Labdane Diterpenoids from *Marrubium globosum* ssp. *globosum*

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A new labdane type diterpenoid, marrubiglobosin, was isolated from the aerial parts of *Marrubium globosum* ssp. *globosum* together with the known diterpenoids, marrubiin and marrubinone B. The structure of this new compound was elucidated by spectral methods.

Key words *Marrubium globosum* ssp. *globosum*; Labiatae; marrubiglobosin; marrubinone B; marrubiin; labdane

The genus *Marrubium* (Labiatae) is known to contain labdane type diterpenoids such as marrubiin (**2**).^{1,2)} The extract of *Marrubium vulgare*, which is the most popular species in the genus, is known to contain **2**,²⁾ and is used in cases of lack of appetite, stomach diseases, coughs, dyspeptic complaints and catarrhs of the respiratory tract.³⁾ During the course of studies on the constituents of Turkish medicinal plants, we have investigated the diterpenic constituents of the aerial parts of *Marrubium globosum* ssp. *globosum* and isolated a new diterpenoid, marrubiglobosin (**1**) together with the known compounds marrubiin (**2**)²⁾ and marrubinone B (**3**).⁴⁾ This paper describes the structural elucidation of the new compound by spectroscopic means.

Compound **1**, $[\alpha]_D^{25} +47.0^\circ$ (CHCl₃), was obtained as an amorphous powder and the molecular formula was determined as C₂₀H₃₀O₅ based on its negative-ion high resolution (HR) FAB-mass spectrum. It showed absorption bands due to hydroxyl groups (3275 cm⁻¹), a five-membered ketone group (1740 cm⁻¹) and a double bond (1600 cm⁻¹) in the IR spectrum. The ¹H-NMR spectrum (in C₆D₆) showed, in addition to signals due to a secondary methyl group [δ 0.49 (3H, d, $J=6.8$ Hz)] and two tertiary methyl groups [δ 0.93 and 1.17 (each 3H, s)], signals due to primary carbonyl protons vicinal to a quaternary carbon atom [δ 2.98 and 4.14 (each 1H, d, $J=11.1$ Hz)], a methylene group with an oxygen atom which might be included in the five membered ring [δ_H 4.26 and 4.58 (each 1H, d, $J=10.4$ Hz)], a methylene group located between a ketone group and a quaternary carbon atom [δ 1.95 and 2.41 (each 1H, d, $J=18.3$ Hz)], a secondary methine group with an oxygen atom [δ 4.20 (1H, m)] and a *cis*-double bond in the dihydrofuran ring [δ 4.78 and 6.23 (each 1H, d, $J=2.6$ Hz)]. The ¹H-NMR spectrum measured in CDCl₃ (see Experimental) also showed the presence of the above mentioned functional groups. The ¹³C-NMR spectrum (Table 1) indicated the presence of four methylene carbon atoms, two methine carbon atoms and four quaternary carbon atoms, two of which bear an oxygen atom, in addition to the carbon atoms which correspond to the above mentioned functional groups. These spectral data, coupled with the results of comparisons of spectral data with those of **3**, suggested that **1** has a labdane skeleton of the **3**⁴⁾ type, and features a bis-spiro-structure at C-9 and C-13. The location of the primary hydroxyl group was deduced to be at C-19 from comparison of chemical shifts (δ 69.0) with that (δ 68.1) of **4**,⁵⁾ and the re-

sults of the heteronuclear multiple bond connectivity (HMBC) spectrum, shown in Fig. 1 and differential nuclear Overhauser enhancement (NOE) experiments, shown in Fig. 2. A secondary hydroxyl group was deduced to be at C-6 from analysis of the ¹H–¹H correlation spectroscopy (COSY) spectrum. Thus, the methine proton assignable to H-5 (δ 1.63) had a cross peak with the signal at δ 4.20, which then crossed peaks with H₂-7 (δ 1.50 and 1.73). The protons at C-7 further showed cross peaks with the signal at δ 2.21 (H-8),

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Table 1. ^{13}C -NMR Data (δ) for **1** in C_6D_6 ^{a)}

C		C	
1	33.9 (33.7)	11	40.6 (40.6)
2	18.9 (18.4)	12	212.1 (213.8)
3	41.0 (40.6)	13	93.1 (92.8)
4	39.1 (39.0)	14	103.3 (102.9)
5	49.4 (49.0)	15	150.9 (150.8)
6	65.8 (65.5)	16	78.2 (78.0)
7	38.8 (38.3)	17	16.0 (15.7)
8	31.8 (31.4)	18	27.3 (27.1)
9	86.7 (86.8)	19	69.0 (68.9)
10	42.4 (42.1)	20	20.2 (20.1)

a) The figures in parentheses are the data for CDCl_3 solution.

