Amberinone, a new guaianolide from *Amberboa ramosa*

Muhammad Ibrahim\(^{a,b}\), Iqbal Hussain\(^d\)*, Nusrat Hussain\(^b\), Arruje Hameed\(^a\), Tahir Farooq\(^a\), Amjad Hussain\(^b\), Muhammad Sajid Hamid Akash\(^c\) and Shabbir Hussain\(^b\)

\(^a\)Department of Applied Chemistry & Biochemistry, Government College University, Faisalabad 38000, Pakistan; \(^b\)HEJ Research Institute of Chemistry, International Centre for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan; \(^c\)College of Pharmaceutical Sciences, Institute of Pharmacology, Toxicology and Biochemical Pharmaceutics, Zhejiang University, Hangzhou, China; \(^d\)Department of Botany, Government College University, Faisalabad 38000, Pakistan

(Received 8 February 2015; final version received 12 April 2015)

The *Amberboa* is a medicinally important genus present in the family *Asteraceae*; members of this genus are mainly distributed in Pakistan and India. It has been used in different systems of traditional medicines for different diseases. Amberinone (1), a new sesquiterpene lactone, has been isolated from the ethyl acetate (EtOAc) soluble fraction of *Amberboa ramosa* together with chrysin (2), quercitine (3), eriodictyol (4) and keamferol (5). This is the first report of these compounds from this species. The structures of the isolated compounds have been elucidated by 1D and 2D \(^1\)H \(^{13}\)C NMR spectroscopy.

**Keywords**: *Amberboa ramosa*; amberinone; *Asteraceae*; guaianolide

1. Introduction

Guaianolides are naturally occurring sesquiterpene lactones that exhibit important biological activities. Their structure consists of the tricyclic 5, 7, 5-ring system. Plants with guaianolides as active constituents have been used in traditional medicine for treating of rheumatic pains, increase of bile production and pulmonary disorders (Devreese et al. 1980; Connolly & Hill 1991).

In continuation of our studies on medicinal plants especially *Amberboa ramosa* (Ibrahim et al. 2010; Ibrahim et al. 2012; Ibrahim, Farooq et al. 2013; Ibrahim, Hussain et al. 2013), we isolated a new guaianolide amberinone (1). *A. ramosa* is an annual herb which is mainly found in Pakistan and India. *A. ramosa* has tonic, aperient, febrifuge, deobstruent, cytotoxic and antibacterial activities (Akhtar et al. 1993). The chloroform soluble fraction of *A. ramosa* has...
been reported with butyrylcholinesterase inhibitory activity (Khan et al. 2005). The literature survey revealed the presence of triterpenoids, flavonoids, steroids and sesquiterpene lactones from this plant (Harrison & Kulshrestha 1984; Khan et al. 2005). The current phytochemical study also resulted in four known compounds, including chrysin (2) (Shen et al. 1993), quercitine (3) (Batterham & Highet 1964), eriodictyol (4) (Wagner et al. 1976) and keamferol (5) (Markham et al. 1978). Structures of the compounds were deduced using different spectroscopic techniques such as $^1$H NMR, $^{13}$C NMR, 2D NMR, ESI-MS and EI-MS.

2. Results and discussion

Amberinone (1) was isolated as a white powder with $[\alpha]_{D}^{20} + 46.1$ ($c = 0.02$, CHCl$_3$) and mp 152–154°C. The HR-ESI-MS exhibited a pseudomolecular ion peak at $m/z$ 323.3068 [M + H]$^+$, corresponding to the molecular formula C$_{17}$H$_{22}$O$_6$. Prominent peaks in EI-MS were observed at $m/z$ 280.2, 262.2, 19.1 and 193.1 (Figure 1).

The IR spectrum showed characteristic absorption bands at 3447, 1755, 1735 and 1656 cm$^{-1}$ for hydroxyl, $\gamma$-lactone, ester and olefinic functionalities, respectively. The UV absorption maxima at 197 and 205 nm were typical of a $\gamma$-lactone (Khan et al. 2005). Further spectral data showed close agreement with a guaianolide-type sesquiterpene (Marco et al. 1993; Ibrahim, Farooq et al. 2013; Ibrahim, Hussain et al. 2013).

