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Bufadienolides. VII.[1] Ring-Opening Reaction of 14,15-α,β-Epoxides and 5,6-α,β-Epoxides with Hydroiodic Acid

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Resibufogenin (IX), which is 14β,15β-epoxy compound in bufadienolides, occurs in nature, but the corresponding 14α,15α-epoxy compound is unknown. In order to examine the ring-opening reaction of 14,15-epoxy function with hydroiodic acid, 14α,15α-epoxy-3β-hydroxy-5β-bufa-20,22-dienolide (VI) and its 3-acetate (VII) were synthesized (Chart 1).

Reaction of 14β,15β-epoxy compound (IX) or 14α,15α-epoxy compound (VI) with hydroiodic acid gave Δ^{14}-anhydrobufalin (II), which was a normal reduced product, 14α-artebufogenin (XI), and 14β-artebufogenin (XIII), respectively. A similar reaction of two acetates (X or VII) gave the corresponding acetates, IV, XII and XIV, respectively (Chart 2). The formation of 14α- and 14β-artebufogenin indicates that the acid–catalyzed ring–opening reaction occurred at a time.

Based on these results, reaction of 5,6-epoxy function was reexamined. As was excepted, reaction of 5β,6β-epoxy compound (XV) or 5α,6α-epoxy compound (XVI) afforded 3β-acetoxy-5,6β-dihydroxy-5α-cholestane (XIX), besides cholesteryl acetate (XVII) and cholesterol (XVIII).

Mōri[3] first demonstrated that 5β,6β-epoxy group in cholestane series was easily reduced to give 5,6-double bond group *via* iodohydrin by treatment with zinc dust in the presence of hydroiodic acid. 5,6-Iodohydrin has been isolated by Barton[4] by the reaction with hydroiodic acid at low temperature, and he described that more prolonged treatment gave only 5,6-double bond compound. A little later, Mōri[5] also reported that chloesteryl acetate β-epoxide (3β-acetoxy-5,6β-epoxy-5β-cholestane) (XV) was reduced to give cholesteryl acetate by treatment with only hydroiodic acid. Morita[6] has also observed the formation of 5,6- double bond group with 3β,17β-diacetoxy-androst-5-ene. On the other hand, Fajkos and co-workers[7] have observed the formation of 16,17-iodohydrin with 15β-brome-16β,17β-epoxy-steroid. However, the reaction of epoxides in the other position has not been applied, and the reaction by the acid–catalysis of hydroiodic acid has been reported only with 16β-methyl-16,17-epoxy-steroids by Syhora[8].

In this paper the author wishes to report the reaction of 14α,15α- and 14β,15β-epoxides bufadienolides, in addition, the reexamination of 5α,6α- and 5β,6β-epoxides in cholestane series and the isolation of a new product by the acid–catalysis.

Although resibufogenin (14β,15β-epoxy-3β-hydroxy-5β-bufa-20,22-dienolide) (IX), which is 14β,15β-epoxy compound in bufadienolides, occurs in nature, the corresponding 14α,15α-epoxy compound is unknown. Therefore, as shown in Chart 1, 14α,15α-epoxy-3β-hydroxy-bufa-20,22-dienolide (VI) and its 3-acetate (VII) were prepared from bufalin (3β,14β-hydroxy-5β-bufa-20,22-dienolide) (I) *via* Δ^{14}-anhydrobufalin (3β-hydroxy-5β-bufa-14,20,22-trienolide)

1) Bufadienolides. VI: Y. Kamano, Y. Tanaka and M. Komatsu, *Chem. Pharm. Bull.* (Tokyo), **17**, 1706 (1969).
2) Location: *No. 34-1, Takata 3-chome, Toshimaku, Tokyo, 170-91, Japan.*
3) S. Mōri, *Nippon Kagaku Zasshi*, **72**, 475 (1951).
4) D.H. Barton, E. Miller, and H.T. Young, *J. Chem. Soc.*, **1951**, 2598.
5) S. Mōri, *Nippon Kagaku Zasshi*, **73**, 505 (1952).
6) K. Morita, *Nippon Kagaku Zasshi*, **78**, 1705 (1957).
7) J. Fajkos, J. Joska, and F. Sorm, *Collection Czech. Chem. Commun.*, **27**, 64 (1962).
8) K. Syhora, *Tetrahedron Letters*, **1960**, 34.

(II). When II was refluxed in methanol in the presence of hydroiodic acid, a crystalline mixture of two isomers of anhydrobufalin was afforded in satisfactory yield.

The mixture was separated by recrystallization into two isomers, II, mp 195—196°, and III ($\Delta^{8(14)}$-anhydrobufalin), mp 213—215°, in 67.7 and 8.2% yields, respectively. Acetylation of II with acetic anhydride and pyridine yielded the corresponding acetate (IV), mp 192—

