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Photochemistry of Bufadienolides. I.[1] Irradiation of Bufalin, Bufotalin and Gamabufotalin[2]

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The results of photolysis of bufadienolides, which have an α-pyrone ring on a C_{17}-position of the steroidal moiety, particularly those of 14β-hydroxy compounds such as bufalin (I), bufotalin (X), gamabufotalin (XVI) and their acetates (II, XI and XVII), are described. Photolysis of I or II in ethanol gave the photoproduct (III), or (IV). The spectral data suggested III and IV had a partial structure either A or B (Chart 1). Then the structure of III and IV were finally proved to be identical with the authentic samples which were prepared from I. Photolysis of I or II in methanol gave V or VI. By hydrolysis III and V were easily converted into the corresponding carboxylic acid (VII), from which methyl ester (V) was prepared by the treatment with diazomethane (Chart 2). Photolysis of X or XI, having 16-β-acetoxyl groups besides 14β-hydroxyl group, in methanol, afforded XII or XIII, respectively (Chart 3). When XVI or XVII, 11α-hydroxy and 11α-acetoxy compounds, were irradiated in methanol, XVIII or XIX were obtained, respectively (Chart 4).

These results indicate that photolysis of the other 14β-hydroxy compounds in bufadienolides might be considered to afford the photoproducts having the same moiety as above.

A plausible mechanism for the formation of III, V, XII, XVIII, and *etc. via* the photo-pyrone (c),[6] the formyl ketene (d) and enol from (f) is depicted in Chart 5.

Recently, the photochemistry of α-pyrone ring system received much attention. Esterle and co-workers[4] have observed a photolytic methanolysis with 2,4-dimethyl-α-pyrone. Photo-dimerization reaction products have been isolated by Mayo and Yip[5] by the irradiation of the above compound in a benzene solution, whereas Corey and Streith[6] have achieved intramolecular photocyclization of α-pyrone by irradiating a dilute ether solution at low temperature.

In this paper we wish to describe photochemical studied of bufadienolides, particularly those of 14β-hydroxy compounds such as bufalin (I), bufotalin (X), gamabufotalin (XVI) and their acetates (II, XI and XVII). All these compounds have an α-pyrone ring on a C_{17}-position of the steroidal moiety.

A solution of bufalin (I) in ethanol was irradiated in a Pyrex vessel with a high-pressure mercury arc lamp under a current of nitrogen. The reaction was followed by thin-layer chromatography which, after ten hours, showed a main spot of *Rf* 0.61 (solvent A, Table I). The reaction mixture was then subjected to column chromatography on silica gel to afford a photoproduct (III), mp 171—173°, as colorless needles from acetone. Elemental analysis

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[1] This paper constitutes Part V of the series entitled "Bufadienolides," Part IV: Y. Kamano, H. Yamamoto, and M. Komatsu, *Chem. Pharm. Bull.* (Tokyo), 17, 1251 (1969).

[2] This work was reported at the 86th Annual Meeting of Pharmaceutical Society of Japan, Sendai, Oct. 1966 and 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.*

[4] J.G. Esterle, P.M. Girling, P. De Mayo, and R.H. Wiley, unpublished observations cited by the P. De Mayo, in Advances in Organic Chemistry, Vol. II, R. Raphael, E. Taylor, and H. Whynberg, eds., Interscience, New York, 1960, p. 394.

[5] P. De Mayo and R.W. Yip, *Pro. Chem. Soc.*, **1964**, 84.

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

and molecular weight determination indicated the molecular formula $C_{26}H_{38}O_4$. The UV spectrum showed a characteristic absorption maximum at 305 mμ (log ε 4.53), which may be compared with the λ_{max} for the starting material, bufalin (I) (300 mμ) except for (see Fig. 1). Compared with bufalin (I), the IR spectrum of the product (III) was devoid of these bands occurring at 1730, 1634 and 1538 cm^{-1} (α-pyrone ring system), but instead possessed new bands at 1695 cm^{-1} (α,β-unsaturated ester), and 1612 and 1595 cm^{-1} (conjugated double bond) (see Fig. 2). In addition, its IR spectrum showed a sharp hydroxyl band at 3490 cm^{-1}, a broad strong C–O band at 1165—1155 cm^{-1} and a typical $R_1CH=CHR_2$ (*trans*) bands at 975, 910 and 840 cm^{-1}. Whereas compound I showed pyrone ring proton signals in NMR spectrum (Fig. 3) at 2.14 τ (doublet, 22-H̲), 2.70 τ (singlet, 21-H̲) and 3.74 τ (doublet, 23-H̲), the product (III) isolated was found to possess a quite similar pattern in a high field of 2.78 τ (doublet), 3.44 τ (singlet) and 4.38 τ (doublet), suggesting the presence of a –CH=C–CH=CH–CO– moiety. The fact that the signals are sharper in III compared with I indicated the disappearance of long-range coupling between the δ-proton and the α- as well as β-proton signals. These results could best be interpreted as the occurrence of α-pyrone ring cleavage reaction. Moreover, the compound showed ethoxy signals at 5.69 and 5.92 τ (AB quartet) and 8.70 τ (triplet).

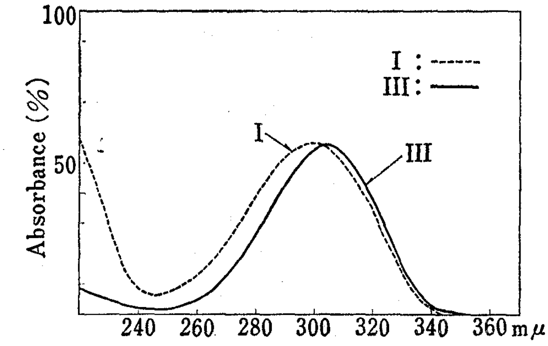


Fig. 1. Ultraviolet Absorption Spectra of Bufalin (I) and Its Photo-Product (III) in MeOH

