FULL PAPER

Lyciumaside and Lyciumate: A New Diacylglycoside and Sesquiterpene Lactone from *Lycium shawii*

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One new diacylglycoside named lyciumaside (**1**) and a new sesquiterpene lactone named lyciumate (**2**) were isolated from *Lycium shawii* Roem. & Schult. The structures of the two new compounds were elucidated based on 1D- (^{1}H- and ^{13}C-NMR and NOE) and 2D-NMR (COSY, HSQC, and HMBC) spectroscopic techniques, and mass spectrometry (ESI-MS). Preliminary evaluations demonstrated lyciumaside (**1**) possesses strong antioxidant activity with an IC_{50} = 30 μg/ml (80% inhibition) while it was inactive in α-glucosidase and urease enzymes assays.

Keywords: Natural product, *Lycium shawii*, Diacylglycoside, Sesquiterpene lactone, Antioxidant activity.

Introduction

Lycium (boxthorn) belongs to the Solanaceae family consisting of *ca*. 90 species of thorny shrubs. It was noted earlier that plants of the genus *Lycium* demonstrated antioxidant, anticancer, and antidiabetic activities, and also some plants were used as diuretics and laxatives and in the treatment of jaundice [1]. *Lycium shawii* Roem. & Schult is a native plant of the Arabian Gulf region and is used in traditional medicine to treat mouth sores, backache, coughs, constipation, stomach ache, and certain fevers in livestock [2][3].

Moreover, *L. shawii* extracts have been used as a hypotensive, antidiabetic agent, and also possessed anti-inflammatory, anticancer (toward the HEK293 and MCF7 cancer cell lines), hypoglycemic, hepatoprotective, antitrypanosomal, antiplasmodial, and cytotoxic activities [1][3][4]. These biological activities prompted our group to further investigate the phytochemical composition of *L. shawii* which resulted in the isolation and characterization of one new diacylglycoside named lyciumaside (**1**) and a new sesquiterpene lactone named lyciumate (**2**). Preliminary activity assays showed lyciumaside (**1**) to possess strong antioxidant activity with an IC_{50} = 30 μg/ml.

Results and Discussion

Phytochemical investigation of *L. shawii* provided two new compounds *viz*., diacylglycoside (**1**) and sesquiterpene lactone (**2**; *Fig. 1*).

The molecular formula of lyciumaside (**1**) was established to be $C_{22}H_{22}O_{11}$ based on the molecular ion peak in the ESI-MS spectrum and detailed NMR spectroscopic analysis. The IR spectrum suggested the presence of a OH (3380 cm^{-1}), ester (1715 cm^{-1}), and aryl ring (1610 cm^{-1}). The ^{1}H-NMR spectra (*Table*) illustrated the signals for one galloyl group (δ(H) 7.05 (*s*, H–C(2″), H–C(6″))), which was further confirmed by the typical galloyl signals

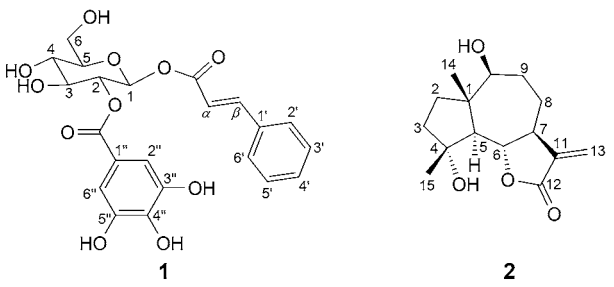

Fig. 1. Structures of lyciumaside (**1**) and lyciumate (**2**).

