Marrubinones A and B, New Labdane Diterpenoids from *Marrubium astracanicum* (Labiatae)

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Two new labdane diterpenoids, marrubinones A (1) and B (2), were isolated from aerial parts of *Marrubium astracanicum* collected in Turkey. Compounds 1 and 2 were mostly characterized by two-dimensional NMR. For determination of the absolute configuration, the modified Mosher's method was applied to the 12-hydroxy forms of 1 and 2. Compounds 1 and 2 are pentacyclic diterpenoids, which differ from each other only in the stereochemistry at C-13, and they were identified as 9α,13*S*:15,16-diepoxy-12-oxo-14-labden-19,6β-olide and 9α,13*R*:15,16-diepoxy-12-oxo-14-labden-19,6β-olide, respectively.

Key words *Marrubium astracanicum*; marrubinone; labdane; diterpenoid; modified Mosher's method

The genus *Marrubium* (family Labiatae) consists of perennial plants, which grow mainly along the Mediterranean Sea, and also in the temperate zone of the Eurasian Continent. As chemical constituents of *Marrubium* sp., flavonoids[1] such as the apigenin and luteolin families and labdane diterpenoids, *i.e.*, premarrubiin[2] and preperegrinine,[3] have been reported so far. In our continuing survey of diterpenoids from the Labiatae plants, we collected *M. astracanicum* during a field survey on traditional medicines and medicinal plants in Turkey and isolated two new labdane diterpenoids, marrubinones A (**1**) and B (**2**), from the aerial parts of the plant as bitter principles (Fig. 1). In this paper, the isolation and structure determination of **1** and **2** are described.

Experimental

General Methods Optical rotations were measured with a JASCO DIP-360 digital polarimeter at room temperature. IR and UV spectra were recorded on Shimadzu IR-435 and UV 2200 spectrophotometers. All NMR experiments were performed on Bruker AC-300, ARX-500 and AM-600 spectrometers. Samples were dissolved in $CDCl_3$ containing tetramethylsilane as an internal standard. HPLC was performed on Shimadzu LC-6A and 8A systems using YMC packed octadecyl silica (ODS) columns (YMC Co., Ltd.), AM-313 (6 mm i.d. × 250 mm) for analytical HPLC and SH-345 (20 mm i.d. × 250 mm) for semi-preparative HPLC. FAB-MS was carried out on a JEOL JMS-HX/HX110A mass spectrometer. *m*-Nitrobenzyl alcohol was used as a matrix. Samples were bombarded with 7 kV xenon atoms. TLC was performed on silica gel (Kieselgel 60 F254, Merck). For column chromatography, Silica gel 60 (70—230 mesh, Nacalai Tesque) and Cosmosil 140 C_{18}-OPN (Nacalai Tesque) were used.

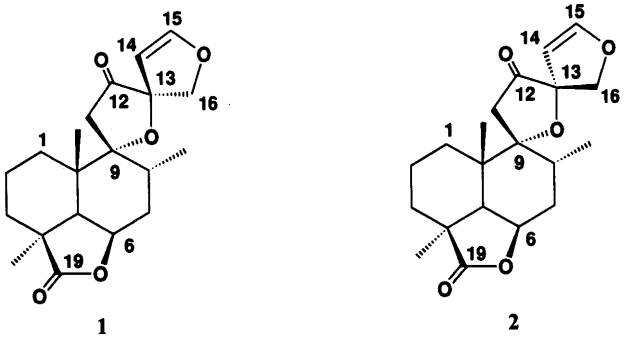
Isolation of Marrubinones A (1) and B (2) The methanolic extract (222 g) from the aerial parts (2.4 kg) of *M. astracanicum*, collected in Amasya Province (Turkey) in July 1991, was partitioned between 90% MeOH (1.2 l) and *n*-hexane (1 l) four times. The residue (179 g) from the MeOH layer was suspended in 1.2 l of H_2O and extracted with 1 l (×4) of EtOAc. After evaporation of the EtOAc layer, the residue (29 g) was chromatographed on silica gel (1.5 kg) and eluted with CH_2Cl_2–MeOH (25:1, v/v) to afford a mixture (2.1 g) containing compounds **1** and **2**. The mixture was purified by silica gel chromatography twice (diethyl ether:*n*-hexane = 3:2 and *n*-hexane:acetone = 8:2, respectively) and then by reversed-phase column chromatography (acetonitrile:H_2O = 7:3) to give a syrupy residue showing a single spot on TLC. The residue was finally purified by reversed-phase HPLC [conditions: mobile phase, acetonitrile–H_2O (40:60, v/v); flow rate, 7 ml/min; UV detection, 210 nm; column, YMC packed column SH-345-5 (20 mm i.d. × 250 mm); column temperature, 40 °C] to afford **1** (222 mg, t_R = 56.9 min) and **2** (160 mg, t_R = 63.7 min) as amorphous compounds.