concentration I: 0.04% in MeOH
III: (0.04×1/5) % in MeOH

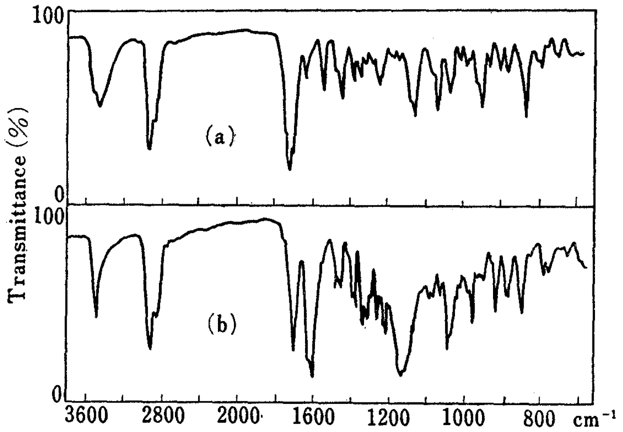


Fig. 2. Infrared Spectra of Bufalin(I)(a) and Its Photo-Product(III)(b) in KBr

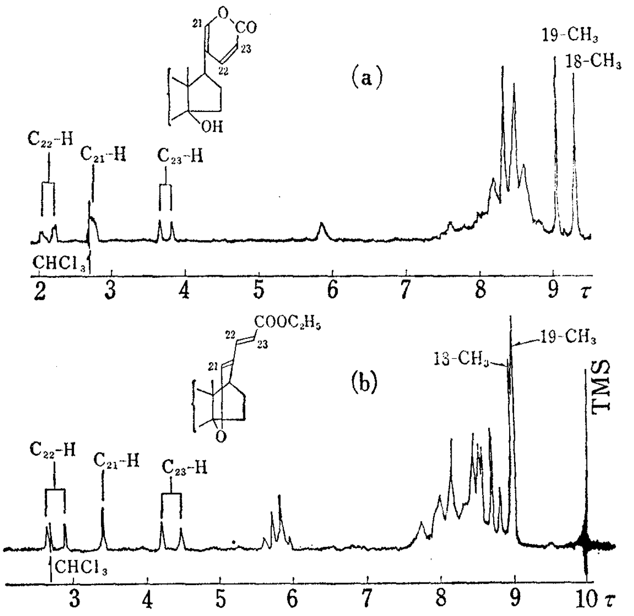
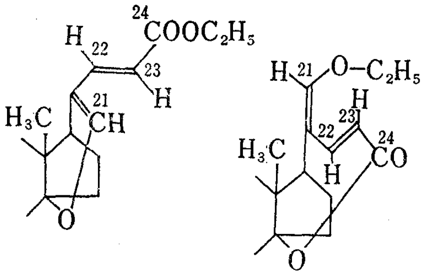


Fig. 3. Nuclear Magnetic Resonance Spectra of Bufalin(I)(a) and Its Photo-Product(III)(b) in $CDCl_3$ at 60Mc

On the other hand, acetylation of III with acetic anhydride in pyridine gave an acetate (IV), mp 166—168°, $C_{28}H_{40}O_5$, which was proved to be identical with the compound prepared by the irradiation of acetyl-bufalin (II) under the same conditions. This acetate (IV) showed characteristic UV absorption similar to III, but had no hydroxyl band in the IR spectrum. Thus a hydroxyl group at C_{14} was assumed to enter the reaction. The presence of an acetoxyl group in IV was also supported from NMR spectrum. The above data suggested photolysis products III and IV had a partial structures either A or B.

Fortunately structure A is known to exist in the so-called "isobufogeninic acid ester".[7] Accordingly we converted I to isobufogeninic acid ester structure by hydrolysis with potassium hydroxide in aqueous ethanol, followed by acidification under the conditions of Wartburg and Renz,[8] as shown in Chart 2. This hydrolysis product (III) was proved to be identical with the photoproduct (III), by mixed meltint point and comparison of the IR and NMR spectra and *Rf* values (TLC), this alternative structure (B) was ruled out. The same conclusion as this was also obtained based on the comparison of the acetate (IV).



A B

Chart 1

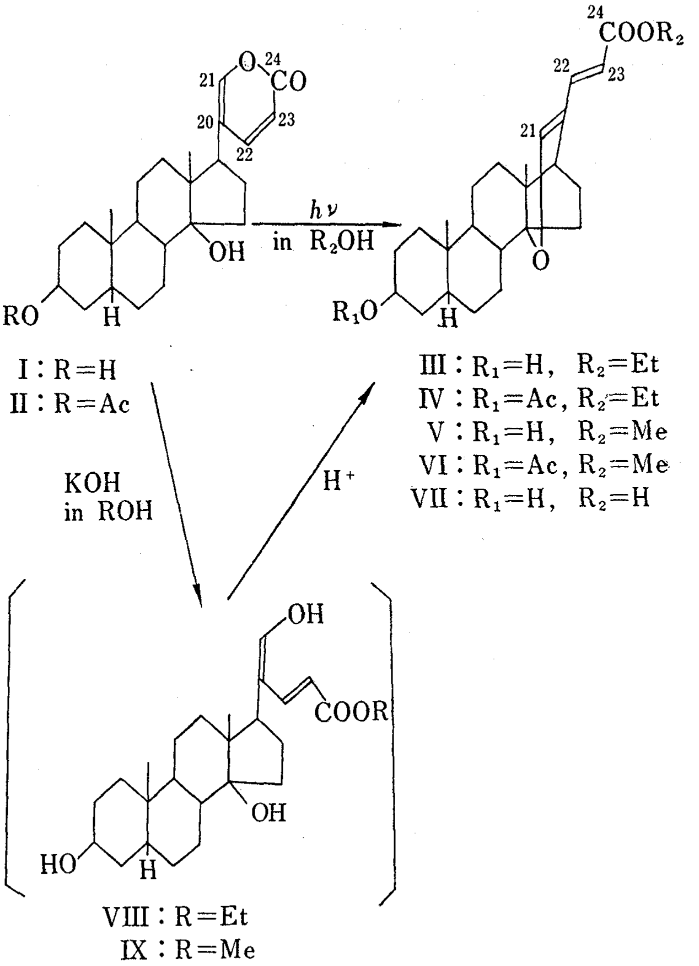


Chart 2

Photolysis of bufalin (I) or acetyl-bufalin (II) in methanol in the same way as described above gave the photoproduct (V), mp 210—213°, $C_{25}H_{36}O_4$, or VI, mp 153—155°, $C_{27}H_{38}O_5$, respectively. The NMR spectra of both V and VI showed three-proton singlet (COOMe) typical signals at 6.29. The assignment of the structures was achieved by their elemental analysis, similarity of their UV, IR and NMR spectra to those of III and V. The structure of V and VI were finally proved to be identical with the authentic samples which were prepared from I according to the similar hydrolysis procedure described above. Acetylation of V in the usual manner afforded VI.

