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Photochemistry of Bufadienolides. II.[1] Irradiation of Resibufogenin and 14α-Artebufogenin[2]

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Although in the photolysis of 14β-hydroxy compounds, as described in the previous paper,[1] ring formation reaction between C_{21} and 14β-hydroxy group is observed, in the case of resibufogenin(I) and 14α-artebufogenin(IV), which are 14β,15β-epoxy- and 15-oxo-compounds, the photolysis products (A and B, in Fig. 1) were unstable and could not be isolated. However, it should be noted that by treatment with acid the photolysis products were converted into the stable 21-OCH_3 structure, *i.e.* methyl 3-hydroxy-21-methoxy-15-oxo-5β,14α-chola-20(21),22-dienoate(II) and methyl 14β,15β-epoxy-3β-hydroxy-21-methoxy-5β-chola-20(21),22-dienoate(IV). The fact that the epoxy ring was intact during photolytic conditions was indicated by thin-layer chromatography (Fig. 1) in the irradiation of I by the appearance of spot A which had different *Rf* value compared with that of spot B, a photolysis product of IV.

Furthermore, the progress of the photo-reaction was followed by UV spectra measurement (Fig. 4) and a plausible mechanism for the formation of II and IV is depicted in Chart 2.

Previously we have described[1] on the photolysis of bufalin, bufotalin, gamabufotalin and their acetates, bufadienolide compounds having 14β-hydroxy group. In the present study the irradiation of resibufogenin and 14α-artebufogenin, which are 14β,15β-epoxy- and 15-oxo-compounds, respectively, is reported.

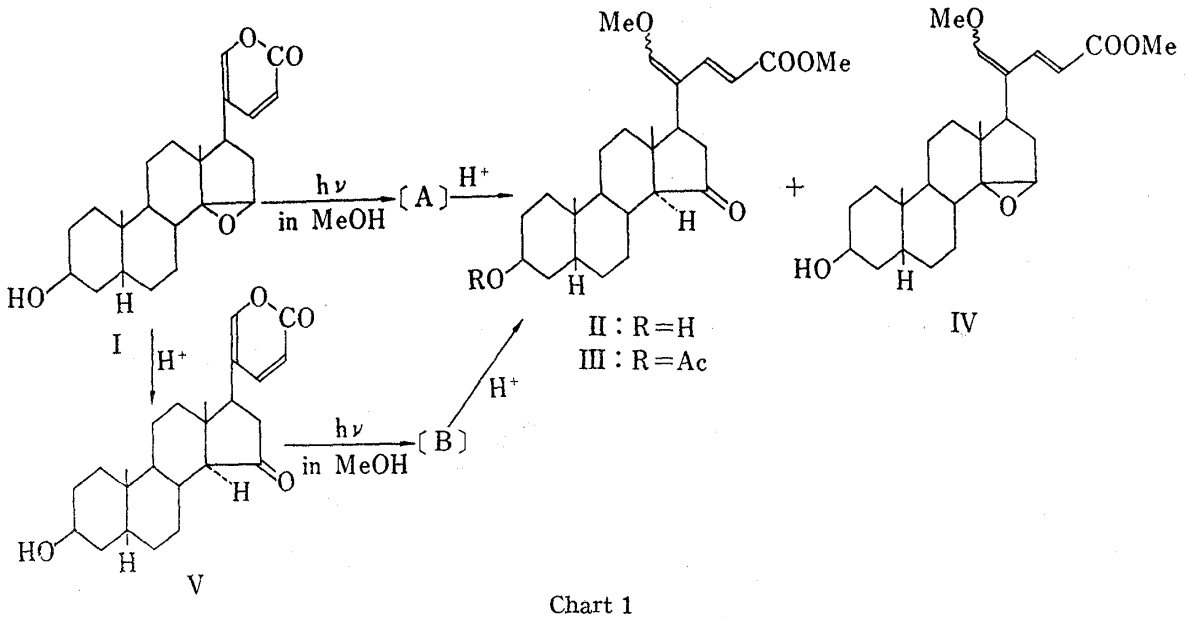

Chart 1

1) This paper constitutes Part VI of the series entitled "Bufadienolides"; Part V (Photochemistry of Bufadienolides. I): Y. Kamano and M. Komatsu, *Chem. Pharm. Bull.* (Tokyo), **17**, 1698 (1969).
2) This work was reported at the Photochemical Meeting of the Chemical Society of Japan, Sendai, Oct. 14, 1968.
3) Location: *No. 34-1, Takata 3-chome, Toshimaku, Tokyo, 170-91, Japan.*

