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A TRIFOLIRHIZIN MALONATE FROM *SOPHORA FLAVESCENS* VAR. *ANGUSTIFOLIA* AND ITS STABILITY

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Key Word Index—*Sophora flavescens* var. *angustifolia*; Leguminosae; callus culture; trifolirhizin 6″-malonate; maackiain.

Abstract—Trifolirhizin 6″-*O*-malonate [(−)-maackiain 3-*O*-(6-*O*-malonyl-β-D-glucopyranoside)] was isolated both from the roots and callus cultures of *Sophora flavescens* var. *angustifolia*, and identified by HPLC, MS and NMR.

INTRODUCTION

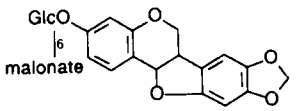
Dry roots of *Sophora flavescens* Solander ex Aiton var. *angustifolia* Kitagawa (Leguminosae) have been reported to contain prenylated flavonoids (kurarinone, kuraridine, etc) pterocarpans (maackiain, trifolirhizin) [1], and quinolizidine alkaloids [2]. Recently, we have found that callus cultures of *S. flavescens* var. *angustifolia* also produced prenyl flavonoids and pterocarpans [3]. In the latter study, an unknown compound which was easily changed to trifolirhizin during Soxhlet-extraction was observed. We describe the characterization and the stability of the compound.

RESULTS AND DISCUSSION

Compound **1** exhibited $[M-H]^-$ at *m/z* 531 in the negative FAB-MS, which is bigger than that of trifolirhizin by *m/z* 86. A triplet at δ41.3 and two singlets at δ166.7 and 167.9 in the ^{13}C NMR indicate that **1** contains a malonyl group. Its location on a glucose moiety is indicated because the difference of −190 between the two fragments at *m/z* 487 and 297 $[aglycone-H]^-$ in the FAB-MS shows a loss of a glucose (−162) bearing a formyl group (−29) released by elimination of CH_2–CO_2H from the malonyl group. The chemical shift of C-6′ (δ64.1) is lower than that of trifolirhizin (δ61.8) by 2.3 ppm, which indicates that the malonyl group is attached at C-6 of the glucose through an ester bond. The CD result agreed well with that of (−)-maackiain. Therefore, **1** was characterized as (−)-maackianin 3-*O*-(6-*O*-malonyl-β-D-glucopyranoside) (trifolirhizin 6″-*O*-malonate).

The heat stability of **1** was examined and the results are shown in Fig. 1. Compound **1** (10 μmol) dissolved in methanol (5 ml) was quantitatively hydrolysed to trifolirhizin by reflux within 15 hr. On the other hand, maackiain was not detected after refluxing for 15 hr, which showed that trifolirhizin itself is heat stable.

Lyophilized roots and callus tissues of *S. flavescens* var. *angustifolia* were extracted with methanol by ultrasonication and subjected to HPLC analysis. Pterocarpan in the roots accumulated mainly as a malonylated glycoside,



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but maackiain was not detected (Table 1). Trifolirhizin was always present in the roots and accounted for 5–10% of the total pterocarpan, but it may be an artifact derived from **1** during extraction. In callus tissues, **1** was a main component as in intact roots. After Soxhlet extraction for 4 hr, **1** was completely hydrolysed to trifolirhizin. It is noteworthy that maackiain was always detected in the callus tissues in the range of *ca* 5 50% of the total pterocarpan derivatives (Table 1) [3], which may be caused by the different activities of glucosyltransferase and glucosidase. In callus tissues, the former activity may be lower, while the latter one stronger than those in intact plants.

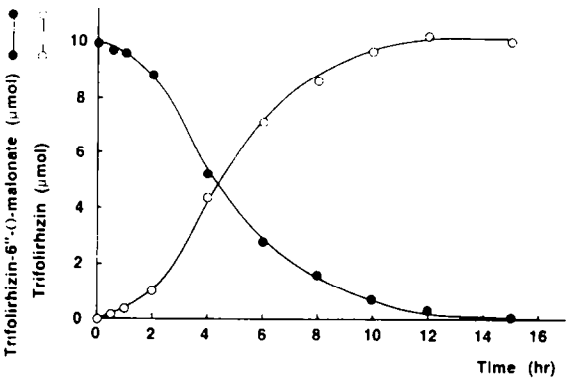

Fig. 1. Stability of trifolirhizin 6″-*O*-malonate against heating.

Table 1. Contents (μmol g^{-1} dry wt) of maackiain, trifolirhizin and its malonyl ester in roots and callus cultures of *Sophora flavescens*

	Maackiain	Trifolirhizin	Trifolirhizin 6″-*O*-malonate
Roots (ultrasonication)†	n.d.*	0.248	3.247
Callus cultures (ultrasonication)	5.046	0.426	5.441
Callus cultures (Soxhlet-extraction)‡	4.921	5.428	n.d.