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in the ^{13}C-NMR spectrum (δ(C) 166.6 (CO), 120.3 C(1″), 110.5 C(2″), C(6″), 146.5 C(3″), C(5″), 140.6 C(4″)) [5 – 7]. Moreover, the ^{1}H-NMR spectrum of compound **1** illustrated *trans*-coupled olefinic signals and aromatic signals for a cinnamoyl group (δ(H) 7.74 (*d*, *J* = 15.6, H–C(α)); δ(C) 147.0; 6.55 (*d*, *J* = 15.6, H–C(β)); δ(C) 118.4; 7.57 – 7.59 (*m*, H–C(2′), H–C(6′)); δ(C) 129.3; 7.38 – 7.39 (*m*, H–C(3′), H–C(5′)); δ(C) 129.9; 7.38 – 7.39 (*m*, H–C(4′)); δ(C) 131.5; δ(C) 167.1 (CO); δ(C) 135.6 C(1′)) [5 – 7].

Further analysis of the ^{1}H-NMR spectrum showed signals for a glucose moiety (δ(H) 5.87 (*d*, *J* = 8.4, H–C(1)); δ(C) 94.0; δ(H) 5.17 (*dd*, *J* = 8.4, 9.6, H–C(2)); δ(C) 74.3; δ(H) 3.54 – 3.56 (*m*, H–C(3)); δ(C) 79.1; δ(H) 3.54 – 3.56 (*m*, H–C(4)); δ(C) 71.2; δ(H) 3.77 – 3.78 (*m*, H–C(5)); δ(C) 76.0; δ(H) 3.92 (*dd*, *J* = 1.8, 12.6, H_a–C(6)); 3.76 (*dd*, *J* = 6.0, 12.6, H_b–C(6)); δ(C) 62.2). Importantly, the relatively large *J* value of 8.4 Hz for the anomeric H-atom indicated a β-configuration of the glucose moiety. Furthermore, the complete structure assignment of the lyciumaside (**1**) was determined by COSY and HMBC experiments (*Fig. 2*).

The locations of the galloyl and cinnamoyl groups were confirmed from the lower chemical shifts of the glucose H-atom signals *viz*., H–C(1) (δ(H) 5.87 (*d*, *J* = 8.4)) and H–C(2) (δ(H) 5.17 (*dd*, *J* = 8.4, 9.6)). Furthermore, the ^{3}J HMBCs of H–C(1) from (δ(H) 5.87) to δ(C) 167.1 and H–C(2) from (δ(H) 5.17) to δ(C) 166.6 indicated that cinnamoyl and galloyl groups were bound

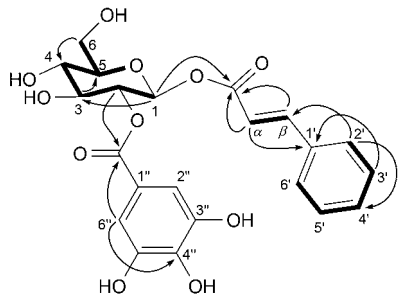

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Fig. 2. Key ^{1}H,^{1}H-COSY (▬) and HMBC (H → C) correlations of lyciumaside (**1**).

to C(1) and C(2), respectively. Therefore, compound **1** was identified as 1-*O*-cinnamoyl-2-*O*-galloyl-β-D-glucopyranoside.

The molecular formula of lyciumate (**2**) was confirmed to be $C_{15}H_{22}O_4$ based on ESI-MS and the proposed five degrees of unsaturation. The IR spectrum illustrated absorptions for an OH group (3415 cm^{-1}), an α,β-unsaturated-γ-lactone moiety (1760 cm^{-1}), and an ester group (1720 cm^{-1}) [8]. The presence of an exomethylene-γ-lactone ring was recognized due to the typical CH_2(13) H-atom signals at δ(H) 6.11 and 5.44, and their HMBCs to C(7), C(11), and C(12), and were further confirmed from the ^{13}C-NMR spectral signals (*Table*) for an exomethylene (δ(C) 118.1 C(13), 138.0 C(11)) and lactone

Table. NMR data[a–c]) of lyciumaside (**1**) and lyciumate (**2**). δ in ppm, *J* in Hz.