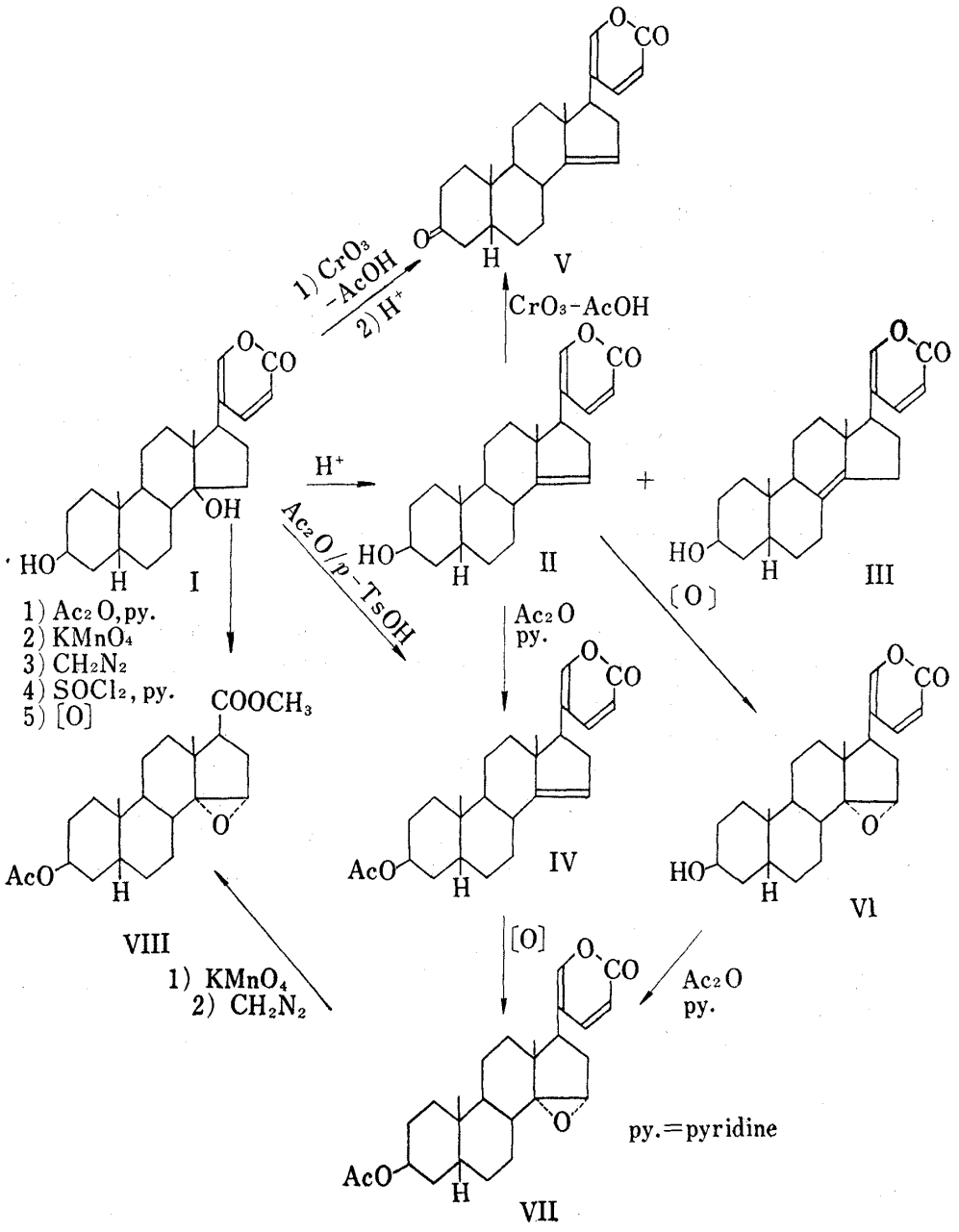

Chart 1

193°. The same compound was also obtained at one step by the new method from I in good yield by treatment with *p*-toluenesulfonic acid in acetic anhydride at room temperature. Oxidation of II with chromium trioxide gave V, mp 245—247°, which was also prepared from I. In the infrared (IR) and nuclear magnetic resonance (NMR) spectra, II,IV or V indicated the presence of $R_1R_2C{=}CHR_3$ moiety, but III did not indicate.[9]

9) Komatsu has already observed that Δ^{14}-anhydrogamabufotalin indicated the presence of $R_1R_2C{=}CHR_3$ moiety, but $\Delta^{8(14)}$-anhydroisomer did not indicate, in the IR and NMR spectra (M. Komatsu, *Yakugaku Zasshi*, **84**, 77 (1964)).

Thus, II or IV was oxidized with perbenzoic acid to the corresponding epoxy derivatives, VI, mp 236—238°, or VII, an amorphous solid, in good yields. VII was also obtained from VI by usual acetylation. Stuctural assignment for compounds VI and VII was based upon the spectral and chemical evidence similar to that for 14β,15β-isomers (IX andX). VII was converted by potassium permanganate oxidation and esterification of an acid thus obtained into methyl 3β-acetoxy-14α,15α-epoxy-5β-androstane-17-carboxylate (methyl 3β-acetoxy-14α,15α-epoxy-etianate) (VIII). The identity was established by comparison with the authentic samples, which was prepared independently from I according to the method of Meyer[10] and Lardon, *et al.*[11]

When resibufogenin (IX) was treated with hydroiodic acid in acetone at room temperature for 60 min, the three spots were observed on thin-layer chromatogram, as shown in Fig. 1. Although a spot of *Rf* 0.48 was agreed with that of Δ^{14}-anhydrobufalin (II), which was the expected reduction product, two spots of *Rf* 0.24 and 0.31 were accorded with those of 14α- and 14β-artebu-

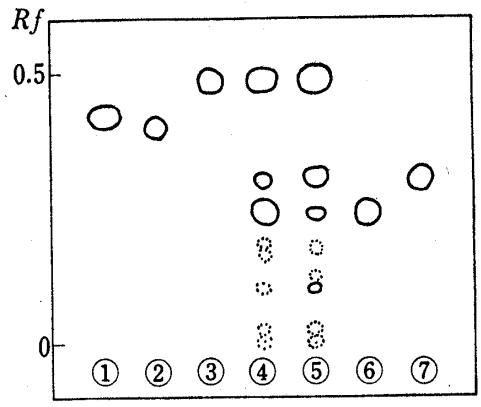

Fig. 1. Thin-Layer Chromatograms of 14α,-15α- and 14β,15β-Epoxy Compounds

solvent: acetone–$CHCl_3$–*n*-hexane (3:3:4) (A)
samples:
①: resibufogenin (IX)
②: 14α,15α-epoxy-3β-hydroxy-5β-bufa-20,22-dienolide(VI)
③: Δ^{14}-anhydrobufalin (II)
④: the reaction mixture of IX by treatment with HI
⑤: the reaction mixture of VI by treatment with HI
⑥: 14α-artebufogenin (XI)
⑦: 14β-artebufogenin

