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Labdane diterpenes from *Marrubium velutinum* and *Marrubium cylleneum*

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Abstract

From the aerial parts of *Marrubium velutinum* and *Marrubium cylleneum*, seven labdane diterpenes, velutine A, 15-*epi*-velutine A, velutine B, 15-*epi*-velutine B, velutine C, cyllenine A and 15-*epi*-cyllenine A, have been isolated together with five known diterpenes and four known flavones. The structures of the isolated compounds were established by means of NMR [^{1}H–^{1}H–COSY, ^{1}H–^{13}C–HMQC, HMBC, HMQC–TOCSY, NOESY] and MS spectral analyses. Complete NMR assignments are reported for known compounds.

Keywords: *Marrubium velutinum*; *Marrubium cylleneum*; Lamiaceae; Labdane diterpenes

1. Introduction

The genus *Marrubium* comprises around 30 species, indigenous in Europe, the Mediterranean and Asia (Mabberley, 1997). In previous communication (Karioti et al., 2003), we have reported various flavonoid and phenylethanoid glycosides obtained from the methanol extract of the aerial parts of *Marrubium velutinum*. In continuation of our phytochemical investigations into *Marrubium* species of the Greek flora, we report on the isolation and identification of twelve labdane diterpenes, four methoxylated flavones from the dichloromethane extracts from the aerial parts of *M. velutinum* and *Marrubium cylleneum*.

2. Results and discussion

From the dichloromethane extract of the aerial parts of *M. velutinum* four (**1**–**4**) known and five (**5**–**9**) new diterpenes were isolated. Compounds **5**, **6** and **7**, **8** were obtained as a C-15 epimeric mixture and their structures were elucidated on the basis of spectroscopic evidence. From the dichloromethane extract of the aerial parts of *M. cylleneum* three (**10**–**12**) diterpenes were isolated. Compounds **11** and **12** were also a C-15 epimeric mixture and were major metabolites of the plant.

Compounds **5/6** were isolated as an inseparable mixture (2:1) of two hemiacetals. The MALDI-HRMS spectrum of compounds **5/6** revealed a pseudomolecular peak at *m/z* 387.7177 $[M + Na]^+$, indicating a molecular weight corresponding to a molecular formula of $C_{20}H_{28}O_6$. However, from the ^{1}H and ^{13}C NMR data it was evident that there were far too many resonances to be consistent with the estimated molecular formula. In particular, the ^{13}C NMR spectrum showed 20 pairs

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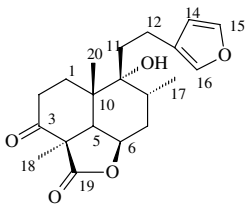
of signals. For each pair, one signal is more intense than the other. It seemed likely that **5/6** was a mixture of two epimers. All signal assignments in 1D and 2D spectra were made on this basis.

ding to protons H-2, which give a multiple signal centered approximately at δ 2.56. The ^{1}H spin systems H-2/H-1a/H-1b and H-5/H-6/H-7/H-8/H-17 were assigned by COSY. Long ^{1}H–^{13}C correlations (HMBC) between



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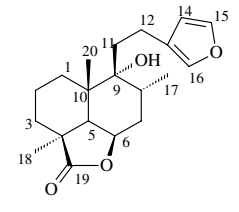

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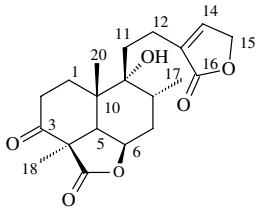

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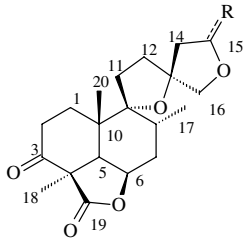
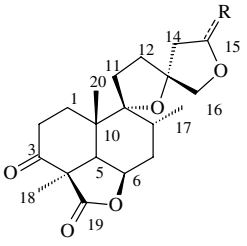


