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FLAVONOIDS FROM *ISODON ORESBIUS*

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Key Word Index—*Isodon oresbius*; Labiatae; flavone; flavanone; oresbiusin.

Abstract—A new flavanone, oresbiusin, in addition to seven known flavonoids, was isolated from the whole dried plant of *Isodon oresbius* (Labiatae) and identified using spectroscopic and chemical methods. The structure of the new flavanone was established as 6,7,8-trihydroxy-5-methoxyflavanone.

INTRODUCTION

Isodon oresbius (W. W. Smith) Kudo is a shrub found in the open dry rocky areas of Yunnan and Sichuan, China. It has been used in traditional Chinese folk medicine to treat blood clots in internal organs of the body [1]. No previous chemical work has been carried out on this species. As a continuation of our study on the biologically active constituents of *Isodon* species, we now report on the chemical components of *I. oresbius*.

RESULTS AND DISCUSSION

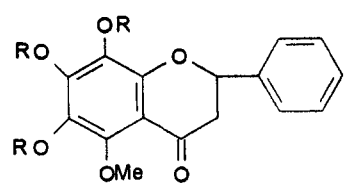
The chloroform-soluble fraction of the whole plant of *I. oresbius* was separated by repeated column chromatography and recrystallization (see Experimental) to give a new flavanone, oresbiusin (**1**), and seven known flavonoids (**2–8**).

The known compounds were determined as pinostrobin (**2**), 5-hydroxy-7,8-dimethoxyflavanone (**3**), dihydrowogonin (**4**), sakuranetin (**5**), chrysoeriol (**6**), apigenin (**7**) and luteolin (**8**), respectively, from their IR, mass and ^{1}H NMR spectra, which were identical to the reported data [2–7].

Oresbiosin (**1**) was crystallized from $CHCl_3$ as pale yellow needles. HR-mass spectrometry gave the $[M]^+$ peak *m/z* 302.2811 corresponding to the molecular formula $C_{16}H_{14}O_6$ (calcd 302.2806). ^{1}H NMR showed three double doublets at δ 5.40 (1H, *dd*, $J = 13.0$, 2.3 Hz, H-2), 3.10 (1H, *dd*, $J = 17.0$, 13.0 Hz, H-3 *trans*) and 2.80 (1H, *dd*, $J = 17.0$, 2.3 Hz, H-3 *cis*), indicating the flavanone skeleton of compound **1**. Other features in the ^{1}H NMR spectrum were a single methoxyl (δ 3.96, *s*), three D_2O exchangeable signals (δ 6.58, 6.05, 5.90) assignable to phenolic hydroxyls and a phenyl group (δ 7.40, 5H, *m*). In the EI-mass spectrum, the unsubstituted nature of the B-ring of **1** was readily apparent from the appearance of two prominent peaks at *m/z* 225 ($[M-77]^+$; $[M-C_6H_5]^+$) and *m/z* 198 ($[M-104]^+$; $[M-C_6H_5-CH{=}CH_2]^+$) corresponding to the loss of phenyl and styrene fragments, respectively, from the $[M]^+$ ion; it further indicated that all the substituents were present in the A-ring.

The ^{13}C NMR of compound **1** gave 16 signals, including five oxygenated aromatic carbons (δ 154.1, *s*; 152.2, *s*; 147.7, *s*; 140.3, *s*; 137.8, *s*) in a flavanone skeleton, which confirmed that the carbons of the A-ring of **1** were all substituted. A methoxyl group at C-5 was concluded as no chelated hydroxyl group was observed in the ^{1}H NMR spectrum. Consequently, three hydroxyls were deduced at the 6, 7 and 8-positions.

Further confirmation of the A-ring substitution pattern was provided by methylation compound of **1** with Me_2SO_4 to yield **1a**, which was identical to an authentic sample of kanakugin [8] in all respects (mass spectrum, ^{1}H and ^{13}C-NMR spectra). Thus, oresbiusin (**1**) was identified as 6,7,8-trihydroxy-5-methoxyflavanone.



