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NOTE

A secondary metabolite amberin from *Amberboa ramosa*

Muhammad Ibrahim[a], Muhammad Imran[b], Bakhat Ali[c], Riaz Hussain[b], Fouzia Sultana Rehmani[a]* and Abdul Malik[b]

[a]*Department of Chemistry, University of Karachi, Karachi 75270, Pakistan;* [b]*International Centre for Chemical and Biological Sciences, HEJ Research Institute of Chemistry, University of Karachi, Karachi 75270, Pakistan;* [c]*Department of Chemistry, University of Science and Technology Bannu, Bannu 28100, Pakistan*

Amberin (**1**), a new sesquiterpene lactone, has been isolated from the ethyl acetate soluble fraction of *Amberboa ramosa* together with apigenin **(2)**, jaseocidine (**3**), crysoeriol (**4**), and 3β, 8α-dihydroxy-11α-methyl-1αH, 5αH, 6βH, 7αH, 11βH-guai-10(14), 4 (15)-dien-6, 12-olide (**5**). The structures of the isolated compounds have been elucidated by 1D and 2D NMR spectroscopy.

Keywords: amberin; guaianolide; *Amberboa ramosa*; Compositae

1. Introduction

The genus *Amberboa* belongs to the family Compositae and comprises six species. *Amberboa ramosa* is an annual herbaceous plant that is mainly found in Pakistan and India. *A. ramosa* has tonic, aperient, febrifuge, deobstruent, cytotoxic, and antibacterial properties [1]. The butyryl-cholinesterase inhibitory activity of the chloroform soluble fraction has also been reported previously [2]. Literature survey revealed that triterpenoids, flavonoids, steroids, and sesquiterpene lactones have previously been reported from this species [1,3]. The chemotaxonomic and ethno-pharmacological significance of the genus *Amberboa* prompted us to reinvestigate the constituents of *A. ramosa*. As a result, we herein report the isolation and structural elucidation of one new sesquiterpene lactone named as amberin (**1**). In addition, the previously reported compounds apigenin (**2**) [4], jaseocidine (**3**) [5], crysoeriol (**4**) [6], and 3β, 8α-dihydroxy-11α-methyl-1αH, 5αH, 6βH, 7αH, 11βH-guai-10(14), 4 (15)-dien-6, 12-olide (**5**) [2] have also been isolated and characterized.

2. Results and discussion

Amberin (**1**) was isolated as white crystals with $[\alpha]_D^{20} + 51.1$ ($c = 0.02$, $CHCl_3$) and mp 137–139°C. The HR-ESI-MS exhibited a pseudomolecular ion peak at *m/z* 469.2068 $[M - H]^-$, corresponding to the molecular formula $C_{23}H_{33}O_{10}$. Further prominent peaks at *m/z* 308 [M^+-162], 290 [M^+-162-H_2O], 265 [M^+-162-$COCH_3$], 250 [M^+-162-$COCH_3$–Me], and 247 [M^+–$COCH_3$–H_2O] were present.

The IR spectrum showed characteristic absorption bands at 3447, 1755, 1735, and 1656 cm^{-1} for hydroxyl, γ-lactone, ester, and olefinic functionalities, respectively. The UV absorption maxima at 197 and 205 nm were typical of a γ-lactone [6].

*Corresponding author. Email: fsrehmani@uok.edu.pk

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Further spectral data showed close agreement with a guaianolide-type sesquiterpene [3,7,8].

The ^{1}H NMR spectrum showed the signals for a trisubstituted double bond at δ 5.50. It also showed signals for the oxymethine protons at δ 5.08 (ddd, $J = 8.3$, 6.0, 5.5 Hz) and 4.33 (dd, $J = 10.0$, 9.0 Hz). The latter was assigned to the proton geminal to the lactone oxygen atom. The larger coupling constant allowed us to assign β and axial orientation which is characteristic of all guaianolides of the genus *Amberboa* and *Ixeris* [9]. It showed ^{1}H–^{1}H correlations with the vicinal protons at δ 2.78 (dd, $J = 9.0$, 8.5 Hz) and δ 2.87 (ddd, $J = 10.0$, 9.5, 8.3 Hz), which could subsequently be assigned to H-5 and H-7, respectively. The larger coupling constants suggested *trans*-diaxial disposition among H-5, H-6 (β), and H-7, providing conclusive evidence for an α-orientation of both H-5 and H-7 [9].

