

PHOTOCHEMISTRY OF BUFADIENOLIDE: OCCURRENCE OF THREE TAUTOMERS IN THE PHOTOLYSIS OF RESIBUFOGENIN

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Abstract - The photolysis of resibufogenin (**1**) in MeOH was found to give a fairly labile mixture of three tautomers (**2a-c**) by the ¹H nmr spectral analysis including difference NOE experiments.

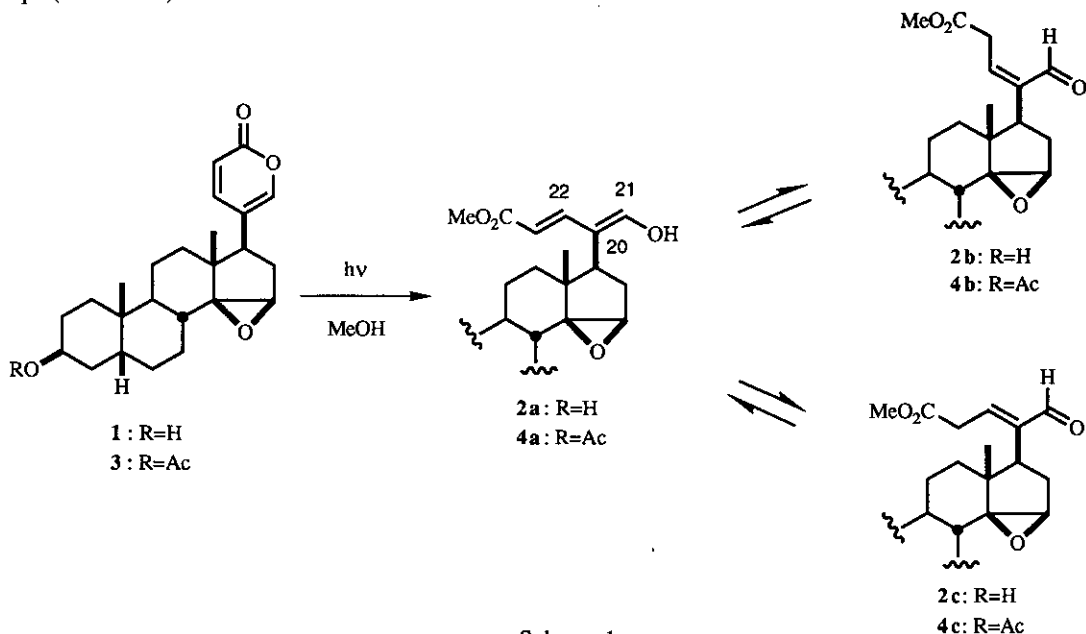
Much attention on the photochemistry of α -pyrone ring system led to the observation of photolytic methanolysis,² photo-dimerization,³ and intramolecular photo-cycloaddition of α -pyrone.⁴ Although the photolysis of naturally occurring bufadienolides linking α -pyrone moiety to steroid skeleton was also investigated,⁵ a genuine photo-product has never isolated because of lability and two mixtures of enol methyl ethers were characterized after acidic treatment of a reaction mixture in the case of the photolysis of resibufogenin (**1**).⁶ In a previous paper, we reported photochemical transformation of the bufadienolide glycoside, proscillaridin, leading to 14 β , 21-epoxy-21-methoxychole-4, 20(22)-diene skeleton.⁷ Thus, we undertook the photolysis of resibufogenin (**1**) to obtain the genuine photo-product and to reveal the photoreaction pathway of α -pyrone ring in the course of our chemical studies on bufadienolide.⁸ In this paper, we describe occurrence of the three tautomers (**2a-c**) in the photolysis of resibufogenin (**1**).

