Novel Highly Sweet Secodammarane Glycosides from *Pterocarya paliurus*†

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Two novel, potently sweet 3,4-secodammaranoid saponins designated pterocaryosides A (**1**) and B (**2**) have been isolated from the leaves and stems of the Chinese tree *Pterocarya paliurus*. The structures of these compounds were established as (23*E*)-(12*R*,20*S*)-12,20,25-trihydroxy-3,4-secodammara-4(28),23-dien-3-oic acid 12-*O*-β-D-quinovopyranoside and (23*E*)-(12*R*,20*S*)-12,20,25-trihydroxy-3,4-secodammara-4(28),23-dien-3-oic acid 12-*O*-α-L-arabinopyranoside, on the basis of spectroscopic measurements, especially 1D and 2D NMR data. The methylated peracetates of pterocaryosides A and B were prepared and used for structural confirmation. Preliminary evaluations constituted by mouse acute toxicity and bacterial mutagenesis determinations supported the safety of **1** and **2**, and subsequently the compounds were rated by a human taste panel as about 50 and 100 times sweeter than 2% sucrose, respectively.

Keywords: *Pterocarya paliurus; Juglandaceae; saponins; secodammarane type; sweet-tasting constituents; pterocaryosides A and B*

INTRODUCTION

As part of our continuing efforts to identify potently sweet plant-derived compounds of natural origin, we have examined the Chinese tree *Pterocarya paliurus* Batal. (Juglandaceae). The Chinese common name for this plant, "tian ye shu", translates as "sweet-leaf tree", suggesting that the plant contains sweet-tasting compounds. Furthermore, in remote areas of Enshi County, Hubei Province, People's Republic of China, the leaves of this plant are used by local populations to sweeten foods in cooking.

The dried leaves of one member of this genus, *P. tonkinensis*, have reported application in the People's Republic of China as an insectide and fish poison (Pei, 1985), although the constituents responsible for these activities have not yet been established. The types of secondary metabolite so far reported among plants of the genus *Pterocarya* include an alkaloid of the spermidine type (Meurer *et al.*, 1988b), a flavonoid (Meurer *et al.*, 1988a), naphthoquinones (Aynehchi *et al.*, 1973; Hirarawa *et al.*, 1986; Pedersen, 1978), tannins (Nonaka *et al.*, 1989), and seed oils (Koyama *et al.*, 1970). In the case of *P. paliurus*, however, the phytochemistry has not been well studied, with only one dammarane saponin, cyclocaryoside A, having been described to date (Yang *et al.*, 1992). Cyclocaryoside A was reported to be potently sweet (Yang *et al.*, 1992). In an attempt to isolate additional sweet-tasting compounds from this species, the present study was conducted. This paper describes the isolation of two novel, potently sweet saponins based on the secodammarane carbon skeleton, which have been accorded the trivial names pterocaryosides A (**1**) and B (**2**).

MATERIALS AND METHODS

^{1}H-NMR and ^{13}C-NMR spectra were measured with TMS as an internal standard by employing a Varian XL-300 instrument operating at 299.9 and 75.6 MHz, respectively. ^{1}H–^{1}H COSY and ^{1}H–^{13}C HETCOR NMR experiments were conducted on a Varian XL-300 spectrometer (using standard Varian pulse sequences) and a GE Omega 500 MHz NMR spectrometer. ^{1}H–^{1}H HOHAHA and additional ^{1}H–^{13}C HETCOR NMR experiments were performed on the GE Omega 500 MHz instrument, operating at 499.9 MHz for ^{1}H NMR and at 124.9 MHz for ^{13}C NMR. Selective INEPT and DEPT NMR experiments were conducted on a Nicolet NT-360 NMR spectrometer, operating at 90.8 MHz for ^{13}C NMR. ^{13}C-NMR multiplicity was determined using APT and DEPT experiments. FABMS and HRFABMS were obtained on a Finnigan MAT-90 instrument. Samples were dispersed in glycerol and bombarded with a beam of Xe atoms with an acceleration of 8 kV. IR spectra were taken as KBr pellets on a Midac Collegian FT-IR spectrometer, and UV spectra were measured in MeOH solutions using a Beckman DU-7 spectrophotometer. Optical rotations were measured with a Perkin-Elmer Model 241 polarimeter. Melting points were determined using a Fisher-Johns melting point apparatus and are uncorrected.

TLC analyses were performed on Kieselgel 60 F254 (Merck), with compounds visualized with 10% H_2SO_4 followed by charring. Sugars were detected using anisaldehyde spray reagent [0.5 mL of anisaldehyde, 10 mL of glacial acetic acid, 85 mL of methanol, and 5 mL of concentrated sulfuric acid (Wagner *et al.*, 1984)]. Si gel, Merck 60 Å, 230–400 mesh ASTM (Aldrich Chemical Co.) was used for flash chromatography and vacuum-liquid chromatography (VLC). Low-pressure column chromatography (LPCC) was carried out on a Lichroprep RP-18 (40–63 μm) prepacked column. GC/MS analyses of the silylated sugars were performed on a Finnigan 4510 automated gas chromatograph/mass spectrometer, using a 60 m × 0.25 mm (i.d.) DB-1 column (J&W Scientific, Folsom, CA): film thickness, 0.25 mm; temperature programmed from 100 to 270 °C at 4 °C min^{-1}; He, 2 mL min^{-1}; injector temperature, 220 °C; ion source temperature, 120 °C; electron energy, 70 eV.

