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# TRIFOLIRHIZIN 6′-MONOACETATE, A NEW GLYCOSIDE FROM THE ROOTS OF *SOPHORA SUBPROSTRATA**

MANKI KOMATSU, ICHIRO YOKOE, YOSHIAKI SHIRATAKI and JULING CHEN

Faculty of Pharmaceutical Sciences, Josai University, Keyakidai 1-1, Sakado Machi, Saitama, Japan

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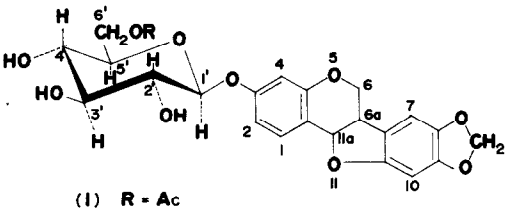
**Key Word Index**—*Sophora subprostrata*; Leguminosae; (−)-pterocarpin; (−)-maackiain; 7,4′-dihydroxyflavone; trifolirhizin 6′-monoacetate; trifolirhizin; sitosterol-β-D-glucoside.

Previous papers [2–8] have reported the isolation and the structural elucidation of eleven new flavonoids from the root of *S. subprostrata* Chun et T. Chen, the Chinese crude drug Guang-Dou-Gen. Further studies on the constituents of this drug resulted in the isolation of a further compound from the EtOAc soluble fraction of the methanolic extract. The present paper deals with the elucidation of the structure of this compound.

The compound (**1**) was obtained as colorless needles, mp 223–225°, $M^+ = 488$, $C_{24}H_{24}O_{11}$, $[\alpha]_D^{22} - 175°$ (AcOH). It gave absorption bands of hydroxyl and ester in IR and the UV suggested a pterocarpan structure. NMR of **1** (TMSi ether, in $CCl_4$) exhibited one acetyl group [$\delta$ 2.02 (3H, *s*, −OAc)], anomeric proton [$\delta$ 4.77 (1H, *d*, *J* 7Hz, $C_1'$ − H)] and a methylenedioxy group [$\delta$ 5.87 (2H, *d*, *J* 1Hz, –O–$CH_2$–O–)].

Saponification of **1** with 1% KOH afforded trifolirhizin, $C_{22}H_{22}O_{10}$, mp 145° (Decomp), identified by comparison with an authentic sample. Hydrolysis of **1** with β-glucosidase gave (−)-maackiain and D-glucose.




(I) R = Ac

trifolirhizin : R = H

From these data, compound (**1**) was considered as trifolirhizin monoacetate. The position of acetyl group on the glucose moiety was shown to be at C-6 as follows: acetylation of one hydroxyl at C-2, C-3 or C-4 of glucose gave in the NMR one proton shifted downfield by 1.0–1.2 ppm, whereas substitution at C-6 shifted two protons downfield by 0.5 ppm [9]. In the case of **1**, two protons were shifted from 3.7 to 4.2 ppm and hence compound (**1**) can be formulated as trifolirhizin 6′-monoacetate.

## EXPERIMENTAL

All mp's were uncorrected. NMR were taken at 100 MHz in $CCl_4$ with TMS as an internal standard. The chemical shifts were given in $\delta$ values. Abbreviations: *s* = singlet, *d* = doublet, *t* = triplet, *q* = quartet, *m* = multiplete and *br* = broad.

*Isolation of compound* **1**. Guang-Dou-Gen (5.2 kg) was extracted 3× boiling MeOH, the residue was successively extracted with $Et_2O$ and EtOAc. The EtOAc soluble part (42 g) was chromatographed on Si gel. The column was eluted with $CHCl_3$ to afford (−)-pterocarpin (9 mg), (−)-maackiain (79 mg), and subsequently with a mixture of $CHCl_3$–MeOH (24:1) to afford 7,4′-dihydroxyflavone (30 mg), **1** (70 mg), $CHCl_3$–MeOH (47:3) to afforded trifolirhizin (765 mg) and sitosterol-β-D-glucoside (12 mg).