1: R = H
1a: R = Me

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EXPERIMENTAL

General. Mps: uncorr; IR: KBr; NMR: ^{1}H at 400 MHz, ^{13}C at 100 MHz, $CDCl_3$; MS: ZAB-HS mass spectrometer.

Plant material. Isodon oresbius (W. W. Smith) Kudo was collected from Lijiang country, Yunnan province, China. The species was authenticated by Prof. Li Hsi-weng, Kunming Institute of Botany, Academia Sinica, where a voucher specimen has been deposited.

Extraction and separation. The air-dried powdered whole plant (2.8 kg) was continuously extracted with boiling 95% EtOH and the extracts concd *in vacuo*. The residue (640 g) was suspended in H_2O (100 ml) and the mixt. was successively extracted with petrol, $CHCl_3$ and *n*-BuOH. The combined $CHCl_3$ layers were concd to dryness to give a $CHCl_3$ fr. (95 g), which was subjected to CC (silica gel) with petrol, petrol–EtOAc or petrol–$CHCl_3$–Me_2CO as eluants. Frs were monitored by TLC. All components were further purified by prep. TLC silica gel, yielding, in order of increasing polarity: pinostrobin (2.5 g), **2**; 5-hydroxy-7,8-dimethoxyflavanone (65 mg), **3**; oresbiusin (48.0 mg), **1**; dihydrowogonin (15.5 mg), **4**; sakuranetin (26.6 mg), **5**; chrysoeriol (20.0 mg), **7**; luteolin (50.0 mg), **8**. All of the known compounds were identified by direct comparison of their mp, IR, ^{1}H NMR and (or) ^{13}C NMR data with an authentic sample, and lit. data [2–7].

Oresbiusin (**1**). Pale yellow needles ($CHCl_3$). Mp 172–173°. HREI-MS *m/z* 302.2811 (calcd 302.2806 for $C_{16}H_{14}O_4$). EI-MS *m/z* 302 ($[M]^+$, 100), 287 (92), 269 (90), 241 (58), 225 (20), 209 (65), 198 (90), 155 (85), 139 (80), 125 (60), 104 (70), 92 (75), 77 (80), 69 (60). IR ν_{max}^{KBr} cm^{-1}: 3200, 3100, 1675, 1600, 1460, 750. ^{1}H NMR ($CDCl_3$): δ 3.96 (3H, *s*, OMe), 5.40 (1H, *dd*, $J = 13.0$, 2.3 Hz, H-2), 3.10 (1H, *dd*, $J = 17.0$, 13.0 Hz, H-3 *trans*), 2.80 (1H, *dd*, $J = 17.0$, 2.3 Hz, H-3, *cis*), 7.40 (5H, *m*, Ar-H). ^{13}C NMR ($CDCl_3$): see Table 1.

Methylation of oresbiusin. Compound **1** (20 mg) was treated with Me_2SO_4 (1.2 ml) and K_2CO_3 (1 g) in dry Me_2CO (20 ml) under reflux for 3 hr. After filtration of inorganic ppts, the soln was concd and the residue purified by silica gel CC using petrol–EtOAc (3:1) as eluant to give the known flavanone **1a**, kanakugin (10 mg).

Table 1. ^{13}C NMR Chemical shifts and assignments for compounds **1** and **1a** (in $CDCl_3$, δ-values)

C	1	1a
2	79.5	79.4
3	45.9	45.9
4	189.4	189.6
4a	113.5	111.6
5	155.3	152.5
6	137.9	138.7
7	154.0	153.4
8	140.3	141.1
8a	153.0	150.2
1′	137.8	137.9
2′, 6′	128.7	128.8
3′, 5′	126.0	125.9
4′	128.6	128.6
O-Me	61.5	61.5, 61.6

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