Fig. 1. Selected Data from the HMBC Spectrum for **1** (in C_6D_6 , $J=8$ Hz) Fig. 2. The Results of Differential NOE Experiments for a CDCl_3 Solution of **1**

which crossed peaks with the signal at δ 0.49 due to the secondary methyl group. The planar structure was thus elucidated as a 12-oxo-derivative of premarrubenol (**5**),⁶ which was supported by the results of the HMBC spectrum shown in Fig. 1. The relative stereochemistry was determined as shown based on the results of differential NOE experiments (Fig. 2). The absolute stereochemistry was the same as that of **3**, since in the circular dichroism (CD) spectrum, **1** showed an extreme at 237 nm ($\Delta\epsilon+4.20$) which was essentially the same as that (237 nm, $\Delta\epsilon+5.20$) of **3**. Thus, the structure **1** was elucidated for marrubiglobosin.

Experimental

^1H - and ^{13}C -NMR spectra (400 and 100 MHz, respectively) were recorded on a JEOL JNM EX-400 or α -400 spectrometer, with tetramethylsilane as an internal standard. Mass spectra were obtained on a JEOL JMS SX-102 spectrometer. FAB-MS were recorded using PEG-400 as a matrix. IR spectra

were taken on a Shimadzu IR-400 spectrophotometer. Specific rotations were determined using a JASCO DIP-360 digital polarimeter. For purification, the following were used: Silica gel (Merck, 230–400 mesh) and pre-coated Silica gel 60 F₂₅₄ TLC plates (Merck, 0.25 and 0.5 mm in thickness).

Plant Material The plant material was collected in the suburbs of Akseki, Turkey on 16th, July, 1995 and identified as *Marrubium globosum* MONTBRET & AUCHER ex BENTHAM ssp. *globosum* by the authors (G. H. and E. S). Voucher specimens (95E 037) are deposited in the Herbaria of the Graduate School of Pharmaceutical Sciences, Kyoto University and the Faculty of Pharmacy, Gazi University.

Extraction and Isolation Dried aerial parts (3.8 kg) of *M. globosum* ssp. *globosum* were extracted with MeOH (541×2) at room temperature for three weeks. The combined MeOH extracts were concentrated *in vacuo* and the residue was dissolved in aqueous 90% MeOH (1.7 l). The solution was washed with *n*-hexane (1 l×3) and the aqueous MeOH layer was concentrated *in vacuo*. The residue was suspended in H₂O (1 l) and the suspension was extracted with EtOAc (1 l×3). After drying over anhydrous MgSO_4 , the EtOAc extract was concentrated *in vacuo* to give a residue (63.3 g) which was chromatographed over silica gel (1.3 kg) with a mixture of CHCl_3 and Me_2CO with increasing Me_2CO content. 5 liters of each of 0, 5, 10, 15 and 20% Me_2CO – CHCl_3 were passed successively, with 500 ml fractions being collected (Column I). Fractions 7–12 gave a residue (11.0 g) which was rechromatographed over silica gel (500 g) with CHCl_3 as eluent, collecting 200 ml fractions. Sub-fractions 12–19 gave a residue (4.61 g) which was recrystallized from MeOH to give **2** (1.22 g). The mother liquor gave a residue (1.92 g) which was separated by repeated silica gel column chromatography with CHCl_3 as eluent to give **3** (96 mg).

Fractions 13–16 of column I gave a residue (7.52 g) which was separated by repeated silica gel column chromatography with the following solvent systems [CHCl_3 – Me_2CO with increasing Me_2CO content, *n*-hexane– Et_2O with increasing Et_2O content and Et_2O] to give **1** (24 mg) as an amorphous powder [R_f 0.35 (solvent: Et_2O)].

