Marrusidins A and B, New Epimeric Labdane Diterpenes from *Marrubium anisodon*

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Marrusidins A (**1**) and B (**2**), two new labdane-type diterpenes, were isolated from the $CHCl_3$-soluble subfraction of *Marrubium anisodon*, along with polyodonine. Their structures were assigned with the aid of ^{1}H- and ^{13}C-NMR spectra and by COSY, HMQC, NOESY, and HMBC experiments.

Introduction. – The genus *Marrubium* (Lamiaceae) comprises 30 species which are mainly found in Europe, the Mediterranean region, and Asia [1]. One of these is *Marrubium anisodon* which is a herb growing wildly in the Balochistan Province of Pakistan [2]. It possesses tonic, aromatic, stimulant, diaphoretic, and diuretic properties [3]. Previously, diterpenes, flavonoidal glycosides, and essential oils have been reported from this species [4–6]. The ethnopharmacological and chemotaxonomic importance of the genus *Marrubium* prompted us to reinvestigate the chemical constituents of *Marrubium anisodon*. As a result, we isolated two new epimeric labdane-tye diterpenes, named as marrusidins A[1])(**1**) and B[1]) (**2**) (*Fig. 1*), along with polyodonine (=(2′*R*,2″a*S*,5′*R*,5″a*S*,7″*R*,8″a*R*,8″b*R*)-3″,4″,5″,5″a,7″,8″,8″a,8″b-octahydro-2″a,5″a,7″-trimethyldispiro[furan-3(2*H*),2′(5″*H*)-furan-5′,6″-[6*H*]naphtho[1,8-*bc*]-furan] [7], reported for the first time in this species.

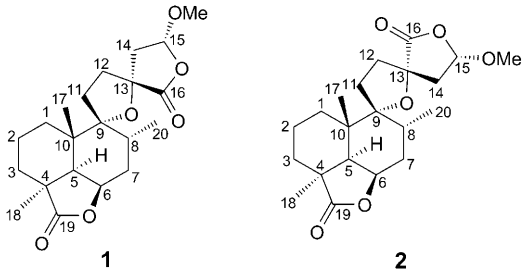

Fig. 1. *Compounds* **1** *and* **2***, isolated from Marrubium anisodon*[1])

[1]) Trivial atom numbering (IUPAC); for systematic names, see *Exper. Part.*

Results and Discussion. – The MeOH extract of *Marrubium anisodon* was divided into hexane-, $CHCl_3$-, AcOEt-, BuOH-, and H_2O-soluble fractions. A subseries of chromatographic techniques applied to the $CHCl_3$-soluble subfraction furnished the diterpenes **1**, **2**, and polyodonine.

Marrusidin A (**1**) was obtained as an optically active white amorphous solid. The molecular formula $C_{21}H_{30}O_6$ was deduced by HR-EI-MS, showing the molecular-ion peak at *m/z* 378.1340. The IR spectrum showed characteristic absorption bands at 1780 and 1765 cm^{-1} for γ-lactones and 1250 cm^{-1} for the MeO group. The broad-band-decoupled and DEPT ^{13}C-NMR spectra showed 21 signals, comprising four Me, seven CH_2, and four CH groups, and six quaternary C-atoms (*Table*). The low-frequency signals at δ(C) 183.6 and 177.3 were assigned to γ-lactone moieties. Two O-bearing quaternary C-atoms resonated at δ(C) 93.1 and 82.9, while the O-bearing CH moieties were observed at δ(C) 101.1 and 76.4. The MeO group gave a signal at δ(C) 56.8. A

Table. *^{1}H-* and *^{13}C-NMR Data* (400 and 100 MHz, resp.; $CDCl_3$) *of Compounds* **1** *and* **2**[a]). δ in ppm, *J* in Hz.