3 R=Hβ, OHα
4 R=Hα, OHβ



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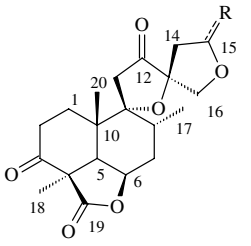
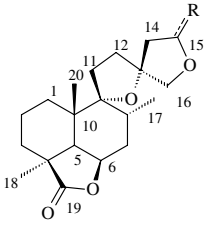
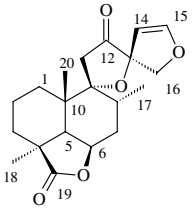
6 R=Hα, OHβ



7 R=Hβ, OHα
8 R=Hα, OHβ



11 R=Hβ, OHα
12 R=Hα, OHβ



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The IR spectrum of **5/6** contained absorption bands characteristic of hydroxyl groups (3468 cm^{-1}), γ-lactone (1782 cm^{-1}) and keto (1708 cm^{-1}) groups.

The ^{13}C NMR spectrum exhibited duplicate resonances of two tertiary methyl groups (at δ 20.5 and 19.1), one secondary methyl group (at δ 17.2/17.0), seven methylenes (at δ 29.1/29.0, 34.1/34.0, 31.6/31.5, 29.4 × 2, 34.7/37.0, 46.0/47.2 and 76.9/77.4), four methines (at δ 47.4/47.3, 74.9/74.5, 31.1 × 2 and 98.8/98.5), as well as six quaternary carbons (at δ 54.18/54.17, 206.2 × 2, 90.7/90.3, 39.3 × 2, 89.9/89.3 and 174.4 × 2), two belonging to an ester and ketone-carbonyl group at (δ 174.4 × 2 and 206.2 × 2, respectively).

Detailed examination of the spectra showed that velutine A (**5**) and 15-*epi*-velutine A belong to hemiacetalic labdane group of diterpenes. COSY, HSQC and HMBC experiments revealed the presence of a labdane skeleton bearing a keto-group at C-3, a hydroxyl group on C-9 and a 19, 6 lactone function. The presence of a carbonyl group at position 3 causes a significant deshielH-1, H-2/C-3, H-18/C-3, C-4, C-5, H-5/C-4 and H-8, H-17, H-20, H-11/C-9, C-10 established the planar structure of **5/6**. COSY and HSQC experiments revealed the presence of the methylenes 11 and 12. From the HMBC spectrum it was evident (H-11, H-12/C-10, C-13) that these two methylenes should be placed between two tertiary oxygenated carbons forming a tetrahydrofuran ring. From COSY and HSQC experiments the doublets at δ 4.15 (J = 9.0 Hz), 3.60 (J = 9.0 Hz) and 3.87 (J = 8.6 Hz), 3.83 (J = 8.6 Hz) were assigned to protons H-16 (**5/6**, respectively). In the same way, the signals at δ 5.44 and 5.59 were assigned to H-15 (**5/6**). Long ^{1}H–^{13}C correlations (HMBC) between H-15, H-14/C-16 and H-16, H-14/C-13 revealed the presence of a second tetrahydrofuran ring. Two sets of NMR signals mainly differing in the tetrahydrofuran ring 13–14–15–16 together with C-15 at δ 98.8/98.5 indicate the presence of an anomeric mixture of **5/6**.

