A New Rosmarinic Acid Derivative from *Isodon oresbius*

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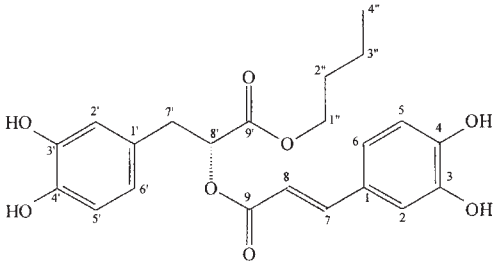
Abstract: **Phytochemical reinvestigation of *Isodon oresbius* afforded, in addition to oleanolic acid, ursolic acid, sodoponin, astragalin, and quercetin-3-*O*-glucoside, three known (oresbiusin A, rosmarinic acid and methyl rosmarinate) and a new rosmarinic acid derivative as well as an *ent*-kaurene diterpenoid, neoangustifolin, characterized as a separated component for the first time. By a combination of 1D- and 2D-NMR techniques the structure of the new compound was established as butyl rosmarinate. The *in vitro* antifungal assay showed that neoangustifolin was active against *Candida albicans* with the MIC being 50 μg/ml.**

Isodon species (Labiatae) are abundantly distributed throughout the southwestern China. *Isodon oresbius* (W. W. Smith) Kudo is a small, about 0.6 m tall perennial shrub commonly found in dry places, especially in open dry rocky areas. It has been used to tre t blood clots in internal organs of the body (1). Previous phy ochemical studies on this plant from Lijiang County, Yunnan Province, China, have provided flavonoids and other phenolic compounds (2, 3). In our continued investigations of biologically active and/or structurally novel substances from *I. oresbius* (3), we reexamined the constituents of the plant obtained from Diqing County of the same province. The results are discussed in this paper.

Two flavone glycosides were identified as astragalin and quercetin-3-*O*-glucoside by comparing their spectral data (IR, EIMS, ^{1}H- and ^{13}C-NMR) with those in the literature (4, 5). The structures of sodoponin, oleanolic acid, and ursolic acid were ascertained by their spectral data (6–8), and those of oresbiusin A, rosmarinic acid, and methyl rosmarinate by the comparison (IR, NMR, TLC) with authentic samples available in our laboratory.

The ^{1}H- and ^{13}C-NMR spectrum of compound **1** demonstrated that it was a rosmarinic acid derivative (2, 9). The carbon resonances at δ 66.7 t, 32.1 t, 20.5 t and 14.4 q in the ^{13}C-NMR spectrum indicate the presence of a butoxy group. The HMBC spectrum of **1** exhibited a long range correlation of the methylene proton (H-1″) at δ 3.92 with the carbonyl carbon (C-9′) at δ 172.3, which defined the linkage of the butoxy group on C-9′. Thus, the structure of compound **1** was determined to be *n*-butyl rosmarinate. The optical rotation of **1** was found to be +56° establishing the *R*-(+) absolute configuration at the chiral center (10).



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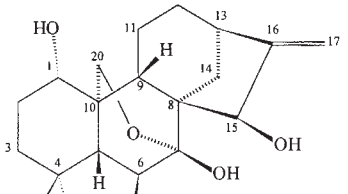

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The spectral data (IR, FABMS, ^{1}H- and ^{13}C-NMR) of compound **2** demonstrated that it was neoangustifolin, characterized previously as a subunit of the complex DCRA consisting of neoangustifolin-epinodosinol (1 : 1) binding together by an intermolecular hydrogen bond (11). This is the first time that neoangustifolin (**2**) was obtained as a pure natural product. Furthermore, a combination of two-dimensional NMR spectra of **2** (COSY, HMQC, HMBC, and NOESY) allowed the unequivocal assignment of all proton and carbon signals. Unexpectedly, the chemical shift of C-2 was shown to be 30.7 ppm, strikingly differently from the previous assignment (24.33 ppm) for C-2 of neoangustifolin, but close to that (30.18 ppm) for C-2 of epinodosinol (11). Accordingly, assignments for the C-2 signal of neoangustifolin and epinodosinol in that reference should be interchanged. *In vitro* antifungal assays against *Aspergillus flavus*, *A. niger*, *Geotrichum candidum*, *Candida tropicalis*, and *C. albicans* showed that compound **2** inhibited the growth of *C. albicans* (MIC: 50 μg/ml), but it had no activities against the other fungi at a concentration of 150 μg/ml (the MICs of the positive control, fluconazole, ranged from 0.5 to 1.0 μg/ml).

Materials and Methods

Melting points were measured on a YANACO MP-S2 apparatus and are uncorrected. IR spectra were taken on a Perkin-Elmer 577 instrument and recorded in KBr pellets. Mass spectra were obtained on a VG QUATTRO mass spectrometer. All NMR spectra were recorded on a Bruker DRX-400 NMR spectrometer with TMS as int. standard. Column chromatography

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was performed on silica gel (200–300 mesh), and the TLC analyses were carried out using glass precoated silica gel plates. In the *in vitro* antifungal bioassay, a previously described procedure was used (12).

The aerial parts of *Isodon oresbius* (W. W. Smith) Kudo were collected in October 1996 in Diqing County, Yunnan Province, P. R. China. The species was authenticated by Prof. Li Xi-wen, Kunming Institute of Botany, Academia Sinica, where a voucher specimen (DQ-96/1014) has been deposited.

