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Bufadienolides. IV.[1] A New Route to Pregnane Series from Resibufogenin[2]

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In the previous paper,[1] it was reported that the treatment of resibufogenin (I) with sodium borohydride gave 14β,15β-epoxy-5β-chol-20(22)-en-3β,21,24-triol (III) in a good yield. Therefore, it is considered that the cleavage reaction of a side chain double bond of III might be led to pregnane derivatives. After III was acetylated, the triacetate (IV) obtained was ozonized at $-60°$ to give 14β,15β-epoxy-20-oxo-5β-pregnane-3β,21-diyl diacetate (V). This was confirmed by the elemental analyses and spectral data. Then, partial hydrolysis of V with potassium bicarbonate yielded 3-monoacetate (VI). The treatment of VI with periodic acid, followed by usual methylation gave the known methyl 3β-acetoxy-14β,15β-epoxy-5β-androstane-17-carboxylate (VII). The identity was established by comparison with the authentic sample, which was prepared independently from acetyl-resibufogenin (II) by the method of Linde and Meyer.[6] The success of the three-step conversion of resibufogenin to a pregnane derivatives (V), which proceeded in a 50% overall yield, opened a new route to pregnane derivatives having A/B- and C/D-*cis* ring system.

Although many synthetic pathways have been devised for the synthesis of steroidal hormones, conversion of bufadienolides to pregnane derivatives did not receive serious attention. Only Meyer[4] has reported the isolation of etianates by the oxidative degradation of bufadienolides.

The present paper describes the preparation of new pregnane derivatives, 14β,15β-epoxy-20-oxo-5β-pregnane-3β,21-diyl diacetate (V) and its 3-monoacetate (VI), both having A/B- and C/D-*cis* ring system from a naturally occurring bufadienolide, resibufogenin (I) (Chart 1).

In the previous paper,[1] it was reported that the reduction of resibufogenin (I) with sodium borohydride gave 14β,15β-epoxy-5β-chol-20(22)-en-3β,21,24-triol (III) in a good yield. Then, it is assumed that the cleavage reaction of a side chain double bond of III might be led to pregnane derivatives.

Triol (III) was first converted to triacetate (IV) by the treatment with acetic anhydride and pyridine. Ozonolysis of triacetate (IV) at $-60°$ in ethyl acetate gave ketodiacetate (V), $C_{25}H_{36}O_6$, mp 165—168°, in a 95% yield, whose IR spectrum (Fig. 1 (a)) showed strong C=O stretching vibration at 1708 cm^{-1} and ester C=O bands. The NMR spectrum (Fig. 2 (a)) did not show vinyl proton signals but had a sharp methylene signal at 5.12 τ (2H, singlet) suggesting the presence of $-CH_2OH$ grouping. In addition, appearance of two signals at a higher field of 7.85 τ (3H, singlet) and 7.95 τ (3H, singlet) indicated the presence of two acetyl groups assignable to C_{21}-and C_3-positions, respectively. A singlet at 6.42 τ (1H) suggested

1) Bufadienolides. III: Y. Kamano, H. Yamamoto and M. Komatsu, *Chem. Pharm. Bull.* (Tokyo), **17**, 1246 (1969).
2) This work was reported at the 88th Annual Meeting of Pharmaceutical Society of Japan, Tokyo, Apr., 1968.
3) Location: *No. 34-1 Takata 3-chome, Toshima, Tokyo, 170-91, Japan.*
4) K. Meyer, *Helv. Chim. Acta*, **32**, 1599 (1949) (Ref.: S. Mōri and K. Morita, *Yakugaku Kenkyu*, **27**, 605 (1955); M. Okada, "Seibutsu Kagaku Saikin no Shimpo," Vol. 3, ed. by K. Kodama, *etc.*, Gihodo Inc., Tokyo, 1957).