Marrubinone A (1) $C_{20}H_{26}O_5$. High-resolution FAB-MS *m/z*: 347.1852 (MH^+, Calcd mass = 347.1858). $[\alpha]_D$: −54.4° (*c* = 1.0, MeOH) −51.2° (*c* = 1.0, $CHCl_3$). IR (KBr): 1760—1750 (γ-lactone and C=O, overlapping), 1590, 1160, 1100, 940 cm^{-1}. UV λ_{max}^{MeOH} nm (ε): 221 (1260), 293 (110).

Marrubinone B (2) $C_{20}H_{26}O_5$. High-resolution FAB-MS *m/z*: 347.1861 (MH^+). $[\alpha]_D$: +51.4° (*c* = 1.0, MeOH) +67.3° (*c* = 1.0, $CHCl_3$). IR (KBr): 1770 (γ-lactone), 1750 (C=O), 1590, 1160, 1100, 940 cm^{-1}. UV λ_{max}^{MeOH} nm (ε): 217 (1970), 297 (90).

Reduction of 1 and 2 with $NaBH_4$ A solution of 16 mg of **1** in MeOH (1.5 ml) was reduced with $NaBH_4$ (2.0 mg) at room temperature. After 1 h, the solution was subjected to reversed-phase HPLC [conditions: mobile phase, acetonitrile–H_2O (45:55, v/v); flow rate, 10 ml/min; UV detection, 210 nm; column, YMC packed column SH-345-5 (20 mm i.d. × 250 mm); column temperature, 40 °C] to afford the epimeric 12-hydroxy forms, **3a** (8.8 mg, t_R = 13.8 min for (12*R*)-form) and **3b** (4.5 mg, t_R = 16.4 min for (12*S*)-form). Similarly, **2** (20 mg) gave the corresponding 12-hydroxy forms, **4a** (8.0 mg, t_R = 14.9 min for (12*S*)-form) and **4b** (9.5 mg, t_R = 17.2 min for (12*R*)-form).

(*R*)- and (*S*)-MTPA Esters of 3a and 4a [MTPA = α-Methoxy-α-(trifluoromethyl)phenylacetic] To stirred solutions of two 2.5-mg aliquots of **3a** in CH_2Cl_2 (0.5 ml) were added successively (*S*)-(+)- and (*R*)-(−)-MTPA chloride (2 μl each), 4-dimethylaminopyridine (1 mg each) and triethylamine (1.1 μl each) at room temperature. After 5 h, the esters were purified by preparative TLC (*n*-hexane:EtOAc = 6:4) to give the (*R*)- and (*S*)-MTPA esters (**5a, b**) of **3a**. The epimeric MTPA esters



1 **2**

Fig. 1. Structures of Marrubinones A (**1**) and B (**2**)

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(6a, b) of 4a were also obtained in the same manner.

