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NEW ALKALOID GLYCOSIDES FROM *SELAGINELLA DOEDERLEINII*

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ABSTRACT.—Three new alkaloid glycosides have been isolated from *Selaginella doederleinii*, hordenine-*O*-α-L-rhamnopyranoside [**1**], *N*-methyltyramine-*O*-α-L-rhamnopyranoside [**2**], and hordenine-*O*-[(6″-*O*-*trans*-cinnamoyl)-4′-*O*-β-D-glucopyranosyl-α-L-rhamnopyranoside] [**3**]. Their structures were elucidated by spectroscopic methods.

Selaginella doederleinii Hieron. (Selaginellaceae) is a small perennial Pteridophyte growing throughout south and southwestern China at low altitude (1). The whole plant seems to be more and more used in traditional Chinese medicine as a bactericide, an anticancer agent, and in the treatment of cardiovascular diseases (2). Recent screening for antitumor properties of the crude drug has shown a strong inhibition ratio of growth of HeLa cells in vitro (3).

The only previous paper dealing with the chemical constituents of this species described the isolation of shikimic acid (4). We have now isolated various flavonoids, lignans, and alkaloids from *S. doederleinii*, and we wish to describe here the structure determination of the alkaloids since, in contrast with the related *Lycopodium* genus (5), the isolation of alkaloids from *Selaginella* species has not been so far reported.

Extraction of the whole plant yielded four major alkaloids isolated after column chromatography. The least polar one has been identified as the well known alkaloid hordenine from its physical constants and spectral data (6) and by direct comparison with an authentic sample. The three other compounds are novel alkaloid glycosides.

The first novel compound has been isolated as a colorless, amorphous solid, $[\alpha]^{20}D = -96°$ (MeOH, $c=1$). The empirical formula has been established by combustion analysis as $C_{16}H_{25}NO_5$. No significant molecular ion could be detected in mass spectrometry using electron impact or chemical ionization techniques. In contrast, when submitted to desorption chemical ionization ms (reagent gas NH_3), it exhibited a molecular ion $(M+H)^+ = 312$ accompanied with a strong fragmentation ion at *m/z* 166 characterizing an hordenine unit. These elements suggested a structure of hordenine-*O*-glycoside. The nature of the sugar moiety could be deduced from the ^{1}H-nmr spectrum (see Experimental section) and ^{13}C-nmr spectrum (see Table 1) which exhibited all the characteristic signals of a hordenine unit (6, 7) and of an α-rhamnose unit (8-10). Of particular interest was the small coupling constant ($J=1$ Hz) between H-1′and H-2′ indicating an α-configuration of the rhamnose unit. Upon acetylation this compound led to a tri-*O*-acetyl derivative. These elements permitted depicting the structure of this alkaloid as hordenine-*O*-α-L-rhamnopyranoside [**1**]. This substance had not been previously described though the corresponding hordenine-*O*-β-D-glucopyranoside is a known natural product (7, 11) and has also been prepared synthetically (12).

The second novel glycoside has been obtained as an amorphous solid, $[\alpha]^{20}D = -131°$ (MeOH, $c=0.3$). The general features of its various spectra were essentially similar to those of **1**. Nevertheless, it differed significantly from **1** by its dci mass spectrum which gave a molecular ion $(M+H)^+ = 298$, instead of 312, and a strong fragmentation ion at *m/z* 152, instead of 166. These elements suggested a structure

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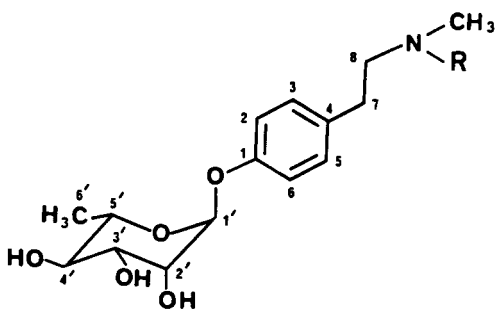
TABLE 1. ^{13}C-nmr Spectra of Compounds **1** and **3** (50 MHz, spin echo)

Carbon Atom	Compounds	
	1	**3**
1	154.2	154.0
2	116.2	116.3
3	129.3	129.3
4	133.6	133.7[a]
5	129.3	129.3
6	116.2	116.3
7	32.2	32.1
8	60.7	60.6
Me_2	44.8	44.8
1′	98.6	98.6
2′	70.1	70.1
3′	70.4	70.4
4′	71.7	80.9
5′	69.2	69.6
6′	17.7	17.6
1″		104.6
2″		73.7[b]
3″		76.0
4″		70.3
5″		73.6[b]
6″		64.0
1‴		133.6[a]
2‴		128.0
3‴		128.7
4‴		130.1
5‴		128.7
6‴		128.0
7‴		144.4
8‴		117.7
9‴		165.9

