14β-hydroxy compound. In our case, it is interesting that 14β,15β-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 14β-hydroxy or 14β,15β-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).

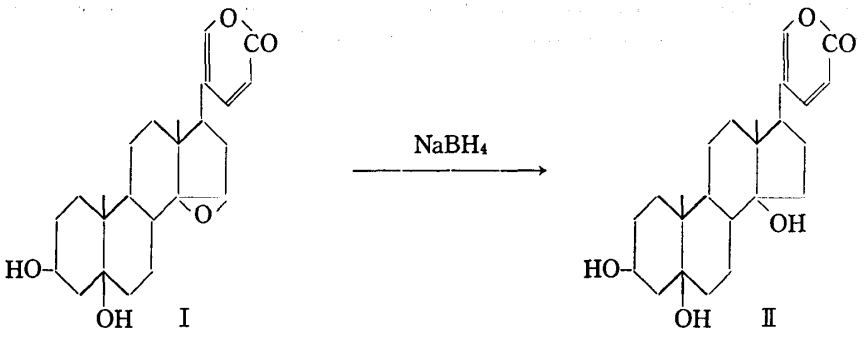


Chart 1

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 promted 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 14β, 15β-epoxy-5β-chol-20(22)-ene-3β,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).

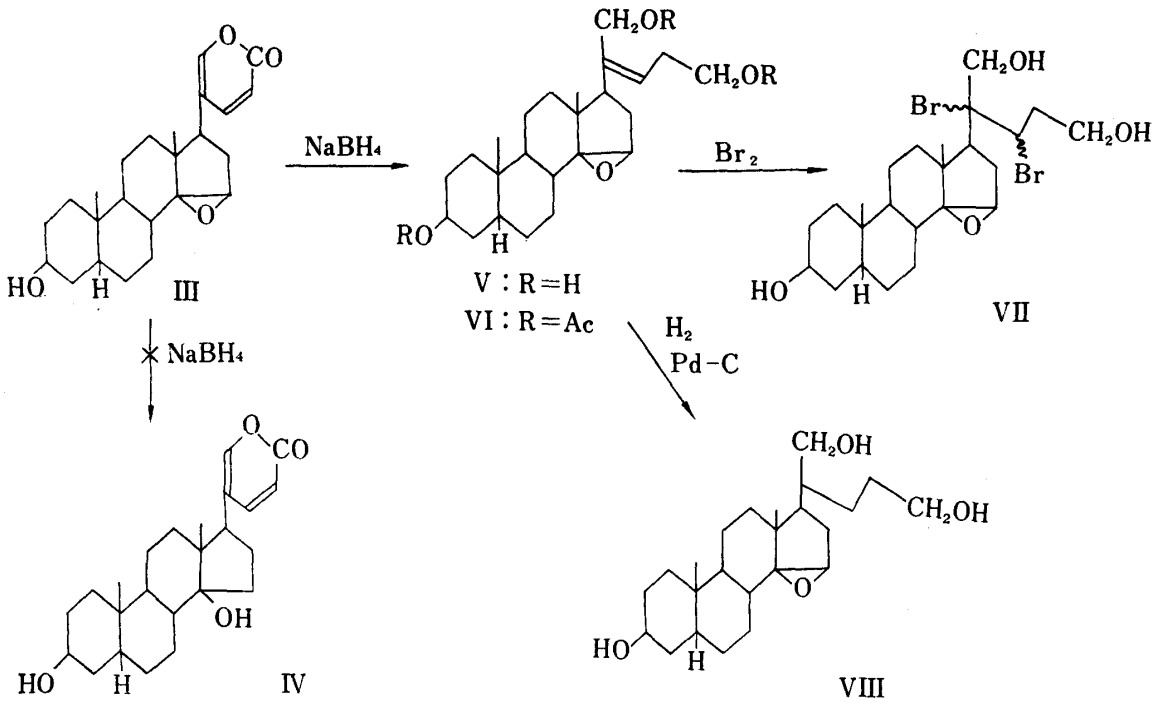


Chart 2

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)).

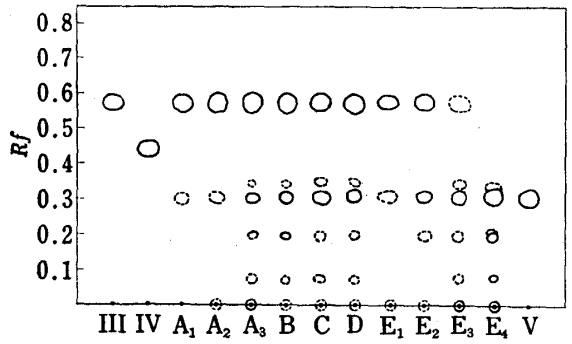


Fig. 1. Thin-Layer Chromatograms of the Reaction Solutions and Authentic Substances