NOESY experiment as well as chemical shifts and coupling constants of the nonoverlapped protons estab-

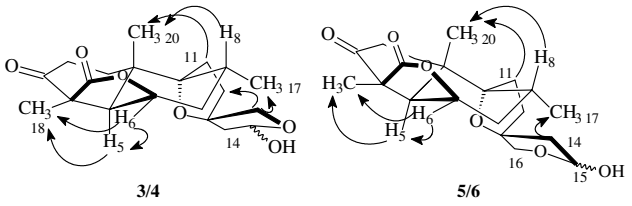

Fig. 1. Selected NOE correlations of compounds **3/4** and **5/6**.

lished the relative stereochemistry of velutine A (**5**) and 15-*epi*-velutine A (**6**) (Fig. 1). In particular, observed NOE between H-20 and H-8 on one hand, and lack of NOE crosspeak between H-5 and H-20 on the other, proved the *trans* fusion of rings A and B in both structures. The coupling constant of proton H-6 (*t*, J = 4.7 Hz) showed its equatorial position, while NOE crosspeaks between H-5/H-6, H-6/H-18, H-5/H-18 indicated these are on the same side (α). In the same spectrum, interactions between H-2/H-20, H-11/H-20, H-8/H-20 proved the β configuration of these protons. Moreover, the orientation of C-11 with respect to ring B was determined to be equatorial, as revealed by NOE crosspeaks between H-11b/H-1b and H-11/H-20. The double doublet at δ 5.44 (J = 9.0, 5.50 Hz) is assigned to H-15β on the basis of NOE between H-15/H-12a (Fig. 2), while the double doublet at δ 5.59 (J = 5.1, 2.7 Hz) is assigned to H-15α.

Finally, the relative stereochemistry on C-13 was determined as shown in Fig. 1 based on NOE crosspeaks between H-14/H-17 and H-16a/H-1b.

Compounds **3/4** have been previously isolated from *Leucas neufliseana* (Khalil et al., 1996) but the C-13 relative stereochemistry has not been determined. NOESY measurements clearly showed that compounds **3/4** are the C-13 epimers of velutine A (**5**) and 15-*epi*-velutine A (**6**). Diagnostic NOE between H-16a and H-17 (Fig. 1) proved that **3/4** have opposite relative stereochemistry compared to **5/6**. Detailed ^{1}H NMR and ^{13}C NMR data are reported for compounds **3/4**.

Velutine B (**7**) and 15-*epi*-velutine B (**8**) were isolated as an inseparable mixture (1:1) of two isomers. Their MALDI-HRMS mass spectrum gave a pseudomolecular peak at *m/z* $[M + Na]^+$ 401.1569, consistent with

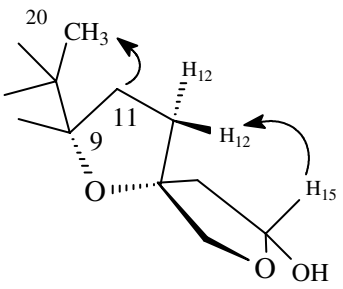

Fig. 2. Diagnostic NOEs for compounds **5/6**.

the molecular formula $C_{20}H_{26}O_7$, indicating 8 degrees of unsaturation. Its IR spectrum showed absorptions due to hydroxyl (3470 cm^{-1}), γ-lactone (1778 cm^{-1}) and keto (1715 and 1750 cm^{-1}) functionalities. Careful analysis of 1D and 2D spectra showed that compounds **7/8** belong to the group of hemiacetals with the exception of a different substitution pattern on ring C. Compounds **7** and **8** are characterized by the presence of a labdane skeleton with a 6, 19 lactone function, a carbonyl group at C-3, an oxygen group at C-9 and, finally, a secondary methyl group at C-8. Long ^{1}H–^{13}C correlation (HMBC) between H-11/C-12 revealed the presence of an extra carbonyl group at position 12. As a result, protons H-11a and H-11b are shifted downfield to δ 2.78 and 2.60, respectively. It is interesting to point out that the geminal coupling between H-11a and H-11b is 19.8 Hz. We could explain this unusually large coupling constant in terms of a special geometry of the 9,13-tetrahydrofuran ring. The above feature is in full agreement with the literature data (Iida et al., 1995; Hatam et al., 1995). The relative stereochemistry of compounds **7** and **8** was established by NOESY experiment and is similar to the relative stereochemistry of the rest of the labdane diterpenes of *Marrubium velutinum*. Interactions between H-5/H-6, H-6/H-7b, H-6/H-18 and H-5/H-1a indicated that these are on the same side (α), while, NOEs between H-20/H-8, H-11a/H-20 and H-11b/H-20 indicated that these are on the opposite side (β). NOE signals between H-16a, H-16b and H-17 revealed the relative stereochemistry at C-13 in both substances. The deshielding of proton H-15 in structure **7** could be attributed to its β orientation within the 15, 16 tetrahydrofuran ring. It seems likely that H-15 β is affected by the presence of the C-12 carbonyl group on the adjacent ring. This assumption is in full agreement with former literature data (Khalil et al., 1996). According to Khalil et al. (1996), β orientation of the 15-OH causes a downfield shift of H-16β in such a way that protons H-16a and H-16b coincide, as they are equally affected by the oxygen groups on C-9 and on C-15. This suggestion is confirmed in all the above structures (**3/4**, **5/6**, **7/8**). When 15-OH has a β orientation H-16a and H-16b have almost identical chemical shifts. C-12 shifts could also be explained in the same way: the β orientation of the 15-OH in compounds **4** and **6** causes a downfield shift of the methylene carbon C-12 of 2.3–3.0 ppm, compared to compounds **3** and **5**, respectively.