When resibufogenin (I) is irradiated in methanol under the same conditions as previous report,[1] a photolysis product (A) is detected by the thin-layer chromatogram (Fig. 1). The compound was unstable and could not be isolated in pure state by chromatographical separation. By refluxing the photolysis solution at pH 2.5 with hydrochloric acid, following by chromatographical separation, colorless needles, mp 202—203°, were obtained in small amount. Structure II could be assigned to this substance based on the following evidence. Elemental analysis and molecular weight determination indicated a molecualr formula $C_{26}H_{38}O_5$. In the IR spectrum the compound showed bands at 1740 cm^{-1}, 1708 cm^{-1} and 1606 cm^{-1} assignable to an ester CO, a ketone and a conjugated C=C absorptions (Fig. 2). When the NMR spectrum of II was compared with that of resibufogenin (I), a proton signal at 6.48 τ due to 14β,15β-epoxy group in I disappeared and instead two signals due to a methoxyl and a carboxyl methyl groups appeared as

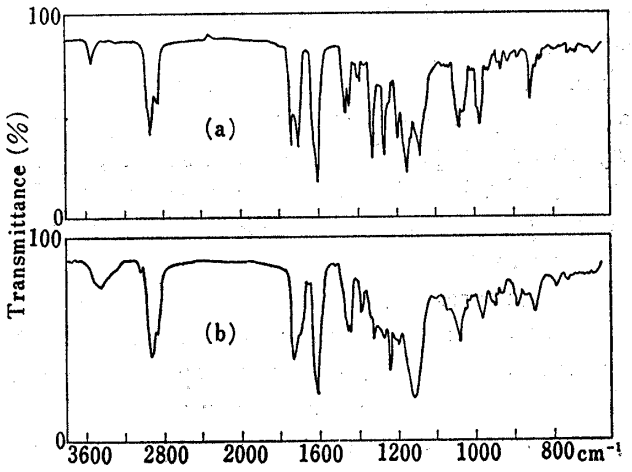

Fig. 2. Infrared Spectra of II(a) and IV(b) in KBr

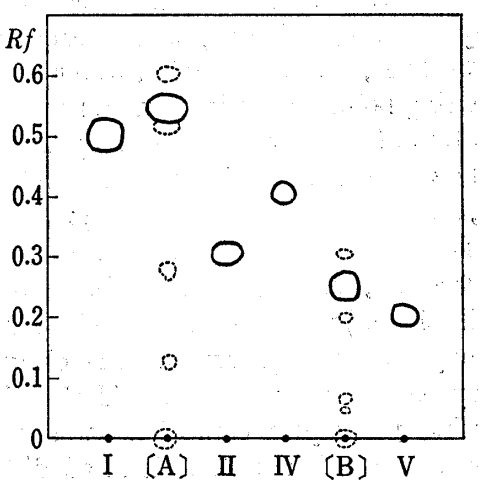

Fig. 1. Thin-Layer Chromatograms of the Reaction Solutions and Authentic Substances

I : resibufogenin
II : methyl 21-methoxy-3-hydroxy-15-oxo-5β, 14α-chola-20(21),22-dienoate
IV : methyl14β,15β-epoxy-21-methoxy-3-hydroxy-5β-chola-20(21),22-dienoate
V : 14α-artebufogenin
[A]: the irradiated solution of resibufogenin (I)
[B]: the irradiated solution of 14α-artebufogenin (V)
solvent system: acetone-$CHCl_3$-*n*-hexane (3:3:4)
(A)