The coupling pattern of the proton signals for H-1 and H-9 supported the guaianolide structure [10]. The entire sequence of protons attached to the guaianolide skeleton was established by correlation spectroscopy (COSY) and spin decoupling experiments. Irradiation of the H-5 proton at δ 2.78 simplified the double doublet of H-6 at δ 4.33 into a doublet and the doublet of double doublets of H-1 into a double doublet.

Irradiation of H-7 at δ 2.87 simplified the doublet of quartet at δ 2.34 into a quartet. Irradiation of H-11 at δ 2.34 simplified the doublet of the methyl group at δ 1.23 into a singlet, confirming the presence of a methyl group at C-11. Irradiation of H-3 at δ 5.50 turned the double doublets at δ 2.26 (H-2α) and 2.37 (H-2β) into doublets. The signal at 1.80 could be assigned to methyl protons at C-4 and further confirmed by ^{2}J and ^{3}J correlations with C-4 (δ 144.0), C-3 (δ 127.1), and C-5 (δ 55.0). The *O*-acetyl group was assigned to C-10, on the basis of the strong correlation in HMBC spectrum between the methyl protons at C-10 resonating at δ 1.16 and the carbonyl carbon at δ 181.0 as shown in Figure 1. The hydroxyl group could be assigned to C-10 based on its lower frequency shift compared to unsubstituted guaianolides and supported by HMBC experiment, in which the methyl protons at C-14 at δ 1.16 showed ^{2}J and ^{3}J correlations with C-10 at δ 81.1, C-9 at δ 42.2, and C-1 at δ 53.0.

The ^{13}C NMR spectrum (broadband and DEPT) showed 23 signals comprising four methyl, three methylene, twelve methine, and four quarternary carbons. The low frequency region showed four signals at δ 181.0, 172.1, 144.0, and 127.1, which could be assigned to *O*-acetyl, lactone, ester, and trisubstituted olefinic carbons. One oxygenated quaternary and two oxygenated methine carbons resonated at δ 81.1, 75.4, and 82.3, respectively. The position of substituents could be confirmed by HMQC, HMBC, and COSY experiments.

The relative stereochemistry at various chiral centers of amberin (**1**) was assigned through NOESY experiments [11–13] (Figure 2), which revealed *trans*/anti/*cis*-fusion of the α-methyl-γ-lactone moiety, the seven member ring at C-7 and C-6, the five member ring at C-5 and C-1. The *cis*-fusion of guaianoloid is also proven by the

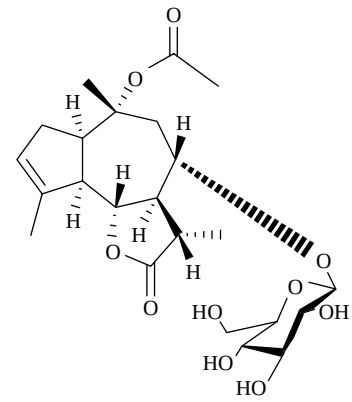

Figure 1. Structure of amberin (**1**).

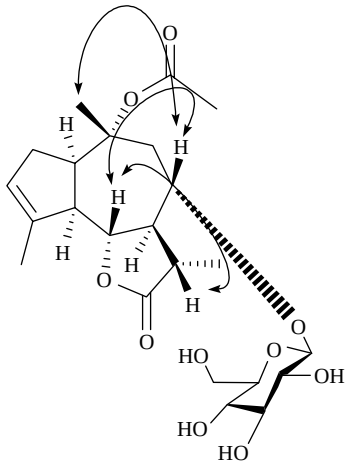

Figure 2. Important NOESY correlations of amberin (**1**).

strong correlation between the H-1 at δ 2.71 and H-5 at δ 2.78. The α-orientation of the acetate group at C-10 could also be deduced through strong interaction in NOESY experiment between the methyl protons attached to C-10 resonating at δ 1.16 and H-8 at δ 5.08, and the methyl group revealing its β-orientation.