Velutine C (**9**) exhibited a pseudomolecular peak at *m/z* $[M + Na]^+$ 385.1625 in its MALDI-HRMS mass spectrum, consistent with the molecular formula $C_{20}H_{26}O_6$, indicating 8 degrees of unsaturation. Its IR spectrum contained absorptions due to γ-lactone (1774 cm^{-1}) and keto (1718 cm^{-1}) functionalities. The ^{13}C NMR spectrum exhibited duplicate resonances of two tertiary methyl groups (at δ 20.5 and 18.1), one secondary methyl group (at δ 16.3), six methylenes (at δ

28.8, 34.1, 31.7, 31.2, 20.7 and 68.8), four methines (at δ 46.7, 74.9, 32.1 and 144.7), one olefinic (144.7), as well as six quaternary carbons (at δ 207.0, 53.7, 74.3, 40.2, 134.2, 174.9 and 174.8), two belonging to an ester (174.9 and 174.8) and one to a ketone-carbonyl group (207.0).

Detailed examination of 1D and 2D NMR (COSY, HSQC, HMBC) spectra revealed the presence of a labdane skeleton containing a butenolide ring. The doublet at δ 4.80 (J = 1.4 Hz) belongs to a methylene group and was assigned to H-15, while the singlet at δ 7.14 coupled to H-15 was assigned to H-14. Long range ^{1}H–^{13}C couplings (HMBC) between H-14/C-16 and H-15/C-13, C-14 revealed the presence of an extra carbonyl moiety at δ 174.9 (C-16). Further, HMBC correlations between methylene protons H-12, H-11 and olefinic carbon C-13, as well C-10, showed that the butenolide ring is linked to the rest of the labdane skeleton through these methylene groups. The NOESY experiment established the relative stereochemistry of velutin C: NOEs between H-5/H-6, H-6/H-18, H-6/H-7b and H-7b/H-17 showed these are orientated in the same side, while interactions between H-20/H-8 and H-20/H-1b indicated that these are on the opposite side.

Compounds **11** (cyllenine A) and **12** (15-*epi*-cyllenine A) were isolated as an inseparable equilibrium mixture of two isomers, structurally related to compounds **3** and **4**. A detailed search in the literature revealed the isolation from *M. vulgare* of analogous hemiacetals (Fulke et al., 1968). However, their spectroscopic data are insufficient to establish the anomeric center, as well as the relative stereochemistry of the compounds. Diagnostic NOEs between H-16 and H-17 proved compounds **11/12** to have the same relative stereochemistry on C-13, as **3/4** and therefore the anomeric center to be C-15. Chemical displacements of protons, as well NOESY measurements clearly showed that compounds **11** and **12** share the same relative stereochemistry as marrubiin.

