Tetrahedron Vol. 45, No. 9, pp. 2551 to 2556, 1989
Printed in Great Britain.

0040-4020/89 $3.00+.00

PTAQUILOSIN, THE AGLYCONE OF A BRACKEN CARCINOGEN PTAQUILOSIDE: CHEMICAL DERIVATION FROM PTAQUILOSIDE AND THE REACTIVITY

HIDEO KIGOSHI, AKIHIKO SAWADA, YOSHIFUMI IMAMURA, HARUKI NIWA, and KIYOYUKI YAMADA*

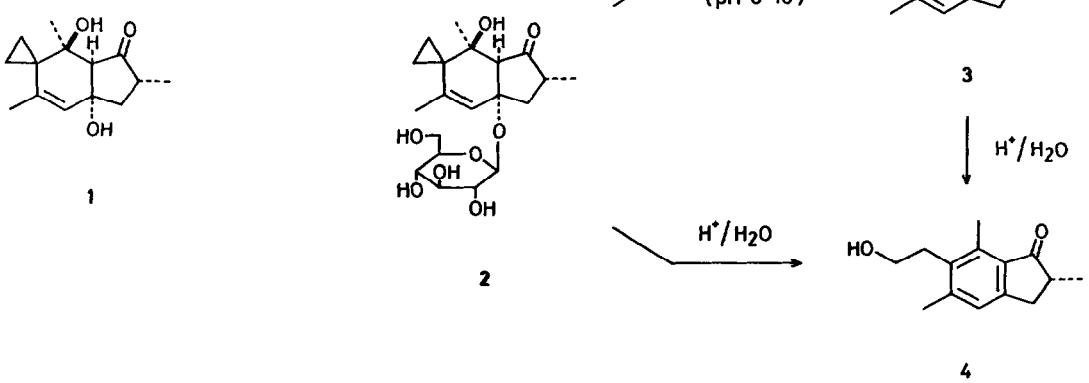
Department of Chemistry, Faculty of Science, Nagoya University, Chikusa, Nagoya 464 Japan

(*Received in Japan* 9 *January* 1989)

Abstract - From a bracken carcinogen ptaquiloside (**2**), the aglycone ptaquilosin (**1**) has been prepared by a series of chemical reactions. Action of weak base on ptaquilosin (**1**) caused dehydration to generate the highly reactive dienone (**3**) the ultimate form of **2**.

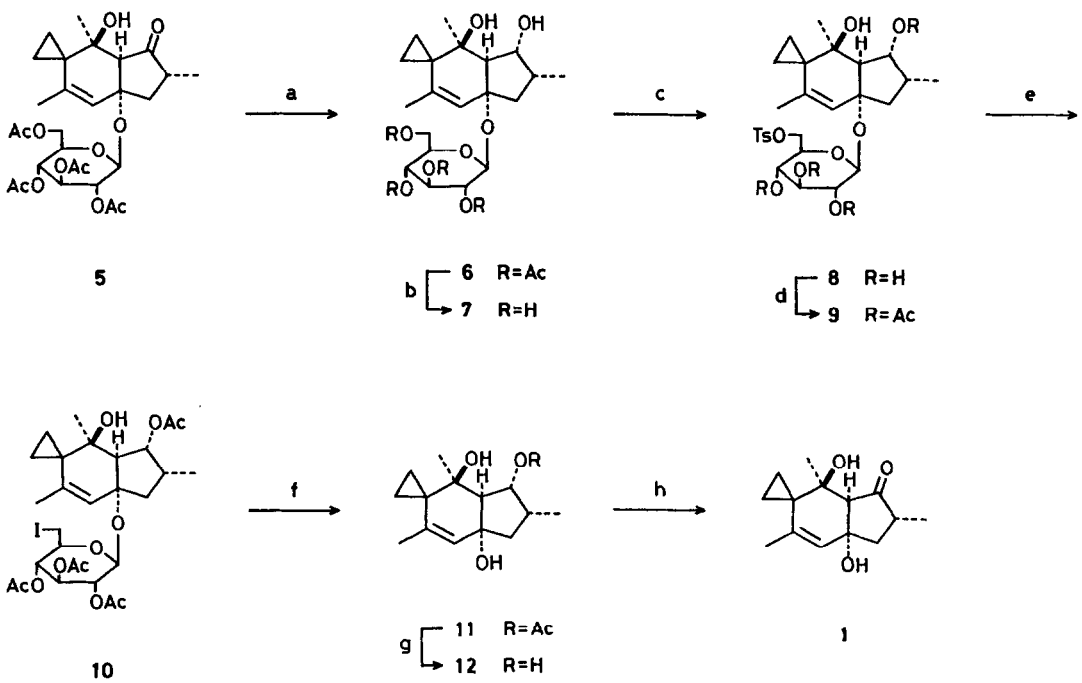
Bracken fern (Pteridium aquilinum) is widely distributed throughout the world and its lethal properties to cattles were first reported in the late 19th century.[1] Cattles which consumed bracken fern exhibited the syndrome known as "cattle bracken poisoning", the features of which are the generalized hemorrhage, anorexia, extensive intestine damage, ulceration, and pyrexia.[1] In connection with the studies on "cattle bracken poisoning" the carcinogenicity of bracken fern was discovered in 1960.[1] In search for the carcinogenic