RESULT AND DISCUSSION

A solution of resibufogenin (**1**) in MeOH was irradiated in the pyrex vessel with a high-pressure mercury arc lamp (400 W) at room temperature under nitrogen atmosphere to afford a photo-product showing one major spot on thin layer chromatographic analysis. The photo-product was very labile and submitted to silica gel column chromatography to give a complex mixture of secondary modified compounds. Separation of the crude-product was, therefore, carried out by ODS reversed-phase hplc to give three peaks in a chromatogram without silica gel column chromatography. The ¹H nmr spectrum of each peak in C₆D₆ (benzene-d₆) was fairly similar one another indicating the presence of three pairs of signals.⁹ Furthermore, the hplc analysis of the ingredient corresponding to each peak showed three peaks again in nearly same proportions before hplc separation, even if the hplc analysis was carried out just after the first hplc separation. On the basis of these findings, the photo-adduct of **1** was presumed to be the mixture of three tautomers. The presumption was confirmed by detailed analysis of integration curve of the ¹H nmr spectrum in which three tautomers were shown in a ratio of 36 : 20 : 44 (Scheme 1).

The photo-product from resibufogenin (**1**) proved to include compounds which have the molecular formula of C₂₅H₃₆O₅ from electron ionization mass spectrometry (EI-ms) and high-resolution EI-ms spectrum.

Additionally, infrared (ir) spectrum of the product showed absorptions due to hydroxyl (3439 cm^{-1}), ester-carbonyl (1740 cm^{-1}), conjugated ester-carbonyl (1715 cm^{-1}), conjugated formyl ($1685, 1680\text{ cm}^{-1}$), and enol groups (1597 cm^{-1}).



Scheme 1

With regard to tautomer A (**2a**), the ^1H proton signal was observed at 10.7 ppm (d, $J=13.0\text{ Hz}$) coupling to the signal at 6.61 ppm (d, $J=13.0\text{ Hz}$). On treatment with D_2O , the former signal disappeared and the latter one was displaced to singlet signal indicative of presence of trisubstituted enol moiety in **2a**. Additionally, the ^1H nmr spectrum of **2a** exhibited the signal due to three olefinic protons (δ 7.56, d, $J=15.4\text{ Hz}$; δ 6.61, d, $J=13.0\text{ Hz}$; δ 5.76, d, $J=15.4\text{ Hz}$), methoxyl protons (δ 3.58), and two tertiary methyl protons.¹⁰ Taking the chemical shifts of olefinic protons and the coupling constants into consideration, a *trans*-olefin conjugated carbomethoxyl group was constructed. NOE enhancements between 21-H and 22-H, 17-H and 23-H indicated the *Z*-configuration of C20-C21 double bond and *E*-configuration of C22-C23 double bond (Figure 1). The structure of tautomer (**2a**) was, therefore, elucidated to be methyl 20(*Z*), 22(*E*)-3 β , 21-dihydroxy-14 β , 15 β -epoxy-5 β -chola-20, 22-dienoate.

Tautomer B (**2b**) had the signals ascribable to a formyl proton (δ 9.86, s, 21-H) and an olefinic proton (δ 7.08, dd, $J=7.7, 8.1\text{ Hz}$, 22-H) in the ^1H nmr spectrum. Furthermore, the signal due to methylene protons (δ 3.12, d, $J=8.1\text{ Hz}$; δ 3.11, d, $J=7.7\text{ Hz}$, 23-H)¹¹ located between an enal and a carbomethoxyl moieties was observed as well as one methoxyl proton signal (δ 3.26). In the difference NOE experiments, irradiation of the formyl, (21-H) and the methylene proton (23-H) caused NOE enhancements to each other. These observations defined the geometry of C20-C22 olefin as *Z*. Thus, the tautomer B (**2b**) was established as methyl 20(*Z*)-14 β , 15 β -epoxy-3 β -hydroxy-21-oxo-5 β -chola-20(22)-enoate.

Tautomer C (**2c**) had enal and carbomethoxyl functions by analysis of the ^1H nmr spectrum like tautomer B (**2b**): one formyl (δ 9.19, br s), one olefin (δ 6.53, br s), and one methoxyl proton (δ 3.38) signals appeared. This spectral feature suggested that tautomer C was geometric isomer of **2b**. Observation of NOE between the formyl

proton (21-H) and the olefinic proton (22-H) revealed the *E*-configuration of C20-C22. Consequently, the tautomer (2c) proved to be methyl 20(*E*)-14 β , 15 β -epoxy-3 β -hydroxy-21-oxo-5 β -chola-20(22)-enoate.

