Tetrahedron Letters,Vol.24,No.38,pp 4117-4120,1983 0040-4039/83 $3.00 + .00
Printed in Great Britain

PTAQUILOSIDE, A NOVEL NORSESQUITERPENE GLUCOSIDE FROM BRACKEN, PTERIDIUM AQUILINUM VAR. LATIUSCULUM

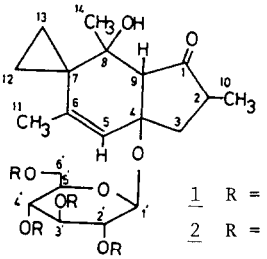
Haruki Niwa,[1] Makoto Ojika,[1] Kazumasa Wakamatsu,[1] Kiyoyuki Yamada,*[1] Iwao Hirono,[2] and Kazuhiro Matsushita[3]

1) Department of Chemistry, Faculty of Science, Nagoya University, Chikusa, Nagoya 464 Japan
2) Department of Carcinogenesis and Cancer Susceptibility, Institute of Medical Science, University of Tokyo, Shirokanedai, Minato-ku, Tokyo 108 Japan
3) NMR Application Laboratory, JEOL Ltd., Akishima, Tokyo 196 Japan

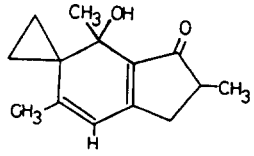
Abstract. *An unstable norsesquiterpene glucoside with a novel illudane skeleton, ptaquiloside (1) has been isolated from bracken fern, Pteridium aquilinum var. latiusculum and the planar structure has been established on the basis of spectral and chemical means.*

The carcinogenicity of bracken, Pteridium aquilinum var. latiusculum was demonstrated most clearly by the experiment of Evans and Mason in 1965.[1] Subsequently this result was confirmed by other workers.[2,3] In connection with the carcinogenic property chemical studies on bracken have so far been carried out extensively.[4] We have examined the constituents of this plant and performed fractionation of the aqueous extract by means of the assay based on carcinogenicity. From the fraction exhibiting carcinogenicity we have isolated an unstable norsesquiterpene glucoside of illudane type named ptaquiloside, the structural elucidation of which is described in the present paper.

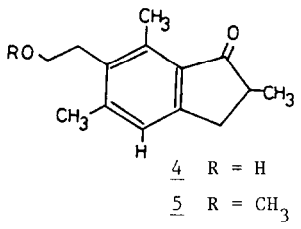
The dried powdered bracken (1 kg) was extracted with boiling water (3 x 10 l, 10 min. each) and the combined aqueous extracts were treated with the resin XAD-2. The portion adsorbed on the resin was eluted with methanol and repeatedly partitioned (n-BuOH - H_2O). The n-butanol fraction was separated by chromatography on silica gel [$CHCl_3$ - MeOH (5:1)] to give crude ptaquiloside (1). Further purification was made by preparative HPLC,[5] affording ptaquiloside (1), amorphous powder (210 mg, 0.02%), $C_{20}H_{30}O_8$,[6] $[\alpha]_D^{22}$ -188° (c 1.00, MeOH); IR (KBr) 3400 (broad), 1724, 1640 (weak) cm^{-1}; ^{1}H-NMR (Table 1); ^{13}C-NMR (Table 2); SIMS m/z 421 $(M + Na)^+$. Ptaquiloside (1) is unstable at room temperature under both acidic and basic conditions, and underwent aromatization to give 1-indanone derivatives such as pterosin B (4)[7] and pterosin O



1 R = H
2 R = $COCH_3$



3

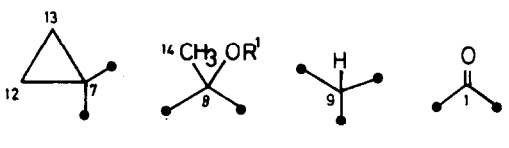


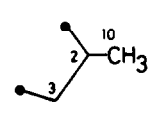
4 R = H
5 R = CH_3

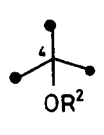
Table 1. ^{1}H-NMR Spectral Data[a)]

