Phlomisamide and Phlomisteriod: A New Ceramide and a New Stigmasterol Derivative from *Phlomis cashmeriana*

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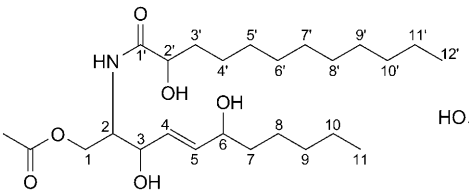
Phlomisamide (**1**), a new ceramide, and a new stigmasterol derivative, phlomisteriod (**2**), have been isolated from *Phlomis cashmeriana*. Their structures were elucidated by comprehensive analysis of their 1D- (^{1}H- and ^{13}C-NMR) and 2D-NMR (COSY, HMQC, HMBC), and HR-EI-MS data.

Introduction. – *Phlomis* is a genus of the family Lamiaceae (Labiatae) consisting of *ca.* 100 species of herbs or shrubs, many of which are highly variable [1]. Some *Phlomis* species are used as tonics and stimulants in Anatolian folk medicine [2]. Different classes of glycosides comprising diterpenoids, iridoids, phenylpropanoids, phenylethanoids, and flavonoids had been identified from genus *Phlomis.* Many of the phenylpropanoids isolated from genus *Phlomis* showed significant biological activities, *e.g.*, cytotoxic, cytostatic, anti-inflammatory, immunosuppressant, and anti-microbial effects [3]. As part of our systematic search for new bioactive lead molecule from Pakistani medicinal plants, one new ceramide, phlomisamide (**1**), and phlomisteriod (**2**), a new stigmasterol derivative, have been isolated from *Phlomis cashmeriana.*



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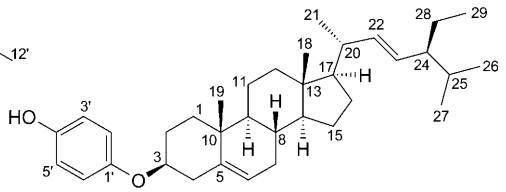

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Results and Discussion. – Dried and powdered samples of whole plant of *P. cashmeriana* were extracted with MeOH at room temperature. The residue obtained after evaporation of the solvent was subjected to conventional purification procedures, resulting in the isolation of two constituents, *i.e.*, one new ceramide, **1**, and one new stigmasterol derivative, **2**.

Compound **1** was isolated as an oil. The molecular formula was determined to be $C_{25}H_{47}NO_6$ by HR-EI-MS. The IR spectrum showed absorption bands at 3630 (OH),

3630 and 1621 (amide), and 2919, 2850, and 1465 cm^{-1} (aliph.), suggesting that **1** is a fatty acid amide. The ^{1}H-NMR spectrum (in $CDCl_3$; see *Exper. Part*) displayed signals for two terminal Me groups (δ(H) 0.85 (Me(12′), Me(11))), aliphatic CH_2 groups (δ(H) 1.29–1.31), a CH_2 group (δ(H) 3.69 (*dd*, J = 6.4, 10.5, H_a–C(1)), 3.64 (*dd*, J = 6.4, 10.5, H_b–C(1)), three CH groups (δ(H) 5.28–5.36 (*m*, H–C(2′)), 5.17–5.19 (*m*, H–C(3)), and 5.29–5.32 (*m*, H–C(6))), H-atoms of a disubstituted olefinic C=C bond (δ(H) 5.32 (*dt*, J = 6.1, 15.0, H–C(4)), 5.13 (*dt*, J = 6.1, 15.0, H–C(5))), an amide H-atom (δ(H) 8.20 (*d*, J = 7.8)), and a CH H-atom (δ(H) 5.16–5.18 (*m*, H–C(2))).

The ^{13}C-NMR spectrum (see *Exper. Part*) exhibited characteristic signals due to an amide CO group at δ(C) 173.5 and a CH group C-atom linked to amide N-atom at δ(C) 51.3. These spectral data and the molecular formula suggest that compound **1** is a ceramide [4]. The (*E*)-configuration of the C(4)=C(5) bond was evidenced by the large coupling constant (J = 15.5 Hz).