(Figure 1)

Similarly, the photolysis of 3-*O*-acetylresibufogenin (3) afforded a mixture of three tautomers (4a-c) in a ratio of 33 : 39 : 29 in 72 % yield.

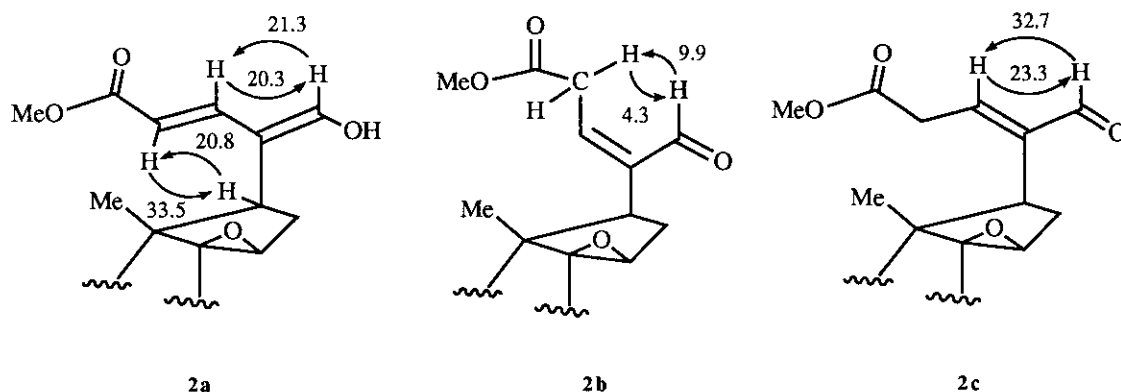
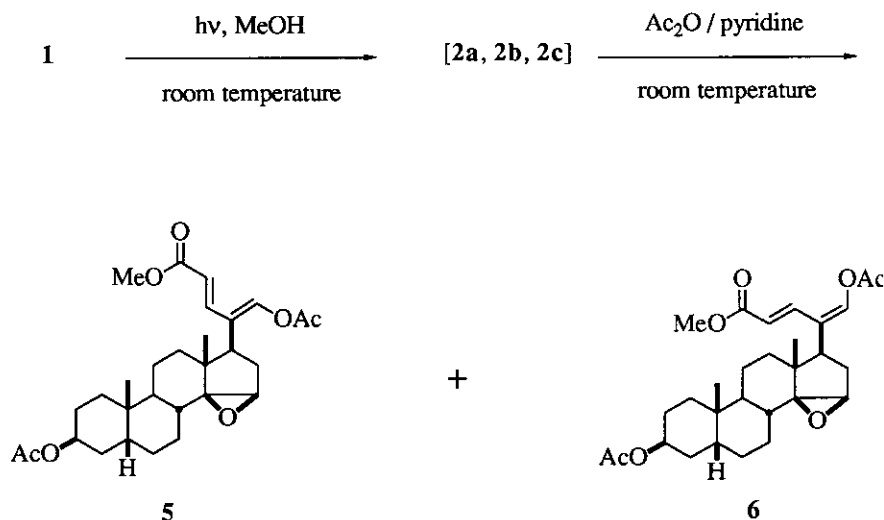


Figure 1 NOEs (%) observed in NOE difference spectra of 2a-c

Finally, photolytic methanolysis of **1** followed by acetylation was performed to confirm the structures of three tautomers chemically. After irradiation of resibufogenin (**1**) in MeOH with pyrex vessel, the product was treated with acetic anhydride in pyridine. Purification using hplc afforded two acetates (**5** and **6**) in 28 % and 20 % yields respectively. (Scheme 2)



Scheme 2

Both products were obtained as stable crystalline powder and the molecular formula were determine to be C₂₉H₄₀O₇ by elemental analyses. The ¹H nmr spectra of **5** and **6** showed the presence of dienol acetate moiety

conjugated with a carbomethoxyl group. As for diene portion, one was a trisubstituted olefin and the other was a disubstituted *E*-olefin. However, these were a little difference in olefinic proton signals between **5** and **6**. Thus, the compounds (**5** and **6**) were assumed to be geometrical isomers of the diene moiety. In the case of **5**, observation of NOEs in two pairs of protons, two olefin protons (21-H and 23-H), the methine proton (17-H) and the olefin proton (22-H), suggested existence of the 20(*Z*), 22(*E*)-diene moiety (Figure 2). Thus, the compound (**5**) was established as methyl 20(*Z*), 22(*E*)-3 β , 21-diacetoxy-14 β , 15 β -epoxy-5 β -chola-20, 22-dienoate.