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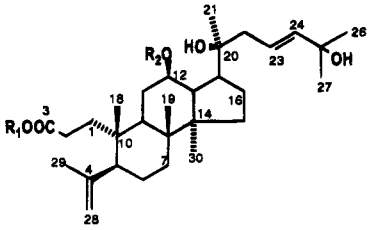

	R_1	R_2
1	H	D-quinovose
2	H	L-arabinose
3	CH_3	D-quinovose-2',3',4'-triacetate
4	CH_3	L-arabinose-2',3',4'-triacetate
5	CH_3	Ac

Figure 1. 3,4-Secodammaranoid derivatives.

Plant Material. The stems and leaves of *P. paliurus* were collected in Hubei Province (Enshi County), People's Republic of China, in August 1993 and identified by Dr. W. Zhu of the Wuhan Institute of Botany, Academia Sinica, Wuhan, People's Republic of China. A voucher specimen has been deposited at the Wuhan Institute.

Extraction Procedure. The air-dried, milled stems and leaves (3 kg) were extracted exhaustively with 70% EtOH (30 L) and concentrated under reduced pressure to yield an EtOH extract (429 g). The resulting residue was redissolved in 80% MeOH (1 L) and defatted using petroleum ether (3 × 1 L). The MeOH layer was then concentrated to remove most of the MeOH, with H_2O then added to the solution (to 1 L), which was then partitioned with $CHCl_3$ (3 × 1 L). The remaining aqueous solution was partitioned with *n*-BuOH (3 × 1 L), resulting in four extracts of differing polarity, namely, petroleum ether, $CHCl_3$, *n*-BuOH, and H_2O.

Isolation and Charaterization of Sweet-Tasting Compounds 1 and 2. A portion (20 g) of the $CHCl_3$ fraction was adsorbed onto Si gel (40 g) and separated over additional Si gel (120 g) by vlc, using a gradient of 1–20% MeOH in $CHCl_3$, and fractions containing materials of similar R_f were combined to provide seven pooled fractions. The sixth combined fraction, eluted with about 15% MeOH, contained two major components which were further purified by repeated flash chromatography using EtOAc–MeOH–H_2O (81:11:8), followed by RP-18 LPCC using a gradient of 50–80% MeOH in H_2O, to yield pure **1** (20 mg; 0.0007% w/w yield) and **2** (42 mg; 0.0014% w/w yield) (structures are given in Figure 1).

Pterocaryoside A [(23*E*)-(12*R*,20*S*)-12,20,25-Trihydroxy-3,4-secodammara-4(28),23-dien-3-oic Acid 12-*O*-β-D-Quinovopyranoside (1)]: amorphous white powder; mp 149–151 °C (dec); $[\alpha]^{22}_D$ +3.0° (*c* 0.71, MeOH); ^{1}H- and ^{13}C-NMR data in CD_3OD presented in Tables 1 and 2; ^{1}H NMR (300 MHz, C_5D_5N) δ 6.22 (1H, m, H-23), 6.00 (1H, d, *J* = 15.5 Hz, H-24), 4.97 (2H, bs, H-28a and H-28b), 4.92 (1H, d, *J* = 7.5 Hz, C-1′), 4.45 (1H, m, H-12), 4.17 (1H, t, *J* = 8.3 Hz, H-2′), 4.07 (1H, t, *J* = 8.5 Hz, H-3′), 3.62 (2H, m, H-4′ and H-5′), 1.87 (3H, s, CH_3-29), 1.55 (3H, d, CH_3-6′), 1.54 (6H, s, CH_3-26 and CH_3-27), 1.45 (3H, s, CH_3-21), 1.34 (3H, s, CH_3-19), 1.08 (3H, s, CH_3-30), 0.93 (3H, s, CH_3-18); UV λ_{max} (MeOH) 207 nm (end absorption); IR υ_{max} (KBr) 3424, 2963, 2932, 1707, 1634, 1456, 1385, 1279, 1165 1067, 1009, 893, 519 cm^{-1}; FABMS *m*/*z* (M − H)$^-$ 635, (M − $C_6H_{10}O_4$) 489; HRFABMS *m*/*z* found 635.41461, calcd for $C_{36}H_{59}O_9$, 635.41591.