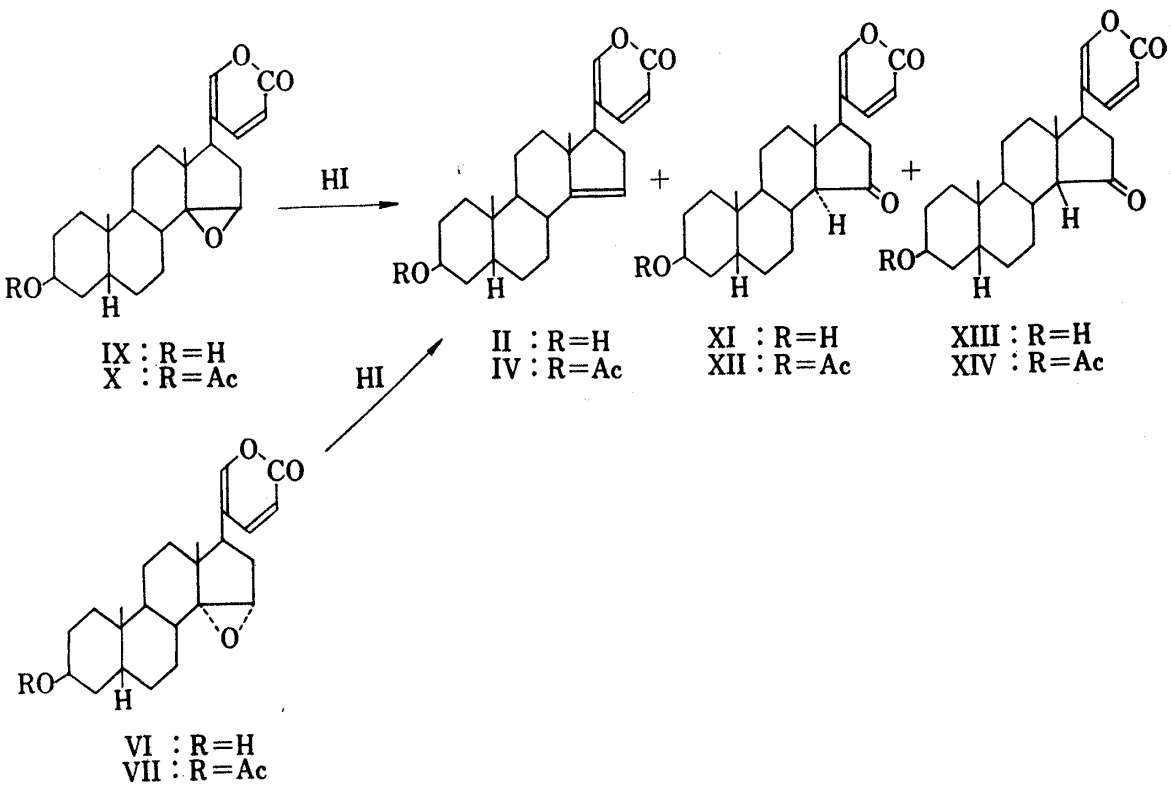

Chart 2

10) K. Meyer, *Helv. Chim. Acta*, **32**, 1238 (1949).
11) A. Lardon, H.P. Sigg, and T. Reichstein, *Helv. Chim. Acta*, **42**, 1457 (1959); H. Ishii, T. Tozyo, and D. Satoh, *Chem. Pharm. Bull.* (Tokyo), **11**, 576 (1963).

fogenin (XI and XIII), which were the 15-oxo compounds. The reaction mixture was chromatographed on silica gel to afford II, mp 193—195°, XI, mp 264—266°, and XIII, mp 127—131°, in 59.8, 24.0 and 6.0% yields, respectively, which were found to be identical with the authentic samples. Treatment of acetyl-resibufogenin (X) with hydroiodic acid in acetone gave, after the column chromatographical separation, the three acetates, IV, mp 191—193°, XII, mp 220—222°, and XIV, mp 234—236°, in 57.5, 28.8 and 5.8% yields, which were also obtained by acetylation of II, XI and XIII in the usual way.

On the other hand, when 14α,15α-epoxy compound (VI) was submitted to the similar reaction as described above, II, XI and XIII were obtained in 57.7, 6.4 and 19.2% yields, respectively. Then, a similar treatment sequence was applied to the reaction of VII. The resulting three acetates, IV, XII and XIV were also afforded in 56.0, 5.6 and 28.0% yields.

In the case of the reaction on 14β,15β-epoxides, 14α-compounds were always obtained in a better yield than 14β-compounds, whereas in the case of that on 14α,15α-epoxides, the latter was better than the former in yield.

Formation of the unexcepted 14α- and 14β-artebufogenin can be explained by the acid-catalysis of hydroiodic acid. Therefore, the author reexamined the reaction of cholesteryl acetate α- and β-eposides, as illustrated in Chart 3.

When cholesteryl acetate 5β,6β-epoxide (XV) was treated with aqueous hydroiodic acid in chloroform-acetone at room temperature for 15 min, the reaction solution showed two spots corresponding to XXI and XIX on thin-layer chromatogram, but the products were isolated by the preparative thin-layer chromatography. Compound XIX was found to be identical with the material, which was obtained by the following more strong treatment. Compound XXI, which was obtained in a small amount, could not be recrystallized by reason of an unstable compound in solvents, but was assumed to have the projected structure, since XXI had a positive Beilstein's test. More prolonged treatment afforded, after the column chroma-

