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ECDYSTEROIDS FROM *PFAFFIA IRESINOIDES* AND REASSIGNMENT OF SOME ^{13}C NMR CHEMICAL SHIFTS*

NOBUSHIGE NISHIMOTO, YOSHINORI SHIOBARA, MASUMI FUJINO, SHUN-SUKE INOUE, TSUNEMATSU TAKEMOTO, FERNANDO DE OLIVEIRA,† GOKITHI AKISUE,† MARIA KUBOTA AKISUE,† GORO HASHIMOTO,‡ OSAMU TANAKA,§ RYOJI KASAI§ and HIROMICHI MATSUURA¶

Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro-cho, Tokushima 770, Japan; †Departmento de Farmácia da Faculdade Ce Ciências Farmacêuticas da Universidade de São Paulo, São Paulo 30786, Brasil; ‡Centro de Pesquisas História Natural, Vila Leopoldina, São Paulo 05318, Brasil; §Institute of Pharmaceutical Sciences, Hiroshima University, School of Medicine, Minami-ku, Hiroshima 734, Japan; ¶Central Research Laboratories, Wakunaga Pharmaceutical Co., Ltd., Koda-cho, Takata-gun, Hiroshima 729-64, Japan

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Key Word Index—*Pfaffia iresinoides*; Amaranthaceae; Brazil ginseng; ecdysteroid; triterpenoid saponin; ecdysterone; polypodine B; pterosterone; chikusetsusaponin IVa.

Abstract—From the crude drug 'Brazil ginseng', the roots of *Pfaffia iresinoides*, a large amount of ecdysterone has been isolated together with polypodine B and pterosterone. The ecdysterone content of each part of the plant was determined by high pressure liquid chromatography quantitative analysis. A triterpenoid saponin, chikusetsusaponin IVa has also been isolated.

INTRODUCTION

The crude drug 'Brazil ginseng' has been used medicinally in Brazil, chiefly as a tonic, an aphrodisiac and as an antidiabetic [1]. In the course of our investigation of the chemical constituents of 'Brazil ginseng', we found that at least two kinds of plants, *Pfaffia paniculata* Kuntze and *P. iresinoides* Spreng., were used for the preparation of the crude drug. From the former plant, pfaffic acid and its glycosides which showed inhibitory effects on the growth of cultured tumour cells were isolated [2, 3].

In this paper, we wish to report the isolation and identification of three ecdysteroids and a triterpenoid saponin from the roots of *P. iresinoides*.

RESULTS AND DISCUSSION

The roots of *P. iresinoides* were extracted with cold methanol. From the crude extract, ecdysterone (**1**) [4], polypodine B(**2**) [5] and pterosterone (**3**) [6] were isolated by a combination of liquid–liquid partition and column chromatography. Each compound was identified by comparison with an authentic sample [^{1}H NMR (Experimental), ^{13}C NMR (Table 1) spectra and mixed melting point test]. The present assignments of the spectra were performed by means of ^{1}H–^{1}H and ^{1}H–^{13}C shift correlated two dimensional NMR spectroscopy. Some of the previous ^{13}C assignments [7–9] have been revised.

The yield of **1**, the major component of the root increased to 0.31% from the dried roots by extracting with hot methanol. Further, high pressure liquid chromatography quantitative analysis of the ecdysterone content of each part of the plant showed high values (% yield based on dried plant): leaves (0.92%), stems (0.17%), roots (0.62%).

Since our first isolation of **1** from the plant kingdom in 1966, it has become feasible to investigate some pharmacological activities for **1** in mammals [10, 11]. More recently, Suksamrarn *et al.* reported the isolation of **1** from the bark of *Vitex glabrata* (Verbenaceae) in high yield [12 and A. Suksamrarn, personal communication]. *P. iresinoides*, as well as the above plant, is therefore a convenient source of **1**.

Chikusetsusaponin IVa (**4**) [13], known as a component of *Panax japonicus* (Araliaceae) was also isolated and identified by direct comparison with an authentic sample.

EXPERIMENTAL

Mps uncorr; ^{1}H and ^{13}C NMR spectra were measured on a JEOL JNM-GX400 instrument. TLC was performed on Kieselgel $60F_{254}$ precoated silica gel plates (Merck) and developed in $CHCl_3$–MeOH–H_2O (65:35:10, lower phase). Spots were visualized by UV light or by spraying with 10% H_2SO_4 and heating at 150°.

Plant materials. Roots of *P. iresinoides* were collected in the Mato Grosso area of Brazil and samples were deposited in the Herbarium of the Institute of Pharmacognosy, Tokushima Bunri University. Fresh plants were cultivated in the botanical garden of Tokushima Bunri University and were collected in June.