Scheme I



principle of bracken fern a great deal of chemical work on bracken fern has been carried out:[1,2] we isolated a carcinogen ptaquiloside (**2**) from bracken fern in 1983, elucidated the structure,[3] and proved its carcinogenicity.[4] Ptaquiloside (**2**) is unstable in both acidic and basic solutions at room temperature, and is converted to 1-indanone compounds such as pterosin B (**4**)[3a,3d] (Scheme I). Under weakly basic conditions ptaquiloside (**2**) is readily transformed with liberation of D-(+)-glucose into the dienone (**3**),[3a,3d] which is the active form of **2** and is capable of cleaving DNA base-specifically.[5] The dienone (**3**) is instantly converted to **4** in the weakly acidic solution.[3a,3d] Attempts were made to prepare ptaquilosin (**1**), the aglycone of ptaquiloside (**2**) by enzymatic hydrolysis of **2** for the purpose of obtaining the information on the reactivity of **1**. However, under the conditions of enzymatic hydrolysis (emulsin, citrate buffer (pH 5.0), room temperature, 21 h) aromatization of **2** took place prior to the hydrolysis of the glycosidic linkage to afford pterosin B (**4**) exclusively, and ptaquilosin (**1**) was not obtained at all.

Scheme II[a]

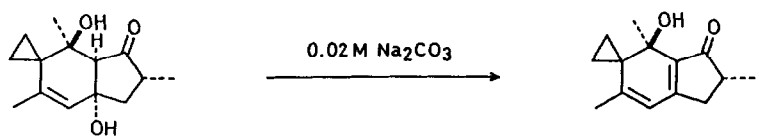


[a](a) $NaBH_4$, THF/EtOH(1:1); (b) KOH, MeOH; (c)(i) Bu_2SnO, MeOH (ii) TsCl, dioxane; (d) Ac_2O, pyridine; (e) KI, DMF; (f) Zn, NH_4Cl, EtOH; (g) K_2CO_3, MeOH; (h) PCC, CH_2Cl_2.

Thus, we have attempted to prepare **1** from **2** by chemical means, and actually have developed a new, mild method for the removal of the glucose moiety of **2** to produce the aglycone **1**. The present method will generally be applicable to the cleavage of the glycosidic bond of glucosides which are labile under acidic conditions.

Ptaquiloside tetraacetate (**5**)[3a,3d] was reduced with $NaBH_4$ to afford the diol **6**[6] (78%) (Scheme II). Deacetylation of **6** under alkaline conditions gave the hexaol **7**, which was selectively tosylated at the 6'-position of the glucose moiety according to the Tsuda's method[7] to provide the tosylate **8** (81% overall). Acetylation of **8** yielded the tetraacetate **9**, which on reaction with KI afforded the iodide **10** (85% overall). The cleavage of the glycosidic linkage of **10** was effected by reducing the iodide **10** with Zn (NH_4Cl, EtOH, reflux) to give the diol **11** (68%). The acetate group in **11** was hydrolyzed to afford the triol **12** (92%), which was subsequently oxidized with PCC to provide ptaquilosin (**1**) (48%). Thus, ptaquilosin (**1**) was prepared from ptaquiloside (**2**) in 9 steps in 16% overall yield.

