Picrasinoside H, a New Quassinoid Glucoside, and Related Compounds from the Stem Wood of Picrasma ailanthoides

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PICRASINOSIDE H, A NEW QUASSINOID GLUCOSIDE, AND RELATED COMPOUNDS FROM THE STEM WOOD OF *PICRASMA AILANTHOIDES*

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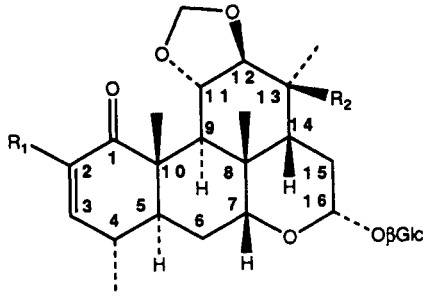
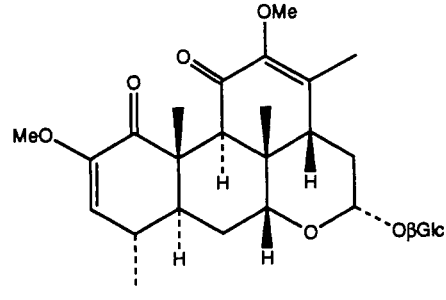
ABSTRACT.—A new quassinoid glucoside, picrasinoside H [**1**], and five known quassinoids have been isolated from the stem wood of *Picrasma ailanthoides*. The structures of these quassinoids were elucidated on the basis of spectral evidence.

Takahashi and co-workers (1–6) and Hikino *et al.* (7) have reported the isolation of more than 20 quassinoids from *Picrasma ailanthoides* Planchon (Simaroubaceae). We described recently (8) the isolation and structural elucidation of seven new quassinoid glucosides, picrasinosides A, B [**2**], C [**3**], D [**4**], E [**5**], F, and G [**6**], as well as two new quassinoid hemiacetals, pirasinols A and B, from the stem bark of *P. ailanthoides*. Further study on the stem wood of this plant has led to the isolation and characterization of a new quassinoid glucoside, picrasinoside H [**1**], along with the five known



1 R_1=OMe, R_2=OAc
3 R_1=OMe, R_2=H



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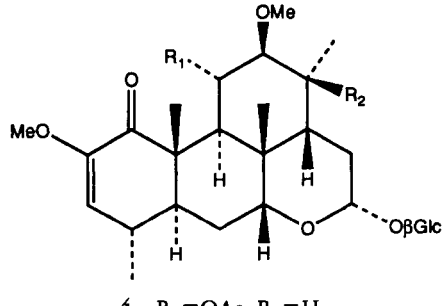

4 R_1=OAc, R_2=H
5 R_1=OAc, R_2=OH
6 R_1=R_2=OH

compounds picrasinosides B [**2**], C [**3**], D [**4**], E [**5**], and G [**6**]. We report herein on the results of this study.

Compounds **1–6** were isolated as colorless amorphous solids. Compounds **2–6** were identified as picrasinosides B, C, D, E, and G, respectively, by comparing their R_f and Rt values, as well as ir spectra, with the authentic compounds that were isolated previously from the stem bark of the same plant (8). The identity was also substantiated by their ^{1}H- and ^{13}C-nmr spectral evidence (Tables 1 and 2) except for **4**, which was isolated only in trace amounts. Compound **1** was assumed to be a new quassinoid because its ir, ^{1}H-nmr, and ^{13}C-nmr spectra did not coincide with any one of the quassinoids **2–6** mentioned above.

Picrasinoside H [**1**] has the empirical formula $C_{30}H_{44}O_{13}$ as determined by hrms and fdms. It contains one glucosyl and one acetyl group as evidenced by mass peaks at *m/z* 432.2150 $[M-C_6H_{12}O_6]^+$ and 372.1917 $[M-C_6H_{12}O_6-C_2H_4O_2]^+$.

