6β-Propionyloxy-12α- and 12β-methoxy respectively-8,12-epoxyeremophila-1(10),7(11)8-triene (**10** and **11**). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1740 (CO_2R); MS *m/z* (rel. int.): 318.184 $[M]^+$ (4) (calc. for $C_{19}H_{26}O_4$: 318.183), 244 $[M-RCO_2H]^+$ (17), 229 $[244-Me]^+$ (20), 57 $[RCO]^+$ (100).

REFERENCES

1. Pinhey, J. T., Simpson, R. F. and Bately, I. L. (1971) *Aust. J. Chem.* **27**, 2621.
2. Bohlmann, F. and Zdero, C. (1978) *Phytochemistry* **17**, 1135.
3. Bohlmann, F. and Zdero, C. (1979) *Chem. Ber.* **107**, 2912.
4. Bohlmann, F., Suwita, A. and Mahanta, P. (1976) *Chem. Ber.* **109**, 3570.
5. Hafez, S., Jakupovic, J., Bohlmann, F., Sarg, T. M. and Omar, A. A. (1989) *Phytochemistry* **28**, 843.
6. Bohlmann, F., Zdero, C., Berger, D., Suwita, A., Mahanta, P. and Jeffrey, C. (1979) *Phytochemistry* **18**, 79.
7. Bohlmann, F., Zdero, C., King, R. M. and Robinson, H. (1984) *Phytochemistry* **23**, 1979.

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DENNSTOSIDE A, AN ANALOGUE OF PTAQUILOSIDE, FROM *DENNSTAEDTIA SCABRA*

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Key Word Index—*Dennstaedtia scabra*; Pteridaceae; illudane-type sesquiterpene glycoside; dennstoside A.

Abstract—A new illudane-type sesquiterpene glycoside, named dennstoside A, was isolated from *Dennstaedtia scabra* and characterized as an analogue of ptaquiloside, the carcinogenic principle of *Pteridium aquilinum*, on the basis of spectral analyses and chemical conversion to pterosin A.

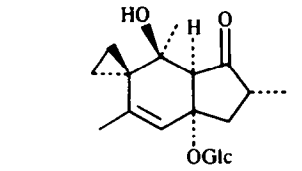
INTRODUCTION

Ptaquiloside (aquilide A) (**1**) is a carcinogen and bovine bracken poison which has been isolated from bracken fern (*Pteridium aquilinum*), and characterized as a novel glycoside of a *nor*-illudane-type sesquiterpene [1–3]. Using biological and chemical methods for the assay of ptaquiloside (**1**) and related compounds, *ca* 30 species of the Pteridaceae have been examined and the widespread occurrence of such compounds revealed [4, 5]. In a previous paper [6], we have reported the isolation of ptaquiloside (**1**) from *Pteris cretica* and *Histiopteris incisa*, as well as the isolation and the structure determination of several analogues, hypolosides A (**2**), B (**3**) and C (**4**) from *Hypolepis punctata*, and B and C from *Dennstaedtia hirsta*. We now report the isolation and characterization of a further ptaquiloside analogue from *Dennstaedtia scabra*.

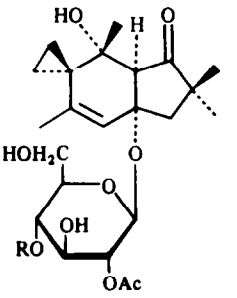
RESULTS AND DISCUSSION

In the course of chemotaxonomical work on the Pteridaceae by Murakami's group [7], the indan-1-one-type sesquiterpenes (2*S*)-pterosin A (**5**), (2*S*)-4-hydroxypterosin A (onitisin) and pterosin V were isolated and pterosins K and F were detected by GC-MS from the terrestial part of *Dennstaedtia scabra*. The application of our screening test to this fern [5] suggested the presence of a ptaquiloside-like substance. This compound, named dennstoside A (**6**), was isolated by a modification of the method used for hypolosides [6], using TLC [5] for monitoring. The molecular formula of **6** was established as $C_{23}H_{34}O_{10}$, having one more oxygen atom than hypoloside A (**2**), by the examination of FAB-MS and other spectral data. The ^1H and ^{13}C NMR signals (Tables 1 and 2) of dennstoside A (**6**) were nearly the same as those of hypoloside A (**2**) except for those due to C-2, C-3, C-10a and C-10b. The presence of a hydroxymethyl group instead of the methyl group at C-10 was clearly demonstrated by DEPT experiments. These facts showed that one of the methyl groups at C-10 of **2** is replaced by a hydroxymethyl group. On treatment with acid, alkali, heat or on standing at room temperature, dennstoside A (**6**) gave pterosin A (**5**), an indan-1-one [8], as in the case of the formation of pterosin B (**7**) from ptaquiloside (**1**) [2, 3] and pterosin Z (**8**) from hypolosides (**2**–**4**) [6]. Thus, the plane structure of **6** was established.