Extraction and separation. The air-dried roots (20 kg) were crushed and extracted with MeOH (200 l) for 5 days. Evaporation of the solvent yielded a brown syrup (1 kg). H_2O (2 l) was added and the mixture was treated with *n*-BuOH (4 × 1 l). The organic layer was evaporated to yield a brown viscous syrup (140 g), which was chromatographed on silica gel (650 g) and eluted successively with AcOEt (2 l), AcOEt–MeOH (98:2, 9 l) and AcOEt–MeOH (95:5, 9 l). The eluant was collected as 300 ml fractions. Fractions 14–26 were concd and the residue (5.3 g) was rechromatographed on silica gel (70 g) using $CHCl_3$–MeOH–H_2O (80:20:10, lower phase) and 30 ml fractions were collected. The residue obtained from fractions 13–15

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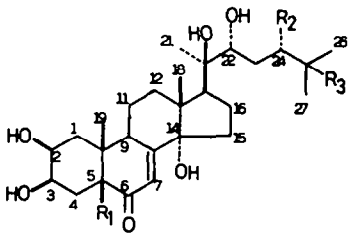

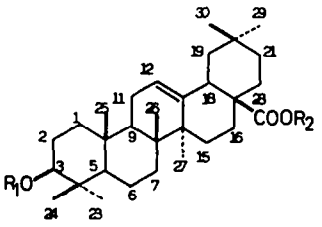

1 R_1=R_2=H, R_3=OH

2 R_1=R_3=OH, R_2=H

3 R_1=R_3=H, R_2=OH

4 R_1=ß-D-glucuronopyranosyl
R_2=ß-D-glucopyranosyl

Table 1. ^{13}C NMR data* of compounds 1–3

Carbon No.	1	2	3
1	37.5	34.9‡	37.7
2	68.0	68.1	68.1
3	67.9	69.9	68.0
4	32.2	36.1	32.4
5	51.2	79.9	51.3
6	204.0	201.0	203.8
7	121.5	119.9	121.6
8	166.4	167.0	166.3
9	34.2	38.4‡	34.3
10	38.5	44.8	38.6
11	20.9	22.1‡	21.0
12	31.8‡	32.2‡	31.9
13	47.9	48.2	48.0
14	84.1	84.1	84.1
15	31.5‡	31.8‡	31.6
16	21.3†	21.4‡	21.4§
17	49.9	50.1	49.9
18	17.8	18.0‡	17.9
19	24.2	17.3‡	24.4
20	76.9	76.9	76.8
21	21.5†	21.7	21.6§
22	77.5	77.7	77.4§
23	27.2	27.6	35.7
24	42.3	42.3	76.7§
25	69.8	69.7	33.8
26	29.6	30.1	17.0
27	29.8	30.2	19.5

*Measured at 100 MHz with C_5D_5N as solvent. Chemical shifts are given δ values in ppm from TMS.

†,‡,§ Previous assignments (†: ref. [7], ‡: ref. [8], §: ref. [9]) are revised.

was recrystallized from AcOEt–MeOH to give pterosterone (**3**, 180 mg). Fractions 27–57 were concd and the crystalline residue was recrystallized from AcOEt–MeOH to afford ecdysterone (**1**, 30 g). The residue obtained from the mother liquor was chromatographed on silica gel (100 g) and eluted successively with $CHCl_3$–MeOH–H_2O (80:20:10, lower phase, 1 l) and $CHCl_3$–MeOH–H_2O (75:25:10, lower phase, 3 l) and 50 ml fractions were collected. Fractions 9–11 were concd and the residual solid was recrystallized from aq. Me_2CO to give polypodine B (**2**, 340 mg). Fractions 12–30 were combined and treated in the same manner described above to afford an additional amount of **1** (7 g). The residue (3.5 g) obtained from fractions 46–78 was rechromatographed on Sephadex LH-20 (180 g) using MeOH and 30 ml fractions were collected. Fractions 32–39 were concd and the crystalline residue was recrystallized from 50% MeOH to afford chikusetsusaponin IVa (**4**, 550 mg).

Ecdysterone (**1**). Mp 240–242° (lit. mp 242° [4]); Found: C, 67.4; H, 9.2. Calc. for $C_{27}H_{44}O_7$: C, 67.5; H, 9.2%. IR ν_{max}^{KBr} cm^{-1}: 3400, 1650; ^{1}H NMR (400 MHz, C_5D_5N): δ1.02 (3H, *s*, H-19), 1.17 (3H, *s*, H-18), 1.39 (6H, *s*, H-26 and H-27), 1.60 (3H, *s*, H-21), 2.42 (1H, *q*, J = 10. 3Hz, H-16), 2.52 (1H, *dt*, J = 12.8 and 4.3 Hz, H-12), 2.96 (2H, *m*, H-5 and H-17), 3.56 (1H, *br t*, J = 8.6 Hz, H-9), 3.87 (1H, *br d*, J = 9.1 Hz, H-22), 4.21 (1H, *br dt*, J = 11.8 and 3.4 Hz, H-2), 4.25 (1H, *br s*, H-3), 6.23 (1H, *d*, J = 2.2 Hz, H-7).