The length of the fatty acid was determined by the characteristic peaks at *m*/*z* 57 ($[C_4H_9]^+$), 85 ($[C_6H_{13}]^+$), 113 ($[C_8H_{17}]^+$), 141 ($[C_{10}H_{21}]^+$), 171 ($[C_{11}H_{23}O]^+$), 199 ($[C_{12}H_{23}O_2]^+$), 214 ($[C_{12}H_{24}NO_2]^+$), 287 ($[C_{15}H_{29}NO_4]^+$), 300 ($[C_{16}H_{30}NO_4]^+$), 356 ($[C_{19}H_{34}NO_5]^+$), 428 ($[C_{23}H_{42}O_6]^+$), and 457 ($[C_{25}H_{47}NO_6]^+$) in the EI-MS [4–14]. The ^{1}H,^{1}H and ^{1}H,^{13}C connectivities were supported by the ^{1}H,^{1}H-COSY and HMQC spectra. This assembly of spectral data and the molecular formula suggest that compound **1** is a ceramide. The position of the C=C bond between C(4) and C(5) in the long chain base was confirmed by HMBC.

The positions of the three OH groups in the long chain base were further confirmed from the mass-fragmentation pattern (*Fig. 1*) as well as from the HMBCs (*Fig. 2*). We have named the compound phlomisamide after the producing organism, *Phlomis cashmeriana.*

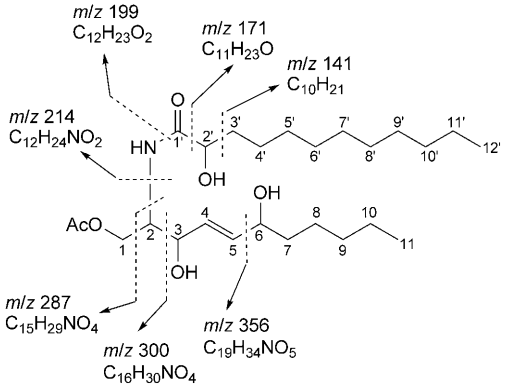

Fig. 1. *Mass-fragmentation pattern for phlomisamide* (**1**)

The configuration at the stereogenic centers at C(2), C(2′), C(3), and C(6) could not be established without chemical transformations that would require much more material [11].

The molecular formula of **2** determined as $C_{35}H_{52}O_2$ by HR-EI-MS indicated ten degrees of unsaturation. The IR spectrum displayed absorptions at 3428 and 1037 cm^{-1} due to the OH and olefinic functions, respectively.

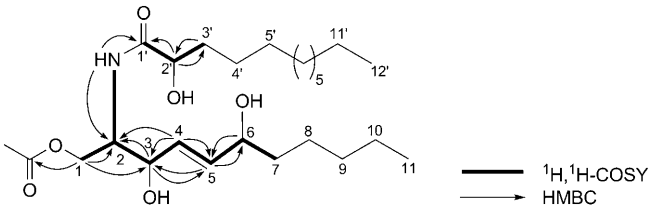

Fig. 2. *^{1}H,^{1}H-COSY and HMBC correlations for phlomisamide* (**1**)

In the ^{1}H-NMR spectrum of **2** (see *Exper. Part*), the signal of the H-atom of one of the trisubstituted C=C bond was observed at δ(H) 5.21 (br. *s*, H–C(6)), and the signals of an (*E*)-configured, 1,2-disubstituted C=C bond were observed at δ(H) 5.03 (*dd*, $J = 15.2$, 7.5, H–C(23)), 5.12 (*dd*, $J = 15.2$, 7.5, H–C(22)). This is further confirmed by typical signals at δ(C) 119.7 for C(23), 119.6 for C(22), and 132.0 for C(6), respectively, in the ^{13}C-NMR spectrum. The angular Me(18) and Me(19) groups resonated at δ(H) 0.83 (*s*, Me(19)) and 0.70 (*s*, Me(18)), typical of a $\Delta^{9(11)}$ sterol [15]. The steroidal secondary Me-group signals were observed as *doublet*s at δ(H) 0.99, 0.97, 0.81, and 0.89, with coupling constants of 6.7–8.0 Hz. Finally, the *triplet* at δ(H) 0.84 (*t*, $J = 8.0$) was assigned to Me(29). The most-downfield O-bearing CH group signal at δ(H) 3.60–3.62 (*m*) showed a connectivity with four neighboring H-atoms in the ^{1}H,^{1}H-COSY spectrum, and, hence, was assigned to H–C(3). A signal at δ(H) 3.88 with significant multiplicity was attributed to H_α–C(3) for a steroid. The ^{1}H-NMR spectrum of **2** further indicated the presence of a phenyl moiety, attached through a ether linkage at C(3), as indicated by δ(H) 6.50–6.52 (*m*, H–C(2′), H–C(3′), H–C(5′), H–C(6′)). The ^{1}H-NMR spectrum also implied the presence of OH moiety at δ(H) 7.24 (*s*).