The $^1$H NMR spectrum showed the signals for four methyl protons at $\delta$ 0.97 (s), $\delta$ 1.35 (d, $J = 6.0$ Hz), $\delta$ 1.84 (s) and $\delta$ 2.09 (s). It also showed signals for the oxymethine protons at $\delta$ 5.08 (ddd, $J = 8.3$, 6.0, 5.5 Hz) and $\delta$ 5.27 (d, $J = 10.0$ Hz). The latter was assigned to the proton geminal to the lactone oxygen atom. The $\beta$- and axial stereochemistry of this oxymethine proton was assigned based on larger coupling constant values, which is characteristic of all guaianolides of the genus Amberboa and Ixeris. It also showed COSY correlations with the vicinal proton at $\delta$ 2.73 (ddd, $J = 10.0$, 9.5, 8.3 Hz) which could subsequently be assigned to H-7. The larger coupling constants suggested trans-diaxial disposition among H-6 ($\beta$) and H-7, providing conclusive evidence for an $\alpha$ orientation of H-7 (Seto et al. 1986).

The coupling pattern of the proton signals for H-1 and H-9 supported the guaianolide structure (Youssef 1998). The entire sequence of protons attached to the guaianolide skeleton was established by correlation spectroscopy (COSY) and spin decoupling experiments. Irradiation of H-7 at $\delta$ 2.73 simplified the doublet of quartet at $\delta$ 2.34 into a quartet. Irradiation of H-11 at $\delta$ 2.34 simplified the doublet of the methyl group at $\delta$ 1.23 into a singlet, confirming the presence of a methyl group at C-11. The HMBC correlation between H-8 and C-16 was missing, this helped to place the O-acetyl group at C-10. This was further supported by the chemical shift values recorded for C-8 ($\delta$ 72.2) and C-10 ($\delta$ 83.0) (Figure S2). C-8 with O-acetyl group has been
reported with chemical shifts 78–80 ppm (Ibrahim, Farooq et al. 2013; Ibrahim, Hussain et al. 2013), while in this case it was observed at 72.2 ppm. Similarly C-10 having ‘OH’ is observed at 76–78 ppm (Ibrahim et al. 2010), while in this case it was observed at 83.0. The down-field frequency of C-10 and a little up-field frequency for C-8 confirmed the presence of O-acetyl group at C-10.

The $^{13}$C NMR spectrum (broad-band decoupled and DEPT) showed 17 signals comprising four methyl, two methylene, five machines and six quaternary carbons. The down-field frequency region showed five signals at $\delta$ 210.2, 179.2, 171.2, 164.6 and 142.7, which could be assigned to ketone, lactone ester, O-acetyl and substituted olefinic carbons, respectively. One oxygenated quaternary and two oxygenated methine carbons resonated at $\delta$ 83.0, 72.2 and 78.5, respectively. The positions of substituents were confirmed by HMQC, HMBC and COSY experiments.

The relative stereochemistry at various chiral centres of Amberinone (1) was assigned through NOESY experiments (Zdero et al. 1987; Zdero & Bohlmann 1990; Khan et al. 2004) (Figure S1), which revealed trans/anti/cis-fusion of the $\alpha$-methyl-$\gamma$-lactone moiety, the seven-member ring at C-7 and C-6, the five-member ring at C-5 and C-1. The $\alpha$-orientation of the acetate group at C-10 was confirmed from the $\beta$-orientation of the methyl group based on key NOESY interactions between the methyl protons attached to a C-10 resonating at $\delta$ 0.97 and H-1 at $\delta$ 3.40.

On the basis of the above-cited spectroscopic data, the structure of amberinone (1) was unambiguously assigned to be (3S,3aR,4R,6R,6aS,9bS)-4-hydroxy-3,6,9-trimethyl-2,8-dioxo-2,3,3a,4,5,6,6a,7,8,9b-decahydroazuleno [4,5-b]furan-6-yl acetate.