The reactivity of **1** was shown to be similar to that of **2**. On treatment with base (0.02 M Na_2CO_3, room temperature, 30 min) ptaquilosin (**1**) was converted to the dienone (**3**). This finding is significant, because ptaquilosin (**1**) can be a precursor of the highly reactive dienone (**3**), the ultimate form of **2**.



1 **3**

Experimental

Melting points are uncorrected. The UV spectrum was taken on a JASCO UVDEC-510 spectrophotometer. IR spectra were obtained with a JASCO IR-810 spectrophotometer. ^1H NMR spectra were recorded on a JEOL JNM-C675 (270 MHz) instrument: chemical shifts (δ) are reported in ppm downfield from internal tetramethylsilane; coupling constants are reported in herz. The low resolution mass spectra (EIMS and FDMS) and high resolution mass spectra (HREIMS) were measured on a JEOL JMS-LG2000 instrument. Optical rotations were measured on a JASCO DIP-181 polarimeter. Fuji-Davison silica gel BW-820 MH was used for column chromatography. Merck precoated silica gel 60 F_{254} plates, 0.25 mm thickness, were used for thin layer chromatography (TLC). Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl under nitrogen. N,N-Dimethylformamide (DMF) was distilled from CaH_2 under reduced pressure. Dioxane and dichloromethane were distilled from CaH_2 under nitrogen. Methanol and ethanol were distilled from the corresponding magnesium alkoxide, respectively. Organic solutions were washed with saturated NaCl solution, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure by a rotary evaporator.

Diol **6**. To a solution of **5** (103 mg, 0.182 mmol) in THF (2 ml) and EtOH (2 ml) was added $NaBH_4$ (11.4 mg, 0.302 mmol) at 0 °C. The mixture was stirred at room temperature for 1 h and diluted with 0.4 M phosphate buffer solution (pH 7.0, 5 ml), and the stirring was continued for 10 min. The mixture was extracted with EtOAc (4 x 5 ml). The combined

extracts were washed with saturated NaCl solution, dried, and concentrated. The residue was purified by column chromatography on silica gel (3 g) with 2:1 hexane-EtOAc to afford **6** (80.6 mg, 78%) as a colorless amorphous powder: $[\alpha]_D^{25}$ -50.3° (c 1.12, $CHCl_3$); IR ($CHCl_3$) 3450 (broad), 1755, 1650, 1220 (broad), 1110, 1040 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.78 (1H, m), 0.83 (1H, m), 1.00 (1H, m), 1.04 (3H, d, J = 5.9 Hz), 1.09 (1H, m), 1.21 (3H, s), 1.54 (3H, d, J = 1.0 Hz), 1.57-1.69 (3H, m), 1.81 (1H, m), 1.96 (3H, s), 2.00 (3H, s), 2.03 (3H, s), 2.09 (3H, s), 2.57 (1H, dd, J = 10.3, 1.3 Hz), 3.70 (1H, ddd, J = 9.9, 5.7, 2.9 Hz), 3.84 (1H, br s), 3.97 (1H, br dd, J = 10.3, 8.4 Hz), 4.15 (1H, dd, J = 12.0, 2.9 Hz), 4.24 (1H, dd, J = 12.0, 5.7 Hz), 4.79 (1H, d, J = 7.9 Hz), 4.98 (1H, dd, J = 9.6, 7.9 Hz), 5.02 (1H, dd, J = 9.9, 9.6 Hz), 5.21 (1H, dd, J = 9.6, 9.6 Hz), 5.48 (1H, br s); FDMS m/z (rel intensity) 568 (M^+; 100).