Hydrolysis of III and V with potassium hydroxide in aqueous methanol gave the corresponding carboxylic acid (VII), $C_{24}H_{34}O_4$, mp 224—226°, from which methyl ester (V) was prepared by the treatment with diazomethane.

Nextly, photolysis of bufotalin (X) and acetyl-bufotalin (XI) having 16-β acetoxy groups beside 14β-hydroxyl group were examined. Irradiation of X in methanol under the same conditions as above, followed by chromatographical separation gave XII, $C_{27}H_{38}O_6$, mp 118°, which on acetylation in the usual manner gave the corresponding acetate (XIII), $C_{29}H_{40}O_7$, mp 204—205°.

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7) L.F. Fieser and M. Fieser, "Steroids," Reinhold Publ. Corp., New York, N.Y., 1959, pp. 782—787.

8) A. von Wartburg and J. Renz, *Helv. Chim. Acta*, **42**, 1620 (1959).

Similar irradiation of acetylbufotalin (XI) also gave the same acetate (XIII) as shown in Chart 3. The assignment of those structure (XII and XIII) were obtained by the UV, IR and NMR spectra as described in the experimental part. Final structure proof of those compounds were attained by comparison of XIII with the authentic sample synthesized by hydrolysis of bufotalin (X), followed by acidification as shown in Chart 3.

Finally, when gamabufotalin (XVI) or acetyl-gamabufotalin (XVII), 11α-hydroxy and 11α-acetoxy compounds, were irradiated in methanol under the same conditions as above, the photoproduct (XVIII), $C_{25}H_{36}O_5$, mp 118—119°, or (XIX), $C_{29}H_{40}O_7$, mp 202—203°, were obtained, respectively (Chart 4). XVIII was easily converted into XIX by acetylation in the usual way. The structure of XVIII and XIX were confirmed from their elemental analysis, UV, IR and NMR spectral analyses. They were finally confirmed by mixed mp determination and comparison of the spectra and TLC with those of the authentic samples synthesized by hydrolysis of gamabufotalin (XVI), followed by acidification as shown in Chart 4.

Since NMR spectra of the all photoproducts had coupling constant (JAB) bewteen 22- and 23-olefin protons around *ca.* 16 cps, it can be concluded that the 22- and 23-olefin protons have the trans-configuration.

Based on these conclusion, photolysis of the other 14β-hydroxy compounds in bufadienolides might be considered to afford the photoproducts having the same moiety as above.