	1		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
CH_2(1)	1.51–1.53 (*m*, H_a), 1.29–1.30 (*m*, H_b)	28.7	1.48–1.50 (*m*, H_a), 1.30–1.32 (*m*, H_b)	28.2
CH_2(2)	1.78–1.80 (*m*, H_a), 1.71–1.73 (*m*, H_b)	17.9	1.76–1.77 (*m*, H_a), 1.66–1.69 (*m*, H_b)	17.8
CH_2(3)	2.10–2.13 (*m*, H_a), 1.42–1.44 (*m*, H_b)	28.0	2.05–2.06 (*m*, H_a), 1.40–1.43 (*m*, H_b)	27.6
C(4)	–	44.1	–	44.0
H–C(5)	2.05 (*d*, *J* = 4.5)	46.2	2.14 (*d*, *J* = 4.5)	45.7
H–C(6)	4.68 (*t*, *J* = 5.4)	76.4	4.67 (*t*, *J* = 5.2)	76.0
CH_2(7)	2.15–2.17 (*m*, H_a), 1.75–1.77 (*m*, H_b)	31.7	2.21–2.23 (*m*, H_a), 1.70–1.74 (*m*, H_b)	32.2
H–C(8)	2.14–2.15 (*m*)	31.8	2.09–2.11 (*m*)	31.8
C(9)	–	93.1	–	93.5
C(10)	–	39.1	–	39.3
CH_2(11)	2.07–2.08 (*m*, H_a), 1.86–1.87 (*m*, H_b)	29.2	2.24–2.25 (*m*, H_a), 1.87–1.88 (*m*, H_b)	29.1
CH_2(12)	2.37–2.39 (*m*, H_a), 2.17–2.19 (*m*, H_b)	37.6	2.33–2.55 (*m*, H_a), 2.15–2.17 (*m*, H_b)	37.0
C(13)	–	82.9	–	84.0
CH_2(14)	2.41 (*dd*, *J* = 13.4, 5.5, H_a), 2.21–2.24 (*m*, H_b)	44.2	2.55 (*dd*, *J* = 13.6, 5.6, H_a), 2.18–2.19 (*m*, H_b)	43.9
H–C(15)	5.32 (*dd*, *J* = 9.2, 5.7)	101.1	5.36 (*dd*, *J* = 9.0, 5.8)	101.8
C(16)	–	177.3	–	177.0
Me(17)	1.04 (*s*)	24.1	1.02 (*s*)	23.8
Me(18)	1.27 (*s*)	22.8	1.25 (*s*)	23.1
Me(19)	–	183.6	–	183.7
Me(20)	1.10 (*d*, *J* = 6.3)	16.1	0.84 (*d*, *J* = 6.0)	17.3
MeO–C(15)	3.45 (*s*)	56.8	3.48 (*s*)	57.0

[a]) Assignments based on HMBC cross-peaks.

detailed examination of the spectra showed that marrusidin A (**1**) belongs to the labdane group of diterpenes. COSY, HMQC, and HMBC experiments revealed the presence of a labdane skeleton bearing an O-functionality at C(9) and a C(19,6) lactone function. COSY and HMQC experiments revealed the presence of CH_2(11) and CH_2(12) groups. The HMBC spectrum showed ^3J correlations of CH_2(11) with C(10) and C(13). Similarly, CH_2(12) showed a ^3J correlation with C(9), C(14), and C(16), revealing that these two CH_2 groups should be placed between two tertiary O-bearing C-atoms forming a tetrahydrofuran ring. The spectral data showed close resemblance to cyllenine A, except for the absence of a CH_2 group at C(16) revealing the presence of a C(15,16) lactone moiety. The low-frequency O-bearing CH group at δ(H) 5.32 could be ascribed to H–C(15). Its chemical shift indicated the attachment of a MeO group at C(15). This could further be confirmed through HMBC experiments, showing a ^3J correlation of MeO at δ(H) 3.45 with C(15) at δ(C) 101.1. The chemical shifts, coupling constants, and NOESY experiments established the relative configuration of marrusidin A (**1**). In particular, the NOE between H–C(8) and Me(17) on the one hand and the lack of an NOE between H–C(5) and Me(17) on the other hand established the *trans* fusion of rings *A* and *B*. The coupling constant of H–C(6) (*t*, J = 5.4) pointed to an equatorial position, while the NOE cross-peaks H–C(5)/H–C(6), H–C(6)/Me(18), and H–C(5)/Me(18) indicated that these protons are on the same side (α). The NOEs CH_2(2)/Me(17), CH_2(11)/Me(17), and H–C(8)/Me(17) established the β configuration of these H-atoms. Moreover, the orientation of C(11) with respect to ring *B* was determined to be equatorial, as revealed by the NOE cross-peaks H_b–C(11)/H_b–C(1) and CH_2(11)/Me(17). The configuration of H–C(15) was assigned β on the basis of the NOE between H–C(15)/H_a–C(12). Its larger coupling constant provided further support for the configuration of the MeO group at C(15) [8]. The relative configuration at C(13) was assigned *R** based on NOE cross-peaks between CH_2(14) and H_a–C(1). On the basis of these cumulative evidences, the structure of marrusidin A (**1**) could be assigned as *rel*-(6β,8α,13*R*,15*R*)-9,13-epoxy-6,15-dihydroxy-15-methoxy-labdane-16,19-dioic acid di-γ-lactone.

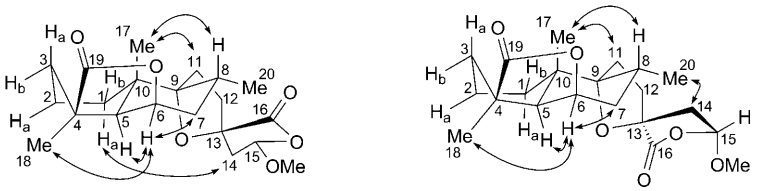

Fig. 2. *Important NOESY* (H ↔ H) *correlations of compounds* **1** *and* **2**[1])