Results

Carbon Skeletons of Marrubinones A (1) and B (2) The isolated compounds, 1 and 2, were found to have the same molecular formula ($C_{20}H_{26}O_5$) from high-resolution FAB-MS ($MH^+ = 347.185$, unsaturation degree = 8). The IR spectra of 1 and 2 revealed the absence of hydroxyl groups and the presence of carbonyl groups in the molecules. In the spectrum of 2, the absorptions at 1770 and 1750 cm^{-1} suggested the presence of a γ-lactone and a five-membered ring ketone in the molecule, respectively. Although two carbonyl peaks were not unambiguously recognized in the IR spectrum of 1 because of overlapping (1760—1750 cm^{-1}), the ^{13}C-NMR spectrum supported the existence of two carbonyl groups in 1 as well as in 2: the chemical shifts of the carbonyl signals in their ^{13}C-NMR spectra indicated that one of the two in each molecule is due to the ester group (1 and 2, 183.3 ppm) and the other, to the ketone group (1, 213.6 ppm; 2, 212.5 ppm). The distortionless enhancement by polarization transfer (DEPT) spectra of 1 and 2 showed that both compounds are composed of three methyl carbons, six methylene carbons including one $-CH_2-O-$ group, five methine carbons including one oxygenated carbon and those from one $-CH=CH-O-$ group and six quaternary carbons including two carbonyl and two oxygenated carbons. At this stage, 1 and 2 were considered to be pentacyclic diterpenoids, based on the presence of three double bonds and the value of the degree of unsaturation. In order to establish the carbon skeletons of 1 and 2, connectivities ($^1J_{CC}$) for these carbons were obtained by means of a two-dimensional incredible natural abundance double quantum transfer experiment[4] (2D-INADEQUATE, Fig. 2a) and both compounds were assigned as a labdane skeleton (Fig. 2b). In addition, the absence of hydroxyl groups in the pentacyclic molecules indicates that the remaining three oxygen atoms are involved in formation of one ester and two ether bonds. Thus, the remaining problem is, which carbons are involved in the γ-lactone and ether bond formation. The DEPT data of 1 and 2 showed that C-6 in each compound is an oxygenated methine carbon. Accordingly, this carbon was concluded to participate in the γ-lactone formation with C-19. Furthermore, the DEPT data indicated that C-9 and -13 in 1 and 2 are both oxygenated quaternary carbons. Considering the presence of the five-membered ring ketone, one ether bond was suggested to be located between the C-9 and -13 carbons. Thus, C-15 and -16, which are both oxygenated, were connected by the remaining one oxygen atom to give another ether bond. Consequently, 1 and 2 were concluded to have the same planar structure. Based on this structure, all ^{1}H-NMR signals were assigned by correlated spectroscopy (COSY), double quantum filtered (DQF)-COSY, relayed coherent transfer (RCT)-COSY and nuclear Overhauser enhancement spectroscopy (NOESY). The chemical shifts of the ^{1}H and ^{13}C signals are summarized in Tables 1 and 2.

Relative Configurations of Marrubinones A (1) and B (2) The relative configurations of 1 and 2 were elucidated from the values of coupling constants, J_{HH}, and intramolecular nuclear Overhauser effect (NOE) correlations observed in the NOESY spectra. Figure 3 shows diagnostic NOEs observed in 1 and 2. In 1 (Fig. 3), H-5 exhibited an NOE cross-peak with H_a-1 due to the 1,3-diaxial arrangement. Thus, it was found that H-5 and H_a-1 have the axial configuration. H_a-1 showed a long-range coupling ($^4J_{HH}$) with H-20 due to W-type coupling[5] in the COSY spectrum. This correlation indicated that C-20 is axial. The result that H-5 and C-20 are axial with respect to