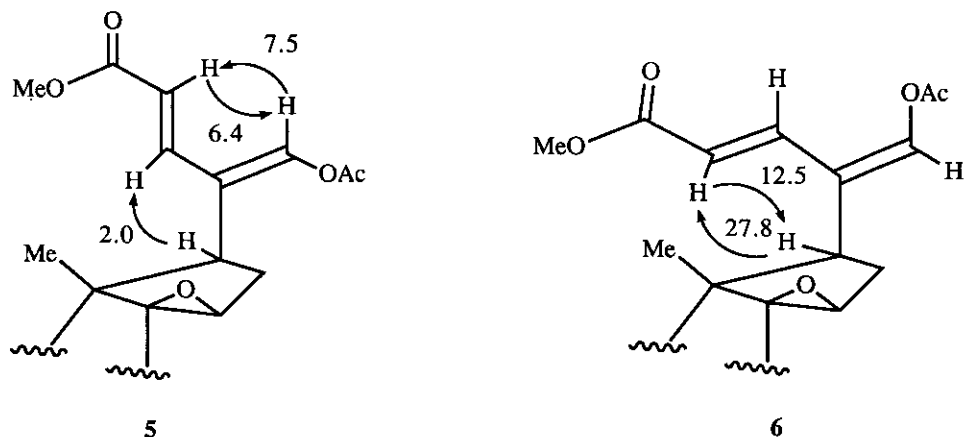


Figure 2 NOEs (%) observed in NOE difference spectra of **5** and **6**.

On the other hand, NOE enhancement between 17-H and 23-H of **6** indicated *E*-configured C22-C23 double bond. The geometry of C20-C21 double bond was determined as follows. Previously, H. H. Wasserman *et al.* reported that a *cis*-allylic proton to a acetoxyl group in an enol acetate resonated in a lower field than that of *trans*-allylic proton because of the steric effect of acetoxyl group.¹² The proton signal due to the methine proton on C17 appeared at 3.05 ppm in the case of **5**, while the ¹H nmr spectrum of **6** exhibited the C17 methine proton signal at 2.62 ppm. This finding indicated *E*-configuration of double bond located between C20 and C21. In addition, no observation of NOE enhancement between 21-H and 22-H strongly supported the *E*-configuration. Consequently, the structure of **6** was elucidated to be methyl 20(*E*), 22(*E*)-3 β , 21-diacetoxy-14 β , 15 β -epoxy-5 β -chola-20, 22-dienoate.

In conclusion, we physicochemically proved occurrence of three tautomers (**2a-c**) in the photolytic methanolysis of resibufogenin (**1**), the naturally occurring bufadienolide with epoxy ring between C14 and C15. It should be noted that the products in the photolytic methanolysis of α -pyrone ring system were physicochemically characterized for the first time.

EXPERIMENTAL

All melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. The ultraviolet (uv) spectra were recorded with a Shimadzu UV-2100 spectrophotometer, and the infrared (ir) spectra with a JASCO IR-810 and a Shimadzu FTIR-8100 spectrophotometers. The ¹H nuclear magnetic resonance (nmr) spectra were measured with JEOL GSX-400 and EX-270 spectrometers using tetramethylsilane as an

internal standard. The following abbreviations are used; s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Optical rotations were measured on a JASCO DIP-140 digital polarimeter. The electron ionization mass spectra (EI-ms) and high-resolution EI-ms spectra were measured with a JEOL DX-300 mass spectrometer. High-performance liquid chromatography (hplc) was performed using a JASCO 880-PU pump, and a Shodex RI, SE-11 differential refractometer. Thin-layer chromatography (tlc) was carried out on Merck precoated Kieselgel 60F254, and spots were detected by illumination with an ultraviolet lamp, or spraying 1 % Ce(SO₄)₂-10 % H₂SO₄ followed by heating. Column chromatography was performed on Silica gel BW-200 (Fuji Davison Chemicals Co., Ltd.). High-pressure mercury arc lamp (400 W) was employed from Shigemi standard Co., Ltd., AHH 400 S.