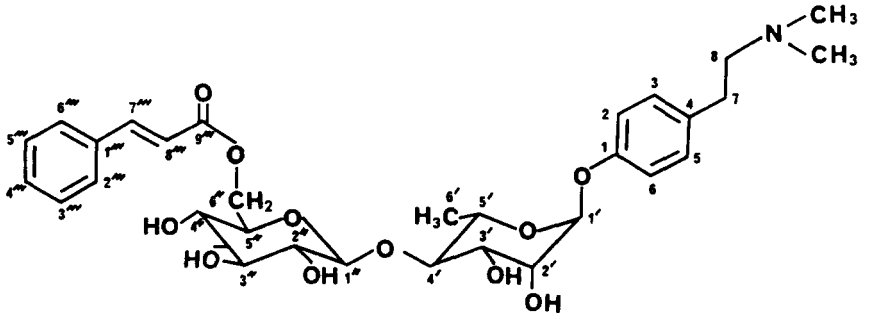
[a,b]Values may be reversed.

similar to that of **1** but where the hordenine unit was replaced by a *N*-methyltyramine unit. In good agreement with this statement, the ^{1}H-nmr spectrum exhibited the same signals as those observed for **1** except for a 3H-singlet at 2.27 ppm (NMe) instead of a 6H-singlet at 2.22 ppm (NMe_2). Consequently, the structure of this compound was established as *N*-methyltyramine-*O*-α-L-rhamnopyranoside [**2**].



1 R=CH_3 **2** R=H

The third new alkaloid glycoside has also been obtained as a colorless amorphous solid, $[\alpha]^{20}D = -84°$ (MeOH, $c=1$). Its empirical formula has been established as $C_{31}H_{41}NO_{11}$ by combustion analysis. Its dci mass spectrum gave a molecular ion $(M+H)^+ = 604$ together with prominent fragmentation ions at *m/z* 474, 312, and 166, suggestive of the presence of cinnamoyl, hexosyl, deoxyhexosyl, and hordenine units. The ^{1}H-nmr spectrum exhibited signals typical of these four units. The identification of the sugars as glucopyranose and rhamnopyranose could be deduced from a thorough study of the chemical shifts and coupling constants observed in the ^{1}H-nmr spectra of the alkaloid glycoside and of its pentaacetylderivative. Two low field anomeric signals at 4.53 ppm ($J=7$ Hz) and 5.24 ppm ($J=1$ Hz) indicated a β-glucose unit was linked to one of the alcoholic oxygens of an α-rhamnose, itself linked to the phenolic oxygen of hordenine. Two doublets ($J=16$ Hz) at 6.63 and 7.69 ppm were consistent with the presence of a *trans*-cinnamoyl unit. Finally, the sequence of the four units could be deduced from the ^{13}C-nmr spectrum (Table 1). A downfield shift of the signal of C-4′ which appears at 80.9 ppm instead of 71.7 ppm in the spectrum of **1** gave evidence for the site of glucosylation on the rhamnose unit (8-10, 13, 14). The location of the cinnamoyl unit at C-6″ on the glucose could be deduced from the downfield shift of C-6″ signal and the upfield shift of C-5″ signal when compared to the values published for various *O*-β-D-glucosides (8, 9, 13-15). On the basis of these observations, the structure of this compound was concluded to be hordenine-*O*-[(6″-*O*-*trans*-cinnamoyl)-4′-*O*-β-D-glucopyranosyl-α-L-rhamnopyranoside] [**3**].



3

The four alkaloids described in the present paper are the first ones to be isolated from a *Selaginella* species. All of them belong to the same biogenetic series. The acylated sugar sequence of compound **3** is very similar to that previously described for a flavonoid glycoside recently isolated from *Ginkgo biloba* L. (14). This latter sequence differs only from that of **3** by the replacement of cinnamic acid with 4-hydroxycinnamic acid. This emphasizes the chemical relationships between Pteridophytes and primitive Gymnosperms.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Optical rotations were measured on a Perkin-Elmer 141 polarimeter. Spectra were recorded on the following apparatus: uv, Unicam SP 800; ir, Beckman 4250; ms, Nermag R 10-10C; ^{1}H nmr, Bruker HX 270; ^{13}C, nmr, Bruker AM 400.

PLANT MATERIAL.—*S. doederleinii* was collected in the Fujian, People's Republic of China, in March and April 1985. Voucher specimens are kept in the Herbarium of the Musée de Matière Médicale de la Faculté de Pharmacie de Paris, France.