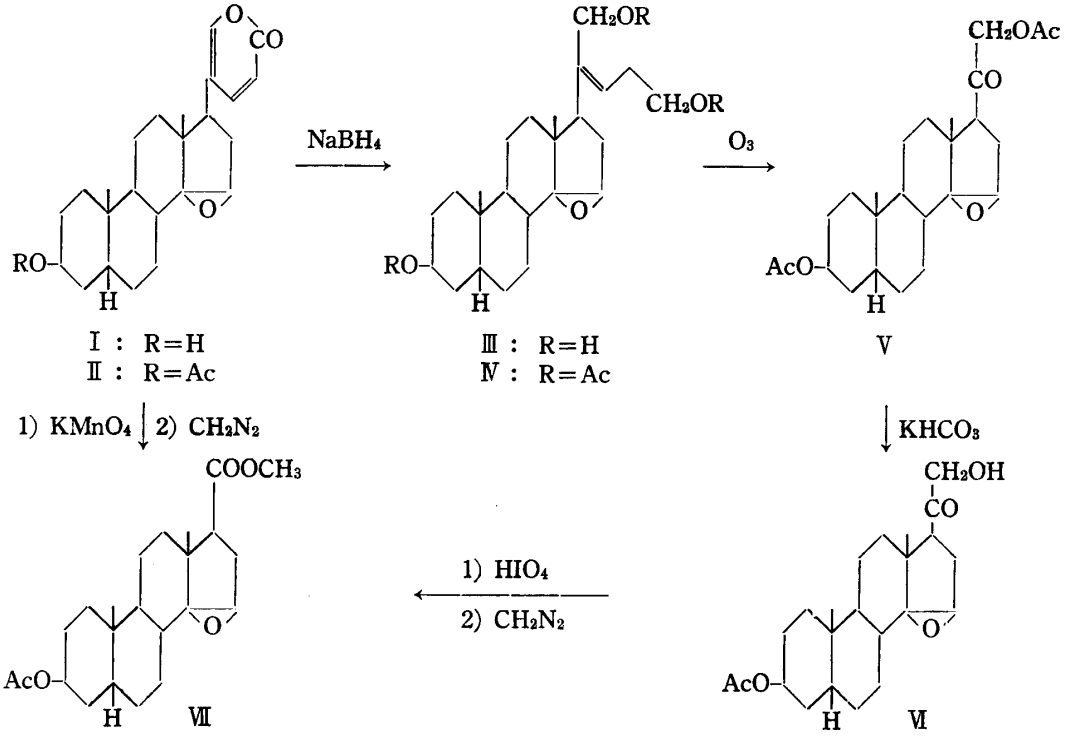

Chart 1

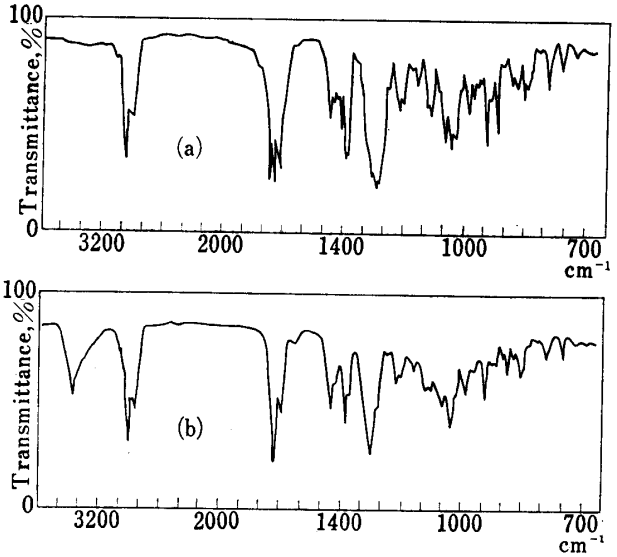

Fig.1. Infrared Spectra of V(a) and VI(b) in KBr

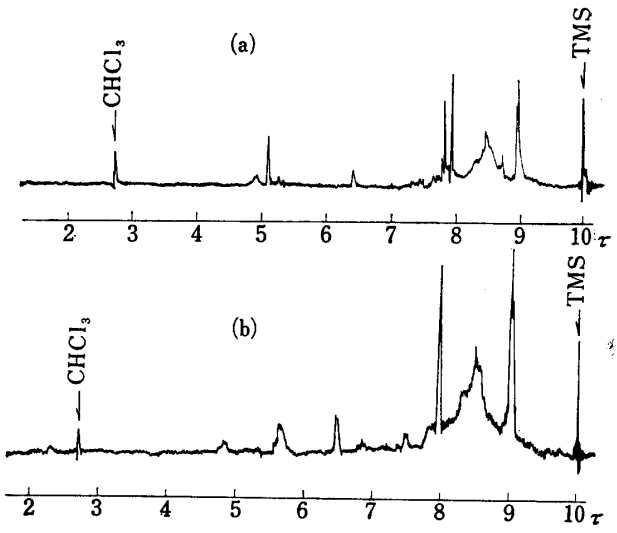

Fig.2. Nuclear Magnetic Resonance Spectra of V(a) and VI(b)in $CDCl_3$ at 60Mc

the presence of a 14β,15β-epoxy group. Based on these results, the structure of V was determined to be 14β,15β-epoxy-20-oxo-5β-pregnane-3β,21-diyl diacetate.