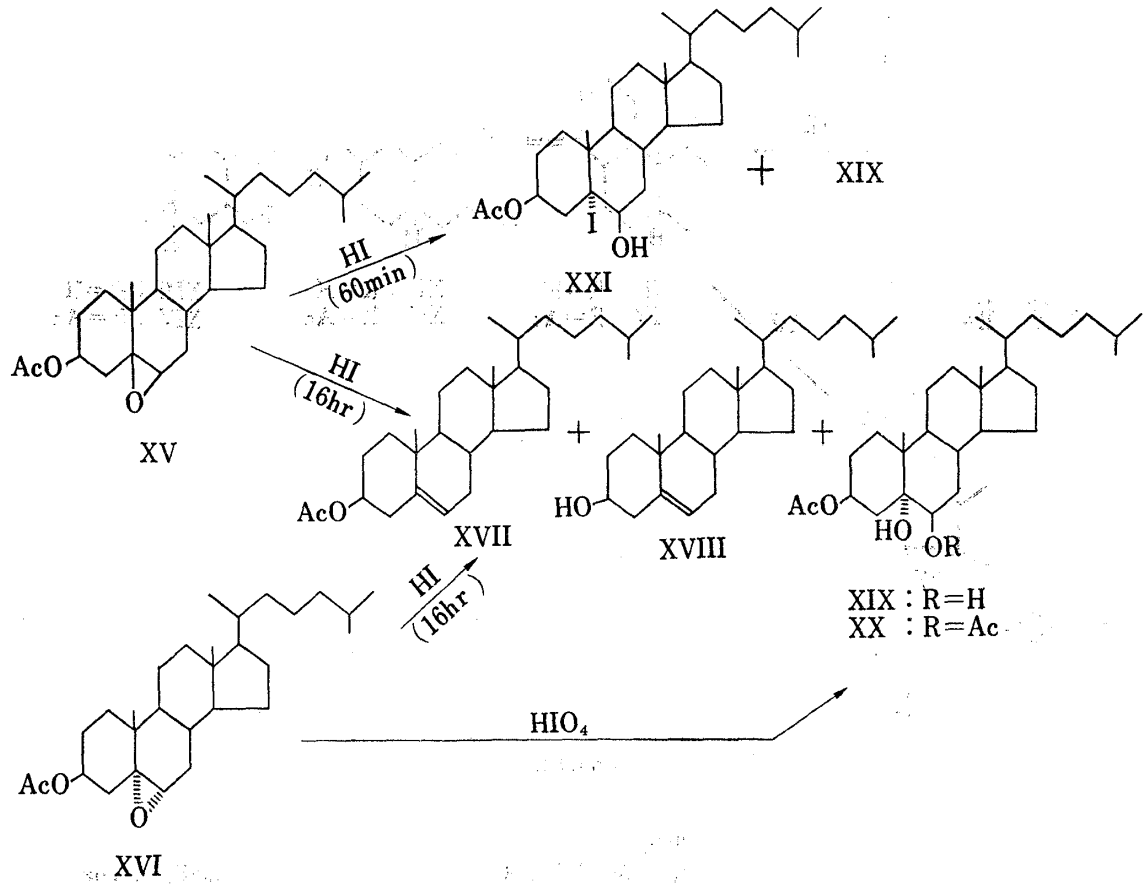

Chart 3

tographical separation, the three crystals, XVII, mp 114—115°, XVIII, mp 146—148°, and XIX, mp 207—208°, respectively. On thin-layer chromatogram, two spots of XVII and XVIII[12] were agreed with those of the authentic samlpes (Fig. 2). Compound XIX showed a negative Beilstein's Test and absorption band at 3490 cm^{-1} and 3420 cm^{-1} suggesting the presence of the two hydroxyl groups in the IR spectrum. An acetate (XX), mp 165.5°, which was obtained by acetylation in the usual way, indicated also the presence of the hydroxyl group at 3485 cm^{-1} in the IR spectrum. Based on these results, the structure of XIX was assigned to 3β-acetoxy-5,6β-dihydroxy-5α-cholestane, and this assignment was confirmed by comparison with an authentic sample which was prepared from XVI according to the similar method as described by Rowland and Nace.[13]

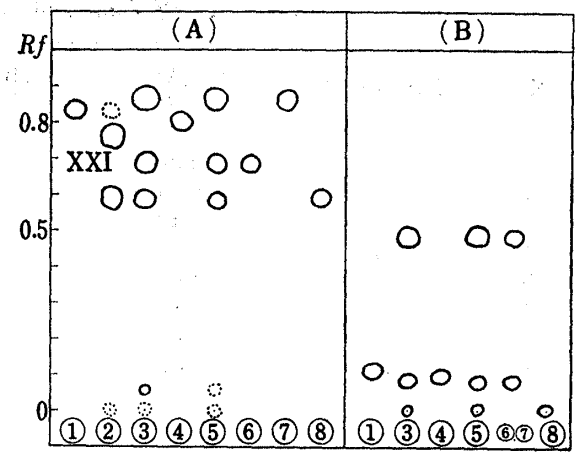

Fig. 2. Thin-Layer Chromatograms of 5α,6α- and 5β,6β-Epoxy Compounds

solvents: (A) acetone–$CHCl_3$–*n*-hexane (3:3:4). (B) C_6H_6.
samples:
①: 3β-acetoxy-5,6β-epoxy-5β-cholestane (XV)
②: the reaction mixture of XV by treatment with HI for 15 min
③: the reaction mixture of XV by treatment with HI for 16 hr
④: 3β-acetoxy-5,6α-epoxy-5α-cholestane (XVI)
⑤: the reaction mixture of XVI by treatment with HI for 16 hr
⑥: cholesterol (XVIII)
⑦: cholesteryl acetate (XVIII)
⑧: 3β-acetoxy-5,6β-dihydroxy-5α-cholestane (XIX)

Moreover, the same compounds were also obtained from α-isomer by the same treatment. Thin-layer chromatography of the reaction solution showed in Fig. 2.

It should be noted that the acid-ring opening reaction was observed with both 14,15-α,β-epoxides and 5,6-α,β-epoxides in steroids in addition to the reduction by treatment with hydroiodic acid.

Experimental[14]

Thin-Layer Chromatography (TLC)——TLC was performed with silicagel G plates according to the procedure described before.[15] The solvent used are (A) acetone–$CHCl_3$–*n*-hexane (3:3:4), (B) C_6H_6. The course of the reactions was followed by TLC, as shown in Fig. 1 and 2. *Rf* values and coloration of the authentic samples are summarized and shown in Table I.

Δ^{14}-Anhydrobufalin (II) and $\Delta^{8\,(14)}$-Anhydrobufalin (III)——To a solution of bufalin (I) (2.8 g) dissolved in 102 ml of MeOH, 5.7 ml of 35%-hydrochloric acid solution was added and the mixture was refluxed for 2 hr. The solution was diluted with H_2O, concentrated *in vacuo* to remove MeOH and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with H_2O, dil. $NaHCO_3$ aq and H_2O, dried over Na_2SO_4, and evaporated *in vacuo* to give 2.7 g of the residue, which was crystallized from acetone to 2.4 g of the mixture of II and III. Recrystallization from ethyl acetate afforded II (1.9 g, 67.7%), flat needles, mp 195—196° and III (0.23 g, 8.2%), needles, mp 213—215°.

