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Bufadienolides. VIII.[1] Epimerization of 14α- and 14β-Artebufogenin[2]

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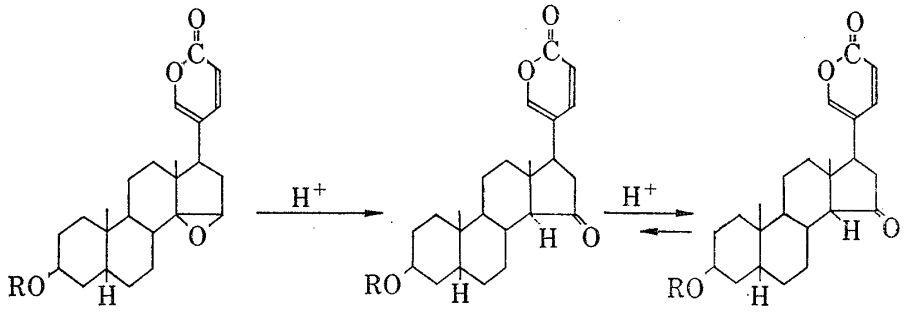
Epimerizations of 14α-artebufogenin (14α-H-15-oxo-bufadienolide, III) and 14β-artebufogenin (14β-H-15-oxo-bufadienolide, V) by acid were examined. From the results of nuclear magnetic resonance and optical rotatory dispersion measurements, the free energy difference between both compounds was calculated to be 0.4 kcal/mole.

Therefore the 14β-isomer (C/D *cis* ring system) was more stable than the 14α-isomer (C/D *trans* ring system).

Although 14α-artebufogenin (14α-H-15-oxo-bufadienolide, III) was first considered to be a component of Ch' an Su (Senso), Lind and Meyer[4] suggested that III was actually derived from resibufogenin (I). They also found[4] that III was epimerized to the corresponding 14β-H-15-oxo-compound, 14β-artebufogenin (V), by the treatment with aluminium oxide. Meyer, *et al.*[5] subsequently, reported that epimerization was easily completed by the treatment with hydrochloric acid (Chart 1).



I: R=H
II: R=Ac

III: R=H
IV: R=Ac

V: R=H
VI: R=Ac

Chart 1

We now wish to report that 14β-artebufogenin (V) can be epimerized back to the 14α-isomer (III) by acid. In addition, the epimerization in both directions was examined quantitatively by the nuclear magnetic resonance (NMR) and optical rotatory dispersion (ORD).

When resibufogenin (I) was treated with perchloric acid in acetone at 15—18° for 30 min under the conditions similar to these used by Meyer, *et al.*,[5] 14β-artebufogenin (V) and 14α-artebufogenin (III) were obtained in 4.8% and 67.5% yields, respectively. A more prolonged treatment (18 hr) afforded V and III in 24.7 and 32.1% yields, respectively.

1) Bufadienolides VII: Y. Kamano, *Chem. Pharm. Bull.* (Tokyo), **17**, 1711 (1969).
2) Part of this work was presented at the 89th Annual Meeting of the Pharmaceutical Society of Japan, Nagoya, April 1969.
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4) H. Linde and K. Meyer, *Helv. Chim. Acta*, **42**, 807 (1959).
5) M.S. Ragab, H. Lind, and K. Meyer, *Helv. Chim. Acta*, **45**, 1794 (1962).

These results suggested that V epimerized into III in the reaction mixture.

In order to examine this possibility, V was treated with hydrochloric acid in acetone or methanol, the formation of III (eventually confirmed) was observed by thin-layer chromatography (TLC) (Fig. 1).

Treatment of III or V with hydrochloric acid in acetic acid gave the acetates, IV and VI. In the course of this reaction, spots corresponding to the four compounds (III, IV, V and VI) were observed on TLC. The same TLC pattern was also obtained by treatment of acetates, IV and VI, with hydrochloric acid in acetone or methanol. The resulting TLC showed the spots of III and V. Similarly reaction of the acetates (IV or VI) in acetic acid yielded the mixture of two acetates (IV and VI) (Fig. 1). Probably these epimerizations correspond to keto⇆enol equilibrium at C_{15}, as shown in Chart 2.