On the basis of ^{1}H and ^{13}C NMR, UV and MS data compounds **1**–**4**, **10** and **13**–**16** were identified as peregrinine (**1**) (Salei et al., 1966), marrubinone B (**2**) (Iida et al., 1995), 9α,13*R*: 15,16-bisepoxy-15α-hydroxy-3-oxo-labdan-6β, 19-olide (**3**) (Khalil et al., 1996), 9α,13*R*: 15,16-bisepoxy-15β-hydroxy-3-oxo-labdan-6β,19-olide (**4**) (Khalil et al., 1996), marrubiin (**10**) (Savona and Piozzi, 1976), ladanein (**13**) (Seshadri and Sharma, 1973), 5,7,4′-scutellarein trimethylether (**14**) (Sumaroyono et al., 1990), 5,6,7,4′-scutellarein-tetramethylether (**15**) (Schneider and Tan, 1973) and salvigenin (**16**) (Ulubelen et al., 1980). Finally, HMQC-TOCSY experiment in peregrinine gave evidence to some differences to previously reported data, i.e. C-1 should be placed at δ 28.6 instead of 34.6 and C-11 should be assigned at δ 34.5 vs 28.7 (Salei et al., 1966; Canonica et al., 1968; Savona et al., 1984).

This is the first detailed report on hemiacetal and furanic labdanes in the genus *Marrubium*. Previous work only reports incomplete spectroscopic data. This work presents full ^{1}H and ^{13}C NMR assignments. Compounds **2**, **7** and **8** could be classified as prefuranic labdanes, due to the presence of a carbonyl group at C-12. However, **7** and **8** belong to an unusual type of labdane differing from all hitherto known labdane hemiacetals and prefuranic labdanes, as they combine both a hemiacetal group together with a carbonyl group at C-12.

3. Experimental

3.1. General experimental procedures

^{1}H, ^{13}C and 2D NMR spectra were recorded in $CDCl_3$ on Bruker DRX 400 and Bruker AC 200 (50.3 MHz for ^{13}C NMR) instruments at 295 K. Chemical shifts are given in ppm (δ) and were referenced to the solvent signals at 7.24 and 77.0 ppm for ^{1}H and ^{13}C NMR, respectively. HR-MALDI mass spectra were measured on an Ionspec Ultima FTMS spectrometer using 2,5-dihydroxybenzoic acid (DHB) as matrix. The $[\alpha]_D$ values were obtained in $CDCl_3$ at 20 °C on a Perkin–Elmer 341 Polarimeter.

Vacuum liquid chromatography (VLC): silica gel 60H (Merck, Art. 7736); HPLC: CE 1100 Liquid Chromatography Pump. Column: Kromasil C_{18} (250 × 10 mm) Column chromatography (CC): silica gel 60 (SDS, 40–63 μm), gradient elution with the solvent mixtures indicated in each case; Sephadex LH-20 (Pharmacia). TLC: Merck silica gel 60 F_{254} (Art. 5554). Detection: UV-light, spray reagent [vanillin-H_2SO_4 on silica gel].

3.2. Plant material

Aerial parts of *Marrubium velutinum* Sibth. and Sm. were collected from Kellaria – Parnassos mountain (Sterea Hellas) in July 1998 and of *M. cylleneum* were collected from Mainalon mountain (Peloponnese) in May 1999. Voucher specimens have been kept in the Herbarium of Patras University (UPA) under the numbers Skaltsa and Lazari 114 and Skaltsa and Lazari 111b-99.