Polypodine B (**2**). Mp 252–254° (lit. mp 254–257° [5]); Found: C, 64.5; H, 9.0. Calc. for $C_{27}H_{44}O_8 \cdot 1/2H_2O$: C 64.2; H, 9.0%. IR ν_{max}^{KBr} cm^{-1}: 3380, 1685; ^{1}H NMR (400 MHz, C_5D_5N): δ1.13 (3H, *s*, H-19), 1.19 (3H, *s*, H-18), 1.35 (6H, *s*, H-26 and H-27), 1.57 (3H, *s*, H-21), 2.43 (1H, *q*, J = 10.2 Hz, H-16), 2.54 (1H, *dt*, J = 12.6 and 4.6 Hz, H-12), 2.96 (1H, *t*, J = 9.0 Hz, H-17), 3.62 (1H, *br t*, J = 8.6 Hz, H-9), 3.84 (1H, *br d*, J = 9.2 Hz, H-22), 4.15 (1H, *br s*, H-3), 4.26 (1H, *br dt*, J = 11.4 and 4.0 Hz, H-2), 6.25 (1H, *d*, J = 2.2 Hz, H-7).

Pterosterone (**3**). Mp 228–230° (lit. mp 229–230° [6]; Found: C, 65.0; H, 9.0. Calc. for $C_{27}H_{44}O_7 \cdot H_2O$: C, 65.0; H, 9.3%. IR ν_{max}^{KBr} cm^{-1}: 3420, 1650; ^{1}H NMR (400 MHz, C_5D_5N): δ1.003 and 1.006 (each 3H, *d*, J = 6.6 Hz, H-26 and H-27), 1.06 (3H, *s*, H-19), 1.21 (3H, *s*, H-18), 1.59 (3H, *s*, H-21), 2.47 (1H, *q*, J = 10.4 Hz, H-16), 2.60 (1H, *dt*, J = 13.0 and 5.0 Hz, H-12), 2.94 (1H, *t*, J = 9.2 Hz, H-17), 3.02 (1H, *dd*, J = 13.0 and 4.3 Hz, H-5), 3.58 (1H, *br t*, J = 9.2 Hz, H-9), 3.94 (1H, *dt*, J = 9.0 and 4.0 Hz, H-24), 4.12 (1H, *br d*, J = 10.6 Hz, H-22), 4.16 (1H, *br dt*, J = 11.8 and 3.4 Hz, H-2), 4.22 (1H, *br s*, H-3), 6.25 (1H, *d*, J = 2.2 Hz, H-7).

Chikusetsusaponin IV*a* (**4**). Mp 218–220° (lit. mp 216–218° [13]), Found: C, 60.8; H, 8.1. Calc. for $C_{42}H_{66}O_{14} \cdot 2H_2O$: C, 60.7; H, 8.5%. IR ν_{max}^{KBr} cm^{-1}: 3400, 1730; ^{13}C NMR (100 MHz, C_5D_5N): δ15.6 (C-25), 17.1 (C-24), 17.6 (C-26), 18.6 (C-6), 23.5, 23.8 and 23.9 (C-11, C-16 and C-30), 26.2 (C-27), 26.7 (C-2), 28.3 (C-15 and C-23), 30.9 (C-20), 32.6 (C-22), 33.2 (C-7 and C-29), 34.1 (C-21), 37.0 (C-10), 38.7 (C-1), 39.6 (C-4), 40.0 (C-8), 41.8 (C-18), 42.4 (C-14), 46.3 (C-19), 47.1 (C-17), 48.1 (C-9), 55.8 (C-5), 62.3 (C-6″), 71.2 (C-4″), 73.5 (C-4′), 74.2 (C-2″), 75.6 (C-2′), 77.8 and 78.2 (C-3′ and C-5′), 79.0 and 79.4 (C-3″ and C-5″), 89.1 (C-3), 95.8 (C-1″), 107.3 (C-1′), 123.0 (C-12), 144.2 (C-13), 173.1 (C-6′), 176.5 (C-28).

Isolation of ecdysterone (**1**) *from hot MeOH extract.* The dried

roots (400 g) were extracted with hot MeOH (3 × 1 l). The extract was evaporated and the residue (7 g) was treated in the same manner described above to afford **1** (1.25 g).

HPLC quantitative analysis of ecdysterone content. Each part (1 g) of the air-dried plants was extracted in a Soxhlet with MeOH (130 ml) for 4 hr. The extract was concd *in vacuo*. To the residue was added H_2O (50 ml) and the mixture was centrifuged at 3000 *g* for 10 min. The supernatant was adsorbed on a Sep-pak C_{18} cartridge (Waters Assoc.) and eluted successively with H_2O(10 ml), 20% MeOH (10 ml) and 50% MeOH (10 ml). The 50% MeOH eluate was used for HPLC analysis. The HPLC conditions were as follows; detector, 245 nm; mobile phase, 40% MeOH; flow rate, 1.5 ml/min; column, 30 cm × 4.0 mm packed with Nucleosil C_{18}; R_t 10.1 min.

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