Marrusidin B (**2**) was obtaind as an optically active white amorphous solid. The HR-EI-MS showed an M^+ peak at m/z 378.1343 corresponding to the molecular formula $C_{21}H_{30}O_6$. The IR spectrum was almost superimposable with that of **1**. In so far, however, as the two compounds differ in optical rotation, these are apparently epimeric. The ^{1}H- and ^{13}C-NMR spectra were also very similar to those of **1**, except for the low-frequency shift at δ(H) 2.55 in the ^{1}H-NMR spectrum and high-frequency

shifts of C(13) at δ(C) 84.0 and C(17) at δ(C) 17.3 in the ^{13}C-NMR spectrum (*Table*). The NOE interactions were similar to those of **1**, except for CH_2(14) now showing correlation with Me(20) instead of H_a–C(1) (*Fig. 2*). Marrusidin B (**2**) is, therefore, the C(13) epimer of **1**, and was assigned the structure of *rel*-(6β,8α,13*S*,15*S*)-9,13-epoxy-6,15-dihydroxy-15-methoxylabdane-16,19-dioic acid di-γ-lactone.

Experimental Part

General. TLC: precoated silica gel F_{254} plates (*E. Merck*, Darmstadt, Germany); detection at 254 nm and by spraying with ceric sulfate reagent. Column chromatography (CC): silica gel (SiO_2; 230–400 mesh; *E. Merck*, Darmstadt, Germany). M.p.: *Gallenkamp* apparatus; uncorrected. Optical rotations: *Jasco-DIP-360* digital polarimeter, 10 cm tube. UV Spectra: *Hitachi-UV-3200* spectrometer. IR Spectra: *Jasco-320-A* spectrometer; in cm^{-1}. ^{1}H- and ^{13}C-NMR and 2D-NMR Spectra: *Bruker-AMX-400* spectrometer with Me_4Si as internal standard: EI-MS: *Finnigan-MAT-12* and *-MAT-312* spectrometers; in *m/z* (rel. %).

Plant Material. The whole plant of *Marrubium anisodon* C. KOCH (Lamiaceae) was collected from Quetta (Pakistan) and identified by Prof. *Rasool Bakhsh Tareen*, Department of Botany, University of Balochistan, where a voucher specimen has been deposited (BU-85).

Extraction and Isolation. The shade-dried plant material (15 kg) was extracted with MeOH (3 × 50 l) at r.t. The extract was evaporated to yield the residue (780 g) which was divided into subfractions soluble in hexane (65 g), $CHCl_3$ (60 g), AcOEt (48 g), BuOH (70 g), and H_2O (41 g). The $CHCl_3$ subfraction was subjected to VLC, and three fractions were obtained on successive elution with hexane, $CHCl_3$, and MeOH, resp. The $CHCl_3$ eluate was subjected to CC (SiO_2, hexane, hexane/$CHCl_3$, $CHCl_3$, $CHCl_3$/MeOH, and MeOH in increasing order of polarity): *Fractions 1–8. Frs. 2–4* showing similar TLC profiles were combined and subjected to CC (SiO_2, hexane and hexane/AcOEt in increasing order of polarity). The elute obtained from hexane/AcOEt 9:1 gave polyodonine as colorless crystals (15 mg). Elution with hexane/AcOEt 7:3 provided a binary mixture **1**/**2**. Compounds **1** and **2** were obtained almost pure with lingering traces of impurities by flash chromatography (SiO_2, hexane/AcOEt 8:2). Final purification was achieved by prep. TLC: **1** (12 mg) and **2** (10 mg).

Marrusidin A (= *rel-(2″a*R,*3*S,*5*S,*5′*S,*5″a*R,*7″*S,*8″a*S,*8″b*S)-*Dodecahydro-5-methoxy-2″a,5″a,7″-trimethyldispiro[furan-3(2*H*),2′(5′*H*)-furan-5′,6′-[6*H*]naphtho[1,8-*bc*]furan]-2,2″(2″a*H*)-dione*; **1**): Amorphous powder. M.p. 228–230°. $[\alpha]_D^{25} = -42.2$ ($c = 0.045$, $CHCl_3$). IR (KBr): 2975, 1782, 1765, 1475, 1180, 1050. ^{1}H- and ^{13}C-NMR: *Table*. EI-MS: 378 (34), 335 (10), 320 (7), 211 (100). HR-EI-MS: 378.1340 ($C_{21}H_{30}O_6^+$; calc. 378.2042).

Marrusidin B (= *rel-(2″a*R,*3*R,*5*R,*5′*S,*5″a*R,*7″*S,*8″a*S,*8″b*S)-*Dodecahydro-5-methoxy-2″a,5″a,7″-trimethyldispiro[furan-3(2*H*),2′(5′*H*)-furan-5′,6′-[6*H*]naphtho[1,8-*bc*]furan]-2,2″(2″a*H*)-dione*; **2**): Amorphous powder. M.p. 230–232°. $[\alpha]_D^{25} = +21.0$ ($c = 0.045$, $CHCl_3$). IR (KBr): 2974, 1780, 1768, 1473, 1178, 1055. ^{1}H- and ^{13}C-NMR: *Table*. EI-MS: 378 (36), 335 (9), 320 (6), 211 (100). HR-EI-MS: 378.1343 ($C_{21}H_{30}O_6^+$; calc. 378.2042).

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