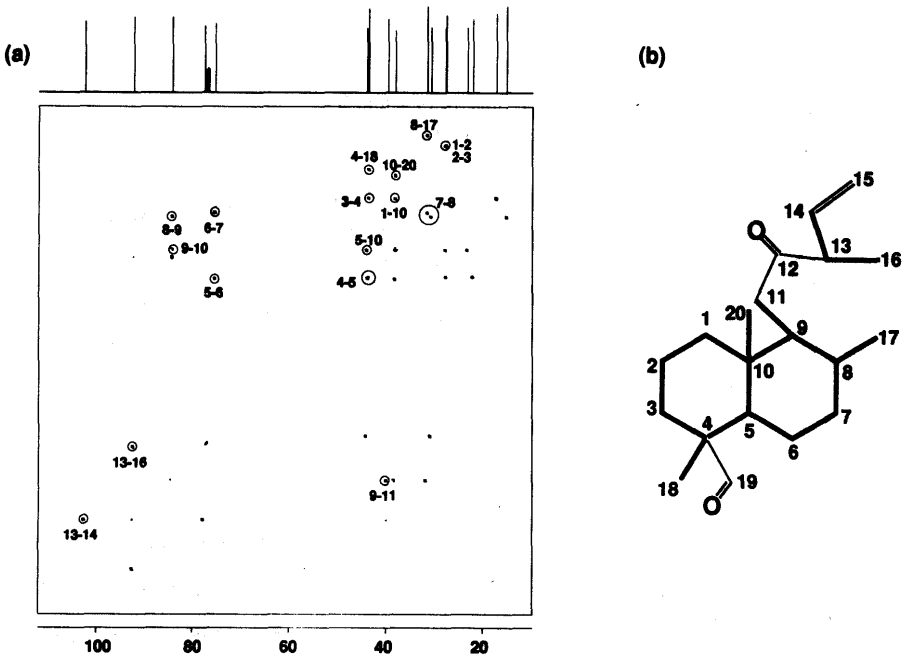


Fig. 2. Part of the Symmetrized 2D-INADEQUATE Spectrum [120 mg in $CDCl_3$ (0.3 ml), 25 °C, 125 MHz, 64 scans] of 2 (a) and the Carbon Skeleton Constructed from $^1J_{CC}$ Connectivities (b)

Seventeen $^1J_{CC}$ connectivities were recognized in the contour plot shown in (a). Bold lines in (b) represent the CC bonds assigned in (a). The remaining connectivities between 4—19, 11—12 and 12—13 do not appear in the region shown in (a).

Table 1. ^{1}H-NMR Data of Compounds **1** and **2** (600 MHz, $CDCl_3$)[a]

H	1	2
	δ, mult., J	δ, mult., J
1α	1.21	1.70
1β	1.21	1.30
2α	1.54	1.59
2β	1.78	1.823 m
3α	1.50	1.511 ddd (4.3, 4.3, 14.8 Hz)
3β	2.104 dd (3.7, 15.2 Hz)	2.13
5α	2.318 (4.6 Hz)	2.277 d (4.4 Hz)
6β	4.764 dd (4.6, 6.4 Hz)	4.743 t-like
7α	1.76	1.73
7β	2.256 dd (6.4, 16.3 Hz)	2.202 dd (5.9, 15.7 Hz)
8β	2.16	2.16
11 (*pro R*)	2.605 d (19.5 Hz)	2.794 d (18.5 Hz)
11 (*pro S*)	2.586 d (19.5 Hz)	2.361 d (18.5 Hz)
14	5.014 d (2.6 Hz)	4.988 d (2.5 Hz)
15	6.666 d (2.6 Hz)	6.614 d (2.5 Hz)
16 (*pro R*)	4.336 d (10.5 Hz)	4.319 d (10.5 Hz)
16 (*pro S*)	4.293 d (10.5 Hz)	4.459 d (10.5 Hz)
17	0.935 d (6.5 Hz)	0.721 d (6.5 Hz)
18	1.312 s	1.318 s
20	1.062 s	1.070 s

a) Chemical shifts observed from two-dimensional spectra are expressed in $\Delta\delta = \pm 0.01$.

Table 2. ^{13}C-NMR Data for Compounds **1** and **2** (125 MHz, $CDCl_3$)

C	1	2
	δ, mult.	δ, mult.
1	28.72 t	28.32 t
2	17.92 t	17.80 t
3	28.23 t	28.17 t
4	43.62 t	44.00 s
5	44.43 d	44.40 d
6	75.74 d	75.69 d
7	31.11 t	31.21 t
8	31.31 d	31.97 d
9	85.10 s	84.63 s
10	39.27 d	38.60 s
11	39.42 t	40.10 t
12	213.61 s	212.54 s
13	93.10 s	92.72 s
14	102.56 d	102.80 d
15	151.94 d	151.29 d
16	79.11 t	77.93 t
17	16.31 q	15.71 q
18	22.61 q	22.56 q
19	183.32 s	183.34 s
20	23.65 q	23.73 q

