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Labdane diterpenes from *Leonurus japonicus* leaves

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Abstract

Three labdane diterpenes 15,16-epoxy-6-hydroxylabda-5,8,13(16),14-tretraen-7-one (leojaponin), (9α,13S);15,16-diepoxy-7β-hydroxy-labd-14-en-6-one (13-*epi*-preleoheterin), and (9α,13R);15,16-diepoxy-6β-hydroxylabd-14-en-7-one (*iso*-preleoheterin) were isolated from the leaves of *Leonurus japonicus*, in addition to the previously reported preleoheterin. The structure elucidations were made based on analysis of their spectroscopic data.

Keywords: *Leonurus japonicus*; Lamiaceae; Diterpenes; Labdanes; Furanoids; Tropics

1. Introduction

Leonurus japonicus Houtt. (syn *L. heterophyllus* Sweet) or Chinese motherwort is a herbaceous annual of pantropical distribution of the Lamiaceae family. In Venezuela it grows in open grassland of temperate regions frequently as thick clusters (pers. obs). Extracts of aerial parts are used in Chinese medicine for various purposes such as heart antiarrhythmic (Hotta et al., 2003), sedative (Widy-Tyszkiewicz and Schminda, 1997), antimicrobial (De Souza et al., 2004), anticoagulant (Lee et al., 1991), antioxidant (Sugaya et al., 1998) and antitumoral (Chinwala et al., 2002), properties, as well as booster of the immuno response (Xu et al., 1992). Several metabolites have been isolated from *L. japonicus* that substantiate the recorded activities. For instance, the iridoid glucoside leonurid, isoquercetin, two phenolic glycosides (Sugaya et al., 1998), melatonin (Chen et al., 2003), β-sitostenone (Hotta et al., 2003) and several diterpenes exclusively of the labdane type have been characterized from its aerial parts (Savona et al., 1982; Hohn et al., 1991, 1993; Satoh et al., 2003; Boalino et al., 2004; Giang et al., 2005a,b). The occurrence of the labdane compounds, and the fast and dense growth of *L. japonicus* makes this plant a renewable source of useful medicinal materials. Typically, these labdane compounds possess a five-membered heterocycle at the end of the two carbon chain stemming from C9, either as dihydrofuran, furan, bis spyrodehydrofuran or γ-lactone mixtures. Representative compounds are hispanolone (**1**), and prehispanolone (**2**) to which the anticoagulant quality of *L. japonicus* extracts, along with preleoheterin (**3**), has been attributed (Xu et al., 1992) (Fig. 1).

The present investigation was the first to examine the components of *L. japonicus* collected in South America. The isolation and characterization of the previously recorded preleoheterin (**3**) (Hohn et al., 1993), and the novel labdanes leojaponin (**4**), epi-preleoheterin (**5**), and *iso*-preleoheterin (**6**) from this plant are the subject of the present report.

2. Results and discussion

All isolates were obtained from the hexane extract of the air-dried leaves of *L. japonicus* collected at 2150 m above

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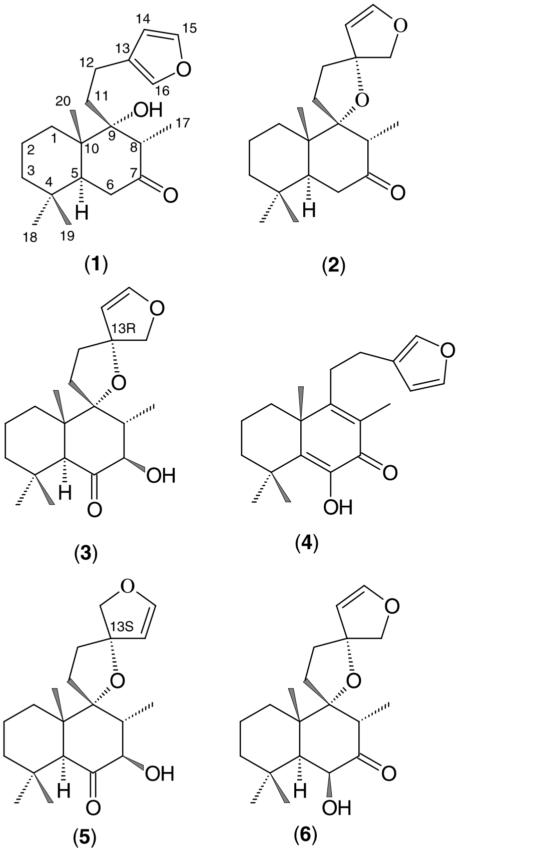