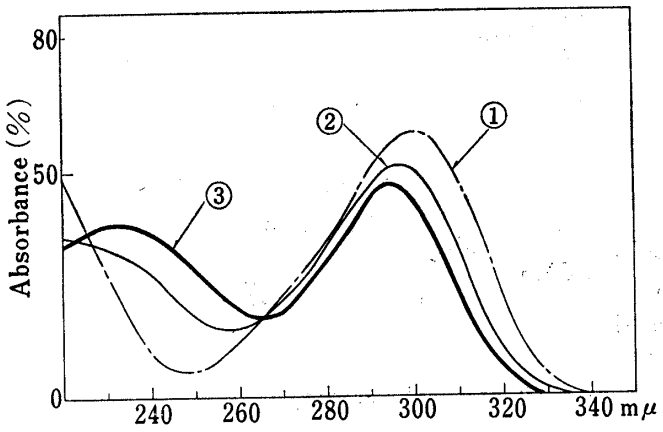

Fig. 4. Ultraviolet Absorption Spectra of Resibufogenin(I) during Irradiation in MeOH

the curve
①: no irradiation (resibufogenin, I)
②: the curve was obtained after irradiation for 1.5 hours
③: the curve was obtained after irradiation for 3 hours

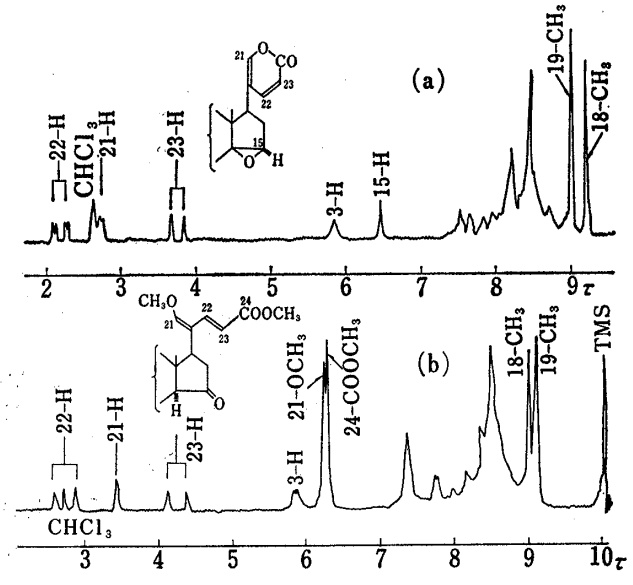

Fig. 3. Nuclear Magnetic Resonance Spectra of Resibufogenin(I)(a) and (II)(b) in $CDCl_3$ at 60Mc

singlets at 6.27 τ and 6.32 τ, respectively (Fig. 3). The typical signals suggesting the presence of the –CH=C(OH)–CH=CH–CO– moiety were observed at 2.76 τ (1H, doublet), 3.48 τ (1H, singlet) and 4.27 τ (1H, doublet) similar to the photolysis product of bufalin described in a previous report.[1] These results suggested the structure of the photolysis product be represented by structure II (Chart 1).

Acetylation of II with acetic anhydride and pyridine in the usual manner yielded the corresponding acetate(III), $C_{28}H_{40}O_6$, mp 159.5—161°.

On the other hand, treatment of the photolysis solution of resibufogenin (I) with dimethyl sulfate, afforded, after chromatographical separation, two products. $C_{26}H_{38}O_5$, mp 203—204°, colorless needles, which was proved to be identical with compound II by the mixed melting point determination and the IR and NMR spectra, and $C_{26}H_{38}O_5$, colorless amorphous material (purity was checked by TLC), which was assigned structure IV on the basis of the following IR and NMR spectra. When the IR spectrum of IV was compared with that of II, a ketone band at 1700 cm^{-1} present in II was not seen in IV but a new band at 3040 cm^{-1} assignable to 15-CH was observed (Fig. 2). The NMR spectrum of IV showed a signal at 6.83 τ (3H, singlet) attributable to 21-OCH_3 group and signals overlapping each other at 6.25 τ (4H, singlet) which are assumed to indicate the presence of 21-methoxy carbonyl and 14β,15β-epoxy groupings (Chart 1). The cleavage reaction of epoxy ring to form 15-oxo compound II may be due work-up with acid after irradiation. The fact that the epoxy ring was intact during photolytic conditions was also indicated by thin-layer chromatography by the appearance of spot A which had different *Rf* value compared with that of spot B, a photolysis product of 15-ketone, 14α-artebufogenin (V) (Fig. 1). Although methoxy group of 24-ester function was introduced during irradiation by methanolysis as before,[1] the treatment with acid was responsible for the methoxyl group at 21-position.