Methylation and Acetylation of 1. An ethereal solution of CH_2N_2 was added to a methanolic solution of **1** (10 mg), with the product (about 10 mg) dissolved in pyridine (1 mL) and Ac_2O (1 mL), and allowed to stand at room temperature overnight. The solvent was then evaporated under nitrogen, and the product was purified by flash chromatography using $CHCl_3$–acetone (10:1) as the solvent system. Similar fractions were pooled to give a pure peracetate methyl ester, methyl (23*E*)-(12*R*,20*S*)-12,20,25-trihydroxy-3,4-secodammara-4(28),-23-dien-3-oate 12-*O*-(2′,3′,4′-*O*-triacetyl)-β-D-quinovopyranoside (**3**) (9 mg), which exhibited the following data: short white needles; mp 90–95 °C; ^{1}H NMR δ 5.60 (1H, m, H-23), 5.57 (1H, d, *J* = 15.5 Hz, H-24), 5.15 (1H, t, *J* = 9.7 Hz, H-3′), 4.87 (1H, m, H-2′), 4.83 (1H, bs, H-28a), 4.80 (1H, t, *J* = 9.6 Hz, H-4′), 4.67 (1H, s, H-28b), 4.50 (d, *J* = 7.8 Hz, H-1′), 3.91 (1H, m, H-12), 3.71 (3H, s, OCH_3), 3.53 (1H, m, H-5′), 2.04 (3H, s, OAc), 2.00 (3H, s, OAc), 1.99 (3H, s, OAc), 1.73 (3H, s, CH_3-29), 1.35 (6H, s, CH_3-26 and CH_3-27), 1.22 (3H, d, *J* = 6.2, CH_3-6′), 1.17 (3H, s, CH_3-21), 1.05 (3H, s, CH_3-19), 1.00 (3H, s, CH_3-30), 0.90 (3H, s, CH_3-18); ^{13}C-NMR data presented in Table 2; IR υ_{max} (KBr) 3443, 2961, 2920, 2872, 1757, 1740, 1638, 1451, 1385, 1254, 1219, 1165, 1113, 1073, 1036 cm^{-1}; EIMS *m*/*z* (relative intensity) 758 (M − H_2O, 0.01%), 740 (1), 468 (0.3), 451 (1), 387 (4), 369 (7), 305 (5), 273 (100), 231 (3), 213 (18), 171 (14), 153 (37), 111 (20).

Table 1. ^{1}H NMR Data for Pterocaryoside A (1)[a]

proton[b]	δ multiplicity (*J*, Hz)	^{1}H/^{1}H relays HOHAHA[c]	COSY[c]
H-1	2.45 d,d,d (5, 13, 13)	1.49, 2.23, 2.66	1.49, 2.23, 2.66
	1.49 m	2.23, 2.45, 2.66	2.23, 2.45, 2.66
H-2	2.66 d,d,d (5, 13, 13)	1.49, 2.23, 2.45	1.49, 2.23, 2.45
	2.23 m	1.49, 2.45, 2.66	1.49, 2.45, 2.66
H-5	1.78 m	1.07, 1.42, 1.63, 1.74	
H-6	1.74 m	1.07, 1.42, 1.63, 1.78	
	1.63 m	1.07, 1.42, 1.74, 1.78	
H-7	1.42 m	1.07, 1.63, 1.74, 1.78	
	1.07 m	1.42, 1.63, 1.74, 1.78	
H-9	1.80 m	1.37, 1.90, 2.35, 4.07	
H-11	2.35 m	1.37, 1.80, 1.90, 4.07	1.37, 4.07
	1.37 m	1.80, 1.90, 2.35, 4.07	1.80, 2.35, 4.07
H-12	4.07 d,d,d (4.5, 10.7, 10.7)	1.37, 1.80, 1.90, 2.35	1.37,1.90, 2.35
H-13	1.90 m	1.37, 1.80, 2.35, 4.07	4.07
H-15	1.56 m	1.31, 1.87, 2.02	
	1.19 m	1.31, 1.54, 1.87, 2.02	
H-16	1.87 m	1.19, 1.56, 2.02	
	1.31 m	1.19, 1.56, 1.87, 2.02	
H-17	2.02 m	1.19, 1.31, 1.56, 1.87	1.31, 1.87
H-18	0.96 s		
H-19	1.09 s		
H-21	1.16 s		
H-22	2.19 m	5.63, 5.71	5.71
H-23	5.71 m	2.19, 5.63	2.19, 5.63
H-24	5.63 d (15.5)	2.19, 5.71	5.71
H-26	1.27 s		
H-27	1.27 s		
H-28	4.69 bs	1.76, 4.84	1.76, 4.84
	4.84 bs	1.76, 4.69	1.76, 4.69
H-29	1.76 s	4.69, 4.84	
H-30	1.04 s		
H-1′	4.35 d (7.5)	1.25, 2.99, 3.20, 3.24, 3.29	3.20
H-2′	3.20 m	1.25, 2.99, 3,24, 3.29, 4.35	2.99, 4.35
H-3′	2.99 t (9.1)	1.25, 3.20, 3.24, 3.29, 4.35	3.20, 3.29
H-4′	3.29 m	1.25, 2.99, 3.20, 3.24, 4.35	2.99, 3.24
H-5′	3.24 m	1.25, 2.99, 3.20, 3.29, 4.35	1.25, 3.29
H-6′	1.25 d (6.0)	2.99, 3.24, 3.20, 3.29, 4.35	3.24

[a] Spectra were run in CD_3OD at 500 MHz, and values are recorded in parts per million with respect to TMS. [b] Assignments were determined from HETCOR and COSY spectra. [c] Only the prominent cross-peaks are listed.