Fig. 1. Representative and novel labdanes from *Leonurus japonicus*.

sea level in the Andean region of Mérida state, western Venezuela. Purification was accomplished by repeated FLC and TLC on silica gel plates as described in the Experimental.

Leojaponin (**4**) was obtained as yellow prisms, m.p. 109–111 °C, with diagnostic bands of an α,β-unsaturated ketone (1619 cm^{-1}) and a hydroxyl group (3350 cm^{-1}) in the FTIR spectrum. The molecular formula was $C_{20}H_{26}O_3$ which was confirmed by analysis of the ^{13}C NMR spectrum (Table 2) and the HREI MS M^+ m/z 314.1878. (Calcd. 314.2062). Twenty carbon signals were observed including four tertiary methyl groups, (δ_H 1.37, 1.38, 1.39, 1.99, all *s*), one of which was linked to an unsaturated carbon. In addition to the ketone at δ_C 181.7, there were eight sp^2 carbons corresponding to two tetrasubstituted olefins and a monosubstituted furan ring. The latter was revealed by typical proton signals at δ_H 6.33 (*m*), 7.29 (*m*), and 7.39 (*t*, $J = 1.5$ Hz) and the carbon spectrum at δ_C 110.5, 138.7, and 143.1. In the mass spectrum, the fragment at m/z 233 (13% int. rel.) showed the loss of this heterocycle and a methylene functionality of the side-chain. One of the tetrasubstituted alkenyl portions appeared to be part of an α,β-unsaturated ketone as revealed by the low field signals at δ_C 165.8 and 181.7, in agreement with the IR interpretation. Attached to this β olefinic carbon was a chain comprising two methylenes, with both units being allylic (δ_H 2.57 (*m*), 2.62 (*dd*, $J_1 = 13.1$, $J_2 = 8.0$ Hz)). This suggested that the furan ring would be at the end of an ethyl group, which joins the furanyl ring to a decalin skeleton, as suggested by the unsaturation number. The position of the double bond was determined by a HMBC connectivity experiment (Fig. 2). Bonded to the second tetrasubstituted double bond had to be a hydroxyl group, as there was no $C(sp^3)$—OH signal in the expected region of the ^{13}C NMR spectrum. The only possible stable arrangement for this enol form, in combination with the data above, was a C=C(OH)—C(=O)—C(CH_3)=C—CH_2—CH_2—furan unit. Due to the absence of additional allylic methynes, both olefinic carbons of this cross-conjugated system had to be bonded to sp^3 quaternary carbons. Based on 2D NMR spectroscopic data, the remaining three vicinal methylenes and three tertiary methyles, along with the rest of the skeleton, should form the labdane type structure as shown (Fig. 1, **4**). These assignments were additionally confirmed by spectroscopic similarities of the known 5,6-dihydro homolog (persianone), which was isolated from *Ballota auchieri* (Rustaiyan et al., 1995). The absolute configuration of the stereogenic carbon C10 shown in **4** was proposed since all labdanes of the *Leonurus* genus so far studied possess this configuration. Thus, the structure of **4** was assigned as 15,16-epoxy-6-hydroxyladba-5,8,13(16),14-tetraen-7-one.