12) It was known already that 3-acetoxyl group was partially saponified to give 3-hydroxyl group.[5,6]
13) A.T. Rowland and H.R. Nace, *J. Am. Chem. Soc.*, **82**, 2833 (1960).
14) All melting points are uncorrected. Infrared Spectra measurements were run on a Nihon Bunko DS01 spectrophotometer. Nuclear Magnetic Resonance spectra were obtained on a Hitachi Model R-20 spectrometer operated at 60 MHz in $CDCl_3$ solution containing tetramethylsilane as internal standard and are reported in δ values. Ultra Violet spectra were taken with a Hitachi automatic spectrophotometer, Model EPS-2u.
15) M. Komatsu, Y. Kamano, M. Suzuki, *Bunseki Kagaku*, **14**, 1049 (1964); Y. Kamano, H. Yamamoto and M. Komatsu, *Chem. Pharm. Bull.* (Tokyo), **17**, 1246 (1969) (Bufadienolides. III).
16) K Meyer, *Helv. Chim. Acta*, **35**, 2444 (1952); H. Linde and K. Meyer, *ibid.*, **42**, 807 (1959); M.S. Ragab, H. Linde, and K. Meyer, *ibid.*, 45, 1794 (1962).

Table I. *Rf* Value and Color of the Compounds

No.	Compounds	(A) *Rf*	(B) *Rf*	Color with conc. H_2SO_4
1	I	0.30		yellow–green–greenish blue
2	II	0.48		blue–greyish blue
3	III	0.46		light blue–greyish blue
4	IV	0.73		blue
5	V	0.65		yellow–yellowish brown
6	VI	0.40		bluish green
7	VII	0.66		bluish green
8	VIII	0.64		bluish green
9	IX	0.42		yellowish green–green
10	X	0.68		green
11	XI	0.24		green
12	XII	0.52		green
13	XIII	0.31		green
14	XIV	0.58		green
15	XV	0.83	0.11	blue–green–greenish brown
16	XVI	0.80	0.09	orange–yellowish brown
17	XVII	0.86	0.48	pinkish purple–purple
18	XVIII	0.68	0.08	pinkish purple–purple
19	XIX	0.59	0.01	brownish orange
20	XX	0.73	0.02	brownish orange
21	XXI	0.76	0.07	yellowish brown–brownish purple

solvents: A) acetone–$CHCl_3$–*n*-hexane (3:3:4) B) C_6H_6

II. $[\alpha]_D^{23}$ +44.0° (c=1.0, MeOH). *Anal.* Calcd. for $C_{24}H_{32}O_3$: C, 78.22; H, 8.75. Found. C, 78.35; H, 8.81. IR ν_{max}^{KBr} cm^{-1}: 3460 (OH), 3030 (C-H), 1745—1690 (conjugated CO), 1635, 1538 (conjugated C=C), 960, 790 (C=C). NMR (10% solution in $CDCl_3$) δ: 7.30 (1H, dd, J=12 and 2 Hz, 22-H), 7.27 (1H, d, J=2 Hz, 21-H), 6.26 (1H, d, J=12 Hz, 23-H), 5.23 (1H, d, J=2 Hz, 15-H), 4.11 (1H, broad peak, 3-H), 0.97 (3H, singlet, 19-CH_3), 0.72 (3H, singlet, 18-CH_3). UV λ_{max}^{MeOH} mμ (log ε): 302 (2.40).

III. $[\alpha]_D^{23}$ +103.9 (c=0.5, MeOH). *Anal.* Calcd. for $C_{24}H_{32}O_3$: C, 78.22; H, 8.75. Found. C, 78.43; H, 8.79. IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1745—1700 (conjugated CO), 1635, 1536 (conjugated C=C), 965, 793 (C=C). NMR (10% solution in $CDCl_3$) δ: 7.30 (1H, dd, J=11.5 and 2 Hz, 22-H). 7.28 (1H, J=2 Hz, 21-H), 6.26 (1H, J=11.5 Hz, 23-H), 4.16 (1H, broad peak, 3-H), 0.85 (3H, singlet, 19-CH_3), 0.71 (3H, singlet, 18-CH_3). UV λ_{max}^{MeOH} mμ (log ε): 301 (2.36).

Acetyl Δ^{14}-Anhydrobufalin (IV)——a) From II: II (500 mg) was acetylated with pyridine (10 ml)–Ac_2O (7 ml) overnight at room temperature to give an acetate (IV) (440 mg), mp 192—193°, as colorless needles from ethyl acetate. *Anal.* Calcd. for $C_{26}H_{34}O_4$: C, 76.04; H, 8.35. Found. C, 76.17; H, 8.24. IR ν_{max}^{KBr} cm^{-1}: 3030 (CH), 1745—1720 (conjugated CO), 1638, 1535 (conjugated C=C), 954, 790 (C=C). NMR (10% solution in $CDCl_3$) δ: 7.32 (1H, dd, J=11.2 and 3 Hz, 22-H), 7.30 (1H, d, J=3 Hz, 21-H), 6.27 (1H, d, J=11.3 Hz, 23-H), 5.24 (1H, d, J=2 Hz, 15-H), 5.08 (1H, broad peak, 3-H), 2.05 (3H, singlet, 3-OCOCH_3), 0.97 (3H, singlet, 19-CH_3), 0.72 (3H, singlet, 18-CH_3). UV λ_{max}^{MeOH} mμ (log ε): 302 (2.39).

b) From I: To a solution of 400 mg of I in 20 ml of AcOH, 300 mg of P-TsOH and 5 ml of Ac_2O were added. The reaction mixture was allowed to stand over night at room temperature, H_2O added and extracted with $CHCl_3$. The $CHCl_3$ solution was washed with dil. Na_2CO_3 solution and H_2O, dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was recrystallized from ethyl acetate to 352 mg of needles, mp 192—193°, which was found to be identical with the compound obtained by the method a) by mixed mp and IR spectrum.

Δ^{14}-Anhydrobufalon (3-Dehydro-Δ^{14}-Anhydrobufalin) (V)——a) From II: To an ice-cold solution of 300 mg of II dissolved in 7.5 ml of AcOH, 3.3 ml of 2% solution of CrO_3 in AcOH was added and the mixture was allowed to stand for 4 hr at room temperature. Excess of CrO_3 was reduced with MeOH, the mixture was diluted with H_2O and extracted with $CHCl_3$. The extract was washed with dil. Na_2CO_3 solution and H_2O, and dried over anhydrous Na_2SO_4. The residue (290 mg) obtained by $CHCl_3$ extraction was crystallized from ethyl acetate to V (201 mg) as prisms, mp 245—247°. *Anal.* Calcd. for $C_{24}H_{30}O_3$: C, 78.65, H, 8.25. Found. C, 78.48; H, 8.35. IR ν_{max}^{KBr} cm^{-1}: 3060 (CH), 1748—1690 (ketone and conjugated CO), 1647, 1540 conjugated C=C), 956, 794 (C=C). NMR (10% solution in $CDCl_3$) δ: 7.35 (1H, dd, J=11 and 3 Hz, 22-H), 7.33 (1H, d, J=3 Hz, 21-H), 6.28 (1H, d, J=11 Hz, 23-H), 5.30 (1H, d, J=2 Hz, 15-H), 1.05 (3H, singlet, 19-CH_3), 0.77 (3H, singlet, 18-CH_3). UV λ_{max}^{MeOH} mμ (log ε): 302 (2.32).