Pterocaryoside B [(23*E*)-(12*R*,20*S*)-12,20,25-Trihydroxy-3,4-secodammara-4(28),23-dien-3-oic Acid 12-*O*-α-L-Arabinopyranoside (2)]: amorphous white powder; mp 138–142 °C; $[\alpha]^{22}_D$ +21.3° (*c* 0.23, MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ 5.68 (1H, m, H-23), 5.62 (1H, d, *J* = 15.5 Hz, H-24), 4.85 (1H, bs, H-28a), 4.72 (1H, bs, H-28b), 4.28 (1H, d, *J* = 6.5 Hz, H-1′), 4.12 (1H, ddd, *J* = 4.5, 10.5, H-12), 3.89 (1H, dd, *J* = 1.5, 13 Hz, H-5′a), 3.79 (1H, m, H-4′), 3.52 (1H, overlapping with H_2O, H-5′b), 3.51 (1H, m, H-2′), 3.50 (1H, m, H-3′), 2.62 (1H, m, H-2a), 2.50 (1H, m, H-1a), 2.40 (1H, m, H-11a), 2.24 (1H, m, H-2b), 2.20 (1H, m, H-22a), 2.04 (1H, m, H-17), 1.95 (1H, m, H-22b), 1.94 (1H, m, H-13), 1.90 (1H, m, H-16b), 1.82 (1H, m, H-9), 1.80 (1H, m, H-5), 1.77 (1H, m, H-6a), 1.77 (3H, s, CH_3-29), 1.61 (1H, m, H-6b), 1.55 (1H, m, H-15a), 1.52 (1H, m, H-1b), 1.42 (1H, m, H-7a), 1.33 (1H, m, H-11b), 1.31 (1H,

Table 2. ^{13}C NMR Data for Compounds 1–5[a]

carbon	1[b]	2[b]	3[b]	4[b]	5[c]
C-1	37.3 t	38.6 t	35.6 t	35.6 t	34.7 t
C-2	30.0 t	31.3 t	29.1 t	29.0 t	28.4 t
C-3	179.1 s	181.0 s	175.0 s	174.9 s	174.0 s
C-4	148.5 s	149.2 s	147.7 s	147.8 s	147.4 s
C-5	49.6 d	50.3 d	49.8 d	49.9 d	50.4 d
C-6	25.1 t	25.9 t	24.7 t	24.8 t	24.8 t
C-7	31.1 t	31.9 t	30.8 t	30.8 t	33.3 t
C-8	40.0 s	40.8 s	39.4 s	39.5 s	39.5 s
C-9	40.6 d	41.4 d	40.1 d	40.2 d	40.8 d
C-10	41.0 s	41.8 s	40.6 s	40.6 s	39.5 s
C-11	33.4 t	33.6 t	33.8 t	33.6 t	29.4 t
C-12	75.7 d	76.1 d	76.8 d	76.8 d	74.4 d
C-13	44.6 d	45.2 d	44.1 d	44.2 d	45.9 d
C-14	50.9 s	51.6 s	50.3 s	50.3 s	52.9 s
C-15	35.0 t	35.8 t	34.5 t	34.5 t	31.4 t
C-16	25.3 t	26.1 t	25.0 t	25.0 t	27.6 t
C-17	52.2 d	52.9 d	51.7 d	51.8 d	52.5 d
C-18	16.1 q	16.9 q	16.3 q	16.1 q	15.4 q
C-19	19.7 q	20.7 q	19.7 q	19.9 q	20.2 q
C-20	75.1 s	75.8 s	74.6 s	74.6 s	73.6 s
C-21	25.6 q	26.4 q	26.5 q	26.6 q	26.8 q
C-22	44.2 t	44.9 t	43.1 t	43.0 t	41.1 t
C-23	123.0 d	123.7 d	121.9 d	122.0 d	123.0 d
C-24	141.2 d	142.0 d	142.4 d	142.4 d	142.2 d
C-25	70.4 s	71.2 s	70.8 s	70.7 s	69.7 s
C-26	29.2 q	30.0 q	30.0 q	30.0 q	30.7 q
C-27	29.1 q	29.9 q	30.0 q	30.0 q	30.7 q
C-28	113.2 t	114.1 t	113.5 t	113.2 t	114.0 t
C-29	23.3 q	24.1 q	23.6 q	23.5 q	23.4 q
C-30	16.2 q	17.1 q	16.4 q	16.5 q	17.4 q
C-1′	99.6 d	100.9 d	97.9 d	98.7 d	
C-2′	74.5 d	72.5 d	73.0 d	69.7 d	
C-3′	76.3 d	74.4 d	73.4 d	70.8 d	
C-4′	76.9 d	70.3 d	71.3 d	68.2 d	
C-5′	72.2 d	67.6 t	69.6 d	63.6 t	
C-6′	17.3 q		17.2 q		
OMe			51.3 q	51.1 q	51.4 q
Me (Ac)			20.8 q	20.7 q	20.8 q
			20.9 q	20.8 q	
			20.9 q	20.9 q	
C=O (Ac)			168.9 s	169.0 s	170.7 s
			169.7 s	170.3 s	
			170.5 s	170.7 s	