3. Experimental
3.1. General experimental procedures
Melting points were determined on a Gallenkemp apparatus. IR spectra were measured on a JASCO 302-A spectrophotometer with KBr cells (Jasco, Tokyo, Japan). UV spectra were obtained on a Hitachi UV-3200 spectrophotometer (U-3200, Hitachi Instruments Inc., Tokyo, Japan). 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 500 MHz for $^1$H and 125 MHz for $^{13}$C NMR (Bruker BioSpin, Rheinstetten, Germany). Electron impact (EI) mass spectra were recorded on JEOL JMS-HX-110 and Varian MAT-311-A mass spectrometers (Jeol, Tokyo, Japan). The HR-ESI-MS was recorded on a Jeol JMS 600H instrument (Jeol, Tokyo, Japan). Silica gel (230–400 mesh, E. Merck, Darmstadt, Germany) was used for column chromatography (CC).

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

3.3. Extraction and isolation
The shade-dried plant material (8 kg) was extracted with MeOH (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, EtOAc and n-butanol.

EtOAc soluble fraction was subjected to CC with silica gel as stationary phase and n-hexane–AcOEt, AcOEt and AcOEt–MeOH (with increase in polarity) as mobile phase that
afforded four major sub-fractions such as sub-fraction A (20 g) \( [n\text{-hexane--EtOAc (8:2)}] \), B (13 g) \( [n\text{-hexane--EtOAc (6.5:3.5)}] \), C (16 g) \( [n\text{-hexane--EtOAc (5.5:4.5)}] \) and D (20 g) \( [n\text{-hexane--EtOAc (4:6)}] \).

Sub-fraction A was chromatographed over silica gel, eluting with a mixture of \( n\text{-hexane--EtOAc} \) (increasing order of polarity) which afforded further sub-fractions A1 (3 g) \( [n\text{-hexane--EtOAc (7:3)}] \), A2 (4.5 g) \( [n\text{-hexane--EtOAc (6:4)}] \) and A3 (4 g) \( [n\text{-hexane--EtOAc (1:1)}] \).

The sub-fraction A2 on further chromatography over silica gel and elution with \( n\text{-hexane--EtOAc} \) (8:2 and 7:3) yielding compound 2 (5.3 mg) and compound 5 (9.7 mg). The sub-fraction A3 was loaded over silica gel and eluted with mixtures of \( n\text{-hexane--EtOAc} \) (6:4 and 1:1) which afforded compound 3 (14.1 mg) and compound 4 (8.7 mg).

3.3.1. *Amberinone* (1)

White crystals, \( [\alpha]^{20}_{\text{D}} = +46.0 \) (c = 0.02, CHCl\(_3\)), mp 152–154°C. IR (KBr) \( \nu_{\text{max}}\text{ cm}^{-1} \): 1735, 3447, 1755 and 1656. UV \( \lambda_{\text{max}}\text{ nm} \): 197 and 215 nm. \(^1\)H NMR (400 MHz, CD\(_3\)OD); 3.40 (1H, dd, \( J = 9.0, 6.5 \) Hz), 2.37 (2H, dd, \( J = 15.5, 9.0 \) Hz), 2.46 (2H, dd, \( J = 15.5, 6.5 \) Hz), 5.27 (6H, d, \( J = 10.0 \) Hz), 2.73 (7H, ddd, \( J = 10.0, 9.5, 8.3 \) Hz), 5.08 (8H, ddd, \( J = 8.3, 6.0, 5.5 \) Hz), 1.87 (9H, d, \( J = 12.3, 5.5 \) Hz), 2.39 (9H, d, \( J = 12.3, 6.0 \) Hz), 2.34 (11H, dq, \( J = 9.5, 6.9 \) Hz), 1.34 (13H, d, \( J = 6.9 \) Hz), 0.97 (14H, s), 1.83 (15H, s), 2.09 (17H, s); \(^{13}\)C NMR (100 MHz, CD\(_3\)OD); 51.1 (C-1), 38.0 (C-2), 210.2 (C-3), 164.6 (C-4), 142.7 (C-5), 78.5 (C-6), 52.4 (C-7), 72.2 (C-8), 51.86 (C-9), 83 (C-10), 41.1 (C-11), 179.2 (C-12), 15.7 (C-13), 21.1 (C-14), 9.36 (C-15), 170 (C-16), 20.3 (C-17) (Table S1). EI-MS: \( m/z \) 322 \([M]^+\), 280.2, 262.2, 19.1 and 193.1. HR-ESI-MS: \( m/z \) 323.3068 \([M+H]^+\) (calcd for C\(_{17}\)H\(_{22}\)O\(_6\)+H\(^+\), 323.3074).