The position of the hexose moiety at C-8 could be confirmed by HMBC correlation of the anomeric proton at δ 4.54 with C-8 (δ 81.1). The larger coupling constant of the anomeric proton allowed us to assign β-configuration to the sugar moiety. The signals of a hexose moiety in broadband spectra at δ 98.4 (C-1′), 75.5 (C-2′), 78.2 (C-3′), 71.8 (C-4′), 77.7 (C-5′), and 62.9 (C-6′) as well as low-frequency shift of C-8 by 81.1 ppm further revealed its presence at C-8. Acid hydrolysis provided D-glucose that could be identified through the sign of its optical rotation and the retention time of its TMS ether in gas chromatography (GC). Thus, the structure of amberin C (**1**) could be assigned as 10α-acetoxy-8α-*O*-(β-D-glucopyranosyl)-1αH, 5αH, 6βH, 8βH, 7αH, 11βH, 11α-methylguaia-3-enolide (Figure 3).

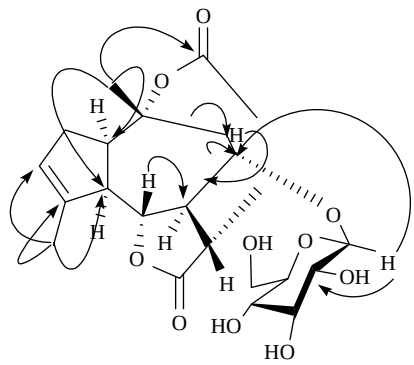

Figure 3. Important HMBC correlations of amberin (**1**).

3. Experimental

3.1 General experimental procedures

Melting points were determined on a Gallenkemp apparatus and are uncorrected. IR spectra were measured on a JASCO 302-A spectrophotometer with KBr cells. UV spectra were obtained on a Hitachi UV-3200 spectrophotometer. Optical rotations were measured on a JASCO DIP-360 polarimeter and the 1D and 2D NMR spectra were recorded on a Bruker AMX-400 Spectrometer operating at 400 MHz for 1H and 100 MHz for 13C. Electron impact (EI) mass spectra were recorded on JEOL JMS-HX-110 and Varian MAT-311-A mass spectrometers. The HR-ESI-MS was recorded on a Jeol JMS 600H instrument. Silica gel (230–400 mesh, E. Merck, Darmstadt, Germany) was used for column chromatography.

3.2 Plant material

The whole plant of *A. ramosa* Jafri (Compositae) was collected in June 2002 from Karachi (Pakistan) and identified by Dr Surraiya Khatoon, Plant Taxonomist, Department of Botany, University of Karachi, where a voucher specimen (no. KU 312 b) has been deposited.

3.3 Extraction and isolation

The shade dried plant material (22 kg) was extracted with methanol (3 × 40 l) for

10 days at room temperature. The solvent was evaporated under reduced pressure and the residue (217 g) was partitioned between *n*-hexane and water. The water soluble fraction was further extracted with chloroform, ethyl acetate, and *n*-butanol.

The column chromatography of the ethyl acetate soluble fraction (90 g) over silica gel and elution with *n*-hexane–ethyl acetate in increasing order of polarity afforded four major fractions: A (20 g) [*n*-hexane–ethyl acetate (8:2)], B (13 g) [*n*-hexane–ethyl acetate (6.5:3.5)], C (16 g) [*n*-hexane–ethyl acetate (5.5:4.5)], and D (20 g) [*n*-hexane–ethyl acetate (4:6)].