In order to clarify the photolytic process, the progress of the reaction was followed by UV spectra measurement (Fig. 4). Namely, the absorption maximum at 300 mμ in resibufogenin (I) shifted to 295 mμ during irradiation and, in addition, a new absorption maximum at 235 mμ was found to develop. This fact suggests the formation of formyl ketone intermediate (d),[4] which is formed *via* photo-pyrone intermediate (c) followed by cleavage of four-membered lactone ring.[1] Methanolysis of (d) afford a tautomeric mixture of methyl esters (e_1—e_4), which then is converted to vinyl methyl ether (f) by further reaction with methanol (Chart 2).

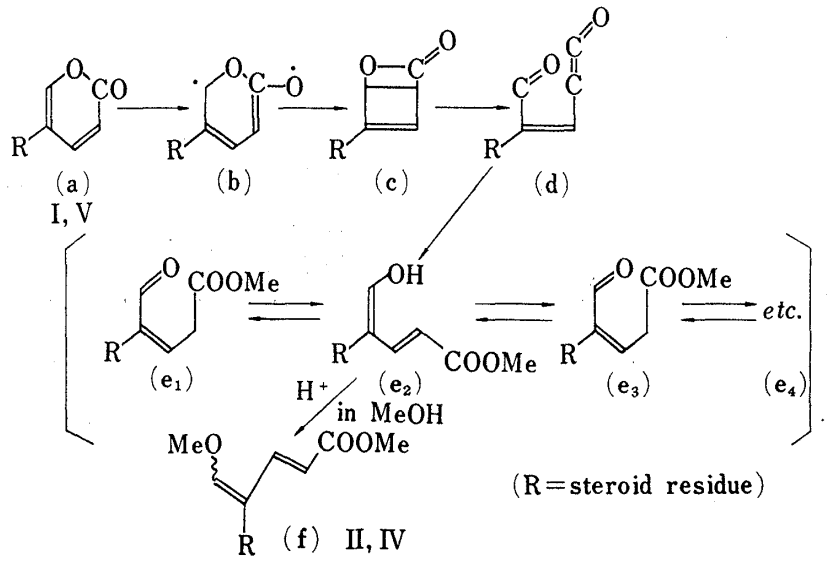

Chart 2

4) E.J. Corey and J. Streith, *J. Am. Chem. Soc.*, **86**, 950 (1964).

When 14α-artebufogenin (V) (Chart 1), which was prepared from resibufogenin (I) by treatment with acid,[5] was irradiated in methanol under the similar conditions described above, followed by treatment with dimethyl sulfate to give a crystalline, mp 203—204°, colorless needles from methanol or acetone in good yield. Intermediate compound (B) (Fig. 1) was not isolated. The compound was proved to be identical with compound II by the mixed melting point determination and comparison of their IR and NMR spectra and TLC.

It should be noted that in the photolysis of 14β-hydroxy compounds ring formation reaction between C_{21} and 14β-hydroxyl group is observed,[1] however, in the case of the 14β,15β-epoxy and 15-oxo compounds the photolysis products were unstable and were converted into 21-OCH_3 structure which was stable, since it had no 14β-hydroxy group.

The coupling constants (JAB) between 22- and 23-olefine protons in II, III and IV were *ca.* 16 cps, the conformation of olefin was assumed to be trans, as shown in the scheme. The same was found to be the case with irradiation products of 14β-hydroxy compounds.