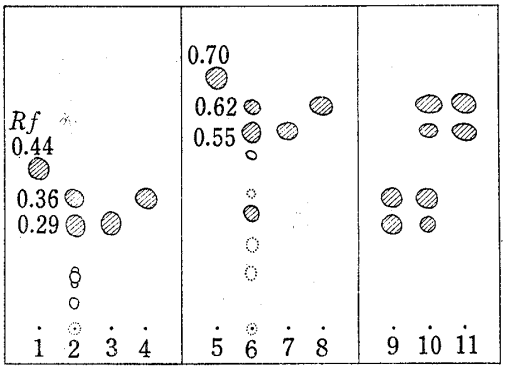

Fig. 1. Thin-Layer Chromatograms of Reactions

samples: 1=resibufogenin (I), 2=reaction mixture of I with $HClO_4$, 3=14α-artebufogenin (III), 4=14β-artebufogenin (V), 5=acetyl-resibufogenin (II), 6=reaction mixture of II with $HClO_4$, 7=acetyl-14α-artebufogenin (IV), 8=acetyl-14β-artebufogenin (VI), 9=reaction mixture of III, IV, V or VI with HCl in MeOH or acetone, 10=The spots of reaction mixture in the case of sample 11 after about 15 min, 11=reaction mixture of III, IV, V or VI with HCl in AcOH
solvent: acetone-$CHCl_3$-*n*-hexane (3:3:4)

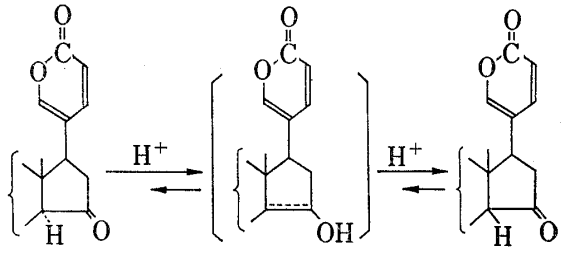

Chart 2

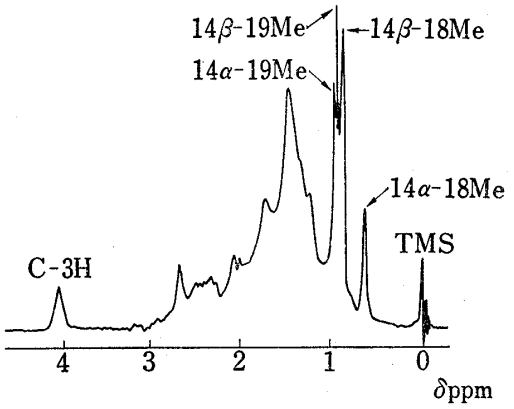

Fig. 2. NMR Spectrum of Equilibrium Mixture

Next, we attempted to establish the composition of the equilibrium mixture by the application of NMR. Fortunately, quantitative analysis was possible on the basis of the integral value of the 18-CH_3 signal of the 14α-isomer (Fig. 2).

A calibration curve prepared by using a known amount of mixture (III and V) (Fig. 3). Mixtures reached equilibrium in about 60 min (Fig. 4). Therefore, after treatment of III or V with acid in methanol, the equilibrium concentration of 14α-artebufogenin (III) was estimated, and 35% of the mixture was found to be III in each case.

This result was further confirmed by using ORD. As shown in Fig. 5, the equilibrium mixture was found to contain 35% III (14α-isomer).

From these results, the free energy difference between these two compounds (14α- and 14β-isomers) was calculated to be 0.4 kcal/mole by application of the equation $\Delta F=-RT\ln K$. This result indicates that the 14β-isomer (C/D *cis* ring system) is more stable than the 14α-isomer (C/D *trans* system). Although Meyer, *et al.*[5] already suspected this possibility they never reported epimerization of the 14β-isomer.

Conformational analysis of the steroid C/D ring has been a subject of many reports but a similar treatment of bufadienolides has not hitherto appeared. Djerassi's group[6] examined

6) N.L. Allinger, R.B. Hermann, and C. Djerassi, *J. Org. Chem.*, **25**, 922 (1960).