13-*epi*-Preleoheterin (**5**) was isolated from the hexane soluble fraction in 0.1% yield from the dry leaves as colorless needles m.p. 95–97 °C, with M^+ m/z 334.2150 (Calcd. 334.2376) corresponding to a molecular formula $C_{20}H_{30}O_4$. Comparison with preleoheterin (**3**) isolated in 0.1% from the same chromatographic run showed several similarities. The mass spectra appeared identical to that of **3**. Most ^{13}C and ^{1}H NMR spectroscopic signals were coincidental (Tables 1 and 2). The *trans* fusion of the AB rings in (**5**) was ascertained by the proton resonances of

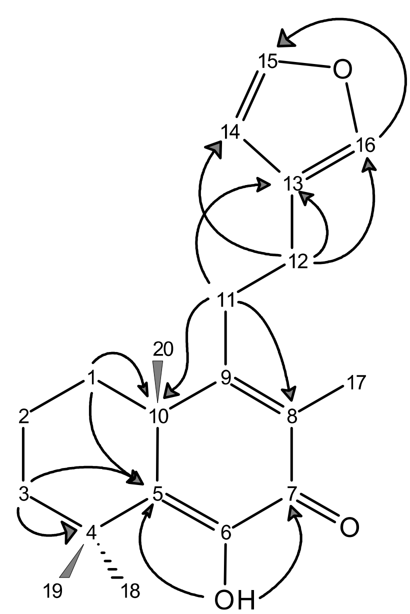

Fig. 2. Selected HMBC correlations of leojaponin (**4**).

Table 1
^{1}H spectroscopic NMR data (δ ppm) of compounds **4**–**6** in $CDCl_3$

H	(**4**)	(**5**)	(**6**)
1	1.55 (*dt*)/2.06 (*ddd*)	1.43 (*m*)	1.39 (*m*)71.55 (*m*)
2	1.70 (*m*)/1.88 (*m*)	1.57 (*m*)	1.55 (*m*)/1.73 (*dt*)
3	1.41 (*dt*)/1.88 (*m*)	1.07 (*dt*)/1.33 (*dt*)	1.21 (*dt*)/1.33 (*m*)
4			
5		2.79 (*d*)	1.61 (*d*)
6			4.33 (*t*)
7		3.85 (*ddd*)	
8		1.82 (*dq*)	3.54 (*q*)
9			
10			
11	2.57 (*m*)/2.67 (*dd*)	1.91 (*m*)/2.04 (*m*)	1.83 (*ddd*)/2.24 (*dt*)
12	2.57 (*m*)	20.4 (*m*)/2.18 (*m*)	2.03 (*m*)
13			
14	6.33	5.22 (*d*)	5.10 (*d*)
15	7.39 (*t*)	6.51 (*d*)	6.39 (*d*)
16	7.29	4.11 (*d*)/4.55 (*d*)	4.03 (*d*)/4.39 (*d*)
17	1.99 (*s*)	1.21 (*d*)	1.00 (*d*)
18	1.37 (*s*)	0.97 (*s*)	1.00 (*s*)
19	1.39 (*s*)	1.26 (*s*)	1.27 (*s*)
20	1.38 (*s*)	0.85 (*s*)	1.42 (*s*)
OH	6.99 (*s*)	3.72 (*d*)	2.20 (*d*)

Table 2
^{13}C NMR spectroscopic data (δ ppm) of compounds **4**–**6** in $CDCl_3$

H	δ (ppm) (**4**)	δ (ppm) (**5**)	δ (ppm) (**6**)
1	29.4	32.3	34.2
2	17.2	18.2	18.9
3	37.2	42.4	43.7
4	35.7	38.1	35.0
5	140.5	56.5	50.2
6	143.1	212.0	76.1
7	181.7	77.7	210.0
8	127.4	46.6	45.7
9	165.8	92.4	96.7
10	43.9	48.4	42.8
11	31.5	29.1	30.2
12	23.8	38.1	37.7
13	124.3	94.2	93.5
14	110.5	107.4	107.1
15	143.1	148.4	148.0
16	138.7	81.1	80.7
17	11.6	13.5	9.3
18	28.1	32.3	32.6
19	27.6	22.2	24.6
20	27.9	19.9	19.7