EXTRACTION AND ISOLATION OF THE ALKALOIDS.—Percolation of the dried plant material (7.5 kg) by MeOH yielded 500 g crude extact. The methanolic extract was then treated by 0.5 N aqueous HCl. The precipitate was filtered off. The acidic solution was basified with concentrated aqueous NH_3 and extracted successively with CH_2Cl_2 and *n*-BuOH. No alkaloids could be detected in the CH_2Cl_2 extract

whereas the butanolic extract (15 g) contained alkaloids accompanied by several secondary products. Column chromatography on Si gel of the butanolic extract yielded successively four alkaloids, hordenine (100 mg), hordenine-*O*-α-L-rhamnopyranoside [**1**] (200 mg), hordenine-*O*-[(6″-*trans*-cinnamoyl)-4′-*O*-β-D-glucopyranosyl-α-L-rhamnoside] [**3**] (150 mg), and *N*-methyltyramine-*O*-α-L-rhamnoside [**2**] (8 mg).

HORDENINE-*O*-α-L-RHAMNOPYRANOSIDE [**1**].—Corlorless, amorphous solid, $[\alpha]^{20}D = -96°$ (MeOH, $c=1$), $C_{16}H_{25}NO_5$ (found, C: 61.50, H: 8.04, N: 4.52, O: 25.60—calcd., C: 61.71, H: 8.09, N: 4.50, O: 25.69); uv λ MeOH max nm (log ε) 230 (3.33), 275 (3.12), 282 (3.06); ir KBr ν max cm^{-1} 3400, 2940, 1620, 1520, 1240, 1130, 1070, 1025, 990, 830; ms (dci NH_3) *m/z* (%) 312 (31) ($M+H^+$), 166 (100), 152 (5); ^1H nmr (270 MHz, CD_3SOCD_3, TMS) δ ppm 7.20 (2H, d, $J=9$ Hz, H-3, H-5), 6.97 (2H, d, $J=9$ Hz, H-2, H-6), 5.35 (1H, d, $J=1$ Hz, H-1′), 5.05 (1H, D_2O exch., OH-2′), 4.88 (1H, D_2O exch., OH-4′), 4.75 (1H, D_2O exch., OH-3′), 3.84 (1H, dd, $J=1.5$ Hz, $J'=1$ Hz, H-2′), 3.65 (1H, dd, $J=9$ Hz, $J'=1.5$ Hz, H-3′), 3.51 (1H, dq, $J=9$ Hz, $J'=6$ Hz, H-5′), 3.24 (1H, t, $J=9$ Hz, H-4′), 2.68 (2H, t, $J=8$ Hz, CH_2-7), 2.44 (2H, t, $J=8$ Hz, CH_2-8), 2.22 (6H, s, NMe_2), 1.14 (3H, d, $J=6$ Hz, CH_3-6′); ^{13}C nmr see Table 1.

HORDENINE-*O*-(2′,3′,4′-TRI-*O*-ACETYL)-α-L-RHAMNOPYRANOSIDE.—To a solution of **1** (12 mg) in pyridine (1 ml) was added Ac_2O (1 ml), and the mixture was left under anhydrous conditions for 72 h at 20°. After removal of reagents in vacuo and column chromatography, the triacetyl derivative was obtained as a glassy foam (10 mg); ^1H nmr (270 MHz, $CDCl_3$, TMS) δ ppm 7.17 (2H, d, $J=9$ Hz, H-3, H-5), 7.00 (2H, d, $J=9$ Hz, H-2, H-6), 5.52 (1H, dd, $J=9$ Hz, $J'=2$ Hz, H-3′), 5.42 (2H, m, H-1′, H-2′), 5.15 (1H, t, $J=9$ Hz, H-4′), 4.00 (1H, dq, $J=9$ Hz, $J'=6$ Hz, H-5′), 2.75 (2H, m, CH_2-7), 2.52 (2H, m, CH_2-8), 2.30 (6H, s, NMe_2), 2.19, 2.07 and 2.03 (3×3H, 3s, 3 OAc), 1.02 (3H, d, $J=6$ Hz, CH_3-6′).

N-METHYLTYRAMINE-*O*-α-L-RHAMNOPYRANOSIDE [**2**].—Colorless, amorphous solid, $[\alpha]^{20}D = -131°$ (MeOH, $c=0.3$); uv λ MeOH max nm (log ε) 231 (3.43), 275 (3.21), 282 (3.16); ms (dci NH_3) *m/z* (%) 298 (100) ($M+H^+$), 152 (35); ^1H nmr (270 MHz, CD_3SOCD_3, TMS) δ ppm 7.15 (2H, d, $J=9$ Hz, H-3, H-5), 6.95 (2H, d, $J=9$ Hz, H-2, H-6), 5.35 (1H, d, $J=1$, 5 Hz, H-1′), 5.04, 4.86 and 4.73 (3×1 H, D_2O exch., OH-2′, 3′ and 4′), 3.82 (1H, t, $J=1.5$ Hz, H-2′), 3.64 (1H, dd, $J=9$ Hz, $J'=1.5$ Hz, H-3′), 3.48 (1H, dq, $J=9$ Hz, $J'=6$ Hz, H-5′), 3.27 (1H, t, $J=9$ Hz, H-4′), 3.22 (1H, br. s, D_2O exch., NH), 2.66 (4H, m, CH_2-7, CH_2-8), 2.31 (3H, s, NMe), 1.13 (3H, d, $J=6$ Hz, CH_3-6′).