Known compounds, **2**²⁾ and **3**⁴⁾ were identified based on direct comparison or comparison of spectral data with those reported.

Marrubiglobosin (1): amorphous powder, $[\alpha]_D^{25} +47.0^\circ$ ($c=1.17$ CHCl_3). IR (CHCl_3) cm^{-1} : 3275, 1740, 1600. ^1H -NMR (C_6D_6) δ : 0.49 (3H, d, $J=6.8$ Hz, H₃-17), 0.88 (1H, m, H_a-1), 0.93 (3H, s, H₃-18), 1.00 (1H, m, H_a-2), 1.08 (1H, m, H_a-3), 1.17 (3H, s, H₃-20), 1.24 (1H, m, H_b-3), 1.33 (1H, m, H_b-2), 1.50 (1H, dt, $J=13.6, 3.2$ Hz, H-7 β), 1.63 (1H, brs, H-5), 1.67 (1H, m, H_b-1), 1.73 (1H, dt, $J=3.2, 13.6$ Hz, H-7 α), 1.95 (1H, d, $J=18.3$ Hz, H₃-11), 2.21 (1H, m, H-8), 2.41 (1H, d, $J=18.3$ Hz, H_b-11), 2.98 (1H, d, $J=11.1$ Hz, H_a-19), 4.14 (1H, d, $J=11.1$ Hz, H_b-19), 4.20 (1H, m, H-6), 4.26 (1H, d, $J=10.4$ Hz, H_a-16), 4.58 (1H, d, $J=10.4$ Hz, H_b-16), 4.78 (1H, d, $J=2.6$ Hz, H-14), 6.23 (1H, d, $J=2.6$ Hz, H-15); (CDCl_3) δ : 0.68 (3H, d, $J=6.8$ Hz, H₃-17), 1.07 (3H, s, H₃-18), 1.30 (3H, s, H₃-20), 1.64 (1H, brs, H-5), 2.34 (1H, m, H-8), 2.34 (1H, d, $J=18.6$ Hz, H_a-11), 2.86 (1H, d, $J=18.6$ Hz, H_b-11), 3.21 (1H, d, $J=11.2$ Hz, H_a-19), 4.24 (1H, m, H-6), 4.26 (1H, d, $J=11.2$ Hz, H_b-19), 4.33 (1H, d, $J=10.3$ Hz, H_a-16), 4.47 (1H, d, $J=10.3$ Hz, H_b-16), 5.01 (1H, d, $J=2.4$ Hz, H-14), 6.59 (1H, d, $J=2.4$ Hz, H-15). ^{13}C -NMR: see Table 1. CD (MeOH, $c=7.14\times 10^{-5}$ M) nm ($\Delta\epsilon$): 237 (+4.20), 205 (–5.33). Negative-ion HR-FAB-MS m/z : 349.1993 [$\text{M}-\text{H}$][–] (Calcd for $\text{C}_{20}\text{H}_{29}\text{O}_5$: 349.2015).

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References

- 1) Al-Hazimi H. M. G., Miana G. A., *J. Chem. Soc. Pakistan*, **16**, 134–153 (1994).
- 2) Appleton R. A., Fulke J. W. B., Henderson M. S., McCrindle R., *J. Chem. Soc. (C)*, **1967**, 1943–1947.
- 3) Knoss W., "Biotech. in Agric. and Forestry, Vol. 43. Medicinal and Aromatic Plants XI," ed. by Bajaj Y. P. S., Springer-Verlag, Berlin, Heidelberg, 1999, pp. 274–289.
- 4) Iida A., Tanaka Y., Mihara T., Tabata M., Honda G., Shingu T., Takeda Y., Takaishi Y., Yesilada E., Sezik E., Fujita T., *Chem. Pharm. Bull.*, **43**, 1454–1457 (1995).
- 5) Savona G., Piozzi F., Hanson J. R., Siverns M., *J. Chem. Soc., Perkin Trans. 1*, **1977**, 497–499.
- 6) Savona G., Piozzi F., Aránguez L. M., Rodríguez B., *Phytochemistry*, **18**, 859–860 (1979).