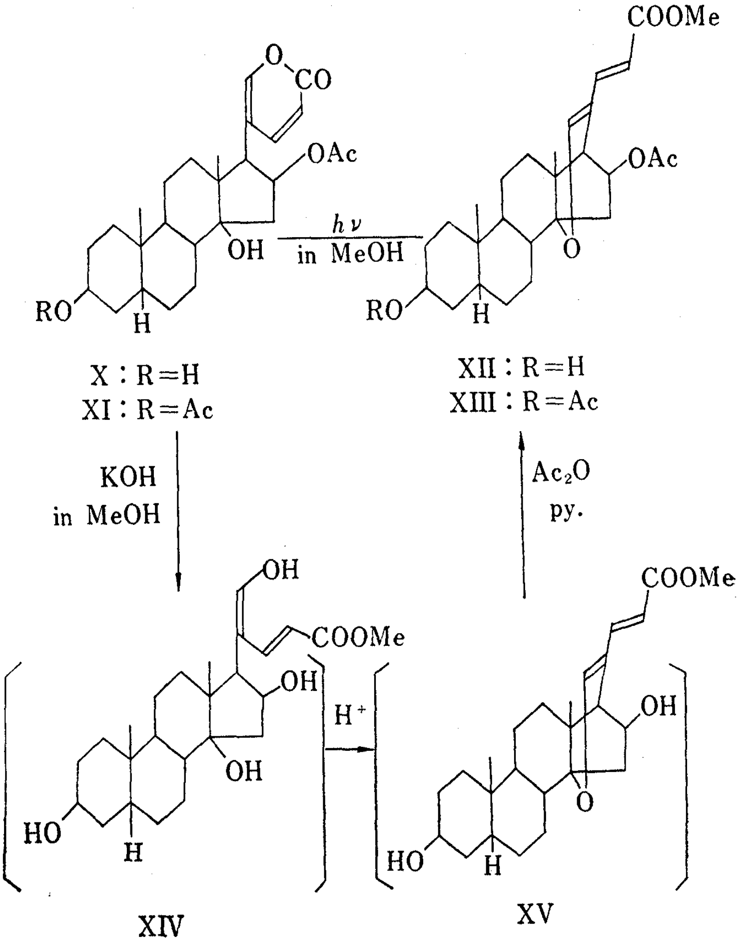

Chart 3

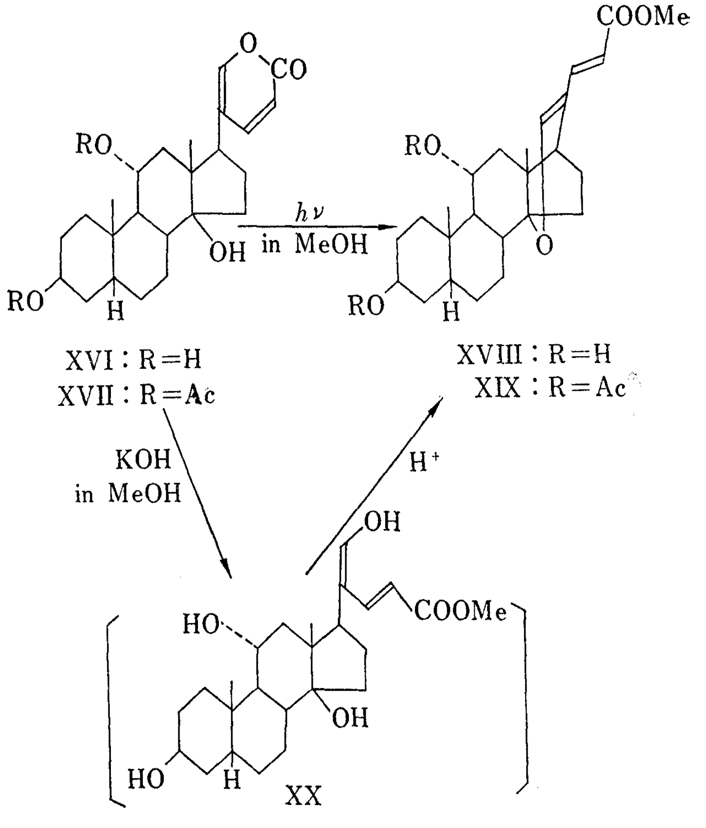

Chart 4

A plansible mechanism for the formation III, V, XII, XVIII, and *etc.* *via* the photo-pyrone (c)[6] is depicted in Chart 5. The photo-pyrone intermediate is assumed to cleave to afford the formyl ketene (d), an optically inactive species, followed by solvolysis to give the formyl ester (e), which *via* enol form (f) is converted to vinyl-ether (g). The formation of the formyl ester (e) is already discussed by Mayo and Yip.[5] However, it is of considerable interest that the formation of the vinyl-ether ring system (g) was observed. It is assumed that 14β-hydroxyl group is located at the sterically favorable position for vinyl-ether ring formation reactions.

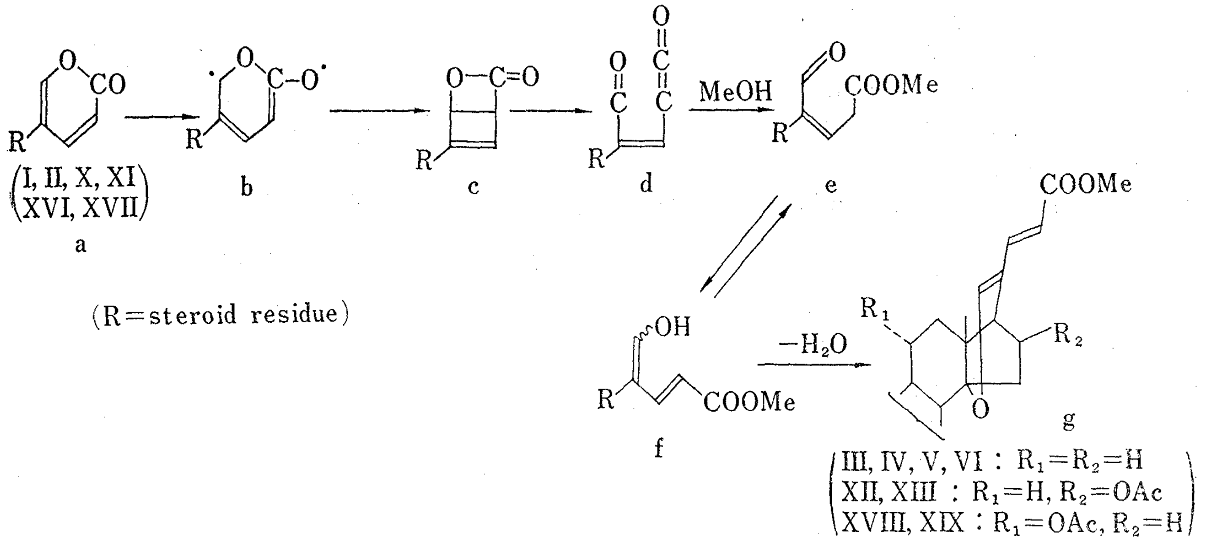

Chart 5

Further other work on the photolysis is now in progress.

Experimental

All melting points are uncorrected. IR spectra were determined in KBr pellets using a Nihon Bunko Model DS301 spectrophotometer. UV spectra were recorded on a Hitachi automatic spectrophotometer, Model EPS-2U, the solvents are indicated. NMR spectra were measured on a Hitachi model R-20 spectrometer using $CDCl_3$ with tetramethylsilane as an internal standard and are reported in τ values. 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.

Thin-Layer Chromatography——Thin-layer chromatography was performed in the same way as reported in the paper[9] 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 *Rf* values of compounds were shown in Table I.