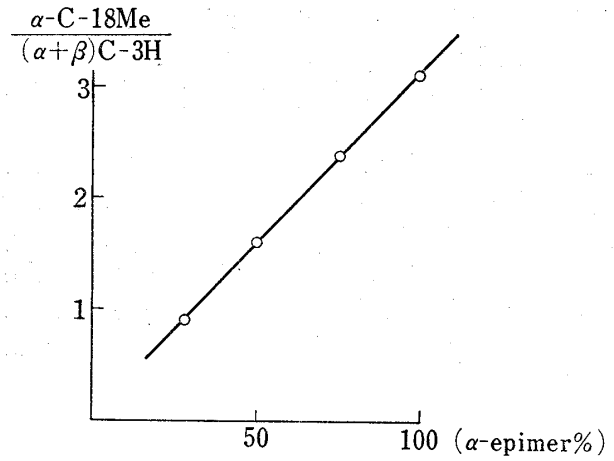

Fig. 3. Integrate Ratio $\frac{\alpha-C-18Me}{(\alpha+\beta)C-3H}$

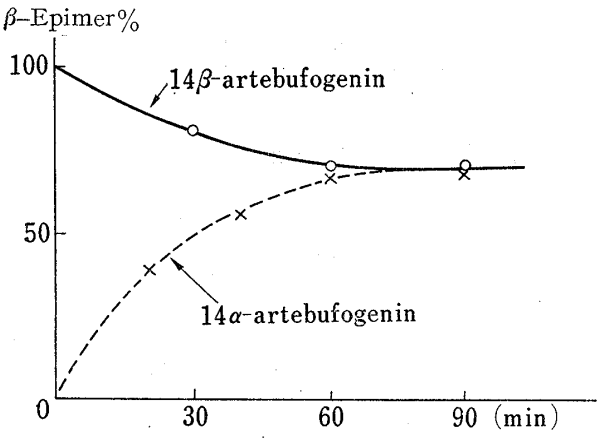

Fig. 4. Epimerization in Acid Methanol Solution

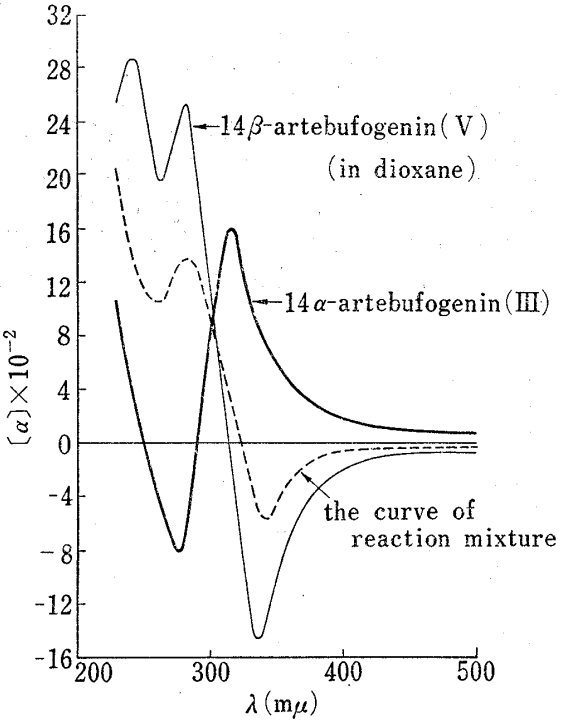

Fig. 5. ORD curve of III, V and their Reaction Mixture

the influence of C_{17}-functional groups of the kinetic and equilibrium measurements of 14-H 15-oxo-steroids, and concluded that 14α-isomers were generally more stable than 14β-isomers. Our result does not agree with this general result. Probably the α-pyrone ring is the cause of discrepancy.

Experimental[7]

Reaction of Resibufogenin (I) with Perchloric Acid——The reaction was carried out by a method similar to that described by Meyer, *et al.*[4]

a) To a solution of I (100 mg) dissolved in acetone (2 ml), 0.1 ml of 60% $HClO_4$ dissolved in acetone (6 ml) was added and the mixture was allowed to stand for 30 min at 18° under cooling with H_2O. The mixture was then poured into ice–water and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated *in vacuo* to dryness. The chromatography of the residue (99 mg) on silica gel with *n*-hexane–acetone (5: 1) and (3: 1) gave 14β-artebufogenin (V), mp 127—130° as colorless prisms from MeOH ,4.8 mg (4.8%) and 14α-artebufogenin (III), mp 264—266° as colorless prisms