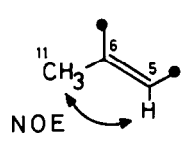
	1 [b)]	2 [c)]	3 [c)]
2	2.23 (ddq, 12.5, 8.0, 6.9)	2.23 (ddq, 12.2, 8.2, 7.0)	2.47 (ddq, 7.6, 6.7, 2.4)
3a	1.93 (t, 12.5)	1.93 (t, 12.2)	2.18 (dd, 18.6, 2.4)
3b	2.49 (dd, 12.5, 8.0)	2.42 (dd, 12.2, 8.2)	2.85 (dd, 18.6, 6.7)
5	5.76 (dq, 1.3, 1.0)	5.73 (quint, 1.5)	6.11 (q, 1.2)
9	2.64 (d, 1.3)	2.53 (d, 1.5)	-
10	1.07 (d, 6.9)	1.07 (d, 7.0)	1.15 (d, 7.6)
11	1.53 (d, 1.0)	1.55 (d, 1.5)	1.74 (d, 1.2)
12a	0.48 (m)[d)]	0.51 (m)[e)]	0.62 (ddd, 9.8, 7.0, 4.3)[f)]
12b	0.86 (m)[d)]	0.87 (m)[e)]	1.06 (ddd, 9.8, 6.7, 4.9)[f)]
13a	0.69 (m)[d)]	0.71 (m)[e)]	0.92 (ddd, 9.8, 7.0, 4.9)[f)]
13b	0.86 (m)[d)]	0.87 (m)[e)]	1.40 (ddd, 9.8, 6.7, 4.3)[f)]
14	1.29 (s)	1.15 (s)	1.24 (s)
1'	4.60 (d, 7.6)	5.07 (d, 7.9)	-
2'	*	4.90 (dd, 9.7, 7.9)	-
3'	*	5.28 (t, 9.7)	-
4'	3.20 (dd, 8.9, 9.1)	4.98 (t, 9.7)	-
5'	*	3.94 (ddd, 9.7, 5.5, 2.5)	-
6'a	3.66 (dd, 11.9, 5.6)	4.17 (dd, 12.2, 5.5)	-
6'b	3.90 (dd, 11.9, 1.3)	4.25 (dd, 12.5, 2.5)	-
Ac	-	1.95, 1.96, 2.02, 2.07	-

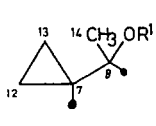
a) Chemical shifts are in ppm relative to TMS. The values shown in parentheses are coupling constants in Hz. Spectra were taken in CD_3OD.
b) Observed at 270 MHz. c) Observed at 400 MHz. d) e) f) Assignments may be interchanged.
* These signals could not be observed by overlapping with a solvent signal.

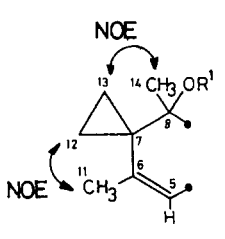










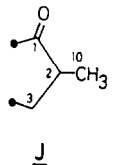


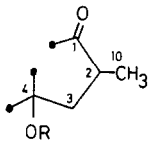
$$\left.\begin{matrix} R^1 \\ R^2 \end{matrix}\right\} = \left\{\begin{matrix} H \\ C_6H_{11}O_5 \text{ (D-glucose)} \end{matrix}\right.$$

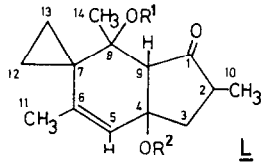
Table 2. ^{13}C-NMR Spectral Data[a)]

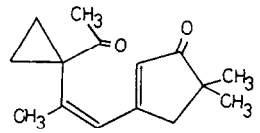
	1 [b)]		2 [c)]	
1	224.9	(s)	221.7	(s)
2	46.1	(d, 120.4)	43.9	(d, 125.0)
3	46.1	(t, 130.8)	44.5	(t, 130.0)
4	83.0	(s)	81.4	(s)
5	124.2	(d, 161.1)	120.0	(d, 159.5)
6	145.3	(s)	145.6	(s)
7	31.0	(s)	29.4	(s)
8	72.9	(s)	70.0	(s)
9	63.5	(d, 129.2)	61.4	(d, 130.0)
10	14.5	(q, 125.5)	13.4	(q, 125.2)
11	20.3	(q, 125.0)	19.6	(q, 126.3)
12	6.8	(t, 159.5)	5.6	(t, 160.0)
13	11.5	(t, 159.5)	10.6	(t, 160.0)
14	27.9	(q, 126.0)	26.4	(q, 126.5)
1'	100.2	(d, 159.9)	95.9	(d, 159.0)
2'	76.2	(d, 143.1)	71.6[e)]	(d, 149.4)
3'	78.7[d)]	(d, 141.2)	71.8[e)]	(d, 149.0)
4'	72.9	(d, 143.5)	69.1	(d, 149.9)
5'	79.2[d)]	(d, 141.5)	73.2	(d, 148.5)
6'	63.9	(t, 143.6)	62.5	(t, 148.9)
			170.4, 170.2	
Ac	-		169.4, 169.0	
			20.8, 20.6	

a) Chemical shifts are in ppm relative to TMS. The values shown in parentheses are $^1J_{C-H}$.
b) Spectra were taken in CD_3OD at 67.8 MHz.
c) Spectra were taken in $CDCl_3$ at 22.5 MHz.
d) e) Assignments may be interchanged.