Photolysis of Bufalin (I)——(a) A solution of 200 mg of I in 100 ml of reagent grade EtOH was irradiated in pyrex vessel using an internal water-cooled higher-pressure mercury are lamp (Ushio, type UM-452, 450-W) under a current of nitrogen. The temperature of the reaction mixture was maintained at 15° to 25° by external water-cooling. After 10 hr the solution showed one spot (*Rf* 0.61 solvent A, Table I) on thin-layer chromatogram. Then the solvent was evaporated *in vacuo* by a rotatory evaporator. The residue was chromatographed on silica gel (Wakogel C-200, 10 g) with acetone–n-hexane (1:5) to afford the photoproduct (III), ethyl 14,21-epoxy-3β-hydroxy-5β-chola-20 (21), 22-dienoate (136 mg), mp 171—173°, as colorless needles from acetone or MeOH. $[\alpha]_D^{20}$ -90.3 ($c=1.1$, $CHCl_3$). UV $\lambda_{\max}^{\mathrm{MeOH}}$ mμ (log ε): 305 (4.53) (UV of the starting material, bufalin I, $\lambda_{\max}^{\mathrm{MeOH}}$ mμ (log ε): 300 (3.54), see Fig. 1). IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3500 (OH), 1695 (conjugated CO), 1612, 1595 (conjugated C=C), 1165—1155 (C–O), 975, 910, 840 (C=C) (see Fig. 2). NMR (10% solution in $CDCl_3$) τ: 2.78 (1H, doublet, $J=16.5$ cps, 22-$\underline{H}$), 3.44 (1H, singlet, 21-$\underline{H}$), 4.38 (1H, doublet, $J=16.5$ cps, 23-$\underline{H}$), 5.69 and 5.92 (2H, AB quartet, $J=8.0$ cps, 24-$COOC\underline{H}_2CH_3$), *ca.* 5.84 (1H, broad peak, 3-$\underline{H}$), 8.70 (3H, triplet, $J=8.0$ cps, 24-$COOCH_2C\underline{H}_3$), 8.96 (3H, singlet, 19-$C\underline{H}_3$), 9.00 (3H, singlet, 18-$C\underline{H}_3$). Mol. wt. 413. *Anal.* Calcd. for $C_{26}H_{38}O_4$: C, 75.32; H, 9.29. Found: C, 75.61; H, 8.99 (see Fig. 3).

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[9] Part III: Y. Kamano, H. Yamamoto, and M. Komatsu, *Chem. Parm. Bull.* (Tokyo), 17, 1246 (1969).

Table I. *Rf* Value and Color of the Photo-Products

No.	Compounds	(A) *Rf*	(B) *Rf*	(C) *Rf*	(D) *Rf*	Color with conc. H_2SO_4
1	III	0.61	0.57	0.56	0.80	yellow→orange→greenish brown
2	IV	0.76	0.73	0.72	0.86	yellow→orange→greenish brown
3	V	0.58	0.54	0.52	0.77	yellow→orange→brown
4	VI	0.71	0.66	0.66	0.83	yellow→orange→brown
5	VII	0.13	0.18	0.20	0.72	purple→greyish brown
6	XII	0.40	0.49	0.48	0.71	purple→brownish purple
7	XIII	0.65	0.70	0.68	0.79	purple→brownish purple
8	XVIII	0.22	0.33	0.40	0.56	yellow→orange→brown
9	XIX	0.67	0.66	0.70	0.76	yellow→orange→brown

solvent: (A) acetone–$CHCl_3$–*n*-hexane (3:3:4), (B) AcOEt–*n*-hexane (7:3)
(C) ether–AcOEt (6:4), (D) AcOEt–$CHCl_3$–HCOOH (2:2:1)

(b) A solution of 105 mg of I in 50 ml of reagent grade MeOH was irradiated under the same conditions described above. After the solvent was evaporated *in vacuo*, the residue was chromatographed on silica gel (Wakogel C-200, 5 g) with acetone–*n*-hexane (1:5) to give methyl 14,21-epoxy-20 (21), 22-dienoate, V (75 mg), mp 210—213°, as colorless needles from acetone. $[\alpha]_D^{19.1}$ -95.0 ($c=0.9$, $CHCl_3$). UV λ_{max}^{EtOH} mμ (log ε): 304 (4.50). IR ν_{max}^{KBr} cm^{-1}: 3500 (OH), 1700 (conjugated CO), 1610, 1598 (conjugated C=C), 1165, 1150 (C=O), 978, 910, 850 (C=C). NMR (10% solution in $CDCl_3$) τ: 2.78 (1H, doublet, $J=15.3$ cps, 22-$\underline{H}$), 3.44 (1H, singlet, 21-$\underline{H}$), 4.39 (1H, doublet, $J=15.3$ cps, 23-$\underline{H}$), 5.88 (1H, broad peak, 3-$\underline{H}$), 6.29 (3H, singlet, 24-$COOC\underline{H}_3$), 9.01 (6H, singlet, 18- and 19-$C\underline{H}_3$). Mol. wt. 402. *Anal.* Calcd. for $C_{35}H_{36}O_4$: C, 74.97; H, 9.06. Found: C, 75.16; H, 8.78.

Acetylation of III——III (20 mg) and freshly distilled Ac_2O (0.3 ml) in dry pyridine (0.5 ml) was set aside at room temperature overnight. The mixture was poured into H_2O and extracted with $CHCl_3$. The extract was washed with dil. HCl and H_2O, dried over Na_2SO_4 and evaporated to give the crude acetate (19 mg). Recrystallization from MeOH afforded the acetate (IV) (16 mg), mp 166—168°, as colorless needles, and was found to be identical with the sample IV, which was obtained by photolysis of II, as described below.

Acetylation of V——In the same way described above, V (25 mg) was acetylated with Ac_2O-pyridine to yield the acetate VI (20 mg), mp 152—154°, as white needles from MeOH, and was found to be identical with the sample VI, which was obtained by photolysis of II, as described below.

Photolysis of Acetyl-bufalin II——(a) A solution of 102 mg of II in 50 ml of reagent grade EtOH was irradiated for 8 hr under the same conditions described above. After the solvent was evaporated *in vacuo*, the residue was chromatographed on silica gel (Wakogel C-200, 5 g) with acetone–*n*-hexane (1:5) to give ethyl 3β-acetoxy-14,21-epoxy-5β-chola-20 (21), 22-dienoate, IV (73 mg), mp 167—169°, as colorless needles from MeOH. $[\alpha]_D^{19.1}$ $-67.7°$ ($c=1.1$, $CHCl_3$). UV λ_{max}^{EtOH} mμ (log ε): 304 (4.45).. IR ν_{max}^{KBr} cm^{-1}: 1738 (shoulder), 1730 (ester CO), 1718 (conjugated CO), 1615, 1600 (conjugated C=C), 1250, 1235, 1215 (ester C–O), 1160—1150 (C–O), 980, 910, 840 (C=C). NMR (10% solution in $CDCl_3$) τ: 2.79 (1H, doublet, $J=15.6$ cps, 22-$\underline{H}$), 3.44 (1H, singlet, 21-$\underline{H}$), 4.38 (1H, doublet, $J=15.6$ cps, 23-$\underline{H}$), 4.91 (1H, broad peak, 3-$\underline{H}$), 5.69 and 5.93 (2H, AB quartet, $J=7.5$ cps, 24-$COOC\underline{H}_2CH_3$), 7.94 (3H, singlet, 3-$OCOC\underline{H}_3$), 8.70 (3H, triplet, $J=7.5$ cps, 24-$COOCH_2C\underline{H}_3$), 8.97 (3H, singlet, 19-$C\underline{H}_3$), 9.00 (3H, singlet, 18-$C\underline{H}_3$). *Anal.* Calcd. for $C_{28}H_{40}O_5$: C, 73.68; H, 8.83. Found: C, 73.84; H, 8.82.