b) From I: To an ice-cold solution of 105 mg of I dissolved in 3.0 ml of AcOH, 1.5 ml of 2% solution of CrO_3 in AcOH was added and the mixture was allowed to stand for 4 hr at *ca.*20°. Excess of CrO_3 was reduced with MeOH, the mixture was diluted with H_2O and extracted with $CHCl_3$. The extract was washed with dil. Na_2CO_3 solution and H_2O, dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue (100 mg) thus obtained was added 5.0 ml of MeOH and 0.2 ml of 35% hydrochloric acid and the mixture was refluxed for 2 hr. The solution was diluted with H_2O. The precipitate was collected by filtration, dried, and crystallized from ethyl acetate to give 36.4 mg of V, mp. 245°, which was found to be identical with the sample obtained by the method a) by mixed mp and IR spectrum.

14α,15α-Epoxy-3β-hydroxy-5β-bufa-20,22-dienolide(VI)——To a solution of II (650 mg) in $CHCl_3$ (12 ml), 4.85 ml of $CHCl_3$ solution of $C_6H_5CO_3H$ (57.7 mg/ml) was added and the mixture was allowed to stand for 2.5 hr at room temperature. The reaction mixture was diluted with $CHCl_3$, washed consecutively with aqueous solutions of KI, $Na_2S_2O_3$ and $NaHCO_3$ and H_2O. The $CHCl_3$ Layer was dried over anhydrous Na_2SO_4 and the solvent was evaporated. Crystallization of the residue (635 mg) so obtained from acetone afforded 556 mg of VI as colorless needles, mp 228—230°, which was recrystallized from the same solvent to give an analytical sample, mp 236—238°, $[\alpha]_D^{17.8}$ =27.6° (*c*=0.75, $CHCl_3$). Anal. Calcd. for $C_{24}H_{32}O_4$: C, 74.97; H, 8.39. Found. C, 74.75; H, 8.22. IR ν_{max}^{KBr} cm^{-1}2460 (OH), 3040 (CH), 1745—1690 (conjugated CO), 1635, 1540 (conjugated C=C), 1245 (epoxy C–C), 963 (C=C), 830 (epoxy C–O), 750 (C=C). NMR (10% solution in $CDCl_3$) δ: 7.21 (1H, d, *J*=2.5 Hz, 21-H), 7.17 (1H, dd, *J*=11.5 and 2.5 Hz, 22-H), 6.26 (1H, d, *J*=11.5, 23-H), 4.1 (1H, broad peak, 3-H), 3.50 (1H, singlet, 15-H), 0.97 (3H, singlet, 19-CH_3), 0.68 (3H, singlet, 18-CH_3). UV λ_{max}^{MeOH} mμ (log ε): 301 (2.21).

3β-Acetoxy-14α,15α-Epoxy-5β-Bufa-20,22-dienolides (VII)——a) From IV: To a solution of IV (540 mg) in $CHCl_3$ (12 ml) was added $C_6H_5CO_3H$–$CHCl_3$ solution (231 mg, 4.0 ml), and the resulting solution was allowed to stand at room temperature for 4 hr. The reaction mixture was worked up in the same way as described above to give 480 mg of a crude product, which was purified by column chromatography on silicagel (Wakogel C-200, 12 g) in *n*-hexane–acetone (9:1) to give 380 mg of VII as a colorless amorphous solid. *Anal.* Calcd. for $C_{26}H_{34}O_5$: C, 73.21, H, 8.03. Found. C, 73.42; H, 8.15. IR ν_{max}^{KBr} cm^{-1}: 3020 (CH), 1760, 1740, 1715—1700 (ester CO and conjugated CO), 1260, 1635, 1540 (conjugated C=C), 1240, 1235 (ester C=O), 955 (C=C), 833 (epoxy C–O), 750 (C=C). NMR (10% solution in $CDCl_3$) δ: 7.14 (1H, dd, *J*=10.5 and 3.5 Hz, 22-H), 7.13 (1H, d, *J*=3.5 Hz, 21-H), 6.24 (1H, d, *J*=10.5 Hz, 23-H), 5.04 (1H, broad peak, 3-H), 3.49 (1H, singlet, 15-H), 2.02 (3H, singlet, 3-$OCOCH_3$), 0.98 (3H, singlet, 19-CH_3), 0.67 (3H, singlet, 18-CH_3). UV λ_{max}^{MeOH} mμ (log ε): 301 (2.07).

b) From VI: VI (180 mg) was dissolved in Ac_2O (5 ml) and pyridine (10 ml) and allowed to stand at room temperature overnight. After usual work-up an oily residue obtained was chromatographed on silica gel (5.4 g) in the same manner as described in a) to give 123 mg of VII, as a colorless amorphous solid. The IR spectrum of VII is superimposable with that of VII obtained from IV, and they show the same Rf values in their TLC.

Methyl 3β-Acetoxy-14α,15α-Epoxy-5β-Androstane-17-Carboxylate (Methyl 3β-Acetoxy-14α,15α-Epoxy-5β-Etianate) (VIII)——a) From VII: To a solution of VII (150 mg) dissolved in 20 ml of acetone, finely powdered $KMnO_4$ (330 mg) was added portion wise at room temperature with stirring. After stirring for 5 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 extracted with $CHCl_3$. The $CHCl_3$ layer was washed with H_2O, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to affored 120 mg of residue, which was esterified with CH_2N_2 in a usual manner. The product thus obtained was chromatographed on silicagel (Wakogel C-200, 3.6 g) with *n*-hexane–acetone (9:1) to give VIII (49 mg) as colorless plates, mp 87—89°, $[\alpha]_D^{23.5}$ +37.5 (*c*=1.0, $CHCl_3$). Mixed mp. and IR spectrum established the identity with an authentic sample of VIII, which was obtained by the method b).

b) From I[10,11]: According to the method of Meyer,[11] bufalin acetate (400 mg), which was obtained by usual acetylation of I with Ac_2O and pyridine, was transformed by oxidation with $KMnO_4$(800 mg), esterification with CH_2N_2 and chromatography on silica gel with *n*-hexane–acetone (7:1) into methyl 3β,14β-dihydroxy-5β-etianate (158 mg), mp 154—156°. The compound (140 mg) was converted into VIII (9.2 mg), mp 88—89°, by dehydration with $SOCl_2$–pyridine, oxidation with $C_6H_5CO_3H$–$CHCl_3$, and chromatography on silica gel according to the method of Lardon, *et al.*[10] Mixed mp on admixture with the sample obtained in a) showed no depression and IR spectra of two samples were identical.