7) All melting points are uncorrected. Analytical thin-layer chromatoplates with a thickness of 0.25 mm of Silica gel H (E. Merck A. G.) were used and the spots were detected by spraying with conc. H_2SO_4 followed by heating. All column chromatographic separations involved the dry method with silica gel (Wakogel C-200). Magnetic resonance spectra were obtained on a Hitachi Model R-20 spectrometer operated at 60 MHz in $CDCl_3$ solution containing tetramethylsilane as the internal standard and are reported in δ values. Optical rotations were measured in dioxane with a model ORD/UV-5 of Japan Spectroscopic Co., Ltd. (Tokyo). The IR and NMR spectra of the compounds has been reported already in Part VII.[1] The compounds were also compared with the authentic samples described in Part VII.[1]

from acetone, 67.5 mg (67.5%). The both compounds were found to be identical with the authentic samples by the IR comparison and mixed mp.

b) To a solution of I (300 mg) dissolved in acetone (10 ml), 0.2 ml of 60% $HClO_4$ dissolved in acetone (20 ml) was added at 15° under cooling with H_2O and the mixture was allowed to stand for 18 hr at 15—22°. The mixture was poured into ice–water and extracted with $CHCl_3$. The extract was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated *in vacuo* to dryness. The chromatography of the residue (296 mg) on silica gel with *n*-hexane–acetone (5: 1) and (3: 1) gave V (74.1 mg, 24.7%), mp 129—131° as colorless prisms from MeOH, and III (96.3 mg, 32.1%), mp 265—267° as colorless prisms from acetone, identical with the authentic samples.

Reaction of Acetyl-Resibufogenin (II) with Perchloric Acid——To a solution of II (200 mg) dissolved in acetone (10 ml), 0.2 ml of 60% $HClO_4$ was added at 15° under cooling with H_2O and the mixture was allowed to stand for 4 hr at 22°. The mixture was poured into ice–water and extracted with $CHCl_3$. The extract was washed with H_2O, dried over anhydrous Na_2SO_4, and concentrated *in vacuo* to dryness. The chromatography of the residue (195 mg) on silica gel with *n*-hexane–acetone (19: 1), (9: 1) and (5: 1) gave acetyl-14β-artebufogenin (VI) (6.2 mg, 3.1%), mp 233—236° as colorless prisms from acetone, acetyl-14α-artebufogenin (IV) (105.2 mg, 52.6%), mp 222—223° as colorless prisms from acetone and 14α-artebufogenin (III) (22.8 mg, 11.4%), mp 264—265° as colorless prisms from acetone.

Treatment of 14α-Artebufogenin (III) with aq. HCl——a) This was carried out according to a method similar to that described by Meyer, *et al.*[5] To a solution of III (130 mg) dissolved in MeOH (10 ml), 4 ml of 2N HCl aq. was added and the mixture was refluxed for 60 min. The mixture was poured into ice–water and extracted with $CHCl_3$. The extract was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated to dryness. The chromatography of the residue (132 mg) on silica gel with *n*-hexane–acetone (7: 1) and (5: 1) gave 14β-artebufogenin (V) (45.9 mg, 35.3%), besides the original 14α-artebufogenin (III) (56.9 mg, 43.8%).

b) III (105 mg) was treated with 2N HCl (3.5 ml) in acetone (10 ml) in the manner described in a). After the column chromatographic separation of the product (106 mg), III (43.5 mg, 43.5%) and V (35.0 mg, 35.0%) were obtained.

c) A mixture prepared from III (100 mg), 2N HCl aq. (5 ml) and AcOH (25 ml) was refluxed. After 15 min, the mixture showed four spots on TLC which are probably due to V, III, VI, and IV, as shown in Fig. 1. After 90 min, the resulting mixture showed two spots of the acetates, VI and IV, was poured into ice–water, and extracted with $CHCl_3$. The extract was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated *in vacuo* to dryness. The chromatography of the residue (97 mg) on silica gel with *n*-hexane–acetone (19: 1) and (9: 1) gave the acetates, VI (28.8 mg, 28.8%) and (38.5 mg, 38.5%), which are identical with the authentic samples.