(b) After a solution of 98 mg of II in 50 ml of reagent grade MeOH was irradiated for 8.5 hr by the same way as A, the mixture was concentrated *in vacuo*. The residue was chromatographed on silica gel (Wakogel C-200, 5 g) with acetone–*n*-hexane (1:5) to give methyl 3β-acetocy-14,21-epoxy-5β-chola-20 (21), 22-dienoate, VI (70 mg), mp 153—155°, as colorless needles from MeOH. $[\alpha]_D^{19.1}$ $-71.3°$ ($c=1.1$, $CHCl_3$). UV λ_{max}^{EtOH} mμ (log ε): 304 (4.40). IR ν_{max}^{KBr} cm^{-1}: 1740, 1735 (ester CO), 1720 (conjugated CO), 1618, 1602 (conjugated C=C), 980, 912, 840 (C=C). NMR (10% solution in $CDCl_3$) τ: 2.79 (1H, doublet, $J=15.5$ cps, 22-$\underline{H}$), 3.44 (1H, singlet, 21-$\underline{H}$), 4.38 (1H, doublet, $J=15.5$ cps, 23-$\underline{H}$), 4.92 (1H, broad peak, 3-$\underline{H}$), 6.29 (3H, singlet, 24-$COOC\underline{H}_3$), 7.95 (3H, singlet, 3-$OCOC\underline{H}_3$), 8.98 (3H, singlet, 19-$C\underline{H}_3$), 9.02 (3H, singlet, 18-$C\underline{H}_3$). *Anal.* Calcd. for $C_{27}H_{38}O_5$: C, 73.27; H, 8.65. Found: C, 73.58; H, 8.66.

14,21-Epoxy-3β-hydroxy-5β-chola-20 (21),22-dienoic Acid (VII)——(a) From III: III (55 mg) dissolved in 5 ml of 3% KOH–MeOH was heated under reflux for 18 hr, H_2O (12 ml) added, and acidified (pH 2—3) with dil. HCl. The mixture was concentrated *in vacuo* by a rotatory evaporator to one-third of the original volume. The precipitate was collected by filtration, washed with H_2O, dried, and recrystallized from acetone and MeOH to give 43 mg of 14,21-epoxy-3β-hydroxy-5β-chola-20 (21), 22-dienoic acid, VII, mp 224—226°, as white needles. $[\alpha]_D^{19.6}$ $-99.8°$ ($c=0.8$, MeOH). UV λ_{max}^{EtOH} mμ (log ε): 298 (4.04). IR ν_{max}^{KBr} cm^{-1}: 3450, 3390 (OH), 3300—3100 (broad, OH of COOH), 1700, 1683 (conjugated CO), 1605 (conjugated C=C), 1170—1150

(C–O), 978, 910, 843 (C=C). NMR (10% solution in $CDCl_3$) τ: 2.69 (1H, doublet, $J=15.6$ cps, 22-$\underline{H}$), 3.38 (1H, singlet, 21-$\underline{H}$), 4.38 (1H, doublet, $J=15.6$ cps, 23-$\underline{H}$), 5.85 (1H, broad peak, 3-$\underline{H}$), 9.00 (6H, singlet, 18- and 19-$C\underline{H}_3$). *Anal.* Calcd. for $C_{24}H_{34}O_4$: C, 74.56; H, 8.84. Found: C, 74.27; H, 8.86.

(b) From V: V (12 mg) dissolved in 1 ml of 3% KOH–MeOH was heated under reflux for 17.5 hr. On work-up procedure described in (a), the crude product obtained was recrystallized from acetone to 8 mg of a white needles, mp 223—225°, which was identified with the sample VII, which was prepared from III, by mixed mp and IR spectrum.

Methylation of VII——VII (18 mg) dissolved in ether solution of the freshly diazomethane was allowed to stand for 2 hr at room temperature, and evaporated to dryness. Recrystallization of the residue from Me_2CO gave III as colorless needles, mp 171—172°, which was identified with the sample III, which was obtained by photolysis of I, by mixed mp and IR spectrum.

Transformation of I into III and IV *via* VIII——The following is an adaption of a published procedure of Wartburg and Renz.[8] I (100 mg) dissolved in 5 ml of 0.1N abs. EtOH–KOH was allowed to stand at room temperature. After 12 hr, the mixture gave only one spot (*Rf* 0.45, solvent A) of the product, which had presumably the structure VIII (ethyl 3β,14β,21-trihydroxy-5β-chola-20(21),22-dienoate), being shown in Chart 2, on thin-layer chromatogram. Without the isolation of VIII, the mixture was acidified with dil. HCl and was set aside at room temperature for 2 hr. Then this was added H_2O and was evaporated *in vacuo* to one-third of the original volume. The precipitate was collected by filtration, was washed with H_2O, dried and recrystallization from acetone and MeOH to give 63 mg of III, mp 172—173°, as colorless needles. The compound was identified with the sample III, which was obtained by photolysis of I, by mixed mp, TLC, and IR and NMR spectra.

Ac_2O–pyridine at room temperature converted III (40 mg) into the acetate IV (32 mg), recrystallizing from MeOH as colorless needles, mp 213°. The mixed mp on admixture with the sample IV which was obtained by photolysis of II showed no depression, and IR and NMR spectra of two samples were identical.