Photolysis of resibufogenin (1)

A solution of resibufogenin (1) (50 mg, 0.13 mmol) in MeOH (5 ml) was irradiated with pyrex vessel with high-pressure mercury arc lamp (400 W) at room temperature (24°C) for 4 h. The reaction mixture was poured into saturated aqueous NaCl and extracted with a EtOAc, and the extract was washed with saturated aqueous NaCl, then dried over anhyd. Na₂SO₄. After removal of the solvent *in vacuo*, the residue was purified by hplc (Develosil ODS-5, 10 mm i. d. x 250 mm, MeOH:H₂O= 3:1) to furnish the tautomers (**2a-c**, 26 mg, 48 %) in a ratio of 36:20:40.

EI-ms *m/z* (%): 416 (*M*⁺, 50), 398 (*M*⁺-H₂O, 63), 380 (*M*⁺-2H₂O, 20), 366 (*M*⁺-H₂O-MeOH, 27), 166 (100). High-resolution EI-ms: Calcd for C₂₅H₃₆O₅: 416.256. Found: 416.258. Ir $\nu_{\max}^{\text{film}}$ cm⁻¹: 1740 (CO₂Me, **2b** and **2c**), 1715 (CO₂Me, **2a**), 1686 (CHO, **2b**), 1680 (CHO, **2c**), 1597 (21-OH, **2a**). ¹H Nmr (400 MHz, C₆D₆, δ): **2a**; 0.78, 0.79, 0.80, 0.83, 0.84, 0.88 (all s, **2a-c**-10-CH₃, 13-CH₃), 2.50 (dd, *J*=2.2, 11.3 Hz, 17-H), 3.15 (s, **2a-c**-15-H), 3.58 (s, 24-OCH₃), 3.82 (s, **2a-c**-3-H), 5.76 (d, *J*=15.4 Hz, 23-H), 6.61 (d, *J*=13.0 Hz, 21-H), 7.56 (d, *J*=15.4 Hz, 22-H), 10.74 (d, *J*=13.0 Hz, 21-OH); **2b**, 3.11 (d, *J*=7.7 Hz, 23-H), 3.12 (d, *J*=8.1 Hz, 23-H), 3.26 (s, 24-OCH₃), 7.08 (dd, *J*=7.7, 8.1 Hz, 22-H), 9.86 (s, 21-H); **2c**, 3.16 (s, 23-H₂), 3.38 (s, 24-OCH₃), 6.53 (br s, 22-H), 9.19 (br s, 21-H).

Photolysis of 3-O-acetylresibufogenin (3)

A solution of 3-O-acetylresibufogenin (**3**) (20 mg, 0.047 mmol) in MeOH (2 ml) was irradiated with high-pressure mercury arc lamp (400 W) at room temperature (24°C) for 4 h. Work up of the reaction mixture was carried out as described above. The crude product was separated by hplc (Develosil ODS-5, MeOH:H₂O=85:15) to furnish the mixture of tautomers (**4a-c**, 11 mg, 50 %) in a ratio of 36 : 20 : 44.