methyls C18, C19, and C20 which were almost identical to those of **3**. The ^{1}H–^{1}H coupling of C7 and C8 methynes were $J = 10.9$ and 11.1 Hz in **5** and **3**, respectively, suggesting that in both compounds the *trans*-C7—C8 arrangement is maintained. The absolute configuration of C10 is likely preserved, as in all compounds of this family isolated so far, with or without the 9,13 epoxy bridge. However, small differences in the ^{13}C and ^{1}H NMR frequencies of C11 and C12 methylenes, (Tables 1 and 2) suggested dissimilarities between **3** and **5**. The only remaining possibility was that **5** was the C13 S epimer of **3**. Indeed, the 0.28 ppm shielding of protons H1 and the simultaneous 0.08 ppm deshielding of protons H17 in **3**, when compared to **5**, was consistent with the repositioning of the C14═C15 double bond in the S configuration. According to energy minimized (MOPAC) molecular models, the dihydrofuran ring, the C7 methyl and the equatorial C1 proton share a common plane. The C(1)—H_{eq}—C14 distance varies from 2.65 Å in **3** to 4.25 Å in **5** whereas the distance between C14 and the C17 methyl changes from 4.38 to 4.03 Å, differences that are consistent with the observed proton shifts. These were confirmed by analysis of the ROESY spectrum. Thus the structure of **5** was determined as (9α,13S)15,16-diepoxy-7β-hydroxylabd-14-en-6-one.

Iso-preleoheterin (**6**) was isolated in higher yield (1.7% of dry leaves) as a colorless oil, with M^+ m/z 334.2145 (Calcd. 334.2376) corresponding to a molecular formula $C_{20}H_{30}O_4$. Characteristic signals in the FTIR permitted assignment of the keto (1708 cm^{-1}) and hydroxyl (3446 cm^{-1}) functional groups. The characteristic frequencies of spiro carbons C10 (δ_C 96.7) and C13 (δ_C 93.5) and the diagnostic frequency of C16 (δ_C 80.7), coupled to the C14═C15 double bond at δ_C 107.1 and 148.0, respectively, permitted us to propose a spirobis-dihydrofuran moiety typical of the various *Leonurus* diepoxy-labdane metabolites. This strongly suggested a labdane skeleton similar to that of preleoheterin (**3**). HMBC heteronuclear correlations of C18 and C19 methyl groups with methylenes C1 and C2 established the structure of ring A, and thus permitted assignment of the hydroxyl and keto functionalities to ring B, with similar features of preleoheterin (**3**). However, protons H-17 (δ_H 1.00, d, $J = 6.7$ Hz) in **6** were shifted upfield by 0.13 ppm when compared to the ^{1}H NMR spectrum of **3**. At the same time, proton H-8 in **6** was strongly shifted downfield (δ_H 3.54, q, $J = 6.7$ Hz) versus the δ_H 1.82 signal (*dq*) ($J_1 = 11.1$; $J_2 = 6.5$ Hz) of compound **3**. Along the same lines, the angular proton C5 was shifted upfield from δ_H 2.72 in **3** to δ_H 1.61 in **6**. Also, proton H-4 appeared at low field (δ_H 4.33) under the deshielding influence of both OH and C═O vicinal functionalities. Hence the resulting arrangement was the novel 6-hydroxy-7-keto-9,13,15,16-diepoxylabdane derivative, namely *iso*-preleoheterin. The permutation of the carbonyl and OH functions caused an important downfield shift of the angular C20 methyl group by 0.61 ppm, with the latter likely caused by the axial 1,4-relationship with the carbonyl. Energy minimized molecular models (MOPAC), showed that the carbonyl group lies above the molecular plane and therefore the methyl group at position 10 is closer and facing the deshielding carbonyl cone. The stereochemistry of chiral carbons in the AB nucleus was deduced as follows. The H5—H6 coupling constant ($J = 2.6$ Hz) placed these protons *cis* to one another in an α,α-axial-equatorial disposition, leaving the hydroxyl group as β-axial. The unusually low-field signal (δ_H 3.54 ppm) of H8 for a α-carbonyl proton could only be explained if this atom was perpendicular to the vicinal carbonyl group above the molecular plane, leaving the C17 methyl as α-equatorial. Similar spectroscopic features were noted for the C17 methyl of hispanolone (Savona et al., 1978) which also showed a methyl-C8-methyne coupling constant of $J = 6.5$ Hz. Axial methyls exhibit a larger coupling constant (8 Hz). Finally, in the absence of a C13S epimer to compare the spectroscopic data with, the NOESY data was necessary to solve the relative stereochemistry of C13 (Fig. 3). The interaction of H14/H1α,H1β on the one hand, and the H16/H17 on the other, confirmed that the configuration C13R in (**6**) was that of preleoheterin (**3**). The foregoing data thus conclusively proved the novel structure *iso*-preleoheterin (**6**) as (9α,13R)15,16-diepoxy-6β-hydroxylab-14-en-7-one. The nearest known derivative is the C6 acetate, recently isolated from *Otostegia fruticosa* (Al-Musayeib et al., 2000) which shares most of our spectroscopic observations.