Transformation of I into V and VI *via* IX——The following is an adaption of a published procedure.[8] I (150 mg) dissolved in 7.5 ml of 0.1N abs. MeOH–KOH was allowed to stand at room temperature. After 12.5 hr, the mixture gave only one spot (*Rf* 0.41, solvent A) of the product, which was considered probably to have the structure IX (methyl 3β,14β,21-trihydroxy-5β-chola-20(21),22-dienoate), as shown in Chart 2, on thin-layer chromatogram. On work-up procedure described above, the product was recrystallized from MeOH to give 105 mg of V, mp 212—213°, as colorless needles. The mixed mp on admixture with the sample V which was obtained by photolysis of I showed no depression, and IR and NMR spectra and TLC of two samples were identical.

By the usual means V (50 mg) was acetylated with Ac_2O (3.5 ml)–pyridine (5 ml) to give a crude acetate (47 mg), which was recrystallized from MeOH to give colorless needles (32 mg), mp 154—156°. The mixed mp on admixture with the sample V which was obtained by photolysis of II showed no depression, and IR and NMR spectra of two samples were identical.

Photolysis of Bufotalin X——After a solution of 300 mg of X in 80 ml of reagent grade MeOH was irradiated for 6.5 hr by the same way described above, the mixture was concentrated *in vacuo*. Column chromatography on 12 g of silica gel (Wakogel C-200) and elution with acetone–*n*-hexane (1:5) gave 210 mg of a white solid. Recrystallization from acetone–MeOH afforded 174 mg of methyl 16β-acetoxy-14,21-epoxy-3β-hydroxy-5β-chola-20(21),22-dienoate, VII, mp 118°, as colorless needles.

UV $\lambda_{\max}^{\mathrm{EtOH}}$ mμ (log ε): 300 (4.38). IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3500, 3400 (OH), 1735—1720 (ester and conjugated CO), 1620, 1610 (conjugated C=C), 1260, 1235 (ester C–O), 1170—1160 (C–O), 973, 910, 843 (C=C). NMR (10% solution in $CDCl_3$) τ: 2.69 (1H, doublet, $J=15.0$ cps, 22-$\underline{H}$), 3.34 (1H, singlet, 21-$\underline{H}$), 4.50 (1H, doublet, $J=15.0$ cps, 23-$\underline{H}$), 5.90 (1H, broad peak, 3-$\underline{H}$), 6.32 (3H, singlet, 24-$COOC\underline{H}_3$), 8.12 (3H, singlet, 16-$OCOC\underline{H}_3$) 9.06 (6H, singlet, 18-$C\underline{H}_3$ and 19-$C\underline{H}_3$). Mol. wt. 460. *Anal.* Calcd. for $C_{27}H_{38}O_6$: C, 70.71; H, 8.35. Found: C, 70.92; H, 8.33.

Acetylation of XII——XII (34 mg) was acetylated with Ac_2O–pyridine in the usual manner to give the acetate (XIII) (28 mg), mp 204—205°, as colorless needles from acetone, which was identified with the sample XIII, which was obtained by photolysis of XI, by mixed mp and IR spectrum.

Photolysis of Acetyl-bufotalin XI——After a solution of 250 mg of XI in 100 ml of reagent grade MeOH was irradiated for 5.5 hr by the same way described above, the mixture was concentrated *in vacuo*. Column chromatography on 11.2 g of silica gel (Wakogel C-200) and elution with acetone–*n*-hexane (1:19) gave 197 mg of a white crystaline. Recrystallization from acetone and MeOH afforded 153 mg of methyl 3β,16-diacetoxy-14,21-epoxy-5β-chola-20 (21),22-dienoate, XIII, mp 204—205.5°, as colorless needles. UV $\lambda_{\max}^{\mathrm{EtOH}}$ mμ (log ε): 295 (4.27). IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 1735 (ester CO), 1715 (conjugated CO), 1616, 1608 (conjugated C=C), 1250, 1236, 1220 (ester C–O), 1160 (broad, C–O), 986, 905, 842 (C=C). NMR (10% solution in $CDCl_3$) τ: 2.67 (1H, doublet, $J=15.3$ cps, 22-$\underline{H}$), 3.29 (1H, singlet, 21-$\underline{H}$), 4.46 (1H, doublet, 23-$\underline{H}$), 4.93 (1H, broad peak, 3-$\underline{H}$), 6.28 (3H, singlet, 24-$COOC\underline{H}_3$), 7.96 (3H, singlet, 3-$OCOC\underline{H}_3$), 8.08 (3H, singlet, 16-$OCOC\underline{H}_3$), 8.99 (6H, singlet, 18-$C\underline{H}_3$ and 19-$C\underline{H}_3$). *Anal.* Calcd. for $C_{29}H_{40}O_7$: C, 69.57; H, 8.05. Found: C, 69.45; H, 8.17.

Transformation of X into XIII *via* XIV and XV——X (399.5 mg) dissolved in 20 ml of 0.1N abs. MeOH–KOH was allowed to stand at room temperature. After 5 hr, the mixture was observed the main spot of the product (XIV) (*Rf* 0.32, solvent A), which is considered assumedly to have the structure XIV (methyl 3β,14β,16β,21-tetrahydroxy-5β-chola-20 (21),22-dienoate) in Chart 3, on thin–layer chromatogram. Then it was acidified (pH 3) with 10% HCl aq. and was set aside at room temperature for 1 hr. The mixture was added 40 ml of H_2O and was concentrated *in vacuo* to one–third of the volume. The white precipitate was collected by filtration, was washed with H_2O, and dried to yield 362 mg of a white powdery, which showed the main spot (*Rf* 0.58, solvent A) on thin–layer chromatogram. This was assumed to have the structure XV (methyl 14,21-epoxy-3β,16β-dihydroxy-5β-chola-20 (21),22-dienoate). Without purification, by the usual means the product (XV) was acetylated in pyridine (8 ml) and Ac_2O (5.6 ml) to yield a crude acetate (350 mg), which was chromatographed on silica gel (Wakogel C-200, 12 g). Elution with acetone–*n*-hexane (1:9) and recrystallization of the eluate from acetone and MeOH gave XIII (165 mg) as colorless needles, mp 204—206°. The mixed mp on admixture with the sample XIII which was obtained by photolysis of XI showed no depression, and IR and NMR spectra and TLC of two samples were identical.