Hexaol **7**. A mixture of **6** (32.5 mg, 0.057 mmol) in a methanolic 0.2 M KOH solution (1.5 ml, 0.3 mmol) was stirred at room temperature for 1 h, neutralized by adding Amberlite IRC-50 (acid form, 0.3 g), then stirred for 10 min, and passed through a column of Amberlite IRC-50 (acid form, 0.3 g) with MeOH. The column was washed thoroughly with MeOH. The filtrate and washings were combined and concentrated. The residue was purified by column chromatography on silica gel (1.2 g) with 10:1 EtOAc-MeOH to give **7** (20.9 mg, 92%) as a colorless amorphous powder: $[\alpha]_D^{24}$ -83.2° (c 1.17, MeOH); IR (KBr) 3400 (broad), 1650, 1075 cm^{-1}; ^1H NMR (270 MHz, CD_3OD) δ 0.55-0.72 (2H, m), 0.91 (1H, m), 0.98 (3H, d, J = 5.9 Hz), 1.14 (1H, m), 1.30 (3H, s), 1.52 (3H, d, J = 1.0 Hz), 1.60 (1H, dd, J = 12.2, 10.5 Hz), 1.63-1.76 (1H, m), 1.83 (1H, dd, J = 10.5, 4.1 Hz), 2.67 (1H, d, J = 10.5 Hz), 3.15 (1H, dd, J = 8.9, 7.9 Hz), 3.20-3.28 (3H, m), 3.65 (1H, dd, J = 11.9, 5.3 Hz), 3.85 (1H, dd, J = 11.9, 2.0 Hz), 3.92 (1H, dd, J = 10.5, 9.0 Hz), 5.62 (1H, br s); FDMS m/z (rel intensity) 400 (M^+; 100).

Tosylate **8**. A mixture of **7** (370 mg, 0.925 mmol) and dibutyltin oxide (249 mg, 1.00 mmol) in MeOH (35 ml) was refluxed for 2 h under nitrogen. After cooling, the resulting clear solution was concentrated. The residual pale yellow resin was dissolved in dioxane (20 ml) and TsCl (190 mg, 1.00 mmol) was added. The mixture was stirred at room temperature for 15 h under nitrogen and concentrated. The residue was purified by column chromatography on silica gel (20 g) with 50:1 EtOAc-MeOH to afford **8** (450 mg, 88%) as a colorless amorphous powder: $[\alpha]_D^{24}$ -60.2° (c 1.05, MeOH); IR (KBr) 3400 (broad), 1655, 1600, 1365, 1175, 1095 cm^{-1}; ^1H NMR (270 MHz, CD_3OD) δ 0.56-0.72 (2H, m), 0.91 (1H, m), 0.99 (3H, d, J = 5.9 Hz), 1.14 (1H, m), 1.27 (3H, s), 1.45-1.57 (1H, m), 1.50 (3H, d, J = 0.7 Hz), 1.60-1.76 (2H, m), 2.45 (3H, s), 2.62 (1H, d, J = 10.2 Hz), 3.12 (1H, dd, J = 9.2, 7.6 Hz), 3.16 (1H, dd, J = 9.6, 9.2 Hz), 3.27-3.35 (1H, m), 3.43 (1H, ddd, J = 9.6, 6.9, 2.0 Hz), 3.91 (1H, dd, J = 10.2, 9.2 Hz), 4.06 (1H, dd, J = 10.6, 6.9 Hz), 4.34 (1H, dd, J = 10.6, 2.0 Hz), 4.47 (1H, d, J = 7.6 Hz), 5.54 (1H, br s), 7.44 (2H, br d, J = 8.4 Hz), 7.81 (2H, br d, J = 8.4 Hz); FDMS m/z (rel intensity) 554 (M^+; 5), 518 (10), 374 (35), 220 (100).

Tetraacetate **9**. A mixture of **8** (62.4 mg, 0.113 mmol), pyridine (1 ml), and Ac_2O (1 ml) was stirred at room temperature for 17 h. Concentration of the mixture afforded a residue, which was purified by column chromatography on silica gel (4 g) with 1:1 hexane-EtOAc to give **9** (78.7 mg, 96%) as a colorless amorphous powder: $[\alpha]_D^{25}$ -55.6° (c 1.05, $CHCl_3$); IR ($CHCl_3$) 3370 (broad), 3025, 1755, 1650, 1600, 1370, 1250, 1035 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.62-0.74 (2H, m), 0.90 (1H, m), 1.00-1.06 (1H, m), 1.01 (3H, d, J = 6.9 Hz), 1.19 (3H, s), 1.53 (3H, d, J = 1.3 Hz), 1.64 (1H, s), 1.77-1.84 (1H, m), 1.94 (3H, s), 1.96-2.09 (2H, m), 1.98 (3H, s), 2.01 (3H, s), 2.06 (3H, s), 2.46 (3H, s), 2.61 (1H, d, J = 7.3 Hz), 3.77 (1H, ddd, J = 10.2, 7.1, 2.8 Hz), 3.99 (1H, dd, J = 10.8, 7.1 Hz), 4.09 (1H, dd, J = 10.8, 2.8 Hz), 4.77 (1H, d, J = 7.9 Hz), 4.85 (1H, dd, J = 10.2, 9.2 Hz), 4.93 (1H, dd, J = 9.6, 7.9 Hz), 5.09 (1H, dd, J = 7.3, 4.6 Hz), 5.18 (1H, dd, J = 9.6, 9.2 Hz), 5.53 (1H, br s), 7.36 (2H, br d, J = 8.3 Hz), 7.79 (2H, br d, J = 8.3 Hz); FDMS m/z (rel intensity) 722 (M^+; 100).