Position	**1**		Position	**2**	
	δ(H)	δ(C)		δ(H)	δ(C)
Glucose			1	–	41.8
1	5.87 (*d*, *J* = 8.4)	94.0	2a	2.06 – 2.08 (*m*)	21.7
2	5.17 (*dd*, *J* = 8.4, 9.6)	74.3	2b	1.53 – 1.55 (*m*)	
3	3.54 – 3.56 (*m*)	79.1	3a	1.60 – 1.63 (*m*)	38.0
4	3.54 – 3.56 (*m*)	71.2	3b	1.78 – 1.80 (*m*)	
5	3.77 – 3.78 (*m*)	76.0	4	–	71.2
6a	3.92 (*dd*, *J* = 1.8, 12.6)	62.2	5	1.84 (*d*, *J* = 11.0)	56.4
6b	3.76 (*dd*, *J* = 6.0, 12.6)		6	4.11 (*t*, *J* = 11.0)	80.9
Cinnamoyl			7	2.60 – 2.55 (*m*)	50.4
CO	–	167.1	8a	1.73 – 1.75 (*m*)	28.9
α	7.74 (*d*, *J* = 15.6)	147.0	8b	1.57 – 1.59 (*m*)	
β	6.55 (*d*, *J* = 15.6)	118.4	9a	1.99 – 2.02 (*m*)	38.9
1′	–	135.6	9b	1.28 – 1.30 (*m*)	
2′, 6′	7.57 – 7.59 (*m*)	129.3	10	3.42 – 3.45 (*m*)	78.3
3′, 5′	7.38 – 7.39 (*m*)	129.9	11	–	138.0
4′	7.38 – 7.39 (*m*)	131.5	12	–	169.6
Galloyl			13a	6.11 (*d*, *J* = 3.4)	118.1
CO	–	166.6	13b	5.44 (*d*, *J* = 3.4)	
1″	–	120.3	14	0.96 (*s*)	13.6
2″, 6″	7.05 (*s*)	110.5	15	1.35 (*s*)	24.4
3″, 5″	–	146.5			
4″	–	140.6			

[a]) Assignments were determined using 2D-NMR (^{1}H,^{1}H-COSY, NOESY, HSQC, and HMBC) spectra. [b]) Multiplicity was determined by DEPT experiments and *J* values are given in parentheses. [c]) Compound **1** spectra measured in CD_3OD and **2** in $CDCl_3$ at 600 (^{1}H-NMR) and 150 MHz (^{13}C-NMR).

C=O signal (δ(C) 169.6 C(12)). Moreover, the ^{1}H-NMR spectrum showed the typical lactone signal (δ(H) 4.11 (*t*, *J* = 10.8, H–C(6)); δ(C) 80.8) and was further supported through its strong ^{1}H,^{1}H coupling with H–C(7) and ^3J HMBC connectivity to C(12) [8].