A comparison of the ^{13}C-nmr spectra (Table 2) of **1**, **2**, **3**, **5**, and **6** indicated that the signals for their C-1–C-10 and the sugar moiety are nearly identical. However, the signals for C-11–C-13 are considerably different between **1** and **2**, **3**, **5**, and **6**. On the other hand, the ^{1}H-nmr spectrum (Table 1) of **1** showed, in addition to the presence of the aforementioned acetoxy group at δ 2.07, a methylenedioxy moiety at δ 5.20 and 5.41. Because the methylenedioxy signals appeared at a somewhat lower field compared to those of **3**, which were seen at δ 5.13 and 5.32, it suggests that **1** possesses the acetoxy group at C-13. This suggestion is also supported by the downfield shift of the 13-Me at δ 1.70, which is due to its attachment to a carbon bearing an acetoxy group, as compared to the methyl groups at the OH-bearing C-13 of **5** and **6**, which appear at the higher field of δ 1.54 and 1.45, respectively. The foregoing evidence, coupled with the co-occurrence of **1** and **2–6** in the same plant, led to the assignment of **1** for picrasinoside H.

TABLE 1. The ^{1}H-nmr Spectra of **1**, **2**, **3**, **5**, and **6**.

Proton	Compound				
	1	**2**	**3**	**5**	**6**
2-OMe	3.49 s	3.48 s	3.46 s	3.51 s	3.46 s
H-3	5.15 d (2)	5.20 d (2.4)	5.12 d (2.4)	5.05 d (1.7)	5.26 d (2.5)
4-Me	0.80 d (7)	0.83 d (6.5)	0.79 d (7)	0.79 d (6)	0.80 d (6.5)
H-7	3.22 brs	3.33 brs	3.25 brs	3.24 brs	3.18 brs
8-Me	1.24 s	0.96 s	1.03 s	1.17 s	1.18 s
H-9	3.16 d (11.5)	3.49 s	3.10 d (11.5)	3.29 d (11.5)	2.85 d (11.2)
10-Me	1.44 s	1.61 s	1.39 s	1.42 s	1.47 s
H-11	4.29 m	—	3.65 dd (11, 8.5)	6.05 dd (9.5, 11.5)	4.40 m
H-12	3.75 d (9.5)	—	3.48 dd (11, 8.5)	3.39 d (9.5)	3.09 d (9)
12-OMe	—	3.73 s	—	3.46 s	3.73 s
13-Me	1.70 s	1.73 s	0.91 d (6.5)	1.54 s	1.45 s
H-15α	1.61 m	1.66 m	1.62 m	1.65 m	1.67 m
H-15β	1.86 m	1.86 m	1.86 ddd (14, 3, 3)	1.85 m	1.81 dd (14, 3)
H-16	5.17 dd (1.5, 10)	5.28 dd (2, 9.8)	5.19 dd (3.4, 8.8)	5.20 d (6.8)	5.18 d (9)
H-1′	5.40 d (7.5)	5.41 d (7.5)	5.39 d (7.6)	5.41 d (8)	5.39 d (7.8)
H-2′	4.11 t (7.5)	4.13 t (7.5)	4.11 t (7.6)	4.13 brs	4.12 brs
OAc	2.07 s	—	—	2.10 s	—
-OCH_2O-	5.20 s	—	5.13 d (1)	—	—
	5.41 s	—	5.32 d (1)	—	—

TABLE 2. The ^{13}C-nmr Spectra of **1**, **2**, **3**, **5**, and **6**.