Iodide **10**. A solution of **9** (78.7 mg, 0.109 mmol) and KI (83 mg, 0.50 mmol) in DMF (1.5 ml) was stirred at 80 °C for 6 h. After cooling, the mixture was diluted with H_2O (5 ml) and extracted with EtOAc (4 x 5 ml). The combined extracts were washed with saturated NaCl solution, dried, and concentrated. The residue was purified by column chromatography on silica gel (4 g) with 2:1 hexane-EtOAc to afford **10** (65.5 mg, 89%) as colorless crystals: mp 93-96 °C (ether-EtOAc); $[\alpha]_D^{25}$ -55.0° (c 1.07, $CHCl_3$); IR ($CHCl_3$) 3470 (broad), 3030, 1755, 1650, 1375, 1250, 1035 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.60-0.74 (2H, m), 0.92

(1H, m), 1.00-1.10 (1H, m), 1.06 (3H, d, J = 6.9 Hz), 1.22 (3H, s), 1.54 (3H, d, J = 1.3 Hz), 1.55 (1H, s), 1.95-2.06 (2H, m), 1.95 (3H, s), 1.99 (3H, s), 2.04 (3H, s), 2.06 (3H, s), 2.24 (1H, m), 2.67 (1H, dd, J = 7.1, 1.0 Hz), 3.11 (1H, dd, J = 10.7, 9.9 Hz), 3.29 (1H, dd, J = 10.7, 2.3 Hz), 3.60 (1H, ddd, J = 9.9, 9.6, 2.3 Hz), 4.82 (1H, d, J = 7.9 Hz), 4.85 (1H, dd, J = 9.6, 9.2 Hz), 5.01 (1H, dd, J = 9.6, 7.9 Hz), 5.10 (1H, br dd, J = 7.1, 4.8 Hz), 5.19 (1H, dd, J = 9.6, 9.2 Hz), 5.79 (1H, br s); FDMS m/z (rel intensity) 678 (M^+; 100). Anal. Calcd for $C_{28}H_{39}IO_{11}\cdot 1/2(C_2H_5)_2O$: C, 50.36; H, 6.20. Found: C, 50.51; H, 6.40.

Diol **11**. Zinc powder (31 mg, 0.47 mmol) and NH_4Cl (40 mg, 0.75 mmol) were added to a solution of **10** (33.0 mg, 0.049 mmol) in EtOH (3 ml) under nitrogen. The mixture was refluxed with vigorous stirring for 30 min and cooled to room temperature. To the mixture was added saturated $NaHCO_3$ solution (0.5 ml). The mixture was concentrated and the residue was suspended in ether (5 ml). The suspension was filtered through a pad of Celite. The filter cake was washed thoroughly with ether. The filtrate and the washings were combined and concentrated. The residue was purified by column chromatography on silica gel (2 g) with 2:1 benzene-EtOAc to afford **11** (9.3 mg, 68%) as a colorless oil: $[\alpha]_D^{25}$ -50.6° (c 0.725, $CHCl_3$); IR ($CHCl_3$) 3600, 3450 (broad), 3010, 1740 (shoulder), 1725, 1655, 1250, 1020 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.59 (1H, ddd, J = 9.2, 6.6, 4.0 Hz), 0.68 (1H, ddd, J = 9.6, 6.6, 4.3 Hz), 0.90 (1H, ddd, J = 9.6, 5.6, 4.0 Hz), 1.04 (1H, m), 1.06 (3H, d, J = 6.6 Hz), 1.38 (3H, s), 1.48 (3H, d, J = 1.3 Hz), 1.61 (1H, dd, J = 12.2, 5.8 Hz), 1.68 (1H, s), 1.94-2.12 (2H, m), 1.98 (1H, s), 2.06 (3H, s), 2.64 (1H, dd, J = 7.6, 1.0 Hz), 5.17 (1H, dd, J = 7.6, 5.3 Hz), 5.48 (1H, br s); EIMS m/z (rel intensity) 280 (M^+; 3), 262 (4), 244 (10), 202 (60), 187 (80), 171 (100) [HREIMS. Found: 280.1692 (M^+). $C_{16}H_{24}O_4$ requires: M^+ 280.1675].