III: resibufogenin
IV: bufalin
V : 14β,15β-epoxy-5β-chol-20 (22)-en-3β,21,24-triol
A[4]: the reaction at—20° at PH 8—9 in 80%EtOH, (A_1, 30 min; A_2, 60 min; A_3, 120 min)
B[5]: the reaction at 20° for 2 hr in MeOH
C : the reaction was added $MgSO_4 \cdot 2H_2O$ 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–H_2O (4:1), (E_1, 30 min; E_2, 4 hr; E_3, 20 hr; E_4, 48 hr)
solvent system: Me_2CO–$CHCl_3$–*n*-hexane (3:2:2) (A)

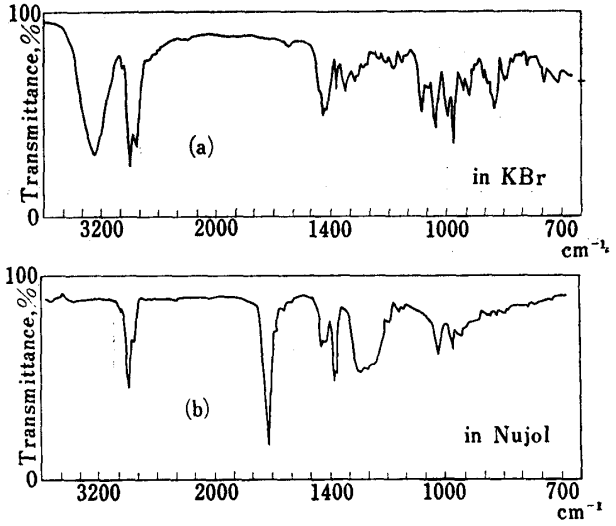


Fig. 2. Infrared Spectra of V (a) and VI (b)

Similar chromatogram was alwasys 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 mμ. 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 $\begin{matrix}R\\R\end{matrix}\!\!>\!C{=}CHCH_2\text{-}CH_2-$. 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}.

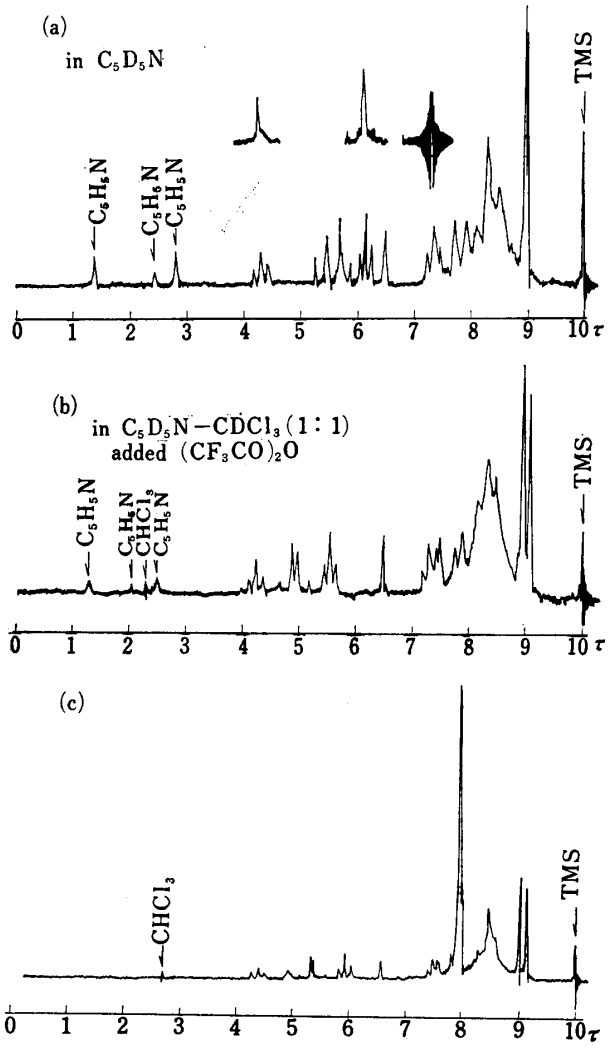


Fig. 3. Nuclear Magnetic Resonance Spectra of V (a,b) and VI (c) at 60 Mc

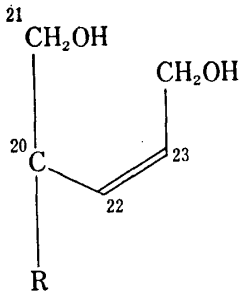
On acetylation with acetic anhydride in pyridine, compound V gave a triacetate VI as a colorless oil (purity checked by TLC). The IR spectrum of VI did not show a hydroxy band and had a C-H stretching vibration band at 3040 cm^{-1} due to a 14β,15β-epoxy grouping (Fig. 2 (b)). The presence of 14β, 15β-epoxy 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 14β,15β-epoxy-5β-chol-20(22)-ene-3β,21,24-triol.