4. Conclusion

The phytochemical investigation on *A. ramosa* resulted in the isolation and characterisation of a new sesquiterpene lactone, Amberinone (1). Four known compounds namely, chrysin (2), quercitine (3), eriodictyol (4) and keamferol (5) were also isolated and characterised.

Supplementary material

Supplementary material relating to this paper is available online, alongside Table S1 and Figures S1–S2.

Acknowledgements

The authors are grateful to H. E. J. Research Institute of Chemistry, University of Karachi, for providing spectro-support in carrying out NMR studies.

Conflicts of interest

The authors declare no conflicts of interest.

Funding

We are thankful to the Higher Education Commission of Pakistan for financial support. This work was partially supported by the Higher Education Commission (HEC), Islamabad, Pakistan [project grant number PD-IPFP/HRD/HEC/2013/1114].
References

Akhtar N, Malik A, Afza N, Badar Y. 1993. Cycloartane-type triterpenes from *Amberboa ramosa*. J Nat Prod. 56(2):295–299. doi:10.1021/np50092a019.

Batterham TJ, Highet RJ. 1964. Nuclear magnetic resonance spectra of flavonoids. Aust J Chem. 17(4):428–439. doi:10.1071/CH640428.

Connolly JD, Hill RA. 1991. Dictionary of terpenoids. Vol. 1. London: Chapman and Hall.

Devreese AA, De Clercq PJ, Vandewalle M. 1980. A general entry to guaianolides: an illustrative synthesis of (±)-compressanolide. Tetrahedron Lett. 21(49):4767–4770. doi:10.1016/S0040-4039(80)88117-2.

Harrison DA, Kulshrestha DA. 1984. Chemical constituents of *Amberboa ramosa*. Fitoterapia. 55:189–1992.

Ibrahim M, Farooq T, Hussain N, Hussain A, Gulzar T, Hussain I, Akash MS, Rehmani FS. 2013. Acetyl and butyryl cholinesterase inhibitory sesquiterpene lactones from *Amberboa ramosa*. Chem Cent J. 7(1):116. doi:10.1186/1752-153X-7-116.

Ibrahim M, Hussain I, Imran M, Hussain N, Hussain A, Mahboob T. 2013. Corniculatin a, a new flavonoidal glucoside from *Oxalis corniculata*. Revista Brasileira de Farmacognosia. 23(4):630–634. doi:10.1590/S0102-695X2013005000059.

Ibrahim M, Khan R, Malik A. 2010. Two new guaianolides from *Amberboa ramosa*. Nat Prod Commun. 5:1865–1868.

Khan SB, Afza N, Malik A, Ul Haq A, Ahmed Z. 2004. Structure determination of ramosine, a guaianolide, by NMR spectroscopy. Mag Reson Chem. 42(12):1063–1065. doi:10.1002/mrc.1461.

Shen C-C, Chang Y-S, Hott L-K. 1993. Nuclear magnetic resonance studies of 5,7-dihydroxyflavonoids. Phytochemistry. 34(3):843–845. doi:10.1016/0031-9422(93)85370-7.

Youssef DTA. 1998. Sesquiterpene lactones of *Centaurea scoparia*. Phytochemistry. 49(6):1733–1737. doi:10.1016/S0031-9422(98)00257-X.

Zdero C, Bohlmann F, Müller M. 1987. Sesquiterpene lactones and other constituents from *Eriocephalus* species. Phytochemistry. 26(10):2763–2775. doi:10.1016/S0031-9422(00)83588-8.

Zdero C, Bohlmann F. 1990. Glaucolides, fulvenoguaianolides and other sesquiterpene lactones from *Pentzia* species. Phytochemistry. 29(1):189–194. doi:10.1016/0031-9422(90)89035-8.