Triol **12**. A mixture of **11** (9.3 mg, 0.033 mmol) and K_2CO_3 (1.5 mg, 0.011 mmol) in MeOH (1 ml) was stirred at room temperature for 1 h and neutralized by the addition of Amberlite IRC-50 (acid form, 50 mg). After stirring for 10 min, the mixture was passed through a column of Amberlite IRC-50 (acid form, 50 mg) with MeOH. The column was washed thoroughly with MeOH. The filtrate and the washings were combined and concentrated. Purification of the residue by column chromatography on silica gel (1 g) with 1:2 hexane-EtOAc afforded **12** (7.2 mg, 92%) as colorless crystals: mp 154-155 °C (hexane-EtOAc); $[\alpha]_D^{15}$ -73.9° (c 0.974, MeOH); IR ($CHCl_3$) 3600, 3450 (broad), 1650, 1100, 1025, 1000 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.62 (1H, m), 0.81 (1H, m), 0.96-1.08 (2H, m), 1.07 (3H, d, J = 6.3 Hz), 1.36 (3H, s), 1.41-1.70 (3H, m), 1.50 (3H, d, J = 1.3 Hz), 1.81 (1H, dd, J = 11.2, 5.3 Hz), 1.90-2.20 (1H, br s), 2.52 (1H, d, J = 9.6 Hz), 3.70-4.30 (1H, br s), 4.04 (1H, dd, J = 9.6, 8.9 Hz), 5.42 (1H, br s); EIMS m/z (rel intensity) 238 (M^+; 30), 220 (35), 149 (100), 135 (50) [HREIMS. Found: 238.1565 (M^+). $C_{14}H_{22}O_3$ requires: 238.1569].

Ptaquilosin (**1**). A mixture of **12** (23.5 mg, 0.099 mmol) and PCC (33 mg, 0.15 mmol) in CH_2Cl_2 (3 ml) was stirred at room temperature for 15 min, diluted with 1 M phosphate buffer solution (pH 7.0, 5 ml), and extracted with EtOAc (4 x 5 ml). The combined extracts were washed with saturated NaCl solution, dried, and concentrated. The residue was separated by TLC on silica gel with 4:1 CH_2Cl_2-EtOAc to give **1** (11.2 mg, 48%) as a colorless oil: $[\alpha]_D^{20}$ -246° (c 0.824, $CHCl_3$); IR ($CHCl_3$) 3600, 3460 (broad), 3010, 1720, 1645, 1030 cm^{-1}; ^1H NMR (270 MHz, C_6D_6) δ 0.19 (1H, ddd, J = 9.6, 6.6, 4.0 Hz), 0.63 (1H, ddd, J = 9.6, 5.5, 4.3 Hz), 0.87 (3H, d, J = 6.9 Hz), 0.90 (1H, ddd, J = 9.6, 6.6, 4.3 Hz), 1.04 (1H, ddd, J = 9.6, 5.5, 4.0 Hz), 1.14 (3H, d, J = 1.3 Hz), 1.15-1.35 (1H, br s), 1.33 (1H, dd, J = 12.2, 10.9 Hz), 1.40 (3H, d, J = 0.7 Hz), 1.73-1.98 (2H, m), 2.24 (1H, d, J = 1.3 Hz), 4.91 (1H, br s), 5.10 (1H, br s); EIMS m/z (rel intensity) 236 (M^+; 80), 221 (10), 218 (10), 205 (70), 149 (100), 135 (95) [HREIMS. Found: 236.1421 (M^+). $C_{14}H_{20}O_3$ requires: 236.1412].