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

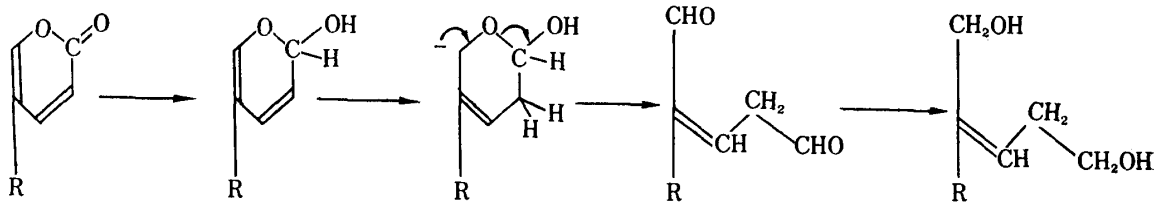
cleavages smoothly. Thus the epoxide ring cleavage observed in marinobufagin is assumed to be an exceptional case possibly ascribable to the structural features. Tamm[6] reported that the treatment of 3-dehydrobufalin with sodium borohydride afforded 3-epibufalin and a by-product of which partial structure (A) was assumed to have $\Delta^{22,23}$-double bond, without experimental evidence.



R=steroid residue
A

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 C_{24}-hydroxyl group, followed by the ring-opening, to furnish the reduced $\Delta^{20,22}$-diol (V) (Chart 3).



R=steroid residue

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–$CHCl_3$–*n*-hexane (3:2:2) and (B) AcOEt–*n*-hexane (9:1). The spots were detected by spraying conc. H_2SO_4 followed by heating. The reaction of resibufogenin III with $NaBH_4$ under the following various conditions was followed by thin-layer chromatography as shown in Fig. 1.

The reaction conditions. A[4]. the reaction at −20° at pH 8—9 in 80% EtOH (A_1, 30 min; A_2, 60 min; A_3, 120 min). B.[5] the reaction at 20° for 2 hr in MeOH. C. the reaction was added $MgSO_4 \cdot 2H_2O$ 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–H_2O (4:1) (E_1, 30 min; E_2, 4 hr; E_3, 20 hr; E_4, 48 hr).

Intensity of a spot with an *Rf* value (0.57), corresponding to resibufogenin III, decreased with time, while a spot with an *Rf* value (0.30) corresponding to compound V in Condition A and E was intensified with time. In neither reactions, a spot of bufalin IV (*Rf* 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–H_2O (4:1), a solution of $NaBH_4$ (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 $NaBH_4$ was decomposed and acidified (pH 3) with dil. H_2SO_4 at 0°, the mixture was poured into 240 ml of H_2O and was concentrated *in vacuo* to one-third of the original volume and extracted with $CHCl_3$. The $CHCl_3$-extract was washed with dil. $NaHCO_3$ and H_2O, dried over Na_2SO_4 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 Me_2CO to yield 0.58 g of 14β,15β-epoxy-5β-chol-20(22)-ene-3β,21,24-triol, V, mp 178—180°, as colorless prisms, $[\alpha]_D^{23}$ +0.9° (c=1.4, $CHCl_3$). UV λ_{max}^{EtOH} mμ: 205. IR ν_{max}^{KBr} cm^{-1}: 3300 (OH), 3010, 1660, 985, 845, 835, 805, 790 (C=C). NMR (10% solution in C_5D_5N) τ: 4.29 (1H, triplet, J=7.5 cps, RRC=CHR), 5.34 and 5.77 (2H, AB quartet, J=12.1 cps, 21-CH_2OH), 6.14 (2H, triplet, J=6.5 cps, 24-CH_2OH), 6.48 (1H, singlet, 15-CH), 7.2—7.6 (2H, multiplet, -CH_2-CH_2OH), 8.98 (3H, singlet, 19-CH_3),

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

9.01 (3H, singlet, 18-CH_3); (in C_5D_5N–$CDCl_3$ (1:1) added $(CF_3CO)_2O$, 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-CH_2OH), 5.57 (2H, triplet, J=6.2 cps, 24-CH_2OH), 6.50 (1H, singlet, 15-CH), 7.2—7.6 (2H, multiplet, –CH_2–CH_2OH), 8.98 (3H, singlet, 19-CH_3), 9.10 (3H, singlet, 18-CH_3). Mol. wt. 392. *Anal.* Calcd. for $C_{24}H_{38}O_4$: C, 73.80; H, 9.81. Found: C, 73.53; H, 9.94. TLC: *Rf* 0.30 (solvent A); 0.20 (solvent B), Color: reddish brown (conc. H_2SO_4).