Experimental[6]

Thin-Layer Chromatography (TLC)——TLC was performed in the same way as reported in the paper[7] with silica gel G plates. The solvent system used are (A) acetone–$CHCl_3$–*n*-hexane (3:3:4), (B) AcOEt–*n*-hexane (7:3), (C) ether–AcOEt (6:4), and (D) AcOEt–$CHCl_3$–HCOOH (2:2:1). The spots were detected by spraying conc. H_2SO_4 followed by heating. The course of the reactions were followed by TLC, as shown in Fig. 1.

TLC of the Starting Materials: resibufogenin (I): *Rf* 0.50 (solvent A); color, green. 14α-Artebufogenin (V): *Rf* 0.21 (solvent A); color, green.

Ultraviolet(UV) Absorption Spectra——UV spectra were determined on a Hitachi automatic spectrophotometer, Model EPS-2U, the solvents are indicated. As shown in Fig. 4, the progress of the photo-reaction of resibufogenin (I) in methanol was followed by UV spectrum. The absorption maximum at 300 mμ in resibufogenin (I) shifted to 295 mμ during irradiation and, in addition, a new absorption maximum at 235 mμ was found to develop (the curve ①→②→③).

Photolysis of Resibufogenin (I) and the Preparation of II and IV——a) A solution of 360 mg of I in 180 ml of reagent grade MeOH was irradiated in pyrex vessel using an internal water cooled higher-pressure mercury arc lamp (Ushio, type UM-425, 450-W) under a current of nitrogen. The temperature of the reaction mixture was maintained at 20° to 28° by external water-cooling. After 12 hr the solution showed a main spot (a photolysis product A; solvent A; *Rf* 0.55; yellowish green with conc. H_2SO_4) on thin-layer chromatogram (Fig. 1). Then the half-volume of the reaction solution was evaporated *in vacuo* by a rotatory evaporator. The residue was chromatographed on silica gel (Wakogel C-200) with acetone–*n*-hexane mixture. However, the compound was unstable and could not be isolated in pure state.

Therefore, the another half-volume of the reaction solution was added a few drops of conc. HCl (pH 2.5) and was boiled under reflux for 30 min. The resulting solution was concentrated *in vacuo*, and the residue was taken up in $CHCl_3$, washed with H_2O and dried over anhyd. Na_2SO_4. The extract was chromatographed on silica gel (Wakogel C-200, 5.5 g) with acetone–*n*-hexane (1:9) to give methyl 3-hydroxy-21-methoxy-15-oxo-5β,14α-chola-20(21),22-dienoate, II (52 mg), mp 202—203°, as colorless micro needles from EtOH. Mol. wt. 432. *Anal.* Calcd. for $C_{26}H_{38}O_5$: C, 72.52; H, 8.90. Found: C, 72.37; H, 9.09. IR ν_{max}^{KBr} cm^{-1}: 3540 (OH), 1740 (ester CO), 1708 (ketone), 1606 (conjugated C=C), 1256 (ester C–O), 970, 858 (C=C). NMR (10% solution in $CDCl_3$) τ: 2.76 (1H, doublet, J=15.6 cps, 22-H), 3.48 (1H, singlet, 21-H), 4.27 (1H, doublet, J=15.6 cps, 23-H), 5.88 (1H, broad peak, 3-H), 6.27 (3H, singlet, 21-OCH_3), 6.32 (3H, singlet, 24-$COOCH_3$), 9.06 (3H, singlet, 19-CH_3), 9.13 (3H, singlet, 18-CH_3). TLC: *Rf* 0.31 (solvent A), 0.48 (solvent B), 0.47 (solvent C), 0.70 (solvent D); color, yellowish green. UV λ_{max}^{EtOH} mμ (log ε): 290 (4.2).