Partial hydrolysis of V with potassium bicarbonate in 80% methanol at room temperature yielded the corresponding 3-monoacetate (VI), $C_{23}H_{34}O_5$, mp 116—118°, in a 60% yield. The IR spectrum (Fig. 1 (b)) exhibited a strong absorption due to primary alcohol at 3470 cm^{-1}. When the NMR spectrum (Fig. 2 (b)) of VI was compared with that of V, 21-methylene signal showed an up-field shift and a sharp signal due to 21-acetyl methyl group disappeared, indicating that the structure of VI should be 14β,15β-epoxy-21-hydroxy-20-oxo-5β-pregnan-3-yl acetate.

The treatment of VI with periodic acid according to the conditions of Meyer, *et al.*,[5] followed by usual methylation gave, after chromatographical separation, methyl 3β-acetoxy-14β,15β-epoxy-5β-androstane-17-carboxylate (VII), $C_{23}H_{34}O_5$, mp 167—169°.[6] The identity was established by comparison with the authentic material, which was prepared independently from acetylresibufogenin (II) by the method of Linde and Meyer[6] (Chart 1).

By these transformations the structure of III was also established as indicated.

The success of the three-step conversion of resibufogenin (I) to a pregnane derivative (V), which proceeded in a 50% overall yield, opened a new route to pregnane derivatives having A/B- and C/D-*cis* ring system.

Experimental

All melting points are uncorrected. IR spectra were determined in KBr pellets using a Nihon Bunko Model DS 301 Spectrophotometer. NMR spectra were taken on a Hitachi Model R-20 Spectrometer with tetramethyl silan as an internal standard and are reported in τ values. The solvents used are $CDCl_3$. Molecular weight were determined using a Hitachi Molecular Weight Apparatus Model 115. The course of reactions and the progress in column chromatography were followed by thin-layer chromatography, which was performed in the same way as reported in the previous paper[1] with silica gel G plates using the solvent systems; (A) acetone–$CHCl_3$–*n*-hexane (3:2:2) and (B) AcOEt–*n*-hexane (9:1).

14β,15β-Epoxy-20-oxo-5β-pregnane-3β,21-diyl Diacetate (V)——Treatment of resibufogenin (I) (2.0 g) with $NaBH_4$ (2.0 g) in dioxane–H_2O (4:1) as reported previously[1] afforded 14β,15β-epoxy-5β-chol-20(22)-en-3β,21,24-triol (III), which was acetylated with Ac_2O–pyridine to yield the triacetate (IV) (1.3 g). Without purification, the triacetate (IV) was dissolved in AcOEt (150 ml) and ozonized at −60°, when 1.5 moles of ozone per mole of steroid was absorbed. To the reaction solution, a solution of $NaIO_4$ (3.5 g), MeOH (100 ml) and H_2O (80 ml) was added and was left to stand at room temperature for 20 hr. After adding H_2O (150 ml), the mixture evaporated *in vacuo* to a half of the original volume and then extracted with $CHCl_3$. The $CHCl_3$–extract washed with H_2O, dried over Na_2SO_4 and evaporated *in vacuo* to give a crystalline substance (1.2 g), which was purified by column chromatography over silica gel (Wakogel C-200, 12 g) using *n*-hexane–acetone (7:1) as an eluent to give 14β,15β-epoxy-20-oxo-5β-pregnane-3β,21-diyl diacetate (V) (980 mg), mp 165—168°, as colorless plates. Mol. wt. 433. *Anal.* Calcd. for $C_{25}H_{36}O_6$: C, 69.42; H, 8.39. Found: C, 69.17; H, 8.27. IR ν_{max}^{KBr} cm^{-1}: 3050 (15–CH), 1755, 1730 (ester CO), 1708 (20–$COCH_2$–), 1245, 1240, 1225, 1210 (C–O). NMR (10% solution in $CDCl_3$) τ: 4.92 (1H, broad peak, 3–C<u>H</u>), 5.12 (2H, singlet, 21–C<u>H</u>$_2$OAc), 6.42 (1H, singlet, 15–C<u>H</u>), 7.85 (3H, singlet, 21–OCOC<u>H</u>$_3$), 7.95 (3H, singlet, 3–OCOC<u>H</u>$_3$), 8.95 (3H, singlet, 18–C<u>H</u>$_3$), 8.98 (3H, singlet, 19–C<u>H</u>$_3$). TLC: Rf 0.67 (solvent A), 0.58 (solvent B); Color: green (conc. H_2SO_4).