b)[4] (Reduction under Condition A). To a solution of III (250 mg) dissolved in 20 ml of 80% EtOH, a solution of $NaBH_4$ (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 H_2SO_4 at 0°, the reaction mixture was poured into 120 ml of H_2O and was concentrated *in vacuo* to a half of the original volume and extracted with $CHCl_3$. The extract was successively washed with 2N Na_2CO_3 and H_2O, dried over Na_2SO_4. The crude product (246 mg) was dissolved in a mixture of C_6H_6–$CHCl_3$ (19:1), chromatographed on a column of Al_2O_3 (5g) eluting successively with C_6H_6–$CHCl_3$ (19:1), C_6H_6–$CHCl_3$ (5:1), C_6H_6–$CHCl_3$ (1:1), $CHCl_3$ and $CHCl_3$–MeOH (98:2). The fraction eluted with C_6H_6–$CHCl_3$ (19:1) and C_6H_6–$CHCl_3$ (5:1) gave 120 mg of the starting material. The fraction eluted with $CHCl_3$ 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 $NaBH_4$ (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 $MgSO_4 \cdot 7H_2O$ (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.

14β,15β-Epoxy-5β-chol-20(22)-ene-3β,21,24-triyl Triacetate (VI)——Acetylation of V with Ac_2O–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 ν_{max}^{Nujol} cm^{-1}: 3040 (15-CH), 1740 (ester CO), 1600, 983, 850, 755 (C=C). NMR (10% solution in $CDCl_3$) τ: 4.39 (1H, triplet, J=7.5 cps, RRC=CH–CH_2–), 4.92 (1H, broad, 3-CH), 5.23 and 5.48 (2H, AB quartet, J=12.0 cps, –CH_2OR), 5.92 (2H, triplet, J=6.5 cps, –CH_2–CH_2OR), 6.56 (1H, singlet, 15-CH), 7.3—7.7 (2H, multiplet, –CH_2–CH_2OR), 7.91 (6H, singlet, 2×–$COCH_3$), 7.96 (3H, singlet, –$COCH_3$), 9.00 (3H, s, 19-CH_3), 9.13 (3H, s, 18-CH_3). TLC: *Rf* 0.56 (solvent A); 0.61 (solvent B), Color: reddish brown (conc. H_2SO_4).

20ξ,22ξ-Dibromo-14β,15β-epoxy-5β-cholane-3β,21,24-triol (VII)——To a stirred solution of 150 ml of V in 45 ml of $CHCl_3$, 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 $Na_2S_2O_3$ (150 mg) dissolved in H_2O. The $CHCl_3$-layer washed fully with H_2O, dried over Na_2SO_4 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 ν_{max}^{KBr} cm^{-1}: 3360 (OH), 1390, 1145, 1080, 1050, 800, 795. NMR (10% solution in $CDCl_3$) τ: 5.5—6.5 (*ca.* 5H, multiplet, ⩾CH and 2×CH_2OH), 7.19 (1H, singlet, ⩾CH), 8.65 (3H, singlet, ⩾CH_3), 9.03 (3H, singlet, ⩾CH_3). *Anal.* Calcd. for $C_{24}H_{38}O_4Br_2$: C, 52.37; H, 6.96; Br, 29.03. Found: C, 52.33; H, 6.98; Br, 29.21. TLC: *Rf* 0.45 (solvent A and B), Color: purple (conc. H_2SO_4).

14β,15β-Epoxy-5β-cholane-3β,21,24-triol (VIII)——A solution of 100 mg of V in 50 ml of EtOH was hydrogenated with H_2 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 H_2, 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 ν_{max}^{KBr} cm^{-1}: 3460—3420 (OH), 3040 (CH), 1445, 1380, 1070, 1050, 1035, 878. NMR (10% solution in $CDCl_3$) τ: 4.7—5.1 (1H, multiplet, CH), 5.90 (1H, broad peak, 3-CH), 6.37 (2H, triplet, J=6 cps, 21-CH_2OH), 6.58 (1H, singlet, 15-CH), 9.03 (3H, singlet, 19-CH_3), 9.10 (3H, singlet, 18-CH_3). *Anal.* Calcd. for $C_{24}H_{40}O_4$: C, 73.43; H, 10.27. Found: C, 73.48; H, 10.29. TLC: *Rf* 0.50 (solvent A); 0.49 (solvent B), Color: brown (conc. H_2SO_4).

Acknowledgement The authors wish to thank Professor Dr. K. Hata and Professor Dr. S. Mōri, Tokyo Metropolitan University, for many helpful discussions and suggestions during this work. They express their deep gratitude to S. Uehara, Managing Director of this company, for his permission to publish this paper, to Dr. S. Ikawa, Managing Director of this company, and to Dr. I. Tanaka, Director of this laboratory, for their continued encouragements throughout the course of this work. They are also grateful to the members of the Central Analysis Room of this laboratory for elemental analyses and spectral data.