3.3. Extraction and isolation

The air-dried powdered aerial parts of *M. velutinum* (0.63 kg) were successively extracted at room temperature with petroleum ether, dichloromethane, EtOAc and MeOH (2 l of each solvent, twice, 48 h). The dried dichloromethane extract (7.96 g) was subjected to VLC over silica gel (10 × 5.5 cm) with CH_2Cl_2:EtOAc mixtures of increasing polarity to yield twelve fractions (A–L) of 500 ml. Fractions C and D (1.33 g; eluted with

CH_2Cl_2:EtOAc 95:5–90:10) were combined and further applied to column chromatography over silica gel using CH_2Cl_2:EtOAc to afford 17 fractions (C'_1–C'_{17}). Fraction I_1 (175.5 mg, eluted with CH_2Cl_2:EtOAc 97:3) was further purified by CC over silica gel with P.E.:CH_2Cl_2 and yielded marrubinone B (**2**) (54.4 mg) and salvigenin (**16**) (12.5 mg). Fraction E (422.9 mg, eluted with CH_2Cl_2:EtOAc 87.5:12.5) was subjected to CC over silica gel with CH_2Cl_2:EtOAc and afforded 13 fractions (E_1–E_{13}). Fractions E_7, E_{10} and E_{11} were further purified by RP-HPLC (ACN:H_2O 50:50, 35:65 and 35:65, respectively) as follows: fraction E_7 yielded an equilibrium mixture (1.1 mg) of velutine B (**7**) and 15-*epi*-velutine B (**8**), as well as a complex mixture of compounds **3**–**6**, fraction E_{10} afforded pure velutine C (**9**), (2.5 mg); fraction E_{11} yielded an epimeric mixture (1.8 mg) of **3** and **4**. Fraction F was purified over Sephadex LH-20 (cyclohexane:CH_2Cl_2:MeOH 7:4:0.5) and afforded peregrinine (**1**) (149.1 mg), as well as ladanein [=7,4′-scutellarein-dimethylether] (**13**) (56.8 mg). Fraction H (268.6 mg, eluted with CH_2Cl_2:EtOAc 80:20–75:25) was applied to Sephadex LH-20 (cyclohexane:CH_2Cl_2:MeOH 7:4:0.5) and afforded pure 5,6,7,4′-scutellarein tetramethylether (**15**). Fraction L (2.61 g, eluted with CH_2Cl_2:MeOH 50:50) was subjected to CC over silica gel with CH_2Cl_2:MeOH and afforded 15 fractions (L_1–L_{15}). Fraction L_1 (247.0 mg, eluted with CH_2Cl_2:MeOH 100:5) was further purified by successive CC over silica gel with CH_2Cl_2:EtOAc and RP-HPLC (ACN:H_2O 35:65) and yielded epimeric mixtures of **3/4** (1.0 mg) and **5/6** (8.1 mg) as well as 5,7,4′-scutellarein trimethylether (**14**) (3.2 mg).

The air-dried powdered aerial parts of *M. cylleneum* (1.3 kg) were successively extracted at room temperature

Table 1
^{1}H NMR data of compounds **1**, **3**–**6**, **7**–**9** and **11/12** (400 MHz, $CDCl_3$, *J* in Hz)