Air-dried and roughly powdered aerial parts of *I. oresbius* (3.5 kg) were extracted with hot 80% EtOH (3 L × 3). After solvent evaporation, the resulting extract (620 g) was dissolved in 900 ml of EtOH-H_2O (1 : 9), and subjected to solvent fractionation with petrol, EtOAc and *n*-BuOH (300 ml × 3, each) to give frs. P (90 g), E (120 g), and N (140 g), respectively. Fr. E was repetitiously chromatographed over silica gel (1.8 kg) eluting with Me_2CO-$CHCl_3$ mixtures (elution volume ca. 4 L) of growing polarities to yield oresbiusin A (120 mg), rosmarinic acid (88 mg), methyl rosmarinate (28 mg), and **1** (34 mg). Fr. N was separated over silica gel (2.0 kg) column eluting with a $CHCl_3$-CH_3OH gradient (ca. 3.5 L) to afford astragalin (54 mg) and quercetin-3-*O*-glucoside (66 mg). Fr. P was repeatedly chromatographed over silica gel (1.5 kg) eluting with $CHCl_3$ containing gradually increased amounts of Me_2CO (total elution volume ca. 4.5 L) to give neoangustifolin (**2**, 68 mg), sodoponin (120 mg), oleanolic acid (1 g) and ursolic acid (110 mg).

n-*Butyl rosmarinate* (**1**): Yellow amorphous powder; $[\alpha]_D$: +56° (*c* 0.857, $CHCl_3$); IR (KBr): ν_{max} = 3400–3200, 1700, 1600, 1500, 1450, 1350, 1000, 970, 800 cm^{-1}; FABMS: *m/z* = 417 $[M + H]^+$; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ = 6.88 (1H, d, *J* = 1.5 Hz, H-2), 6.60 (1H, d, *J* = 8.2 Hz, H-5), 6.79 (1H, dd, *J* = 8.2, 1.5 Hz, H-6), 7.40 (1H, d, *J* = 15.9 Hz, H-7), 6.10 (1H, d, *J* = 15.9 Hz, H-8), 6.55 (1H, d, *J* = 1.5 Hz, H-2′), 6.52 (1H, d, *J* = 8.0 Hz, H-5′), 6.40 (1H, dd, *J* = 8.0, 1.5 Hz, H-6′), 2.86 (2H, d, *J* = 6.4 Hz, H-7′), 4.98 (1H, t, *J* = 6.4 Hz, H-8′), 3.92 (2H, t, *J* = 6.4 Hz, H-1″), 1.35 (2H, m, H-2″), 1.12 (2H, m, H-3″), 0.71 (3H, t, *J* = 7.0 Hz, H-4″); ^{13}C-NMR ($CDCl_3$, 100 MHz) (multiplicities by the DEPT pulse sequence): δ = 128.1 (s, C-1), 114.7 (d, C-2), 150.2 (s, C-3), 146.7 (s, C-4), 123.6 (d, C-5), 116.8 (d, C-6), 147.2 (d, C-7), 115.7 (d, C-8), 168.8 (C-9), 129.1 (s, C-1′), 122.3 (d, C-2′), 148.4 (s, C-3′), 146.7 (s,C-4′), 118.1 (d, C-5′), 117.0 (d, C-6′), 38.4 (t, C-7′), 75.3 (d, C-8′), 172.3 (s, C-9′), 66.7 (t, C-1″), 32.1 (t, C-2″), 20.5 (t, C-3″), 14.4 (q, C-4″).

Neoangustifolin (**2**): Colorless needles; m.p. 195–197 °C; IR (KBr): ν_{max} = 3500–3200, 1723, 1648, 1244, 1037 cm^{-1}; FABMS: *m/z* = 431.5 $[M + Na]^+$; ^{1}H-NMR (C_5D_5N, 400 MHz): δ = 3.90 (1H, dd, *J* = 11.0, 5.2 Hz, H-1β), 1.96 (1H, m, H-2α), 2.08 (1H, m, H-2β), 1.98 (1H, m, H-3α), 1.30 (2H, m, overlapped, H-3β and H-14β), 2.01 (1H, d, *J* = 6.0 Hz, H-5β), 4.50 (1H, d, *J* = 6.0 Hz, H-6α), 2.76 (2H, m, overlapped, H-9 and H-13), 2.05 (1H, m, H-11α), 2.39 (1H, m, H-11β), 2.40 (1H, m, H-12α), 1.70 (1H, m, H-12β), 2.20 (1H, m, H-14α, 5.30 (1H, br s, H-15α), 5.54 (1H, br s, H-17a), 5.26 (1H, br s, H-17b), 1.45 (3H, s, H-18), 4.60 (1H, d, *J* = 11.1 Hz, H-19a), 4.99 (1H, d, *J* = 11.1 Hz, H-19b), 4.98 (1H, d, *J* = 10.0 Hz, H-20a), 4.45 (1H, dd, *J* = 10.0, 1.5 Hz, H-20b), 2.02 (3H, s, OAc); ^{13}C-NMR (C_5D_5N, 100 MHz) (multiplicities by the DEPT pulse sequence): δ = 74.4 (d, C-1), 30.7 (t, C-2), 34.3 (t, C-3), 38.1 (s, C-4), 59.5 (d, C-5), 74.0 (d, C-6), 97.7 (s, C-7), 53.2 (s, C-8), 44.3 (d, C-9), 42.1 (s, C-10), 19.7 (t, C-11), 33.6 (t, C-12), 37.7 (d, C-13), 27.4 (t, C-14), 75.8 (d, C-15), 163.0 (s, C-16), 107.4 (t, C-17), 27.5 (q, C-18), 67.0 (t, C-19), 64.8 (t, C-20), 171.3 (s, OAc), 21.2 (q, OAc).

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