*Compound* **1**. This was recrystallized from MeOH as colorless needles, mp 223–225°, $M^+ = 488$. Found: C, 58.78; H, 4.89. $C_{24}H_{24}O_{11}$ requires: C, 59.01; H, 4.95%. $[\alpha]_D^{22} - 175°$ (AcOH). UV $\lambda_{max}^{EtOH}$ nm (log ε): 279(*sh*) (3.60), 284 (3.67), 311 (3.94). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3400 (OH), 1720, 1240 (−OAc), 1620, 1590, 1503 (arom C=C). NMR (TMSi ether, in $CCl_4$) $\delta$ ppm 2.02 (3H, *s*, −OAc), 3.5 (6H, *m*, $C_{2',3',4',5'}$ − H, $C_6$–$H_2$), 4.05 (1H, *q*, $C_{6a}$–H), 4.25 (2H, *m*, –$CH_2$–OAc), 4.77 (1H, *d*, *J* 7 Hz, $C_1'$–H), 5.43 (1H, *d*, *J* 7 Hz, $C_{11a}$–H), 5.87 (2H, *d*, *J* 1 Hz, –O–$CH_2$–O–), 6.35 (1H, *s*, $C_{10}$–H), 6.5 (1H, *d*, *J* 2 Hz, $C_4$–H), 6.63 (2H, *m*, $C_{7,2}$–H), 7.32 (1H, *d*, *J* 9 Hz, $C_1$–H). MS (*m/e*): 488 ($M^+$), 446 ($M^+$–$CH_2CO$), 284.

*Alkaline hydrolysis*. This was carried out with 1% KOH at room temp. for 24 hr. The soln was acidified with HCl and extracted with EtOAc. The solvent was evaporated to yield trifolirhizin. Recrystallization from MeOH gave colorless needles, mp 145° (decomp), which was identified by mmp and IR with an authentic sample.

*Acetylation of* **1**. **1** with $Ac_2O$–$C_5H_5N$, overnight at room temp gave colorless needles, mp and mmp 190–191° of tetraacetyl trifolirhizin.

*Enzymatic hydrolysis of* **1**. **1** with β-glucosidase for 48 hr, gave colorless needles, mp 180–181°, $[\alpha]_D^{22} - 252°$ (C = 1.0, $Me_2CO$), which was identified as (−)-maackiain (mmp and IR) and D-glucose.

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## REFERENCES

1. Komatsu, M., Yokoe, I. and Shirataki, Y. (1976) *Yakugaku Zasshi* **96**, 254.
2. Komatsu, M., Tomimori, T., Hatayama, K., Makiguchi, Y. and Mikuriya, N. (1969) *Chem. Pharm. Bull. (Tokyo)* **17**, 1299.
3. Komatsu, M., Tomimori, T., Hatayama, K., Makiguchi, Y. and Mikuriya, N. (1969) *Chem. Pharm. Bull. (Tokyo)* **17**, 1302.
4. Komatsu, M., Tomimori, T., Hatayama, K., Makiguchi, Y. and Mikuriya, N. (1970) *Chem. Pharm. Bull. (Tokyo)* **18**, 602.

*Part II in the series "Constituents of *Sophora* species", for part 10 see Ref. [1].

5. Komatsu, M., Tomimori, T., Hatayama, K., Makiguchi, Y. and Mikuriya, N. (1970) *Chem. Pharm. Bull. (Tokyo)* **18,** 741.
6. Kyogoku, K., Hatayama, K., Yokomori, S., Shio, M. and Komatsu, M. (1973) *Chem. Pharm. Bull. (Tokyo)* **21,** 1192.
7. Kyogoku, K., Hatayama, K., Suzuki, K., Yokomori, S., Maejima, K. and Komatsu, M. (1973) *Chem. Pharm. Bull. (Tokyo)* **21,** 1436.
8. Kyogoku, K., Hatayama, K., Suzuki, K., Yokomori, S., Maejima, K. and Komatsu, M. (1973) *Chem. Pharm. Bull. (Tokyo)* **21,** 1436.
9. Erickson, R. L., Pearl, I. A. and Darling, S. F. (1970) *Phytochemistry* **9,** 857.