*Not detected.

†Lyophylized samples (100 mg) were extracted with 3 ml methanol (× 3) by ultrasonication for 15 min.

‡Lyophylized samples (100 mg) were Soxhlet-extracted with 10 ml MeOH for 4 hr.

O-Malonyl glycosides of chalcones [4], flavones [5], flavonols [5], anthocyanidins [6] and isoflavones [7], which, have been isolated recently are regarded as the final products in flavonoid biosynthesis and seem to be accumulated in vacuoles by specific transport systems existing in the tonoplasts [8]. In the present study, we demonstrated that a pterocarpan glucoside also accumulated as a malonate ester both in the intact roots and callus cultures of *S. flavescens* var. *angustifolia*.

Pterocarpans such as maackiain and trifolirhizin are toxic against microorganisms [9] and fungi [1], and presumably are not phytotoxic. Therefore, trifolirhizin in the cells of *Sophora* species may be esterified with malonic acid in order to increase its solubility in water and aid its transport into the cell vacuoles as a stable and detoxified metabolite. On the other hand, the prenylated flavonoids such as sophoraflavanone G and kurarinone in *S. flavescens* var. *angustifolia* callus and roots were heat-stable and unchanged by Soxhlet extraction (data not shown). Furthermore, we could not detect their glucosides in the callus tissues of *S. flavescens* var. *angustifolia* [3]. These results suggest that the prenylated flavonoid compounds exist only as aglycones in the tissues, and are accumulated in a different manner from trifolirhizin 6″-*O*-malonate.

EXPERIMENTAL

Plant materials. Roots of *S. flavescens* var. *angustifolia* were collected in Kawashima-cho, Gifu, Japan in May 1990. The origin and the subculturing of callus cultures of the plant were described previously [3].

Extraction and isolation of compound **1**. Lyophilized callus (100 g) was extracted with Me_2CO by ultrasonication. The concd extract was dissolved in H_2O and re-extracted successively with $CHCl_3$ and EtOAc. The EtOAc extract (2.1 g) was chromatographed on a silica gel column (4 cm i.d. × 40 cm) (silica gel BW-300, Fuji Devison Chem., Japan) using $CHCl_3$ and MeOH as solvents. The fr. eluted with 10% MeOH in $CHCl_3$ was evapd to dryness, and the residue dissolved in MeOH was run on a Sephadex LH-20 column (2 × 30 cm) (MeOH as a solvent), to give **1** as a powder after recrystallization from EtOH (125 mg). Compound **1** was also isolated from the dry roots (100 g) of *S. flavescens* by the same procedures as above (35 mg).

Compound **1** [(−)-maackiain 3-*O*-(6-*O*-malonyl-β-D-glucopyranoside)]. Powder. Negative ion FAB-MS: 531 $[M-H]^-$, 487, 297. UV λ nm: 285, 310. CD (MeOH): $\Delta\varepsilon_{360}+3.6$. ^{1}H NMR ($d_6$-DMSO) δ 3.15–3.63 (*m*, sugar protons and H-6b,6a), 4.11 (1H, *m*, H-6a), 4.30–4.41 (2H, *m*, Glc-6-CH_2), 5.57 (1H, *d*, $J=7$ Hz, H-11a), 5.98 (2H, *m*, OCH_2O), 6.57 (2H, *br s*, H-4 and H-10), 6.73 (1H, *dd*, $J=8$, 2 Hz, H-2), 7.01 (1H, *s*, H-7), 7.41 (1H, *d*, $J=8$ Hz, H-1). ^{13}C NMR (d_6-DMSO) δ 132.9 (C-1), 115.3 (C-2), 159.6 (C-3), 105.2 (C-4), 157.3 (C-4a), 66.9 (C-6), 40.3 (C-6a), 119.3 (C-6b), 106.4 (C-7), 142.2 (C-8), 148.6 (C-9), 96.5 (C-10), 153.6 (C-10a), 77.6 (C-11b), 101.5 (OCH_2O), 102.1 (C-1′), 73.1 (C-2′), 76.3 (C-3′), 69.8 (C-4′), 73.7 (C-5′), 64.1 (C-6′), 166.8, 167.9 (CO), 41.3 (CH_2).

Quantitative analysis of pterocarpans. The lyophylized samples (100 mg) were extracted with MeOH by ultrasonication (15 min × 3). After centrifugation, each supernatant was combined and subjected to reversed-phase HPLC. HPLC analysis was carried out on Capcellpack C_{18} AG-120A (Shiseido, Japan), with 1% HOAC containing MeCN–H_2O gradient of 35–50% MeCN in 10 min, then to 65% MeCN in another 15 min at a flow rate of 1 ml min^{-1} with detection at 294 nm. The quantities of the pterocarpans were calculated from the peak area of the chromatogram.

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