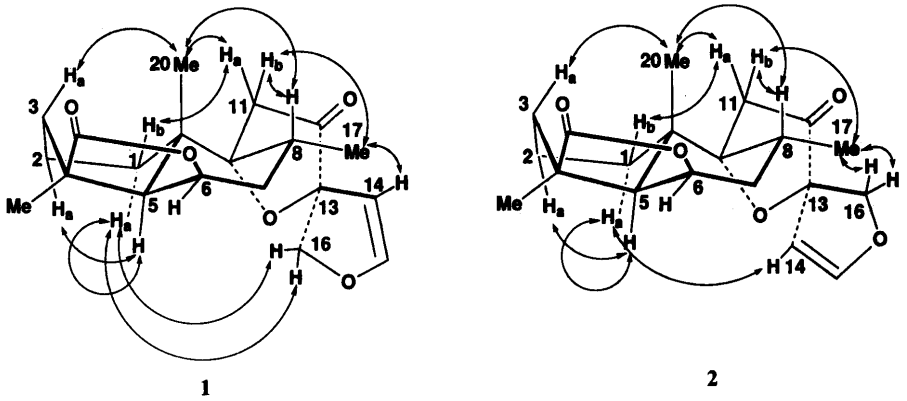


Fig. 3. Diagnostic NOE Correlations Observed in the NOESY Spectra of **1** and **2**
The spectra (600 MHz) were obtained with a mixing time of 1.5 s at 27 °C in $CDCl_3$.

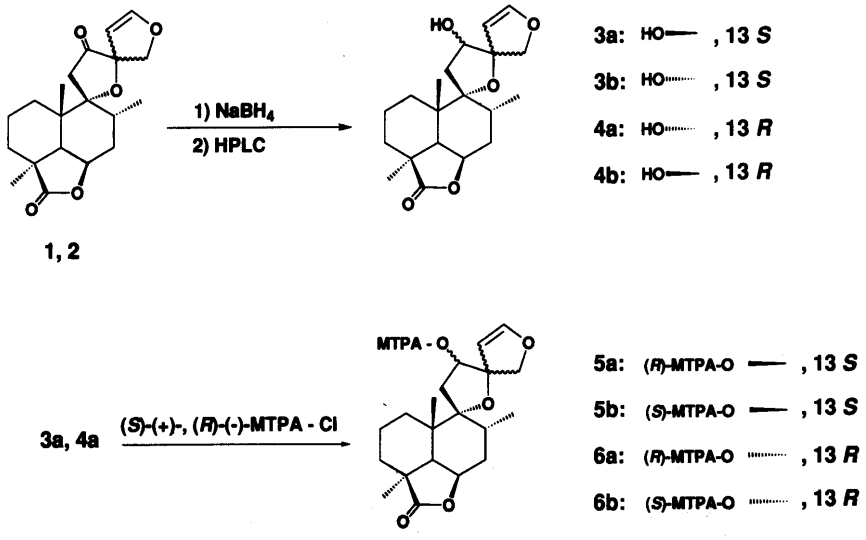
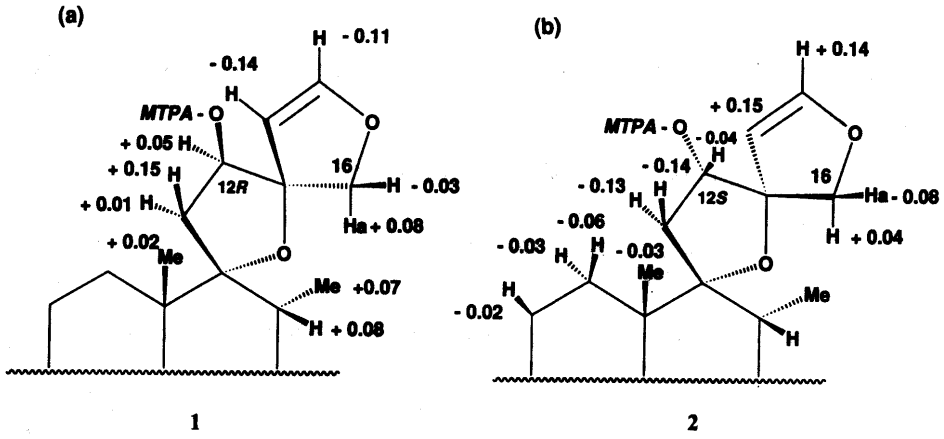


Fig. 4. Route to the MTPA Esters from **1** and **2**
Compounds **3a** and **3b** were obtained from **1**, and **4a** and **4b** from **2**. Compounds **3a** was derived to **5a** and **5b**, and **4a** to **6a** and **6b**.