	Compound **1**	Compounds **3/4**	Compounds **5/6**	Compounds **7/8**	Compound **9**	Compounds **11/12**
H-1a	2.17 *ddd* (*J* = 13.3, 11.3, 4.3)	2.04[a]	1.97[a]	1.64[a]	2.20 *ddd* (*J* = 13.3, 11.3, 4.3)	1.55[a]
H-1b	1.65 *ddd* (*J* = 13.0, 9.9, 4.4)	1.67[a]	1.66[a]	2.01[a]	1.60 *ddd* (*J* = 13.0, 9.9, 4.4)	1.23[a]
H-2a, H-2b	2.60–2.44[a]	2.56[a]	2.59–2.53[a]	2.64–2.61[a]	2.60–2.44	1.71[a]
H-5	2.76 *d* (*J* = 4.4)	2.38 *d* (*J* = 4.4)	2.55 *d* (*J* = 4.3)	2.64–2.61[a]		1.50[a]
H-6	4.58 *t* (*J* = 5.1)	4.57 *dd* (*J* = 4.1, 4.7)	4.56 *t* (*J* = 4.7)	–	–	2.10[a]
H-7a	2.08[a]	2.13[a]	2.15[a]	–	–	1.39[a]
H-7b	1.80[a]	1.74[a]	1.69[a]	2.69 *d* (*J* = 4.1)	2.81 *d* (*J* = 4.4)	1.87 *d* (*J* = 4.4)
H-8	2.04[a]	2.13[a]	2.13[a]	4.64 *dd* (*J* = 5.1, 4.4)	4.62 *t* (*J* = 5.1)	4.67 *dd* (*J* = 4.4, 5.3)
H-11a	1.90–1.73[a]	2.18[a]	2.07[a]	2.19[a]	2.09 *dd* (*J* = 14.4, 5.8)	2.09[a]
H-11b	–	1.88[a]	1.88[a]	1.82[a]	1.80	1.64[a]
H-12a	2.54–2.44[a]	2.15/2.24[a]	2.13/2.23[a]/*dd* (*J* = 12.9, 9.8)	2.19[a]	2.04	2.08[a]
H-12b	–	1.95/2.14[a]	2.07/2.13[a]	2.78 *d* (*J* = 19.8)	1.80	2.06[a]
H-14a	6.23 br*s*	2.27/2.31 *d* (*J* = 13.3)/*dd* (*J* = 13.3, 5.5)	2.27/2.44 br*d* (*J* = 13.7)/*dd* (*J* = 13.3, 5.5)	2.60 *d* (*J* = 19.8)		1.78[a]
H-14b	–	2.02/1.96[a]/*dd* (*J* = 12.0, 1.8)	2.16/2.01[a]	–	2.49	2.09/2.17–2.10
H-15	7.31 br*s*	5.44/5.62 *dd* (*J* = 7.8, 5.2)/br*d* (*J* = 5.2)	5.44/5.59 *dd* (*J* = 9.0, 5.5)/*dd* (*J* = 5.1, 2.7)	–		1.85/2.17–2.10
H-16a	7.19 br*s*	4.22/4.00 *d* (*J* = 9.2)/*s*	4.15/3.87 *d* (*J* = 9.0)/*d* (*J* = 9.0)	2.28/2.36 *dd* (*J* = 13.6, 5.4)/*dd* (*J* = 13.6, 5.1)	7.14 br*s*	2.28/2.30 br*d* (*J* = 12.2)/*dd* (*J* = 14.4, 5.8)
H-16b	–	3.74/4.00 *d* (*J* = 9.4)/*s*	3.60/3.83 *d* (*J* = 8.6)/*d* (*J* = 8.6)	2.20/2.13 *d* (*J* = 14.0)/[a]		2.04/1.88
H-17	0.95 *d* (*J* = 6.5)	0.91/0.86 *d* (*J* = 6.4)/*d* (*J* = 6.4)	0.96/0.92 *d* (*J* = 6.2)/*d* (*J* = 6.3)	5.52/5.60 *dd* (*J* = 10.2, 5.1)/*dd* (*J* = 5.1, 4.4)	4.80 *d* (*J* = 1.4)	5.41/5.61 *m*/br*d* (*J* = 4.3)
H-18	1.46 *s*	1.44/1.45 *s*	1.45/1.44 *s*	4.20/4.16 *d* (*J* = 9.6)/*d* (*J* = 9.2)	–	4.22/3.99 *d* (*J* = 9.2)
H-20	0.87 *s*	0.93 *s*	0.91/0.89 *s*	3.93/4.08 *d* (*J* = 9.9)/*d* (*J* = 9.2)	–	3.71/3.99 *d* (*J* = 9.2)

[a] Signal pattern unclear due to overlapping.