Dienone (**3**). A mixture of ptaquilosin (**1**) (1.1 mg, 0.0047 mmol) in 0.02 M Na_2CO_3 solution (0.05 ml) was stirred at room temperature for 30 min, and extracted with 2:1 hexane-ether (6 x 0.5 ml). The combined extracts were passed through a column of anhydrous Na_2CO_3 and concentrated below 25 °C to afforded an oil (1 mg), which was shown to be a 7:3 mixture of **1** (65%) and **3**[3a,3d] (28%) by ^1H NMR and UV spectral analysis.

Acknowledgments - This work was supported in part by Grant-in-Aid for Special Project Research, Chemical Syntheses for Elucidation of Biological Functions, and by Grant-in-Aid for Encouragement of Young Scientists (No. 60740290 and 62740308 to H. K.) from the Ministry of Education, Science, and Culture, Japan.

References and Notes

1. (a) Evans, I. A. In Chemical Carcinogens, Second Edition; Searle, C. E., Ed.; American Chemical Society: Washington D. C., 1984; Vol. 2, pp 1171-1204. (b) Hirono, I.; Yamada, K. In Naturally Occurring Carcinogens of Plant Origin; Hirono, I., Ed.; Kodansha-Elsevier: Tokyo-Amsterdam, 1987; pp 87-120.

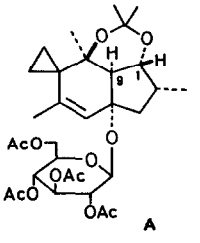
2. (a) Yoshihira, K.; Fukuoka, M.; Kuroyanagi, M.; Natori, S.; Umeda, M.; Morohoshi, T.; Enomoto, M.; Saito, M. Chem. Pharm. Bull. **1978**, 26, 2346 and references cited therein. (b) Fukuoka, M.; Kuroyanagi, M.; Yoshihira, K.; Natori, S. Chem. Pharm. Bull. **1978**, 26, 2365 and references cited therein.

3. (a) Niwa, H.; Ojika, M.; Wakamatsu, K.; Yamada, K.; Hirono, I.; Matsushita, K. Tetrahedron Lett. **1983**, 24, 4117. (b) Niwa, H.; Ojika, M.; Wakamatsu, K.; Yamada, K.; Ohba, S.; Saito, Y.; Hirono, I.; Matsushita, K. Tetrahedron Lett. **1983**, 24, 5371. (c) Ohba, S.; Saito, Y.; Hirono, I.; Niwa, H.; Ojika, M.; Wakamatsu, K.; Yamada, K. Acta Crystallogr., Sect. C, **1984**, 40, 1877. (d) Ojika, M.; Wakamatsu, K.; Niwa, H.; Yamada, K. Tetrahedron, **1987**, 43, 5261.

4. (a) Hirono, I.; Yamada, K.; Niwa, H.; Shizuri, Y.; Ojika, M.; Hosaka, S.; Yamaji, T.; Wakamatsu, K.; Kigoshi, H.; Niiyama, K.; Uosaki, Y. Cancer Lett. **1984**, 21, 239. (b) Hirono, I.; Aiso, S.; Yamaji, T.; Mori, H.; Yamada, K.; Niwa, H.; Ojika, M.; Wakamatsu, K.; Kigoshi, H.; Niiyama, K.; Uosaki, Y. Gann, **1984**, 75, 833. (c) Hirono, I.; Ogino, H.; Fujimoto, M.; Yamada, K.; Yoshida, Y.; Ikagawa, M.; Okumura, M. J. Natl. Cancer Inst. **1987**, 79, 1143.

5. Ojika, M.; Sugimoto, K.; Nozaki, N.; Okazaki, T.; Yamada, K., unpublished result.

6. The stereochemistry of the secondary hydroxyl group in **6** was determined by the ^{1}H NMR spectral analysis: reaction of **6** with 2-methoxypropene (camphorsulfonic acid, benzene) afforded the conformationally rigid isopropylidene derivative **A**, the coupling constant $J_{1,9}$ of which was 9.9 Hz, indicating the trans-diaxial relationship between H-1 and H-9. Thus, the secondary hydroxyl group in **6** was shown to be in the α-configuration.



A

7. Tsuda, Y.; Haque, M. E.; Yoshimoto, K. Chem. Pharm. Bull. **1983**, 31, 1612.