[a] Values are recorded in parts per million relative to TMS. Multiplicity is designated as follows: s, singlet; d, doublet; t, triplet; q, quartet. [b] Spectra were run in CD_3OD at 90.8 MHz; multiplicity was determined by the DEPT ^{13}C NMR experiment. [c] Spectrum was run in C_5D_5N at 22.6 MHz (Aoki *et al.*, 1988).

m, H-16a), 1.28 (3H, s, CH_3-26), 1.28 (3H, s, CH_3-27), 1.21 (1H, m, H-15b) 1.16 (3H, s, CH_3-21), 1.09 (3H, s, CH_3-19), 1.08 (1H, m, H-7b), 1.07 (3H, s, CH_3-30), 0.98 (3H, s, CH_3-18); ^1H NMR (300 MHz, C_5D_5N) δ 6.27 (1H, m, H-23), 6.03 (1H, d, J = 15.6 Hz, H-24), 4.97 (1H, s, H-28a), 4.96 (1H, s, H-28b), 4.84 (1H, d, J = 6.6, H-1′), 4.50 (1H, m, H-12), 4.49 (1H, m, H-2′), 4.25 (1H, d, H-5′a), 4.22 (1H, s, H-4′), 4.09 (1H, m, H-3′), 3.61 (1H, d, H-5′b), 1.85 (3H, s, CH_3-29), 1.57 (6H, s, CH_3-26 and CH_3-27), 1.50 (3H, s, CH_3-21), 1.23 (3H, s, CH_3-19), 1.03 (3H, s, CH_3-30), 0.94 (3H, s, CH_3-18); ^{13}C NMR data presented in Table 2; UV λ_{max} (MeOH) 207 nm (end absorption); IR υ_{max} (KBr) 3424, 2967, 2874, 1701, 1636, 1551, 1456, 1387, 1306, 1254, 1142, 1088, 1001, 893, 781, 654, 534 cm^{-1}; FABMS m/z $(M - H)^-$ 621, $(M - C_5H_8O_4)$; HRFABMS m/z found 621.40043, calcd for $C_{35}H_{57}O_9$, 621.40026.

Methylation and Acetylation of 2. An ethereal solution of CH_2N_2 was added to a methanolic solution of **2** (20 mg), with the product (about 20 mg) dissolved in pyridine (1 mL) and Ac_2O (1 mL), and allowed to stand at room temperature overnight. The solvent was then evaporated under nitrogen, and the product was purified by flash chromatography using $CHCl_3$–acetone (8:2) as the solvent system. Similar fractions were pooled to give a pure peracetate, methyl (23*E*)-(12*R*,20*S*)-12,20,25-trihydroxy-3,4-secodammara-4(28),23-dien-3-oate 12-*O*-α-(2′3′4′-*O*-triacetyl)-L-arabinopyranoside (**4**) (18 mg). Compound **4** exhibited the following data: short white needles; mp 87–91 °C; ^1H NMR (500 MHz, $CDCl_3$) δ 5.74 (1H, m, H-23), 5.70 (1H, m, H-24), 5.20 (1H, m, H-4′), 5.10 (1H, dd, J = 7.3, 10.1 Hz, H-2′), 5.01 (1H, dd, J = 3.4, 10.1 Hz, H-3′), 4.83 (1H, bs, H-28a), 4.67 (1H, bs, H-28b), 4.45 (1H, d, J = 7.3 Hz, H-1′), 4.01 (1H, dd, J = 13.2, 2.2 Hz, H-5′a), 3.95 (1H, dd, J = 10.9, 4.5, H-12), 3.70 (3H, s, OCH_3), 2.18 (3H, s, OAc), 2.03 (3H, s, OAc), 2.01 (3H, s, OAc), 1.74 (3H, s, CH_3-29), 1.35 (6H, s, CH_3-26 and CH_3-27), 1.17 (3H, s, CH_3-21), 1.07 (3H, s, CH_3-19), 1.01 (3H, s, CH_3-30), 0.91 (3H, s, CH_3-18); ^{13}C-NMR data presented in Table 2; IR υ_{max} (KBr) 3488, 2969, 2884, 1748, 1456, 1373, 1252, 1224, 1171, 1105, 1055 cm^{-1}; EIMS m/z (relative intensity) 762 (M^+, 0.02%), 726 (0.3), 663 (0.3), 486 (0.7), 468 (2), 451 (3), 387 (10), 369 (11), 343 (3), 305 (13), 259 (100), 231 (4), 199 (8), 157 (18), 139 (23).