J


k


L


6

(5)[8], depending on the solvent used: the half-life of ptaquiloside (1) in 0.01 M sulfuric acid - methanol at 22° was ca. 2 hours. Acetylation (Ac_2O - pyridine, room temp., 1 h) of 1 gave the tetraacetate (2), $C_{28}H_{38}O_{12}$, mp 173-174° (dec.) (MeOH); IR (KBr) 3450, 1761, 1724 (shoulder), 1640 cm^{-1}; ^{1}H-NMR (Table 1); ^{13}C-NMR (Table 2). Under the particular alkaline conditions [0.01 M Na_2CO_3 solution (pH ca. 11), 22°, 20 min.] ptaquiloside (1) was converted with concomitant elimination of D-(+)-glucose into an unstable conjugated dienone (3), colorless oil, $C_{14}H_{18}O_2$; UV (0.001 M NaOH - MeOH) λ_{max} 321 (ε ca. 10,000), 215 (ε ca. 9,000) nm; IR (CCl_4) 3480, 1667, 1570 cm^{-1}; ^{1}H-NMR (Table 1); MS m/z 218 (M^+). The dienone (3) was extremely unstable in a weakly acidic aqueous solution at room temperature,[9] and immediately converted to pterosin B (4).

The NMR spectral data of ptaquiloside (1) and the derivatives are summarized in Table 1 and 2. Mainly based on the NMR spectral analysis the structure of ptaquiloside (1) was elucidated as follows. The proton noise-decoupled, off resonance, and selective proton decoupled ^{13}C-NMR spectra as well as the ^{1}H-NMR and ^{1}H-NMDR spectra of ptaquiloside (1) revealed the presence of the part structures, A - G. In the ^{13}C-NMR spectrum of 1, three signals at δ_C 31.0 (s, C-7), 6.8 (t, J=159.5 Hz, C-12), and 11.5 (t, J=159.5 Hz, C-13) strongly suggested the presence of the 1,1-disubstituted cyclopropane ring (A) in 1, which was further supported by the ^{1}H-NMR spectrum of 1 [δ_H 0.48 (1H, m, H-12a), 0.69 (1H, m, H-13a), and 0.86 (2H, m, H-12b and H-13b)]. The stereochemistry of the methyl group [δ_H 1.53 (3H, d, J=1.0 Hz, H-11)] and the vinyl hydrogen [δ_H 5.76 (1H, qd, J=1.0 and 1.3 Hz, H-5)] in G was determined to be cis by the differential NOE experiment of 1. The spin decoupling experiments of H-2 [δ_H 2.23 (1H, ddq, J=12.5, 8.0, and 6.9 Hz)] and H-3 [δ_H 1.93 (1H, t, J=12.5 Hz) and 2.49 (1H, dd, J=12.5 and 8.0 Hz)] in E indicated that H-2 was coupled solely to H-3 and H-10 and that H-3 was coupled only to H-2, suggesting that two terminal carbons (C-2 and C-3) in E were connected to quaternary carbons, respectively. Since all carbon atoms in 1 appeared in the part structures, A - G, the remaining problem is the correlation of these part structures. Long range selective proton decoupling experiments (LSPD)[10] were very useful for the correlation of the part structures, A - G, as shown below. In the proton coupled ^{13}C-NMR spectrum of 1 under the gated decoupling experiment, all quaternary carbon signals at δ_C 31.0 (C-7 in A), 72.9 (C-8 in B), 224.9 (C-1 in D), 83.0 (C-4 in F), and 145.3 (C-6 in G) appeared as the broad singlets with fine splittings due to two and/or three bond C-H couplings ($^2J_{C-H}$ and/or $^3J_{C-H}$). LSPD irradiating H-14 (δ_H 1.29) in B collapsed the C-8 (δ_C 72.9) and C-7 (δ_C 31.0) carbon signals to the better defined broad singlets. This means that the protons (H-14) of the methyl group in B are three bonds away from the C-7 quaternary carbon in A, i.e., the cyclopropane ring in A is directly connected to the C-8 carbon in B. Correlation of the part structures, A and B was thus made to give an extended part structure H. The splitting pattern of the C-7 carbon signal in A was also simplified upon irradiation of H-11 (δ_H 1.53) in G: this result indicates that the C-6 carbon is bonded directly to the C-7 carbon, leading to a new, extended part structure I in 1. The presence of the part structure