The absolute configuration of (−)-pterosin A (**5**) has been established as 2*S* [8]. The optical rotation of pterosin A (**5**) ($[\alpha]_D^{20}-36.9°$) obtained from **6** clearly showed 2*S*-configuration. Among the two methylene protons at C-3 (δ1.85 and 2.67), NOE was observed between the higher field proton and the C-10b methyl and



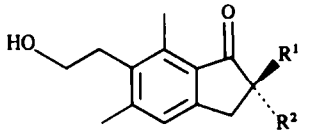
1



2 R = H

3 R = —CO—C(H)=C(H)—C$_6$H$_4$—OH

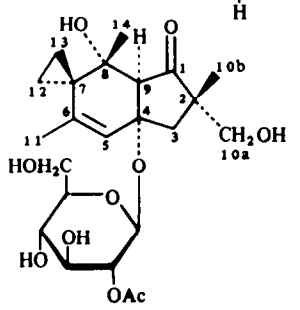
4 R = —CO—C(H)=C(H)—C$_6$H$_4$—OH



5 R^1 = Me, R^2 = CH_2OH

7 R^1 = H, R^2 = Me

8 R^1 = R^2 = Me



6

Table 1. ^{1}H NMR data for compounds **1**, **2** and **6** [chemical shifts (ppm) and coupling constants (Hz) in CD_3OD at 400 MHz]

H	1	2	6
2	2.22 *ddq* (12.7, 8.2, 7.0)		
3α	1.93 *dd* (12.7, 12.7)[a]	2.00 *d* (14.2)[a]	2.67 *d* (14.0)
3β	2.50 *ddd* (12.7, 8.2, 1.2)[a]	2.25 *dd* (14.2, 1.9)[a]	1.85 *d* (14.0)
5	5.76 *m*	5.72 *q* (1.3)	5.79 *m*
9	2.65 *d* (1.2)	2.72 *d* (1.9)	2.68 *br s*
10a	1.07 *d* (7.0)	1.10 *s*	3.24 *d* (10.8)
			3.64 *d* (10.8)
10b		1.10 *s*	1.04 *s*
11	1.53 *d* (1.0)	1.54 *d* (1.3)	1.54 *br s*
12,13	0.48, 0.69, 0.86	0.43, 0.87, 0.87	0.53, 0.82, 0.82
	0.86 *m*	1.20 *m*	1.11 *m*
14	1.29 *s*	1.07 *s*	1.15 *s*
1′	4.61 *d* (8.0)	4.35 *d* (8.4)	4.57 *d* (8.3)
2′	*	4.68 *dd* (8.4, 9.2)	4.67 *dd* (8.3, 9.3)
3′	*	3.41 *dd* (9.2, 9.2)	3.46 *dd* (9.3, 8.9)
4′	*	3.31 *dd* (9.2, 9.2)	3.29 *dd* (8.9, 9.4)
5′	*	3.07 *m*	3.19 *ddd* (9.4, 6.5, 1.7)
6′	3.65 *dd* (10.1, 5.6)	3.63 *dd* (12.3, 6.1)	3.62 *dd* (11.8, 6.5)
	3.90 *dd* (10.1, 1.8)	3.81 *dd* (12.3, 2.3)	3.85 *dd* (11.8, 1.7)
Acetyl Me		2.00 *s*	2.00 *s*

J (Hz) in parentheses.

[a]Assignments may be interchanged.

*3.2–3.4 ppm.

Table 2. ^{13}C NMR data for compounds **1**, **2** and **6** [chemical shifts (ppm) in CD_3OD at 100 MHz]

C	1	2	6
1	224.9	225.2	221.7
2	45.1	46.9	53.6
3	45.1	50.6	44.8
4	82.0	85.1	84.1
5	123.1	125.2	124.9
6	144.4	141.7	142.3
7	30.1	31.8	31.2
8	72.0	73.2	73.2
9	62.4	64.4	65.5
10a	13.6	25.7[a]	67.8
10b		23.7[a]	20.7
11	19.4	19.8	19.8
12,13	5.9, 10.6	6.5, 9.0	8.0, 8.4
14	27.0	27.7	23.3
1′	99.3	97.6	97.4
2′	75.2	74.7	75.1
3′	77.7[a]	76.7	76.6
4′	71.9	71.7	71.8
5′	78.2[a]	78.7	78.6
6′	63.0	62.7	62.8
Acetyl C=O		171.8	171.9
Acetyl Me		21.1	21.2

[a]Assignments may be interchanged.