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# BIOGENESIS AND REVISED STRUCTURE OF ROSELLISIN; STRUCTURE OF ROSELLISIN ALDEHYDE*

M. S. R. NAIR
The New York Botanical Garden, Bronx, NY 10458, U.S.A.

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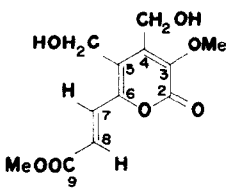
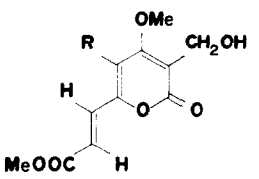
**Key Word Index**—*Hypomyces rosellus*; Hypocreaceae; structure determination; $^{13}C$ acetate incorporation; rosellisin; rosellisin aldehyde.

**Abstract**—On the basis of new data from biogenetic studies on rosellisin, the positions of the 3-OMe and the 4-$CH_2OH$ have been interchanged, resulting in a revised structure. A new metabolite has been identified as rosellisin aldehyde.

In a preliminary communication [1] the structure **1** was proposed for the antibiotic fungal metabolite, rosellisin. The UV, IR, PMR and MS of rosellisin, its acetate and its dihydro derivative indicated two possible structures (**1** and **2**). However, the $^{13}C$ NMR spectrum strongly favored structure **1**: chemical shifts for the ring olefinic carbons were different from those typical for 4-oxy-α-pyrones. [2–4] and the chemical shift of the OMe, 63.2, was higher than would be expected for a methoxyl group in the 4-position [2, 3].




(1)




(2) R ≡ $CH_2OH$

(3) R ≡ CHO

Recent biogenetic studies involving incorporation of $^{13}C$ acetates have led to a reassignment of the $^{13}C$ chemical shifts. When rosellisin was labelled by incorporation of either $^{13}C$-1 or $^{13}C$-2 acetate, a set of four peaks in its $^{13}C$ NMR spectrum were enhanced: in the first instance, those at 125.1, 153.3, 163.8 and 167.7; in the second, those at 113.9, 118.7, 130.5 and 166.2. The previous assignments of these peaks were not compatible with alternate labelling. Accordingly, they have been reassigned as shown in Fig. 1. The observed chemical shift of the 4-OMe carbon, in the new assignment, may can be explained only as due to an abnormal influence of the hydroxyl groups.

Another basis for the original assignment of a 3-methoxyl group in rosellisin was the effect of copper acetylacetonate on the PMR spectrum; the two sharp singlets for the hydroxymethylenes collapsed to broad peaks and little or no broadening of the other peaks occurred. This was attributed to a bidentate complex formation. However, it can be explained on a different basis, which is compatible with structure **2**. Due to the planar nature of the carbon skeleton of the pyrone ring, monodentate complex formation at both the hydroxyls also would result in little broadening of the other peaks.

The biogenetic experiments establish that rosellisin is an acetogenin and not an isoprenoid like nectriapyrone [2], another α-pyrone isolated from a pyrenomycete. The only carbons which were labelled were the alternate ones of the C-2 to C-9 chain. The branch carbons of the hydroxymethylenes presumably originate from the "C1 pool".

A second compound, rosellisin aldehyde (**3**) was isolated in very small amounts from culture liquids of *Hypomyces rosellus*. This compound differs from rosellisin only in having an aldehyde rather than a hydroxymethylene group on C-5. Rosellisin aldehyde, isolated in semi-crystalline form, melted over an extended range, from 50–65°. However, it showed a single spot on TLC, in several solvents. It had MW 268 (CI-MS); $\lambda_{max}^{EtOH}$ 348, 247 and 221 nm; $\nu_{max}$ 3450, 1724, 1700, 1639, 1597 and 1553 $cm^{-1}$; NMR signals at 2.8 (broad, 1H, OH), 3.80 (*s*, 3H, COOMe), 4.2 (*s*, 3H, OMe), 4.61 (*s*, 2H, $CH_2OH$), an AB quartet around 6.96 and 8.10 (1H each, *J* 15.5 Hz, C-8 and C-7 protons) and 10.18 (*s*, 1H, CHO). The downfield shift of the signals for olefinic protons in rosellisin aldehyde as compared to the corresponding signals

* Part 6 of the series, "Metabolites of Pyrenomycetes"; for part 5, see Carey, S. T. and Nair, M. S. R. (1975) *Lloydia* **38,** 448.