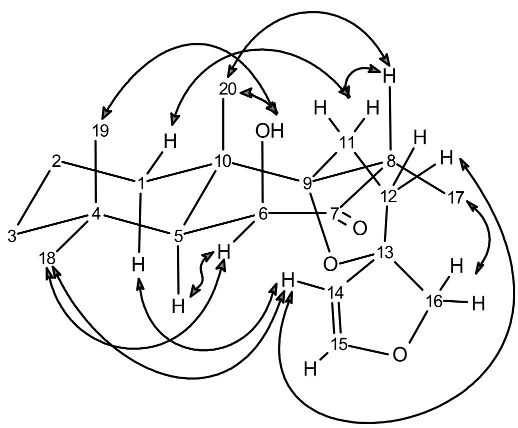

Fig. 3. Selected NOE correlations of *iso*-preleoheterin (**6**).

The *Leonurus* genus is composed of 20 species, of which only five *L. cardiaca* (Papanov et al., 1998a,b), *L. marrubiastrum* (Tschesche and Streuff, 1978; Malakov et al., 1998), *L. persicus* (Tasdemir et al., 1996, 1998; Tasdemir and Sticher, 1997), *L. sibiricus* (Savona et al., 1982; Satoh et al., 2003; Boalino et al., 2004), and *L. japonicus* (Hohn et al., 1991, 1993) (syn *heterophyllus*) (Giang et al., 2005a,b) have been chemically studied. All but *L. marrubiastrum* produce diterpenes of the normal labdane series whereas *L. marrubiastrum* yields clerodane and abietane type diterpenes, a unique feature in the series that does not grant fully recognition of the 9α,13;15,16-diepoxy labdanes as taxonomic markers of the *Leonurus* genus. However, this structural type has been isolated only from *Leonurus* and other genera of the Lamiaceae family. Other labdanes featuring a furanic ring at the end of the C9 chain (e.g. **7**–**11**) have also been reported (Fig. 4) which brings about the question of their origin as artifacts. Either the rupture (Prakash et al., 1979) or the formation (Rustaiyan et al., 1992) of the C9α-C13 epoxy bridge under mild acid conditions, or