Reaction of Resibufogenin (IX) with Hydroiodic Acid——To a solution of IX (800 mg) dissolved in $CHCl_3$ (9 ml) and acetone (50 ml), 2.5 ml of conc. HI solution was added and the mixture was allowed to stand for 60 min. at room temperature. The mixture was then poured into H_2O and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with 10% $Na_2S_2O_3$ solution and H_2O, dried over anhydrous Na_2SO_4, and evaporated *in vacuo*. The residue (795 mg) was chromatographed on silica gel (Wakogel C-200, 24 g) with *n*-hexane–acetone ((5:1) and (3:1)) to give Δ^{14}-anhydrobufalin (II) (480 mg), 59.8%), 14α-artebufogenin (XI) (144 mg, 24.0%), and 14β-artebufogenin (XIII) (36 mg, 6%).

II, mp 194—195° (from AcOEt), colorless needles, was not depressed on admixture with the sample of II obtained above and their IR spectra were identical in all region.

XI, mp 264—266° (from acetone), colorless prisms. *Anal.* Calcd. for $C_{24}H_{32}O_4$: C, 74.97; H, 8.39. Found. C, 74.69; H, 8.32. IR ν_{max}^{KBr} cm^{-1}: 3520 (OH), 1740, 1720—1690 (Ketone and conjugated CO), 1633, 1540 (conjugated C=C), 960 (C=C). NMR (10% solution in $CDCl_3$) δ: 7.30 (1H, d, J=3 Hz, 21-H), 7.22 (1H, dd, J=10.5 and 3Hz, 22-H), 6.29 (1H, d, J=10.5, 23-H), 4.12 (1H, broad peak, 3-H), 0.97 (3H, singlet, 19-CH_3), 0.64 (3H, singlet, 18-CH_3). UV λ_{max}^{MeOH} mμ (log ε): 300 (2.23). The compound was found to be identical with the authentic sample obtained by the method of Linde and Meyer.[16]

XIII, mp 127—131° (from MeOH), colorless prisms. *Anal.* Calcd. for $C_{24}H_{32}O_4$: C, 74.97; H, 8.39. Found. C, 75.09; H, 8.32. IR ν_{max}^{KBr} cm^{-1}: 3500 (OH), 1735, 1720—1690 (conjugated CO), 1635, 1540 (conjugated C=C), 955 (C=C). NMR (10% solution in $CDCl_3$) δ: 7.28 (1H, d, J=3Hz, 21-H), 7.19 (1H, dd, J=10 and 3Hz, 22-H), 6.33 (1H, d, J=10Hz, 23-H), 4.13 (1H, broad peak, 3-H), 0.94 (3H, singlet, 19-CH_3), 0.90 (3H, singlet, 18-CH_3). UV λ_{max}^{MeOH} mμ (log ε): 301 (2.10). The compound was found to be identical with the authentic sample obtained by the method of Linde and Meyer.[16]

Reaction of Acetyl-Resibufogenin (X) with Hydroiodic Acid——To a solution of X (500 mg) dissolved in acetone (25 ml), 2.0 ml of conc. HI solution was added and the mixture was allowed to stand for 1.5 hr at room temperature. The mixture was then poured into H_2O and extracted with $CHCl_3$. The $CHCl_3$ extract was washed 10%-$Na_2S_2O_3$ solution and H_2O, dried over anhydrous Na_2SO_4, and evaporated to dryness. The residue (497 mg) was chromatographed on silica gel (Wakogel C-200, 15 g) with *n*-hexane–acetone (7:1) and (5:1) to give acetyl Δ^{14}-anhydrobufalin (IV) (287 mg, 57.5%), acetyl 14α-artebufogenin (XII) (144 mg, 28.8%) and acetyl 14β-artebufogenin (XIV) (29 mg, 5.8%).

IV, mp 191—193° (form AcOEt), colorless needles, was not depressed on admixture with the sample of II obtained above and their IR spectra were identical in all region.

XII. mp 220—222° (from acetone). colorless prisms. *Anal.* Calcd. for $C_{26}H_{34}O_5$: C, 73.21; H, 8.03. Found. C, 73.34; H, 8.16. IR ν_{max}^{KBr} cm^{-1}: 1750—1720, 1690 (conjugated CO and ester CO), 1642, 1544 (conjugated C=C), 1250, 1225 (ester C–O), 952 (C=C). NMR (10% solution in $CDCl_3$) δ: 7.30 (1H, d, J=2.5Hz, 21-H), 7.23 (1H, dd, J=11.2 and 2.5Hz, 22-H), 6.30 (1H, d, J=11.2Hz, 23-H), 507 (1H, broad peak, 3-H), 0.98 (3H, singlet, 19-CH_3), 0.64 (3H, singlet, 18-CH_3). UV λ_{max}^{MeOH} mμ: 300. The compound was found to be identical with the authentic sample obtained by the method of Linde and Meyer.[16]

XIV. mp 234—236° (from acetone). colorless prisms. *Anal.* Calcd. for $C_{26}H_{34}O_5$: C, 73.21; H, 8.03. Found. C, 73.33; H, 8.00. IR ν_{max}^{KBr} cm^{-1}: 1745—1690 (conjugated CO and ester CO), 1640, 1542 (conjugated C=C), 1260, 1235 (ester C–O), 960 (C=C). NMR (10% solution in $CDCl_3$) δ: 7.27 (1H, d, J=3Hz, 21-H), 7.18 (1H, dd, J=10 and 3Hz, 22-H), 6.31 (1H, d, J=10Hz, 23-H), 5.06 (1H, broad peak, 3-H), 2.03 (3H, singlet, 3-OCOCH_3), 0.94 (3H, singlet, 19-CH_3), 0.91 (3H, singlet, 18-CH_3). UV λ_{max}^{MeOH} mμ: 301. The compound was found to be identical with the authentic sample obtained by the method of Linde and Meyer.[16]

Reaction of VI with Hydroiodic Acid——VI (300 mg) was treated with conc. HI solution (125 ml) in $CHCl_3$ (5 ml)–acetone (25 ml) in the same manner as described in IX. The product (298 mg) was chromatographed on silicagel with the same solvents as described in IX to give II (173 mg, 57.7%), mp 193—194°, XI (19.2 mg, 6.4%), mp 257—260°, and XIII (57.5 mg, 19.2%), mp 128—130°. The three compounds were found to be identical with the samples obtained above.