Furthermore, the NMR data illustrated the presence of one oxymethine tertiary C-atom (δ(H) 3.42 – 3.45 (*m,* C(10)); δ(C) 78.3) and its HMBCs to H–C(1), H–C(2), H–C(5), H–C(8), and H–C(9) confirmed its position to be at C(10). The NMR spectral data also showed the presence of two tertiary Me groups (δ(H) 0.96 (*s*, Me(14)); δ(C) 13.6; δ(H) 1.35 (*s*, Me(15)); δ(C) 24.4) and an O-bearing quaternary C-atom C(4) at δ(C) 71.2. The HMBCs from Me(14) to C(1), C(2), C(5), and C(10) and that from Me(15) to C(3), C(4) and C(5) confirmed their attachments at C(1) and C(4), respectively. Finally, the complete structural confirmation of terpene **2** was achieved by a detailed analysis of the COSY, HSQC, and HMBC spectra (*Fig. 3*). Notably, COSY correlations from H–C(2) to H–C(3), HMBCs from H–C(1) to C(3), C(4), C(5), and C(10), from H–C(1) with C(1), C(4), C(5), C(7), and C(8), along with HMBCs from Me(15) to C(3), C(4), and C(5) supported the left side cyclopentane ring. However, the ^{1}H,^{1}H spin system H–C(5)/H–C(6)/H–C(7)/H–C(8)/H–C(9)/H–C(10) along with HMBCs from H–C(5) to C(8) and from H–C(5) to C(10) lent strong support for the cycloheptane ring and thereby established the sesquiterpene skeleton unambiguously. The relative configuration of lyciumate (**2**) was based on the following NOE correlations *viz*., NOE interactions between H–C(6)/Me(14), H–C(6)/Me(15), and Me (14)/Me(15) all of which strongly suggested that these groups had the same relative orientation (β), NOE interactions between H–C(7) and H–C(5), H–C(10) and H–C(5) and to H–C(8a), indicated they had the same relative orientation (α). Thus, the proposed configuration was further confirmed by the large coupling constants between H–C(5) and H–C(6) (*J* = 11.0 Hz) as well as H–C(6) and H–C(7) (*J* = 11.0 Hz), which indicated that the three H-atoms are axial [8]. Thus, the spectroscopic data when considered holistically lead to the assignment of the structure of lyciumate (**2**) to be determined as (3a*S*,6*S*,6a*S*,9*R*,9a*S*,9b*S*)-6,9-dihydroxy-6a,9-dimethyl-3-methylidenedecahydroazuleno[4,5-*b*]furan-2(3*H*)-one.

Lyciumate (**2**) was tested for DPPH antioxidant activity, urease enzyme inhibition, and α-glucosidase enzyme inhibition. However, it only demonstrated strong antioxidant activity with an IC_{50} = 30 μg/ml (80% inhibition) while it proved to be inactive in α-glucosidase and urease enzyme assays.

Experimental

General

TLC: Pre-coated Al sheets (silica gel *60F-254*; *E. Merck*) were used; visualizations of the TLC plates were achieved under the UV light at 254 and 366 nm and also by spraying with the ceric sulfate reagent followed by heating. Optical rotations: *KRUSS P P3000* polarimeter (*A. Kruss Optronic*, Hamburg, Germany). IR Spectra: *Bruker ATR-Tensor 37* spectrophotometer; $\tilde{\nu}$ in cm^{-1}. Multinuclear and multidimensional NMR spectra: *Bruker* NMR spectrometer operating at 600 MHz (150 MHz for ^{13}C) with cryoprobe prodigy; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. ESI-MS: *Waters Quattro Premier XE* Mass Spectrometer (*Waters*, Milford, MA, USA); in *m*/*z*.

Sample Collection and Identification

The plant *Lycium shawii* was purchased from the local market in May 2015 and was identified by the plant taxonomist at the Department of Biological Sciences and Chemistry, University of Nizwa, the Sultanate of Oman. A voucher specimen (No: BSHR-05/2015) was deposited with the Herbarium of the Department of Biological Sciences and Chemistry.

Extraction and Purification

The air-dried powder (640 g) of the stem bark of *L. shawii* was exhaustively extracted with MeOH for 2 weeks. The resulting MeOH extract (304 g) was suspended in H_2O and

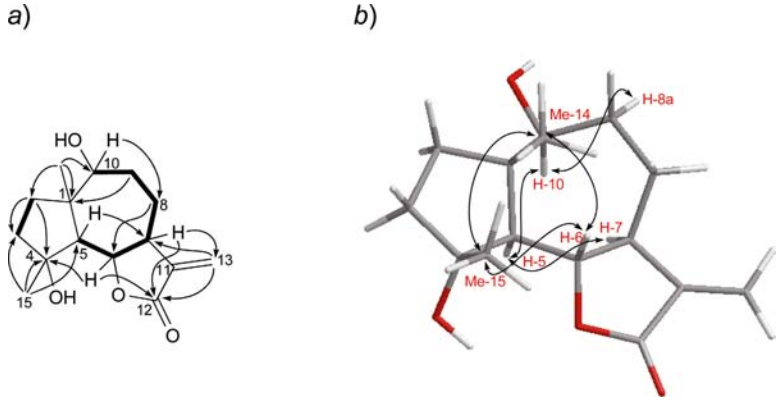

Fig. 3. *a*) Key ^{1}H,^{1}H-COSY (▬) and HMBC (H → C) correlations of lyciumate (**2**). *b*) Key NOESY (H ↔ H) correlations of lyciumate (**2**).