Acid Hydrolysis of 1 and 2. Solutions of **1** or **2** were mixed with 0.5 N HCl and refluxed for 1 h. Each reaction mixture was diluted with water and extracted exhaustively with $CHCl_3$. The $CHCl_3$ layers were dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. Examination of each $CHCl_3$ layer by TLC showed a number of products, which were less polar than the starting materials. The aqueous layer was neutralized with sodium bicarbonate and allowed to dry at room temperature. TLC analysis of the residues using $CHCl_3$–MeOH–H_2O (6:4:1) showed the presence of D-quinovose and L-arabinose as the only sugars for **1** and **2**, respectively. These results were confirmed by GC/MS analysis of the trimethylsilyl derivatives of the sugars. The aqueous residues of **1** and **2** were individually dried in a vacuum desiccator, and in each case about 1 mg was heated with Sigma SIL-A (Sigma Chemical Co., St. Louis, MO) at 70 °C for 1 h. The products were analyzed by GC/MS. TMS-quinovose from **1** was identified by a direct comparison with authentic standard D-quinovose treated in the same manner under the same GC conditions, while TMS-arabinose from **2** was identified by direct comparison with authentic standard L-arabinose treated in the same manner under the same conditions.

Enzyme Hydrolysis of 1. Attempts were made to afford enzymatic hydrolysis of the sugars from the triterpene genin using various enzymes, including naringinase (Kitagawa *et al.*, 1981) and cellulase (Konoshima and Sawada, 1982). However, no observable product was formed in any case.

Acute Toxicity Studies. The initial 70% EtOH extract of *P. paliurus* and compounds **1** and **2** were tested for acute toxicity in male Swiss-Webster mice, administered by oral incubation at dose levels of 1 and 2 g/kg of body weight. The EtOH extract was dispersed in 1% sodium carboxymethylcellulose, and compounds **1** and **2** were dispersed in 10% EtOH and 1% hydroxypropylcellulose, with control groups of animals, treated with the appropriate vehicle alone, being included in each experiment. The procedures and protocols for toxicologic testing were followed as published previously (Choi *et al.*, 1989; Compadre *et al.*, 1987; Fullas *et al.*, 1991). Administration of both the plant extract and compounds **1** and **2** did not cause any mortalities, and body weights recorded on days 0 (prior to administration), 1, 3, 7, and 14 did not differ significantly for treated vs control animals.

Bacterial Mutagenesis Assays. The mutagenic potentials of the EtOH extract and compounds **1** and **2** were evaluated in the dose range 0.2–200 μg/mL according to established protocols employing *Salmonella typhimurium* strain TM677 (Fullas *et al.*, 1991; Pezzuto *et al.*, 1985). Briefly, each test substance was dissolved in DMSO and assayed in duplicate at the given concentration. Each duplicate was plated in triplicate, both in the presence and in the absence of 8-azaguanine (8-AG), and the data were averaged. A compound is regarded as possessing significant mutagenic activity if the induced mutant fraction is greater than 2 times the spontaneous mutant fraction. Neither the plant extract nor compound **1** or **2** was mutagenic, either in the presence or in the absence of a metabolic activating system (S9) derived from the livers of Aroclor 1254-pretreated rats.

Sensory Testing. The ammonium salts of compounds **1** (5 mg) and **2** (5 mg) were made by adding an excess amount of 1 N ammonium hydroxide to each compound. The resulting salts were dried under nitrogen, and their sweetness intensity was compared to that of sucrose by a small taste panel

consisting of three persons, in a manner previously described (Choi *et al.*, 1989; Compadre *et al.*, 1987; Fullas *et al.*, 1991).

RESULTS AND DISCUSSION

The stems and leaves of *P. paliurus* were extracted with 70% EtOH. This extract, determined not to be acutely toxic or mutagenic in preliminary safety tests, was judged to be distinctly sweet. The EtOH extract was then defatted with petroleum ether, and subsequent partitioning yielded three additional portions, namely, $CHCl_3$, BuOH, and H_2O. A sweet saponin, cyclocaryoside A [20,24-epoxydammarane (3β,12β,24*R*)-12-*O*-α-L-rhamnopyranosyl-25-hydroxyl-3-*O*-(5′-*O*-acetyl)-α-L-arabinofuranoside], was earlier isolated from the $CHCl_3$ extract of this same plant that was erroneously identified as *Cyclocarya paliurus* (Batal.) Iljinskaya (Yang *et al.*, 1992). An initial TLC inspection of the four extracts obtained by their chromogenic responses with acid visualization sprays (Wagner *et al.*, 1984) indicated that the $CHCl_3$ extract contained several saponins. Therefore, the $CHCl_3$ fraction of *P. paliurus* was purified further using VLC with a gradient of MeOH in $CHCl_3$, to obtain seven chromatographically distinct fractions. One of the more polar fractions was further purified using repeated Si gel flash chromatography followed by RP-18 LPCC to obtain the two major components of the fraction which have been named pterocaryosides A (**1**) and B (**2**). These compounds gave a positive Liebermann–Burchard color test for saponins (Wagner *et al.*, 1984) and also foamed when shaken with water.