C-14 methyl protons, while the lower-field proton showed NOE with the anomeric proton in the glucose residue and the C-9 proton. Thus, the stereochemistry of C-4, C-8 and C-9 of dennstoside A (**6**) was proved to be the same as in the hypolosides **2**–**4**.

Various illudane-type sesquiterpene glycosides such as ptaquiloside (**1**), hypolosides A–C (**2**–**4**) and dennstoside A (**6**), corresponding to pterosins B (**7**), Z (**8**) and A (**5**) respectively, have so far been characterized from the Pteridaceae. 1-Indanone sesquiterpenes, pterosins and pterosides, occur widely in the family [9]. This fact, in conjunction with the known mutagenicity of the illudane-type sesquiterpene glycosides [10, 11] may suggest the widespread occurrence of carcinogenic constituents in the family.

EXPERIMENTAL

Isolation of dennstoside A (**6**) *from* Dennstaedtia scabra. The terrestrial part of the fern (190 g), collected at Owase, Mie, in August 1988 (kept at −20° and dried at room temp. for 24 hr), was extracted with MeOH (8 l × 3) for 24 hr at 4°. The extract was concd, Me_2CO was added and the ppt. formed removed by centrifugation. The supernatant was concd and the residue chromatographed on a column of silica gel employing a CH_2Cl_2–MeOH gradient system. The frs were checked by 2D TLC [5]. The frs containing dennstoside A (**6**) were pooled and further purified by HPLC using a Develosil 60–5 column (25 × 1 cm) with an RI detector and $CHCl_3$–MeOH (10:1), hexane–Me_2CO (1:1) and hexane–EtOAc (1:7) as the developers to give dennstoside A (**6**) (9.6 mg).

Dennstoside A (**6**). Powder (hexane–Me_2CO), mp 71–76°, $[\alpha]_D^{20}$ −54.2° (MeOH), FAB-MS *m/z* 493.2052 $[M+Na]^+$ ($C_{23}H_{34}O_{10}Na$ requires 493.2056). IR ν_{max}^{KBr} cm^{-1}: 3420, 2880–2990, 1743, 1737, 1380, 1250, 1079, 1042. ^1H NMR (Table 1), ^{13}C NMR (Table 2).

Formation of pterosin A (**5**) *from dennstoside A* (**6**). Compound **6** (9.6 mg) was kept at room temp. for 7 days and then taken up in $CHCl_3$ (5 ml). The $CHCl_3$ soln was purified by prep. TLC using EtOAc–hexane (7:1) as solvent to afford **5** (4.2 mg), mp 118–122°, $[\alpha]_D^{20}$ −36.9° (MeOH). The identity of the product was confirmed by direct comparison with an authentic sample.

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REFERENCES

1. Hirono, I. and Yamada, K. (1987) in *Naturally Occurring Carcinogens of Plant Origin* (Hirono, I., ed.), p. 87. Kodansha, Tokyo and Elsevier, Amsterdam.
2. Ojika, M., Wakamatsu, K., Niwa, H. and Yamada, K. (1987) *Tetrahedron* **43**, 5261.
3. Van der Hoeven, J. C. M., Lagerweij, W. J., Posthumus, M. A., van Deldhuizen, A. and Holterman, H. A. J. (1983) *Carcinogenesis* **4**, 1587.
4. Matoba, M., Saito, E., Saito, K., Koyama, K., Natori, S., Matsushima, T. and Takimoto (née Muramatsu), M. (1987) *Mutagenesis* **2**, 419.
5. Saito, K., Nagao, T., Matoba, M., Koyama, K., Natori, S., Murakami, T. and Saiki, Y. (1989) *Phytochemistry* **28**, 1605.
6. Saito, K., Nagao, T., Takatsuki, T., Koyama, K. and Natori, S. (1990) *Phytochemistry* **29**, 1475.
7. Murakami, T., Owashi, K., Tanaka, N., Satake, T. and Chen, C.-M. (1975) *Chem. Pharm. Bull.* **23**, 1630.
8. Fukuoka, M., Kuroyanagi, M., Yoshihira, K. and Natori, S. (1978) *Chem. Pharm. Bull.* **26**, 2365.
9. Murakami, T. and Tanaka, N. (1988) in *Progress in the Chemistry of Organic Natural Products* Vol. 54 (Herz, W., Griesebach, H., Kirby, G. W. and Tamm, Ch., eds), p. 1. Springer, Vienna.
10. Nagao, T., Saito, K., Hirayama, E., Uchikoshi, K., Koyama, K., Natori, S. and Matsushima, T. (1989) *Mutation Res.* **215**, 173.
11. Matsuoka, A., Hirosawa, A., Natori, S., Iwasaki, S., Sofuni, T. and Ishidate, M. (1989) *Mutation Res.* **215**, 179.