1 2

Fig. 5. Chemical Shift Differences, $\Delta\delta$ ($\delta S-\delta R$), for the (*R*)- and (*S*)-MTPA Esters Derived from **1** (a) and **2** (b)

The differences are expressed in ppm and those for the other signals are not described because they are quite small (less than 0.01 ppm). However, positive and negative values are systematically arranged.

each other led to the conclusion that rings A/B are *trans*. In ring A, H-2a and H-3a showed NOE connectivities with H-5 and H-20, respectively. This observation indicated that the ring takes a twisted form instead of a chair form. The coupling constant ($^3J_{HH}$) between H-5 and H-6 was 4.4 Hz, showing that H-6 is equatorial and, at the same time, the γ-lactone ring is in a diaxial arrangement. An NOE cross-peak was observed between H-20 and H-8. It was, thus, found that H-8 is axial and Me-8 is equatorial. In addition, the orientation of C-11 was determined as equatorial for ring B because H_a-11 had NOE cross-peaks with H_b-1 and H-20, and H_b-11 revealed NOE connectivities with H-8 and H-17. The same relative configuration so far described for **1** was also recognized in **2** (Fig. 3). Thus, the structural difference between **1** and **2** was suggested to result from the configuration at C-13. In **1**, H-16 and H-14 showed NOE connectivities with H_a-1 and H-17, respectively. On the other hand, H-16 and H-14 of **2** had NOE cross-peaks with H-17 and H_a-1, respectively. In addition, H-14 had an NOE cross-peak with H_a-11 in **2**. These NOE correlations proved that **1** and **2** differ from each other in the configuration at C-13 as suggested above.

Absolute Configurations of Marrubinones A (1) and B (2) In order to determine the absolute configurations of **1** and **2**, the modified Mosher's method[6] was applied. The MTPA esters of **1** and **2** were prepared according to the route shown in Fig. 4. Reduction of the ketone group in **1** with $NaBH_4$ gave two epimeric hydroxy forms, which were separated by semi-preparative HPLC to give **3a** and **3b**. The relative configurations of the hydroxyl group at C-12 in **3a** and **3b** were determined by measuring NOESY. The main product **3a** was treated with (*S*)-(+)- and (*R*)-(−)-MTPA chloride for preparation of MTPA esters, **5a** and **5b**. Similarly, **2** was reduced to give the epimeric compounds, **4a** and **4b** in almost the same yield. The alcohol **4a**, which was eluted first, like **3a**, was esterified to afford **6a** and **6b**. After assignment of the ^1H signals of the esters using 2D-NMR, the chemical shift differences, $\Delta\delta$ ($\delta S-\delta R$), for the (*R*)- and (*S*)-MTPA esters were obtained (Fig. 5). In Fig. 5a, a systematic arrangement of positive and negative values for **5a** and **5b** was observed on the right and left sides of the MTPA plane, though one (H_a-16) of the protons on C-16 showed an irregular shift. This result revealed that C-12 of **3a** has the *R*-configuration. Since the relative configuration was already known, the structure of **1** was determined as 9α,13*S*: 15,16-diepoxy-12-oxo-14-labden-19,6β-olide. The irregular shift of H_a-16 can be explained by the hypothesis that the actual MTPA plane is tilted and the MTPA carbonyl group is immediately next to H_a-16. Thus, it is supposed that H_a-16 was affected not only by the anisotropic effect of the benzene ring, but also by the proximity and anisotropic effects of the carbonyl group and showed such a shift. A systematic arrangement of the $\Delta\delta$ values was also observed in the MTPA esters (**6a** and **6b**) of **4a** except for H_a-16 (Fig. 5b). This result indicated that C-12 of **4a** has the *S*-configuration. Therefore, **2** was identified as 9α,13*R*: 15,16-diepoxy-12-oxo-14-labden-19,6β-olide.

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