5) H. Linde and K. Meyer, *Helv. Chim. Acta*, **42**, 807 (1959).
6) All melting points are uncorrected. Molecular weight was determined using a Hitachi Molecular Weight Apparatus Model 115. Infrared Spectra measurements were run on a Nihon Bunko Model DS 301 spectrophotometer. Nuclear magnetic resonance spectra were obtained on a Hitachi Model R-20 spectrometer operated at 60 Mcps in deuteriochloroform solution containing tetramethylsilane as internal standard and are reported in τ values.
7) Bufadienolides. III: Y. Kamano, H, Yamamoto, and M. Komatsu, *Chem. Pharm. Bull.* (Tokyo), **17**, 1246 (1969).

b) A solution of 500 mg of I in 300 ml of reagent grade MeOH was irradiated under the same conditions described above. After 9 hr, the reaction solution was neutralized with 5% $NaHCO_3$ aq. and concentrated *in vacuo* to a small volume. The mixture was taken up in $CHCl_3$. The $CHCl_3$ layer was washed with H_2O, dried over anhyd. Na_2SO_4, and evaporated *in vacuo* to dryness. The residue thus obtained was subjected to silica gel–column chromatography (Wakogel C-200, 14 g) using acetone–*n*-hexane (1:7) as solvent to afford two products, as shown in Fig. 1, II (91 mg), mp 203—204° as colorless needles from acetone, and IV (107 mg) as colorless amorphous material from acetone.

The compound II was found to be identical with the sample obtained above.

The compound IV was assigned to be methyl 14β,15β-epoxy-3-hydroxy-21-methoxy-5β-chola-20(21),22-dienoate, on the basis of the following data. Mol. wt. 431. *Anal.* Calcd. for $C_{26}H_{38}O_5$: C, 72.52; H, 8.90. Found: C, 72.44; H, 8.88. IR ν_{max}^{KBr} cm^{-1}: 3500 (OH), 3040 (15-CH), 1735—1730 (ester CO), 1610 (conjugated C=C), 1235 (ester C–O), 980, 840 (C=C). NMR (10% solution in $CDCl_3$) τ: 2.66 (1H, doublet, J=16.0 cps, 22-H), 3.32 (1H, singlet, 21-H), 4.29 (1H, doublet, J=16.0 cps, 23-H), 5.86 (1H, broad peak, 3-H), 6.25 (4H, singlet, 24-$COOCH_3$ and 15-CH), 6.83 (3H, singlet, 21-OCH_3), 8.76 (3H, singlet, 18-CH_3), 9.06 (3H, singlet, 19-CH_3). UV λ_{max}^{EtOH} mμ (log ε): 290 (4.3). TLC: *Rf* 0.41 (solvent A), 0.58 (solvent B), 0.57 (solvent C), 0.76 (solvent D), color, yellowish green.

Photolysis of 14α-Artebufogenin (V)[5] and the Preparation of II——A solution of 74 mg of V in 35 ml of reagent grade MeOH was irradiated under the same conditions described above. After 7 hr, the reaction solution showed a main spot (a photolysis product B; solvent A, *Rf* 0.41; yellowish green with conc. H_2SO_4) on thin-layer chromatogram (Fig. 1).

Without isolation of the photolysis product, the solution was added a few drops of Me_2SO_4 and was boiled under reflux for 15 min. After neutralization with 5% $NaHCO_3$ aq., the reaction mixture was concentrated *in vacuo* to about one-quarter volume. The resulting precipitate by adding H_2O (10 ml) was collected by filtration, was washed with H_2O, and dried. The precipitate was recrystallized from MeOH to give II (38 mg), mp 203—204°, as colorless needles, which was found to be identical with the sample obtained above by mixed mp, IR and NMR spectra.

Acetylation of II——II (50 mg) was treated with pyridine (1 ml) and Ac_2O (0.7 ml) in the usual manner. Recrystallization from acetone gave III (42 mg) as colorless needles, mp 159.5—161°. *Anal.* Calcd. for $C_{28}H_{40}O_6$: C, 71.16; H, 8.53. Found: C, 71.32; H, 8.48. IR ν_{max}^{KBr} cm^{-1}: 1740, 1730, 1713 (ester CO and ketone), 1619 (conjugated C=C), 1255, 1240 (ester C–O), 990, 855 (C=C). TLC: *Rf* 0.65 (solvent A); color, yellowish green.

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