Carbon	Compound				
	1	**2**	**3**	**5**	**6**
C-1	198.7	198.6	198.5	200.3	206.6
C-2	149.1	148.8	150.0	149.2	148.3
2-OMe	54.8	54.9	54.4	54.6	54.6
C-3	115.2	116.6	115.0	113.5	118.2
C-4	32.3	31.6	31.9	31.9	32.0
4-Me	19.2	19.3	18.9	19.1	18.9
C-5	44.3	44.4	44.0	45.1	44.4
C-6	32.1	33.3	28.6	31.4	31.5
C-7	77.3	77.7	78.2	77.9	77.8
C-8	39.9	38.7	38.8	37.9	38.0
8-Me	23.0	21.7	21.9	29.9	26.3
C-9	38.1	46.9	36.8	37.6	39.2
C-10	47.9	47.3	47.9	47.4	48.6
10-Me	13.0	13.2	12.8	12.4	12.6
C-11	75.2	193.5	78.8	72.1	71.7
C-12	84.5	148.6	82.3	87.7	90.4
12-OMe	—	59.4	—	61.5	62.1
C-13	83.9	118.6	33.0	76.4	76.1
13-Me	22.6	15.4	14.6	23.4	23.6
C-14	47.5	49.6	47.0	52.0	52.0
C-15	26.0	26.2	25.9	25.5	25.3
C-16	99.1	98.8	99.8	99.1	99.3
C-1′	100.7	101.4	100.6	100.6	100.7
C-2′	75.1	75.2	74.8	74.9	75.0
C-3′	78.9	79.0	78.5	78.5	78.6
C-4′	71.5	71.7	71.4	71.4	71.4
C-5′	78.3	78.5	78.0	78.0	78.1
C-6′	62.7	63.0	62.6	62.6	62.6
OAc	170.0	—	—	170.4	—
	21.4			21.3	
-OCH_2O-	95.9	—	95.2	—	—

The stereochemistry of **1** was elucidated based on ^{1}H-^{1}H COSY and ROESY (Rotating Frame Nuclear Overhauser Effect Spectroscopy) nmr analyses. The ^{1}H-^{1}H COSY spectrum of **1** showed significant correlation between pairs of groups, such as H-4 and 4-Me, H-9 and H-11, H-11 and H-12, H-14 and H-15, H-15 and H-16, and H-1′ and H-2′. The ROESY spectrum showed a short distance between the following pairs of groups: 2-OMe and H-3, H-3 and 4-Me, H-4 and 4-Me, H-4 and 10-Me, H-7 and 8-Me, H-7 and H-14, H-7 and H-16, 8-Me and H-11, 8-Me and H-14, H-9 and H-12, H-9 and H-15α, 10-Me and H-11, H-12 and H-13, H-14 and H-15β, H-14 and H-16, and H-15β and H-16. Thus, the conformation of each proton and methyl group in **1** was determined as shown in Figure 1.

Because numerous quassinoids are known to be potent cytotoxic agents (9), a cytotoxicity screening of compounds **1–6** against KB, TE-671, A-549, HCT-8, RPMI, and P-388 tumor cell lines was carried out. The lack of significant cytotoxicity of **1–6** might be due to the absence of a lipophilic side chain as observed in many cytotoxic quassinoids, such as brusatol and bruceantin (9).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on an MRK air-bath-type melting point apparatus and were uncorrected. Specific rotations were obtained on a YANAKO OR-

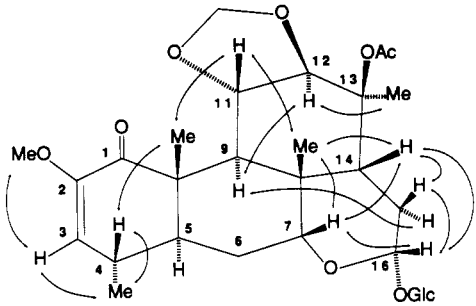

FIGURE 1. The nOe among protons and methyl groups of **1**.

50D polarimeter (L = 0.1 dm). Ir and uv spectra were recorded on a JASCO IR-810 spectrometer and a Hitachi 320-S spectrometer, respectively. ^{1}H- and ^{13}C-nmr spectra were determined on a VARIAN VXR-500 (499.84 MHz for ^{1}H nmr and 125.70 MHz for ^{13}C nmr) in C_5D_5N, using TMS as an internal standard. The results are shown in Tables 1 and 2, respectively. Mass spectra were recorded on a Hitachi M80 instrument. Si gel (Merck, type 60, 70–230 mesh) was used for cc. Precoated Si gel plates (Merck, $60F_{254}$) of 0.25 mm thickness were used for analytical tlc, and plates of 1 mm and 2 mm thickness were used for preparative tlc. Detection of components was made by using a uv lamp. Analytical hplc was performed on a Waters Associates or TOSOH liquid chromatograph equipped with a uv detector at 254 nm and reversed-phase columns (Radial Pak C_{18} and/or TSK-gel ODS-80T_M), using mixed solvents of MeOH-H_2O (1:1) and MeOH-H_2O (4:6). Preparative hplc was carried out on a Gilson liquid chromatograph equipped with reversed-phase columns (M&S PACK C_{18}A and/or Dynamax-60A) at 254 nm. The solvents used for analytical hplc were also used for preparative hplc.