14β,15β-Epoxy-21-hydroxy-20-oxo-5β-pregnan-3-yl Acetate (VI)——After a solution of V (494 mg) in 0.1% $KHCO_3$ (MeOH–H_2O=9:1) (30 ml) was allowed to stand for 20 hr at room temperature, it was neutralized with 0.1N HCl and was concentrated under reduced pressure at room temperature. The $CHCl_3$ extract was washed with H_2O, dried over Na_2SO_4 and then evaporated *in vacuo* to give the crude hydrolysis product (430 mg), which was purified by columm chromatography over silica gel (Wakogel C-200, 13 g) in *n*-hexane–acetone (4:1) to give the 3-monoacetate (VI) (296 mg), mp 116—118°, as colorless plates from acetone Mol. wt. 392. *Anal.* Calcd. for $C_{23}H_{34}O_5$: C, 70.74; H, 8.78. Found: C, 70.47; H, 8.52. IR ν_{max}^{KBr} cm^{-1}: 3470 (OH), 3040 (15–CH), 1740 (ester CO), 1700 (–$COCH_2$–), 1260, 1245, 1238 (C–O). NMR (10% sclution in $CDCl_3$) τ: 4.88 (1H, broad peak, 3–C<u>H</u>), 5.62 (2H, broad peak, 21–C<u>H</u>$_2$OH), 4.45 (1H, singlet, 15–C<u>H</u>), 7.95 (3H, singlet, 3–OCOC<u>H</u>$_3$), 9.00 (6H, singlet, 18– and 19–C<u>H</u>$_3$). TLC: *Rf* 0.56 (solvent A), 0.44 (solvent B); Color: green (conc. H_2SO_4).

Methyl 3β-Acetoxy-14β,15β-epoxy-5β-androstane-17-carboxylate (VII)——a) From VI: To a solution of VI (160 mg) dissolved in dioxane (5.0 ml), a solution of $HIO_4 \cdot 2H_2O$ (210 mg) dissolved in H_2O (1.8 ml) was added and allowed to stand at 18—20° for 5 hr. The mixture was diluted with H_2O and the solvent was distilled off *in vacuo* and extracted with $CHCl_3$. The extract was washed with H_2O, and dried over Na_2SO_4. The product (140 mg) obtained by $CHCl_3$ extraction, without purification, was methylated with CH_2N_2 in MeOH. The crude ester (215 mg) was purified by column chromatography on silica gel (Wakogel C-200, 6.5 g). Elution with *n*-hexane–acetone (9:1) afforded 135 mg of material, which was recrystallized from Me_2CO–ether to give VII (98 mg) as plates, mp 167—169°. *Anal.* Calcd. for $C_{23}H_{34}O_5$: C, 70.74; H, 8.78. Found: C, 70.68; H, 8.83. IR ν_{max}^{KBr} cm^{-1}: 3040 (15–CH), 1753, 1728 (ester CO), 1235, 1173 (C–O). NMR (10% sclution in $CDCl_3$) τ: 4.92 (1H, broad peak, 3–C<u>H</u>), 6.34 (3H, singlet, –COOC<u>H</u>$_3$), 6.52 (1H, singlet, 15–C<u>H</u>), 7.94 (3H,

5) K. Meyer and T. Reichstein, *Helv. Chim. Acta*, **30**, 1508 (1947); A. Lardon, *ibid.*, **32**, 1517 (1949); R. Casanova and C.W. Shoppee, *J. Chem. Soc.*, **1953**, 2983.

6) H. Linde and K. Meyer, *Helv. Chim. Acta*, **42**, 807 (1959).

siglet, 3-OCOC$\underline{H}_3$), 8.91 (3H, singlet, 18-C$\underline{H}_3$), 8.98 (3H, siglet, 19-C$\underline{H}_3$). TLC; *Rf* 0.66 (solvent A), 0.62 (solvent B); Color: blueish green (conc. H_2SO_4). The compound was proved to be identical with an authentic sample of VIII, obtained below b), by mixed fusion, IR, NMR and TLC.

b)[6] From Acetyl-resibufogenin (II): Under stirring, to a solution of II (mp 223—227°) (500 mg) dissolved in acetone (15 ml), finely powdered $KMnO_4$ (1.0 g) was added in portions during 1 hr at room temperature. After additional stirring for 4 hr, the solvent was evaporated to dryness *in vacuo* and the residue thus obtained was extracted with H_2O. The aqueous solution was acidified and then extracted with ether-$CHCl_3$ (4:1). It was washed with H_2O, dried over Na_2SO_4 and then concentrated under reduced pressure to afford an acid, which was methylatad with CH_2N_2. The resulting product was purified by column chromatography on silca gel in the same method as above. The purified product (VIII) (105 mg) was obtained as colorless plates, mp 166—168°, from acetone-ether, and was found to be identical with VII obtained from VI (see above).

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