Fraction B was chromatographed over silica gel, eluting with mixture of *n*-hexane–ethyl acetate in increasing order of polarity to afford sub-fractions B_A (3 g) [*n*-hexane–ethyl acetate (7:3)], B_B (4.5 g) [*n*-hexane–ethyl acetate (6:4)], and B_C (4 g) [*n*-hexane–ethyl acetate (6.5:3.5)]. The fraction B_A on further chromatography over silica gel and elution with *n*-hexane–ethyl acetate (6.5:3.5) yielded compound **3** (9 mg). The chromatography of fraction B_C and elution with *n*-hexane–ethyl acetate (6:4) furnished compound **4** (11.5 mg).

Fraction C was chromatographed over silica gel and eluted with mixtures of *n*-hexane–ethyl acetate to provide two sub-fractions C_A (5.4 g) [*n*-hexane–ethyl acetate (4.5:5.5)] and C_B (6.5 g) [*n*-hexane–ethyl acetate (4:6)]. Fraction C_B on further chromatography over silica gel and elution with *n*-hexane–ethyl acetate (7:3) afforded compound **5** (10 mg).

Fraction D was chromatographed over silica gel and eluted with mixtures of ethyl acetate–methanol in increasing order of polarity to obtain compound **1** [ethyl acetate–methanol (9:1)] (11 mg) and compound **2** [ethyl acetate–methanol (7:3)] (13 mg).

3.3.1 *Amberin (**1**)*

White crystals, $[\alpha]_D^{20} + 51.1$ (*c* 0.02, $CHCl_3$), mp 137–139°C. IR (KBr) ν_{max} cm^{-1}: 1735, 3447, 1755, and 1656. UV λ_{max}: 197 and 215 nm. ^1H and ^{13}C spectral data, see Table 1. EI-MS: *m/z* 470 $[M]^+$, 308, 290, 265, 250, and 247. HR-ESI-MS: *m/z* 469.2068 $[M - H]^-$ (calcd for $C_{23}H_{33}O_{10}$, 469.2074).

Table 1. ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectral data of amberin (**1**) (CD_3OD).

Positions	δ_C	δ_H multiplicity (*J* in Hz)
1	53.0	2.71 ddd (9.0, 8.5, 8.0)
2	34.0	2.37 dd (8.0, 7.0)
		2.26 dd (9.0, 5.0)
3	127.1	5.50 m
4	144.0	–
5	55.0	2.78 dd (9.0, 8.5)
6	82.3	4.33 dd (10.0, 9.0)
7	52.3	2.87 ddd (10.0, 9.5, 8.3)
8	81.1	5.08 ddd (8.3, 6.0, 5.5)
9	42.8	1.87 dd (12.3, 5.5)
		2.39 dd (12.3, 6.0)
10	75.4	–
11	42.1	2.34 dq (9.5, 5.8)
12	172.1	–
13	15.7	1.23 d (6.9)
14	26.1	1.16 s
15	17.7	1.80 s
16	181.0	–
17	21.3	2.06 s
1′	98.4	4.54 d (7.5)
2′	75.5	3.28 m
3′	78.2	3.38 m
4′	71.8	3.17 m
5′	77.7	3.23 m
6′	62.9	3.82 dd (2.1, 12.0)
		3.6 dd (6.0, 12.0)

3.4 *Acid hydrolysis of amberin (1)*

Amberin (**1**) (2 mg) in MeOH (3 ml) containing 1 N HCl (3 ml) was refluxed for 4 h, concentrated under reduced pressure, diluted with water (7 ml) and extracted with ethyl acetate. The residue recovered from the organic phase was a mixture of aglycone products which could not be worked up due to paucity of material. The aqueous phase was concentrated and D-glucose was identified by its optical rotation $[\alpha]_D^{20} = +50.6$ and by comparing

retention times of its TMS ether (α-anomer 3.7 min, β-anomer 5.1 min) with a standard sample in GC. Preparation of TMS ether and its subsequent GC was carried out according to the reported protocol [14].

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