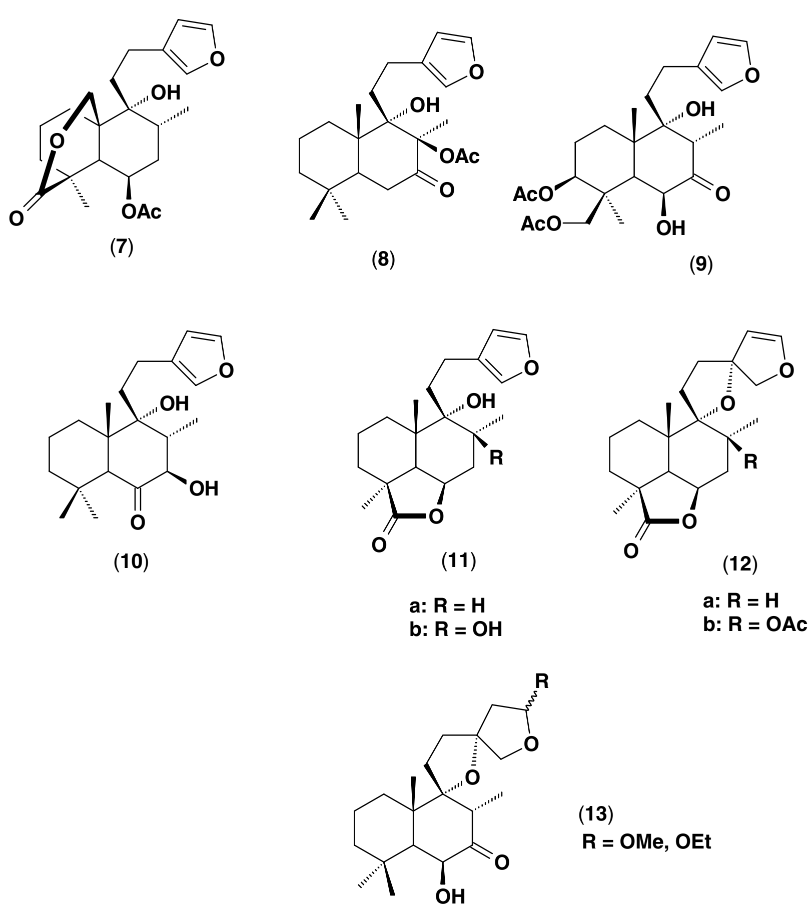

Fig. 4. Structure of some furanic and bis spyrodihydrofuranic labdanes from *Leonurus* sp.

on standing at 4 °C (Tasdemir et al., 1995) are on record and may be explained by straightforward addition/elimination reactions. Furthermore, sibiricones (**13a,b**), which have been isolated from *Ballota aucheri* (Rustaiyan et al., 1992) and *L. sibiricus* (Boalino et al., 2004) using hexane–methanol–ethanol mixtures, might as well be the result of solvent addition to the C14═C15 double bond during isolation. The actual occurrence of 9α,13;15,16-diepoxy labdanes or the furan derivatives in the living plants has been investigated in various growth stages of *Marrubium vulgare* and *Leonurus cardiaca* (Knöss and Zapp, 1997). However, no labdanes were formed in the young plantlets and only after leaf differentiation (12 wks) were premarrubiin (**12a**) in *M. vulagre* and leosibiricin (**12b**) in *L. cardiaca* constitutively synthesized in the leaves. In addition, only premarrubiin was obtained from leaf trichomes of *M. vulgare* whereas neither marrubiin (**11a**) in this species nor leonotine (**11b**) in *L. cardiaca* could be detected in any plant part, thus suggesting possible premarrubiin–marrubiin and leonotine–leosibiricin transformations during plant extraction. However, this result does not rule out that biosynthetic routes from the 9α,13;15,16-diepoxy to the 15,16-epoxy labdanes might exist in other *Leonurus* species. The unique occurrence of isopreleoheterin (**6**) in large quantity in our specimens of *L. japonicus* (1.7%) suggests its role as an intermediate en route to some of the isolated labdanes in this plant. Further systematic studies in living *Leonurus* plants will be necessary before the true natural character of mono-furanic labdanes is ascertained.

3. Experimental

3.1. General experimental procedures

Melting points were determined on a Fischer–Johns apparatus and are not corrected. Optical rotations were measured in EtOAc on a Rudolph Research Autopol III polarimeter. IR spectra were recorded in KBr using a Perkin–Elmer FT-1725X spectrometer. ^{1}H, ^{13}C and two-dimensional NMR spectra were recorded on a Bruker-Avance DRX400 instrument operating at 400 MHz, and using $CDCl_3$ as solvent. EI MS and HREI MS were measured on a Hewlett–Packard 5930A and at the University of California Davis Mass Spectral Facility, respectively, direct inlet, 40 eV. Silica gel 60 (Merck, 70–230 mesh) was used for flash column chromatography (FCC) and precoated silica gel plates (Merck, Kieselgel 60 F_{274}, 0.25 mm) were used for TLC analysis.