The negative ion FABMS of **1** exhibited a pseudomolecular ion $(M - H)^-$ at *m/z* 635, with the molecular formula being established as $C_{36}H_{60}O_9$ from HRFABMS, suggesting that the compound is a triterpene saponin with one hexose unit. A DEPT experiment on **1** (see Table 2) displayed 8 methyl carbons, 9 methylene carbons, and 12 methine carbons, clearly differing from the previously characterized dammarane-type constituent of *P. paliurus*, cyclocaryoside A, characterized by Yang *et al.* (1992). The assignments of the ^{1}H- and ^{13}C-NMR spectra of **1** were facilitated by ^{1}H–^{1}H COSY and ^{1}H–^{13}C HETCOR NMR experiments (see Table 1). Along with these experiments, analysis of the ^{1}H–^{1}H HOHAHA NMR experiment (Bax and Davis, 1985) (see Table 1) suggested that the A-ring of this triterpene was not intact. The ^{1}H-NMR data for **1** indicated that the molecule contains a *trans* disubstituted double bond (H-23 δ 5.71 and H-24 δ 5.63, J = 15.5 Hz) and a terminal methylene [δ 4.69 (bs) and 4.84 (bs)]. The presence of a carboxylic acid functional group in the molecule of **1** was inferred from the ^{13}C-NMR chemical shift at 179.1 ppm. Seven degrees of unsaturation could be calculated from the molecular formula of **1**, with three being olefinic, one being glycosidic, and the remaining three suggestive of a tricyclic triterpene skeleton, with an acyclic side chain, such as can be found in the dammarane skeleton. A literature search on secodammarane compounds afforded information for the 3,4-secodammaranoid model compound, (23*E*)-(12*R*,20*S*)-12,20,25-trihydroxy-3,4-secodammara-4(28),23-dien-3-oate (**5**), which was isolated from the male flowers of *Alnus japonica* (Thunb.) Steudel (Betulaceae) (Aoki *et al.*, 1988). The ^{13}C-NMR data of **1** were very similar to those of **5**, except for the six additional glycosidic carbons (at 99.6, 76.9, 76.3, 74.5, 72.2, and 17.3 ppm) occurring in the former compound. The C-12 and C-20 chiralities of **1** were determined to be *R* and *S*, respectively, on the basis of comparison to the ^{13}C-NMR chemical shift data for **5** and analogous dammaranes (Aoki *et al.*, 1988; Baker *et al.*, 1976; Suga and Hirata, 1979), thus establishing the aglycon of this saponin as (23*E*)-(12*R*,20*S*)-12,20,25-trihydroxy-3,4-secodammara-4(28),23-dien-3-oic acid.

Using both acid- and enzyme-catalyzed methods, attempts were made to hydrolyze **1** to make spectroscopic measurements on the aglycon. However, neither of the enzymes employed, namely, naringinase (Kitagawa *et al.*, 1981) and cellulase (Konoshima and Sawada, 1982), resulted in the production of the genin. Also, even weak concentrations of mineral acid resulted in the formation of many side products due to the apparent acid lability of the triterpene moiety. Structural elucidation, therefore, relied upon spectroscopic data interpretation on the native natural product and on its methylated peracetylated derivative. Methylation and subsequent acetylation of **1** resulted in one major, less polar product, **3**. The ^{13}C-NMR data obtained for **3** showed clearly seven additional resonances (see Table 2), with one at δ 51.3 (q, OMe), confirming the presence of a carboxylic acid on **1**, and the six other new acetate resonances confirming the presence of three secondary hydroxyl groups in the molecule of **1** at C-2′, C-3′, and C-4′. The two tertiary hydroxyl groups, C-20 and C-25, were not acetylated, as has been previously reported for other compounds based on this secodammarane triterpenoid skeleton (Aoki *et al.*, 1988). This acetylation pattern indicated that **1** was glycosylated at position C-12, since the free C-12 hydroxyl group has been acetylated previously for other secodammarane triterpenoids (Aoki *et al.*, 1988). The position of the sugar moiety was confirmed by the selective INEPT (SINEPT) NMR technique (Bax, 1984; Kim and Kinghorn, 1987) in which irradiation of H-12 at δ 4.07 ($^3J_{CH}$ = 5 Hz) showed selective enhancement of C-1′ at δ 99.6 and irradiation of H-1′ at δ 4.35 ($^3J_{CH}$ = 5 Hz) resulted in the selective enhancement of C-12 at δ 75.7. Examination of the ^{1}H-NMR spectrum for H-12 (ddd, J = 10.7, 10.7, and 4.1 Hz) was used to determine the α configuration at this position and also to confirm the absolute configuration of this dammarane C-12 as *R*.