EI-ms *m/z* (%): 458 (*M*⁺, 31), 440 (*M*⁺-H₂O, 24), 398 (*M*⁺-AcOH, 21), 215 (100). High-resolution EI-ms: Calcd for C₂₇H₃₈O₆: 458.267. Found: 458.265. Ir $\nu_{\max}^{\text{film}}$ cm⁻¹: 1732 (OAc, **4a-c**, CO₂Me, **4b** and **4c**), 1700 (sh, CO₂Me, **4a**), 1680 (sh, CHO, **4b** and **4c**), 1597 (21-OH, **4a**). ¹H Nmr (400 MHz, C₆D₆, δ): **4a**; 0.75, 0.78, 0.87, 0.89, 0.90 (all s, **4a-c**-10-CH₃, 13-CH₃), 1.74, 1.74, 1.75 (all s, **4a-c**-OCOCH₃), 2.52 (dd, *J*=2.2, 11.5 Hz, 17-H), 3.16 (s, **4a-c**-15-H), 3.58 (s, 24-OCH₃), 5.15 (s, **4a-c**-3-H), 5.57 (d, *J*=15.4 Hz, 23-H), 6.61 (d, *J*=13.0 Hz, 21-H), 7.57 (d, *J*=15.4 Hz, 22-H); **4b**, 3.11 (d, *J*=7.7 Hz, 23-H), 3.12 (d, *J*=8.1 Hz, 23-H), 3.26 (s, 24-OCH₃), 7.08 (dd, *J*=7.7, 8.1 Hz, 22-H), 9.86 (s, 21-H), 10.7 (d, *J*=13.0 Hz, 22-H); **4c**, 3.17 (s, 23-H₂), 3.34 (s, 24-OCH₃), 6.53 (br s, 22-H), 9.20 (br s, 21-H).

Acetylation of photo-products of 1

Resibufogenin (1) (50 mg, 0.13 mmol) in MeOH (5 ml) was irradiated followed by worked up in the same manner. Ac₂O (1 ml, 10.6 mmol) was added dropwise to an ice-cooled solution of the photo-products in pyridine (2 ml, 25.9 mmol). The mixture was allowed to stand at room temperature for 7 h, then poured into ice water and extracted with EtOAc. The extract was successively washed with 5 % HCl, saturated aqueous NaHCO₃, saturated aqueous NaCl, then dried over MgSO₄. Removal of the solvent under reduced pressure followed by purification by hplc (Develosil ODS-5, MeOH:H₂O=85:15) furnished two products **5** (18 mg, 28 %) and **6** (13 mg, 20 %).

5: a colorless crystalline powder. mp 84-86°C (acetone-isoPr₂O). $[\alpha]_D^{25}$ -4.8° (c=0.3, MeOH). Ir $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 1725 (CO). Uv $\lambda_{\max}^{\text{MeOH}}$ nm (ε): 276.3 (10000). ¹H Nmr (270 MHz, CDCl₃, δ): 0.86 (3H, s, 13-CH₃), 0.99 (3H, s, 10-CH₃), 2.06, 2.17 (each 3H, both s, OCOCH₃ x 2), 3.05 (1H, dd, J=1.8, 9.7 Hz, 17-H), 3.50 (1H, s, 15-H), 3.74 (3H, s, 24-OCH₃), 5.09 (1H, s, 3-H), 6.04 (1H, d, J=15.8 Hz, 23-H), 7.48 (1H, d, J=15.8 Hz, 22-H), 7.49 (1H, s, 21-H). Anal. Calcd for C₂₉H₄₀O₇; C, 69.56; H, 8.06. Found: C, 69.61; H, 8.04.

6: a colorless crystalline powder. mp 78-80°C (acetone-isoPr₂O). $[\alpha]_D^{25}$ -7.4° (c=0.3, MeOH). Ir $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 1730, 1720 (CO). Uv $\lambda_{\max}^{\text{MeOH}}$ nm (ε): 282.4 (19000). ¹H Nmr (270 MHz, CDCl₃, δ): 0.89 (3H, s, 13-CH₃), 0.98 (3H, s, 10-CH₃), 2.03, 2.22 (each 3H, both s, OCOCH₃ x 2), 2.62 (1H, d, J=9.7 Hz, 17-H), 3.50 (1H, s, 15-H), 3.77 (3H, s, 24-OCH₃), 5.03 (1H, s, 3-H), 5.81 (1H, d, J=15.9 Hz, 23-H), 7.64 (1H, s, 21-H), 7.89 (1H, d, J=15.9 Hz, 22-H). Anal. Calcd for C₂₉H₄₀O₇; C, 69.56; H, 8.06. Found: C, 69.58; H, 8.05.

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