3.2. Plant material

L. japonicus Houtt. was collected at the pre-flowering stage in March 2002, near the village of Bailadores, Mérida-Venezuela, located at 2150 m elevation. A voucher specimen (LQE 80) is kept at MERC Herbarium (Facultad de Ciencias, Universidad de Los Andes, R. Romero).

3.3. Extraction and isolation

The leaves of *L. japonicus* were air-dried, ground (500 g) and exhaustively extracted with hexane under sonication for 3 h at 30 °C. Solvents were evaporated in vacuo (<30 °C) yielding a brown yellowish residue (30.0 g). An aliquot of this material (10 g) was subjected to flash column chromatography (FCC), using gradient elution with *n*-hexane/ether (100–10%). Fifteen fractions of 500 mL were obtained and combined (TLC monitoring) to give eight major fractions, A–H. FCC of fraction D (hexane/ether 70:30) gave nine subfractions. Further purification by preparative TLC of fractions D-2 (hexane/ether 97:3), D-4 (hexane/ether 95:5) and D-8 (hexane/ether 90:10), respectively, led to the isolation of leojaponin (33 mg, 0.02%), 13-*epi*-preleoheterin (166 mg, 0.1%) and isopreleoheterin (2.8 g, 1.7%).

3.4. Leojaponin

Recrystalization from *n*-hexane gave yellow prisms, m.p. 109–111 °C; $[\alpha]_D^{20}$: −38.6 (*c* 0.01, EtOAc); IR (KBr) ν_{max}: 3351 (—OH), 2952, 1730 (>C═O), 1619, 1598, 1328, 1030 (C—O—C), 608 cm^{-1}; UV (EtOAc) λ_{max} nm (log ε): 259 (3.99), 306 (3.61); For ^{1}H and ^{13}C NMR ($CDCl_3$) spectra, see: Tables 1 and 2; HREI MS M$^+$ *m/z* 314.1878. Calcd. 314.2062. EI MS *m/z* (% rel. int.): 314 $[M]^+$ (15), 299 $[M^+{-}Me]^+$ (1), 233 $[M^+{-}furan{-}CH_2]^+$ (13), 81$[furan{-}CH_2]^+$ (100).

3.5. 13-epi-Preleoheterin

Colorless needles, m.p. 95–97 °C; $[\alpha]_D^{20}$: +68.4 (*c* 0.01, EtOAc); IR (KBr) ν_{max}: 3467 (—OH), 2928, 1706 (>C═O), 1613, 1464, 1389, 1141, 1072, 1041, 947, 737 cm^{-1}; For ^{1}H and ^{13}C NMR ($CDCl_3$) spectra, see: Tables 1 and 2; HREI MS M$^+$ *m/z* 334.2150 (Calcd. 334.2376). EI MS *m/z* (% rel. int.): 334 $[M]^+$ (4), 82 $[furan{-}CH_3]^+$ (100), 81 $[furan{-}CH_2]^+$ (74).

3.6. Isopreleoheterin

Colorless oil; $[\alpha]_D^{20}$: −39.7° (*c* 0.01, EtOAc); IR (KBr) ν_{max}: 3446 (—OH), 2924, 1708 (>C═O), 1465, 1391, 1035 (C—O—C), 976, 776 cm^{-1}; For ^{1}H and ^{13}C NMR ($CDCl_3$) spectra, see: Tables 1 and 2; HREI MS M$^+$ *m/z* 334.2145 (Calcd. 334.2376). EI MS *m/z* (% rel. int.): 334 $[M]^+$ (5), 252 $[M^+{-}furan{-}CH_3]^+$ (9), 82 $[furan{-}CH_3]^+$ (97), 81 $[furan{-}CH_2]^+$ (100).

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