Photolysis of Gamabufotalin XVI——A solution of 75 mg of XVI in 75 ml of reagent grade MeOH was irradiated under the same conditions described above. After 6 hr, a spot (*Rf* 0.22, solvent A) of the main product (XVIII) was observed on thin–layer chromatogram. Then the solvent was evaporated *in vacuo* by a rotatory evaporator. The residue was chromatographed on silica gel (Wakogel C-200, 1.4 g) eluting with acetone–*n*-hexane mixture with increasing amounts of *n*-hexane to give 34 mg of methyl 14,21-epoxy-3β,11α-dihydroxy-5β-chola-20 (21),22-dienoate, XVIII, mp 118—119°, and colorless small needles from acetone and MeOH. UV $\lambda_{\max}^{\mathrm{MeOH}}$ mμ (log ε): 304 (4.26). IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3400 (OH, broad), 1700 (conjugated CO), 1620, 1610 (conjugated C=C), 1190—1160 (C–O, broad), 985, 910, 845 (C=C). NMR (10% solution in $CDCl_3$) τ: 2.81 (1H, doublet, $J=16.2$ cps, 22-$\underline{\mathrm{H}}$), 3.47 (1H, singlet, 21-$\underline{\mathrm{H}}$), 4.41 (1H, doublet, $J=16.2$ cps, 23-$\underline{\mathrm{H}}$), 5.88 (1H, broad peak, 3-$\underline{\mathrm{H}}$), 6.30 (3H, singlet, 24-$\mathrm{COOC}\underline{\mathrm{H}}_3$), 8.88 (3H, singlet, 19-$\mathrm{C}\underline{\mathrm{H}}_3$), 9.00 (3H, singlet, 18-$\mathrm{C}\underline{\mathrm{H}}_3$). Mol. wt. 414. *Anal.* Calcd. for $C_{35}H_{36}O_5$: C, 72.08; H, 8.71. Found: 72.35; H, 8.68.

Acetylation of XVIII——XVIII (10 mg) was acetylated with Ac_2O (0.15 ml)–pyridine (0.22 ml) under the usual manner. Work-up procedure described above afforded a white solid, which was recrystallized from acetone to yield the acetate (XIX) (7 mg), mp 199—202°, as colorless small needles. The mixed mp on admixture with the sample XIX which was obtained by photolysis of XVII showed no depression, and IR spectra of two samples were identical.

Photolysis of Acetyl-gamabufotalin XVII——XVII (210 mg) of in 100 ml of reagent grade MeOH was irradiated under an atmosphere of dry nitrogen at 20—25° by the same conditions described above. After 6 hr, no XVII remained unchanged. The solvent was concentrated *in vacuo.* The residue (205 mg) was chromatographed on silica gel (Wakogel C-200, 7.5 g) eluting with acetone–*n*-hexane (1:14) to yield 134 mg of methyl 3β,11α-diacetoxy-14,21-epoxy-5β-chola-20 (21),22-dienoate, XIX, mp 202—203°, as colorless needles from acetone. UV $\lambda_{\max}^{\mathrm{MeOH}}$ mμ (log ε): 304 (4.19). IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 1740, 1730 (ester CO), 1710 (conjugated CO), 1620, 1610 (conjugated C=C), 1280—1220 (ester C–O), 1170—1160 (C–O), 985, 913, 842 (C=C). NMR (10% solution in $CDCl_3$) τ: 2.81 (1H, doublet $J=16.0$ cps, 22-$\underline{\mathrm{H}}$), 3.47 (1H, singlet, 21-$\underline{\mathrm{H}}$), (1H, doublet, $J=16.0$ cps, 23-$\underline{\mathrm{H}}$), 4.89 (1H, broad peak, 3-$\underline{\mathrm{H}}$), 6.29 (3H, singiet, 24-$\mathrm{COOC}\underline{\mathrm{H}}_3$), 7.94 (3H, singlet, 3-$\mathrm{OCOC}\underline{\mathrm{H}}_3$), 8.04 (3H, singlet, 11-$\mathrm{OCOC}\underline{\mathrm{H}}_3$), 8.90 (3H, singlet, 19-$\mathrm{C}\underline{\mathrm{H}}_3$), 8.95 (3H, singlet, 18-$\mathrm{C}\underline{\mathrm{H}}_3$). *Anal.* Calcd. for $C_{29}H_{40}O_7$: C, 69.57; H, 8.05. Found: C, 69.43; H, 8.27.

Transformation of XVI into XVIII and XIX *via* XX——XVI (100 mg) dissobved in 5 ml of 0.1N abs. MeOH–KOH was allowed to stand at room temperature. After 5 hr, the mixture gave one spot (*Rf* 0.14, solvent A) of the product (XX), which was considered probably to have the structure XX (methyl 3β,11α,14β,21-tetrahydroxy-5β-chola-20 (21),22-dienoate) in Chart 4, on thin–layer chromatogram. Then it was acidified (pH 2.0—2.5) with 10% HCl aq. and was set aside at room temperature overnight. The mixture was poured into H_2O and the precipitate was collected by filtration, was washed with H_2O, and dried. Recrystallization from acetone gave 65 mg of XVIII, mp 119—120°, as colorless needles. The mixture mp on admixture with the sample XVIII which was obtained by photolysis of XVI showed no depression, and IR and NMR spectra and TLC of two samples were identical.

By the usual means, XVIII (50 mg) was acetylated in pyridine (5 ml) and Ac_2O (3.5 ml) to yield a crude acetate (48 mg). It was recrystallized from acetone to give XIX (37 mg), mp 203—204°, as colorless needles. The mixed mp on admixture with the sample XIX which was obtained by photolysis of XVII showed no depression, and IR and NMR spectra and NMR of two samples were identical.

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