PLANT MATERIAL AND EXTRACTION.—The stem of *P. ailanthoides* was procured and identified by Professor K. Kondo, Laboratory of Plant Chromosome and Gene Stock, Faculty of Science, Hiroshima University. A voucher specimen was deposited in the above department. Half-dried stem of *P. ailanthoides* was separated into bark and wood. The wood (40 kg) was shattered into small chips and was soaked in MeOH (90 liters) at room temperature for a week (2×) to make a MeOH extract. The fatty substance in the MeOH extract was removed by hexane extraction (2×), and the material used for this investigation was obtained by $CHCl_3$ extraction (3×), as a brown resinous substance (143 g).

SEPARATION BY COLUMN CHROMATOGRAPHY.—The resinous substance (100 g) was chromatographed on a Si gel column and eluted with a mixed solvent of $CHCl_3$-MeOH-H_2O (50:14:3) (lower layer, solvent A) and then MeOH to afford 31 fractions. Tlc and hplc analyses of all of these fractions indicated that fractions 16–19 contain quassinoid glycosides.

ISOLATION OF PICRASINOSIDES H [**1**] AND D [**4**].—Fractions 16 (5.47 g) and 17 (5.60 g) were combined and subjected to preparative Si gel tlc (solvent A) to afford a pale yellow solid whose R_f value is 0.54. The solid was subjected to preparative hplc [column Dynamax-60A, solvent MeOH-H_2O (4:6)] to give a colorless amorphous solid (33.5 mg) which was a mixture of two compounds, according to analytical hplc. Careful repeated preparative hplc of these two compounds led to the isolation of picrasinoside H [**1**] (12.7 mg, 0.000045%) and picrasinoside D [**4**] (2.9 mg, 0.00001%) both as colorless amorphous solids.

PICRASINOSIDE H [**1**].—Colorless amorphous solid: mp 270° (dec); $[\alpha]^{20}D$ +20.6° (c = 0.19, pyridine); uv λ max (MeOH) 261 (ε 3820) nm; ir (KBr) 3350 (OH), 1735 (acetate C=O), 1700 (α,β-unsaturated C=O) cm^{-1}; ^{1}H nmr see Table 1; ^{13}C nmr see Table 2; fdms *m/z* $[M+K]^+$ 651, $[M+Na]^+$ 635, $[M+1]^+$ 613; eims *m/z* $[M]^+$ 612 (4.7%), $[M-C_6H_{11}O_6]^+$ 433 (21%), $[M-C_6H_{12}O_6]^+$ 432 (8.3%), $[M-C_6H_{12}O_6-C_2H_4O_2]^+$ 372 (17.6%); hreims *m/z* $[M]^+$ 612.2784 (calcd for $C_{30}H_{44}O_{13}$, 612.2779), $[M-C_6H_{11}O_6]^+$ 433.2256 (calcd for $C_{24}H_{33}O_7$, 433.2224), $[M-C_6H_{12}O_6]^+$ 432.2150 (calcd for $C_{24}H_{32}O_7$, 432.2146), $[M-C_6H_{12}O_6-C_2H_4O_2]^+$ 372.1917 (calcd for $C_{22}H_{28}O_5$, 372.1935).