HORDENINE-*O*-[(6″-*O*-*TRANS*-CINNAMOYL)-4′-*O*-β-D-GLUCOPYRANOSYL-α-L-RHAMNOPYRANOSIDE] [**3**].—Colorless, amorphous solid, $[\alpha]^{20}D = -84°$ (MeOH, $c=1$); $C_{31}H_{41}NO_{11}$ (found, C: 61.44, H: 6.86, N: 2.55, O: 29.49—calcd., C: 61.67, H: 6.85, N: 2.32, O: 29.16); uv λ MeOH max nm (log ε) 226 (4.04), 278 (4.11); ir KBr ν max cm^{-1} 3440, 2950, 1725, 1650, 1525, 1240, 1170, 1070, 1040, 780; ms (dci, NH_3) *m/z* (%) 604(16) ($M+H^+$), 474 (7), 312 (5), 166 (100), 152 (7); ^1H nmr (270 MHz, CD_3SOCD_3, TMS) δ ppm 7.69 (1H, d, $J=16$ Hz, H-7‴), 7.61 (2H, m, H-2‴, H-6‴), 7.38 (3H, m, H-3‴, H-4‴, H-5‴), 7.00 (2H, d, $J=9$ Hz, H-3, H-5), 6.87 (2H, d, $J=9$ Hz, H-2, H-6), 6.63 (1H, d, $J=16$ Hz, H-8‴), 5.24 (1H, d, $J=1$ Hz, H-1′), 5.15, 5.04 and 4.87 (3×1H, D_2O exch., 3 OH), 4.53 (1H, d, $J=7$ Hz, H-1″), 4.44 (1H, dd, $J=12$ Hz, $J'=2$ Hz, H-6″a), 4.24 (1H, dd, $J=12$ Hz, $J'=7$ Hz, H-6″b), 4.02 (1H, dd, $J=1.5$ Hz, $J'=1$ Hz, H-2′), 3.76 (1H, dd, $J=9$ Hz, $J'=1.5$ Hz, H-3′), 3.54 (3H, m, H-4′, H-5′, H-3″), 3.37 (2H, D_2O exch., 2OH), 3.20 (3H, m, H-2″, H-4″, H-5″), 2.62 (4H, m, CH_2-7, CH_2-8), 2.36 (6H, s, NMe_2), 1.11 (3H, d, $J=6$ Hz, CH_3-6′); ^{13}C nmr see Table 1.

HORDENINE-*O*-[6″-*O*-*TRANS*-CINNAMOYL, 2′,3′2″,3″4″-*O*-PENTAACETYL)-4′-*O*-β-D-GLUCOPYRANOSYL-α-L-RHAMNOPYRANOSIDE].—To a solution of **3** (10 mg) in pyridine (1 ml) was added Ac_2O (1 ml) and the mixture was left at 20° for 72 h. After removal of the reagents and column chromatography, the pentaacetyl derivative was obtained as a glassy solid (4 mg); ^1H nmr (270 MHz, $CDCl_3$, TMS) δ ppm 7.72 (1H, d, $J=16$ Hz, H-7‴), 7.48 (2H, m, H-2‴, H-6‴), 7.36 (3H, m, H-3‴, H-4‴, H-5‴), 7.00 (2H, d, $J=9$ Hz, H-3, H-5), 6.91 (2H, d, $J=9$ Hz, H-2, H-6), 6.47 (1H, d, $J=16$ Hz, H-8‴), 5.41 (2H, m, H-1, H-2′), 5.34 (1H, dd, $J=9$ Hz, $J'=2$ Hz, H-3′), 5.20 (1H, t, $J=9$ Hz, H-3″), 5.12 (1H, t, $J=9$ Hz, H-4″), 5.11 (1H, t, $J=9$ Hz, H-2′), 5.01 (1H, t, $J=9$ Hz, H-4′), 4.72 (1H, d, $J=9$ Hz, H-1″), 4.27 (3H, m, H-6″a, H-6″b, H-5′), 3.83 (1H, m, H-5″), 3.14 (4H, m, CH_2-7, CH_2-8), 2.82 (6H, s, NMe_2), 2.15, 2.11, 2.05, 2.03 and 2.00 (5×3H, 5s, 5OAc), 1.15 (3H, d, $J=6$ Hz, CH_3-6′).

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