The loss of 146 amu from the molecular ion in the FABMS suggested the glycosidic portion of **1** to be a deoxyhexose, and a ^{1}H–^{1}H-HOHAHA NMR relay from δ 3.24 (H-5′) to the methyl doublet at δ 1.25 (CH_3-6′) confirmed the presence of a glycosidic methyl substituent. After acid hydrolysis was performed on **1**, the only sugar detected by TLC and GC/MS was D-quinovose. The configuration of the anomeric carbon of the hexose was determined to be β by the coupling constant of H-1′ to H-2′ (J = 7.5 Hz), and the D-conformation of quinovose was inferred according to that most commonly found in nature (Harada *et al.*, 1983). When the ^{1}H NMR of compound **1** was measured in C_5D_5N, the J values of the glycosidic protons were better resolved and showed clearly the *trans*-axial couplings (H-2′, δ 4.17, t, J = 8.3 Hz; and H-3′, δ 4.07, t, J = 8.5 Hz), thus confirming the presence of β-D-quinovopyranoside (Angyal and Pickles, 1972; Gorin and Mazurek, 1975). Therefore, the structure of pterocaryoside A was established as (23*E*)-(12*R*,20*S*)-12,20,25-trihydroxy-3,4-secodammara-4(28),23-dien-3-oic acid 12-*O*-β-D-quinovopyranoside (**1**).

A comparison of the ^{13}C-NMR data of **1** to those of **2** (Table 2) revealed that the two compounds are closely structurally related, except that **2** exhibited five glycosidic carbon signals at δ 100.9, 74.4, 72.5, 70.3, and 67.6,

indicating the presence of a pentose rather than a hexose sugar on a common genin. The FABMS of **2** in the negative ion mode showed a loss of 132 amu from the molecular ion, indicative of the loss of a pentose moiety, while the DEPT NMR experiment showed C-5′ to be a triplet, showing the sugar to be a pyranose rather than a furanose. The ^{13}C-NMR data for the pentose were closely comparable to the literature values reported for arabinose (Mizutani *et al.*, 1980). Furthermore, after acid hydrolysis, the only sugar detected by TLC and GC/MS was L-arabinose. From the coupling constant of H-1′ and H-2′ (J = 6.5 Hz), the α-anomeric configuration of the sugar was evident (Mizutani *et al.*, 1980). Methylation and subsequent acetylation of **2** resulted in **4**, with acetylation on the three secondary hydroxyl groups, C-2′, C-3′, and C-4′, while neither of the tertiary hydroxyls, C-20 and C-25, was acetylated. Again, the position of the sugar moiety could be deduced as C-12 from the acetylation pattern (Aoki *et al.*, 1988), and this position of glycosylation was confirmed by a SINEPT NMR experiment conducted in the same manner as decribed for **1**. In the ^{1}H-NMR spectrum of **4**, the glycosidic protons were better resolved (H-2′, δ 5.10, dd, J = 7.3, 10.1 Hz; and H-3′ δ 5.01, dd, J = 3.4, 10.1 Hz), confirming the presence of the α-L-arabinopyranoside (Durette and Horton, 1971). The structure of pterocaryoside B was therefore established as (23*E*)-(12*R*,20*S*)-12,20,25-trihydroxy-3,4-secodammara-4(28),-23-dien-3-oic acid 12-*O*-α-L-arabinopyranoside (**2**).

Safety evaluations on **1** and **2** showed the compounds were not toxic in preliminary acute toxicity tests in mice (Compadre *et al.*, 1987; Medon *et al.*, 1982; Nanayakkara *et al.*, 1988) and were not mutagenic in forward mutation assays using *S. typhimurium* strain TM677 (Compadre *et al.*, 1987; Medon *et al.*, 1982; Nanayakkara *et al.*, 1988; Pezzuto *et al.*, 1985). The water-soluble ammonium salts of **1** and **2** were prepared for a small human taste panel to evaluate the sweetness potency of these saponins (Choi *et al.*, 1989; Compadre *et al.*, 1987). The panel rated the ammonium salt of **1** as about 50 times sweeter than 2% sucrose, while that of **2** was rated as about 100 times sweeter than 2% sucrose. With both compounds, the onset of sweetness was instantaneous, but both also had a persistent, mildly bitter, off-taste. Compounds **1** and **2** represent the first and second examples of highly sweet secodammarane-type saponins to have been identified and thus represent a new sweet compound chemotype. Interestingly, an number of other dammarane-type saponins have been found to be sweetness inhibitors rather than being sweet *per se*, such as ziziphin from the Chinese jujube tree, *Ziziphus jujuba* P. Miller (Rhamnaceae) (Kurihara *et al.*, 1988), and hodulosides I–V from *Hovenia dulcis* Thunb. (Rhamnaceace) (Yoshikawa *et al.*, 1992). Since **1** and **2** differ only in their glycosidic units, it can be inferred that altering the saccharide unit will affect the perceived sweetness of the compound, and it is possible that derivatives with longer or branched sugar units might be considerably more potent as sweetening agents than either **1** or **2**, as has been found in other sweet glycosides such as the steviol bisglycosides (Darise *et al.*, 1984) and analogs of baiyunoside (Yamada and Nishizawa, 1992). Cyclocaryoside A, previously isolated from *P. paliurus* and reported to be 200 times sweeter than sucrose (Yang *et al.*, 1992), was not obtained in the present investigation.

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