successively portioned to provide hexane (26 g), CH_2Cl_2 (11 g), AcOEt (25 g), and BuOH (26 g) fractions. The AcOEt fraction (25 g) was subjected to column chromatography (CC) and eluted with an eluent of increasing polarity, *viz*., hexane/CH_2Cl_2, CH_2Cl_2, CH_2Cl_2/MeOH, and MeOH to provide 18 fractions (*F1 – F18*). *Fr. 15* (1.2 g) was subjected to further careful CC using AcOEt/hexane (9:1) as eluent to give lyciumaside (**1**, 16 mg), while *Fr. 10* (*F10*, 300 mg) was rechromatographed using AcOEt/hexane (4:6) as eluent to provide lyciumate (**2**, 6.0 mg).

Lyciumaside (= **1-*O*-[(2*E*)-3-Phenylprop-2-enoyl]-2-*O*-(3,4,5-trihydroxybenzoyl)-*β*-D-glucopyranose**; **1**). White solid. IR (KBr): 3380, 1715, 1610, 1450, 1070. UV (CH_2Cl_2): 261 (3.80), 246 (3.84). ^{1}H- and ^{13}C-NMR (600 and 150 MHz, CD_3OD): *Table*. ESI-MS: 485 (70). HR-ESI-MS: 485.1069 ($[M + Na]^+$, $C_{22}H_{22}NaO_{11}^+$; calc. 485.1060).

Lyciumate (= **(3a*S*,6*S*,6a*S*,9*R*,9a*S*,9b*S*)-Decahydro-6,9-dihydroxy-6a,9-dimethyl-3-methylideneazuleno[4,5-*b*]furan2(3*H*)-one**; **2**). White solid. IR (KBr): 3415, 2940, 1760, 1720, 1240, 1075. UV (CH_2Cl_2): 235 (3.58). ^{1}H- and ^{13}C-NMR (600 and 150 MHz, $CDCl_3$): *Table*. ESI-MS: 289 (97). HR-ESI-MS: 289.1429 ($[M + Na]^+$, $C_{15}H_{22}NaO_4^+$; calc. 289.1416).

REFERENCES

[1] A. Gaweesh, A. E. N. B. Sengab, H. M. H, S. M. Osman, A. M. Abdou, *Med. Aromat. Plants* **2015**, *4*, 180.
[2] M. K. Suleiman, N. R. Bhat, S. Jacob, R. R. Thomas, *World J. Agric. Sci.* **2011**, *7*, 26.
[3] P. C. Phondani, A. Bhatt, E. Elsarrag, Y. A. Horr, *J. Tradit. Complement. Med.* **2016**, *6*, 209.
[4] H. Sher, M. N. Alyemeni, *J. Med. Plants Res.* **2011**, *5*, 3387.
[5] Y. Kashiwada, G. I. Nonaka, I. Nishioka, T. Yamagish, *Phytochemistry* **1988**, *21*, 1473.
[6] T. Tanaka, R. Uehara, K. Nishida, I. Kouno, *Phytochemistry* **2005**, *66*, 675.
[7] Z. H. Jiang, Y. Hirose, H. Iwata, S. Sakamoto, T. Tanaka, I. Kouno, *Chem. Pharm. Bull.* **2001**, *49*, 887.
[8] E. M. Maldonado, D. Svensson, S. M. Oredsson, O. Sterner, *Sci Pharm.* **2014**, *82*, 147.

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