Table 2
^{13}C NMR data of compounds **1**–**9** and **11/12** (50.3 MHz, $CDCl_3$, at 295 K)

	Compound **1**	Compounds **3/4**	Compounds **5/6**	Compounds **7/8**[a]	Compound **9**	Compounds **11/12**
1	28.6	29.1	29.1/29.0	28.8	28.8	28.4/28.3
2	34.0	34.1	34.1/34.0	33.8	34.1	17.3/17.5
3	207.0	206.2	206.2	206.0	207.0	27.6/27.7
4	53.0	53.6	54.18/54.17	54.0	53.7	43.6
5	46.4	47.4/48.0	47.4/47.3	46.7	46.7	45.8
6	74.8	74.6/75.0	74.9/74.5	74.2	74.9	75.7
7	30.9	31.4	31.6/31.5	31.2	31.7	30.9
8	31.4	31.6	31.1	31.5	32.1	31.2
9	75.0	90.5/90.2	90.7/90.3	86.1	74.3	91.3/90.0
10	39.8	39.4/39.1	39.3	39.9	40.2	38.5/38.4
11	34.5	29.3	29.4	39.2	31.2	29.1/28.9
12	20.8	34.4/37.7	34.7/37.0	217.0	20.7	34.7/37.2
13	124.6	90.9/90.4	89.9/89.3	89.8	134.2	89.5
14	110.5	45.9/48.0	46.0/47.2	46.3/46.7	144.7	46.0/48.1
15	143.0	99.4/99.2	98.8/98.5	98.9/99.5	68.8	98.7
16	138.5	77.1/77.7	76.9/77.4	75.0/75.8	174.9	76.1/76.4
17	15.8	17.1/16.9	17.2/17.0	15.7	16.3	16.9
18	20.3	20.5	20.5	20.8	20.5	22.6/22.4
19	174.8	174.2	174.4	174.5	174.8	183.6/183.3
20	17.9	19.1	19.15/19.08	18.2	18.1	23.0/23.2

[a] ^{13}C NMR chemical shifts were assigned on the basis of HSQC and HMBC experiments.

with petroleum ether, dichloromethane, EtOAc and MeOH (2 l of each solvent, twice, 48 h). The dried dichloromethane extract (26.8 g) was subjected to VLC over silica gel (8 × 6.0 cm) with CH_2Cl_2:EtOAc mixtures of increasing polarity to yield thirteen fractions (A–M) of 500 ml. Fraction C (5.2 g; eluted with CH_2Cl_2:EtOAc 95:5–90:10) was further applied to VLC over silica gel (8 × 5.5 cm) with cyclohexane:EtOAc mixtures of increasing polarity to yield ten fractions (C_1–C_{10}) of 500 ml. Fraction C_6 (833.0 mg, eluted with cyclohexane:EtOAc 75:25) was further purified by CC over silica gel using cyclohexane:EtOAc and yielded marrubiin (**10**) (235.6 mg). Further purification of fraction D (1.54 g; eluted with CH_2Cl_2:EtOAc 87.5:12.5) by CC over silica gel using cyclohexane:EtOAc afforded an epimeric mixture of compounds **11** and **12** (135.3 mg), as well as ladanein (**13**) (10.3 mg).

3.3.1. Velutine A, 15-epi-velutine A (*5*, *6*)

Colourless oil (8.1 mg); MALDI-HRMS (pos.) *m/z*: 387.7177 (requires for 387.7178); ^1H and ^{13}C NMR spectral data (see Tables 1 and 2).

3.3.2. Velutine B, 15-epi-velutine B (*7*, *8*)

Colourless oil (1.1 mg); MALDI-HRMS (pos.) *m/z*: 401.1569 (requires for 401.1577); ^1H and ^{13}C NMR spectral data (see Tables 1 and 2).

3.3.3. Velutine C (*9*)

Colourless oil (2.5 mg); $[\alpha]_D^{20}$ + 17.6° ($CHCl_3$, *c* 0.12). MALDI-HRMS (pos.) *m/z*: 385.1625 (requires for 385.1628); ^1H and ^{13}C NMR spectral data (see Tables 1 and 2).

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