PICRASINOSIDE D [**4**].—Colorless amorphous solid: mp 144–144.5°; uv λ max (EtOH) 262 (ε 3800) nm; ir (KBr) 3400 (OH), 1735 (acetate C=O), 1690 (α,β-unsaturated C=O), 1635 (C=C) cm^{-1}; fdms *m/z* $[M+K]^+$ 637, $[M+Na]^+$ 621.

ISOLATION OF PICRASINOSIDES B [**2**], C [**3**], D [**4**], E [**5**], AND G [**6**].—Fraction 18 (4.91 g) was subjected to preparative tlc (solvent A) to give a major fraction as a pale brown residue (2348 mg, R_f ca. 0.35–0.40) which showed two major peaks in the analytical hplc. The residue was subjected to a prepara-

tive hplc to give picrasinoside B [**2**] (130 mg) and picrasinoside C [**3**] (124 mg), both as colorless amorphous solids. Fractions 19 (3.31 g) and 20 (1.56 g) were also treated in the same way as fraction 18. Preparative tlc (solvent A) of each fraction gave each major fraction as a pale brown residue [1868 mg, R_f ca. 0.3–0.35 (Residue A) and 1009 mg, R_f ca. 0.25–0.30 (Residue B), respectively]. Analytical hplc of both residues showed that residues A and B had three and two major peaks, respectively. These residues were further subjected to repeated preparative hplc to give picrasinosides B [**2**] (382 mg), E [**5**] (300 mg), and G [**6**] (171 mg) from residue A, and picrasinosides E [**5**] (91 mg) and G [**6**] (73 mg) from residue B. All of them were isolated as colorless amorphous solids.

The combined yields were: picrasinoside B [**2**] (513 mg, 0.0018%), C [**3**] (124 mg, 0.00045%), E [**5**] (391 mg, 0.0017%), and G [**6**] (245 mg, 0.00088%).

PICRASINOSIDE B [**2**].—Colorless amorphous solid: mp 152–153°; uv λ max (EtOH) 251 (ε 10090) nm; ir (KBr) 3400 (OH), 1735 (acetate C=O), 1690 (α,β-unsaturated C=O), 1635 (C=C) cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2; fdms *m/z* $[M-1+Na]^+$ 574, $[M]^+$ 552, $[M-1]^+$ 551.

PICRASINOSIDE C [**3**].—Colorless amorphous solid: mp 162–164°; uv λ max (EtOH) 258 (ε 4420) nm; ir (KBr) 3425 (OH), 2740 (methylenedioxy ring), 1700 (α,β-unsaturated C=O), 1635 (C=C) cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2; fdms *m/z* $[M-1+Na]^+$ 576, $[M]^+$ 554, $[M-1]^+$ 553.

PICRASINOSIDE E [**5**].—Colorless amorphous solid: mp 162–163°; uv λ max (EtOH) 258 (ε 4140) nm; ir (KBr) 3430 (OH), 1720 (acetate C=O), 1700 (α,β-unsaturated C=O), 1635 (C=C) cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2; fdms *m/z* $[M+K]^+$ 653, $[M-1+K]^+$ 652, $[M+Na]^+$ 637, $[M-1+Na]^+$ 636, $[M]^+$ 614, $[M-1]^+$ 613.

PICRASINOSIDE G [**6**].—Colorless amorphous solid: mp 162–164°; uv λ max (EtOH) 269 (ε 5950) nm; ir (KBr) 3450 (OH), 1670 (α,β-unsaturated C=O), 1635 (C=C) cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2.

BIOLOGICAL ACTIVITY.—The in vitro cytotoxicity assay was carried out according to procedures described in Geran *et al.* (10) and Ferguson *et al.* (11). The assay against KB (nasal pharyngeal carcinoma), TE-671 (human medulloblastoma), A-549 (human lung carcinoma), HCT-8 (human colon carcinoma), RPMI (human melanoma), and P-388 (murine leukemia) tumor cells was based on a method reported in Lee *et al.* (12). None of the quassinoids **1–6** demonstrated significant ($ED_{50} \leqq 4$ μg/ml) cytotoxicity against the tumor cell lines mentioned previously.

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