Treatment of 14β-Artebufogenin (V) with aq. HCl——a) V (150 mg) was refluxed in 2N HCl (6 ml) and MeOH (20 ml) in the manner described in III a). After the column chromatographic separation, III (66.0 mg, 44.0%) was obtained besides V (43.2 mg, 29.4%).

b) V (50 mg) was refluxed in 2N HCl (2 ml) and acetone (10 ml) in the manner described in III b). The product (48 mg) was chromatographed as described in III b), and III (21.2 mg, 43.6%) and V (16.8 mg, 33.5%) were obtained.

c) V (85 mg) was refluxed in 2N HCl (3 ml) and AcOH (20 ml) in the manner described in III c). The product (88 mg) was chromatographed on silica gel with the same solvent as described in III c).VI (24.6 mg, 29.0%) and IV (29.4 mg, 34.6%), which were identical with the authentic samples, were obtained. In the course of this reaction (after 15 min), four spots (III, IV, V, and VI) were detected on TLC (Fig. 1).

Treatment of Acetyl-14α-Artebufogenin (IV) with aq. HCl——a) To a solution of IV (100 mg) dissolved in MeOH (15 ml), 4 ml of 2N HCl aq. was added and the mixture was refluxed. After about 20 min, four spots (III, IV, V, and VI) were detected on TLC, and after 120 min, two spots (III and V) were detected. This was poured into ice–water and worked up in the manner as described in III a). After the column chromatographic separation, III and IV were obtained in 43.2 mg (43.2%) and 29.8 mg (29.8%) yields.

b) To a solution of IV (50 mg) dissolved in AcOH (10 ml), 2 ml of 2N HCl aq. was added, and the mixture was refluxed for 120 min. The mixture was poured ice–water and product isolated as described in III c). After the column chromatographic separation, IV and VI were obtained in 21.1 mg (42.2%) and 15.6 mg (31.2%) yields.

Treatment of Acetyl-14β-Artebufogenin (VI) with aq. HCl——a) To a solution of VI (200 mg) dissolved in MeOH (30 ml), 6 ml of 2N HCl aq. was added and the mixture was refluxed for 120 min. After the separation described in IV a), III and V were obtained in 84.8 mg (42.4%) and 61.8 mg (30.9%) yields. In the course of this reaction, four spots of III, IV, V, and VI were detected on TLC (Fig. 1).

b) A similar treatment of VI (200 mg) with 6 ml of 2N HCl aq. in AcOH (30 ml) gave, after the column chromatographical separation, IV (86.2 mg, 43.1%) and VI (60.0 mg, 30%), respectively.

The Preparation of Samples used in the NMR and ORD Data——1) NMR: To a solution of 50 mg of 14α-artebufogenin or 14β-artebufogenin were dissolved in 50 ml of reagent grade MeOH, 10 ml of 2N HCl aq. was added and the mixture was refluxed for one hour (Fig. 4). The solution was diluted with dist. H_2O

(200 ml) and extracted with reagent grade $CHCl_3$ (50 ml×5 times). The extract was washed with dist. H_2O to neutrality, dried over anhydrous Na_2SO_4 and evaporated *in vacuo* to dryness. The residue was dissolved in $CDCl_3$ and the NMR spectra were determined (See Fig. 2, 3, and 4); 14α-artebufogenin (III) (in $CDCl_3$) δ: 4.12 (1H, broad singlet, 3-H), 0.97 (3H, singlet, 19-CH_3), 0.64 (3H, singlet, 18-CH_3); 14β-artebufogenin (V) (in $CDCl_3$) δ: 4.12 (1H, broad singlet, 3-H), 0.94 (3H, singlet, 19-CH_3), 0.90 (3H, singlet, 18-CH_3). These data were reported in full in Part VII.[1]

2) ORD: Each sample was prepared by the method described in a). By the method described in a). the reaction residues were obtained and dissolved in reagent grade dioxane. The ORD of these solutions were determined.

ORD of 14α-artebufogenin (III) (c=0.100, dioxane)
$[\alpha]^{28}$ (nm): +55° (500), +168° (400), +400° (360), +1090° (330), +1580° (317) (peak), +770° (303), 0° (292), −520° (286), −815° (275) (trough), −450° (260), 0° (248), +290° (240), +1065° (230).

ORD of 14β-artebufogenin (V) (c=0.101, dioxane)
$[\alpha]^{28}$ (nm): −58° (500), −198 (400), −395° (370), −790° (347), −1450° (330) (trough), −960° (320), 0° (312), +1400° (300), +2480° (283) (peak), +1920° (260) (trough), +2830° (237), +2610 °(230).

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