Reaction of VII with Hydroiodic Acid——VII (260 mg) was treated with conc. HI solution (0.75 ml) in acetone (20 ml) in the same manner as described in X. The product (255 mg) was chromatographed on silica gel with the same solvents as described in X to afford IV (147 mg, 56%), mp 192—193°, XII (14.8 mg, 5.7%), mp 221—222°, and XIV (72.5 mg, 27.9%) mp 234—235°. The compounds were found to be identical with the samples obtained above.

Reaction of 3β-Acetoxy-5,6β-epoxy-5β-cholestane (XV) with Hydroiodic Acid——a) To a solution of XV (334 mg) dissolved in 1.3 ml of $CHCl_3$ and 7 ml of acetone, 0.6 ml of conc. HI solution was added at room temperature. After 16 hr the mixture was poured into H_2O and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with 10% $Na_2S_2O_3$ solution and H_2O, dried over anhydrous Na_2SO_4, and evaporated *in vacuo* to dryness. The product (325 mg) was chromatographed on silica gel (Wakogel C-200, 13 g) with C_6H_6 to give XVII (117 mg, 35%), XVIII (71.7 mg. 21.5%), and XIX (79.1 mg, 23.7%), respectively.

XVII, mp 114—115.5° (from EtOH), was found to be identical with cholesteryl acetate (XVII) by mixed mp and IR spectrum.

XVIII, mp 146—148° (from EtOH), was found to be identical with cholesterol (XVIII) by mixed mp and IR spectrum.

XIX. mp 217—208° (from acetone and MeOH), colorless needles. *Anal.* Calcd. for $C_{29}H_{50}O_4$: C, 75.27; H, 10.93. Found. C, 75.41; H, 11.02. IR ν_{max}^{KBr} cm^{-1}: 3490 (5α-OH), 3420 (6β-OH), 1720 (ester CO), 1270, 1260 (ester C–O). NMR (10% solution in $CDCl_3$) δ: 5.12 (1H, broad peak, 3-H), 3.52 (1H, broad peak, 6-H), 2.00 (3H, singlet, 3-OCOCH_3), 1.18 (3H, singlet, 19-CH_3), 0.90 (6H, singlet, 2×-CH_3), 0.80 (3H, singlet, -CH_3), 0.67 (3H, singlet, 18-CH_3). The compound was found to be identical with 3β-acetoxy-5,6β-dihydroxy-5α-cholestane (XIX) obtained below.

b) To a solution of XV (50 mg) dissolved in $CHCl_3$ (0.2 ml) and acetone (1.5 ml), 0.1 ml of conc. HI solution was added at room temperature. After 45 min the mixture was submitted to the preparative TLC on silica gel G using solvent (A). The adsorbent of the zone corresponding to the spot (*Rf* 0.76) was eluted

with MeOH and the elute was crystallized from MeOH to give XXI (22 mg) as colorless needles. mp 98—103° (decomp.). Beilstein Test: positive. *Anal.* Calcd. for $C_{29}H_{49}O_3I$: C, 60.83; H, 8.80; I, 22.16. Found: C, 61.28; H, 9.03; I, 22.02. Recrystallization of XXI indicated the formation of the decomposed products by TLC.

Then, XIX (15.7 mg), mp 205—207° (from acetone), was also obtained from the eluate of the corresponding zone (*Rf* 0.59). The compound was found to be identical with the sample obtained in a).

Reaction of 3β-Acetoxy-5,6α-epoxy-5α-cholestane (XVI) with Hydroiodic Acid——XVI (207 mg) was treated with conc. HI solution in $CHCl_3$–acetone solution in the same manner as described in XVa). After chromatographical separation of the crude products (198 mg), XVII (75.5 mg, 36.5%), mp 114—115°, XVIII (47.6 mg, 23.0%), mp 146.5—147.5°, and XIX (52.1 mg, 25.2%), mp 207—209°, respectively. All compounds were found to be identical with the authentic samples by mixed mp and IR spectra.

3β,6β-Diacetoxy-5-hydroxy-5α-cholestane (XX)——XIX (60 mg) was treated with Ac_2O (0.4 ml) and pyridine (0.8 ml) in the usual manner. Recrystallization from MeOH gave XX (54 mg), as colorless needles. mp 165—166°. *Anal.* Calcd. for $C_{31}H_{52}O_5$: C, 73.77; H, 10.38. Found. C, 73.68; H, 10.40. IR ν_{max}^{KBr} cm^{-1}: 3485 (5α-OH), 1740 (6-ester CO), 1723 (3-ester CO), 1270, 1245 (ester C–O). NMR (10% solution in $CDCl_3$) δ: 5.10 (1H, broad peak, 3-H), 4.69 (1H, broad peak, 6-H), 2.06 (3H, singlet, 6-OCOCH_3), 2.00 (3H, singlet, 3-OCHCH_3), 1.14 (3H, singlet, 19-CH_3), 0.90 (6H, singlet, 2×-CH_3), 0.80 (3H, singlet, -CH_3), 0.67 (3H, singlet, 18-CH_3).

Conversion of XVI to XIX——XVI (100 mg) was treated with HIO_4 in acetone for 60 min at room temperature according to the similar method as described by Rawland and Nace.[13] The resulting solution was poured into H_2O. The precipitate was collected by filtration, dried, and recrystallized from acetone of MeOH to give XIX (73 mg), 208—209°, which was found to be identical with the sample obtained above.

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