I in 1 was further supported by the observation of the NOE between H-14 in B and H-13a in A, and between H-11 in G and two proton signals of the cyclopropane ring [H-12a and H-13b (or H-12b)] in A. LSPD irradiating H-10 (δ_H 1.07) in E collapsed only the C-1 carbonyl carbon signal to a simple singlet, indicating that the C-2 carbon in E is connected to the C-1 carbon in D: the part structure E is thus extended into a new part structure J. Since the C-2 and C-3 carbons in E were deduced to be connected to quaternary carbons (vide supra), the remaining quaternary carbon C-4 (δ_C 83.0) in F must be bonded to the C-3 carbon in E: the part structure J is therefore led to a further extended part structure K. LSPD of H-9 (δ_H 2.64) in C dramatically simplified each of three quaternary carbon signals (C-1, C-4, and C-8) to a broad singlet. This observation reveals that the C-9 carbon in C is linked directly to C-1 and C-4 in K and to C-8 in I, leading to the structure L with the illudane skeleton. Further, the planar structure L for 1 was chemically confirmed by the formation of the conjugated dienone (3) and pterosin B (4) from 1 as described above.

The coupling constant of the anomeric proton signal, H-1' [δ_H 4.60 (1H, d, J=7.6 Hz)] and the C-H coupling constant of the anomeric carbon signal, C-1' (δ_C 100.2, J_{C-H}=159.9 Hz) revealed the glycosidic linkage of 1 to be β-configuration. The site of glycosidation was deduced by LSPD of the anomeric proton signal, H-1' in 1. Irradiation of H-1' in 1 eliminated a long range coupling from C-4, revealing that glycosidation site is the hydroxyl group at C-4 and not the one at C-8. Formation of the conjugated dienone (3) accompanied by elimination of D-(+)-glucose on treatment of 1 with aqueous base (vide supra) confirmed unambiguously the location of glycosidation linkage in 1.
From the detailed analysis of ^{1}H- and ^{13}C-NMR spectra coupled with chemical evidence described above, the planar structure of ptaquiloside is established to be represented by the formula 1.

Previously there were isolated more than twenty C_{14} and C_{15}-sesquiterpenes possessing the 1-indanone skeleton named pterosins[4,7,8] as characteristic constituents of this plant. From the biogenetic viewpoint, ptaquiloside (1) can be regarded as a biosynthetic precursor of these pterosins. Further it is interesting to note the structural relation between ptaquiloside (1) and hypacrone (6)[11], the latter being isolated from a fern, Hypolepis punctata Mett. The stereochemistry and biological activities of ptaquiloside (1) are currently under investigation.

References and Notes

1. I. A. Evans and J. Mason, Nature, 208, 913 (1965).
2. A. M. Pamukcu and J. M. Price, J. Natl. Cancer Inst., 43, 275 (1969).
3. I. Hirono, C. Shibuya, K. Fushimi, and M. Haga, J. Natl. Cancer Inst., 45, 179 (1970).
4. a) K. Yoshihira, M. Fukuoka, M. Kuroyanagi, S. Natori, M. Umeda, T. Morohoshi, M. Enomoto, and M. Saito, Chem. Pharm. Bull., 26, 2346 (1978) and references cited therein; b) M. Fukuoka, M. Kuroyanagi, K. Yoshihira, and S. Natori, Chem. Pharm. Bull., 26, 2365 (1978) and references cited therein.
5. HPLC conditions: a column of 10 mm x 25 cm of Develosil ODS-5; H_2O-MeOH (45:55); flow rate 4 ml/min.
6. The molecular formula of ptaquiloside (1) was determined, based on the molecular ion peak at 421 $(M + Na)^+$ in SIMS and the elemental analysis of the tetraacetate (2).
7. a) H. Hikino, T. Takahashi, S. Arihara, and T. Takemoto, Chem. Pharm. Bull., 18, 1488 (1970); b) K. Yoshihira, M. Fukuoka, M. Kuroyanagi, and S. Natori, Chem. Pharm. Bull., 19, 1491 (1971).
8. M. Kuroyanagi, M. Fukuoka, K. Yoshihira, and S. Natori, Chem. Pharm. Bull., 22, 2762 (1974).
9. Whereas this compound (3) is highly unstable under weakly acidic conditions, it survives in a weakly alkaline solution at room temperature at least for 1 hour.
10. H. Seto, T. Sasaki, H. Yonehara, and J. Uzawa, Tetrahedron Lett., 923 (1978).
11. Y. Hayashi, M. Nishizawa, and T. Sakan, Tetrahedron, 33, 2509 (1977).

(Received in Japan 6 June 1983)