The ^{13}C-NMR assignments of various signals in **2** were substantiated by DEPT experiments, which revealed the presence of six Me, nine CH_2, 15 CH groups, and five quaternary C-atoms. The full structure was finally secured by means of ^{1}H,^{1}H-COSY, HMBC, and HMQC experiments. From these data, the structure of **2** was determined and given the trivial name phlomisteriod.

Experimental Part

General. Optical rotations: *Perkin-Elmer 241* polarimeter. UV Spectra: *UV-2101PC* spectrometer. IR Spectra: *NICOLET 510P* FT-IR spectrometer. ^{1}H, 2D ^{1}H,^{1}H-COSY, ^{13}C, 2D-HMQC, and HMBC spectra: *Bruker Avance 500* MHz spectrometer; chemical shifts referenced to internal TMS ($\delta = 0$), and coupling constants *J* in Hz.

Plant Material. P. cashmeriana was collected at Parachinar Kurram Agency, N.W.F.P Pakistan, in 2005, and identified by Dr. *Jahandar Shah* (plant taxonomist), Department of Botany, Islamia College, University of Peshawar, and by Mr. *Elias Naveed* (plant taxonomist). A voucher specimen was deposited with the Herbarium of the Botany Department, Kohat University of Science and Technology, Kohat.

Extraction and Isolation. The roots and aerial parts of the *P. cashmeriana* were soaked in MeOH at r.t. for 7 d (3 ×) then filtered. The filtrate was concentrated under vacuum to give 195 g of crude residue. The crude fraction was then subjected to column chromatography (CC; SiO_2; hexane, hexane/AcOEt, and AcOEt in order of increasing polarity). The fraction which was eluted with 5–30% AcOEt/hexane on further CC afforded phlomisamide (**1**; 18 mg) and phlomisteriod (**2**; 24 mg).

Phlomisamide (=*(4*E*)-3,6-Dihydroxy-2-[(2-hydroxydodecanoyl)amino]undec-4-en-1-yl Acetate*; **1**). Amorphous oil. $[\alpha]_D^{20} = -7.67$ ($c = 0.04$, $CHCl_3$/MeOH). IR ($CHCl_3$): 3630, 2919, 2850, 1621, 1465. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.85 (*t*, *J* = 7.8, Me(12′), Me(11)); 1.29–1.31 (*m*, CH_2), 3.64 (*dd*, *J* = 6.4, 10.5, H_a–C(1)); 3.69 (*dd*, *J* = 6.4, 10.5, H_b–C(1)); 5.13 (*dt*, *J* = 6.1,15.0, H–C(5)); 5.16–5.18 (*m*, H–C(2)); 5.17–5.19 (*m*, H–C(3)); 5.28–5.30 (*m*, H–C(6), H–C(2′)); 5.32 (*dt*, *J* = 6.1, 15.0, H–C(4)); 8.20 (*d*, *J* = 7.8, NH). ^{13}C-NMR (125 MHz, $CDCl_3$): 174.2 (MeCO); 173.83 (C(1′)); 136.7 (C(4)); 118.1 (C(5)); 80.5 (C(2′)); 77 (C(3)); 74.4 (C(6)); 64 (C(1)); 51.3 (C(2)); 36.2 (C(8)); 44.2 (C(6′)); 39.7 (C(3′)); 37.7 (C(4′)); 36.5 (C(5′)); 34.7 (C(10)); 34.5 (C(9)); 31.5 (C(7)); 29.3 (C(7′)); 29.3 (C(8′)); 29.3 (C(9′)); 29.3 (C(10′)); 29.3 (C(11), (C(12′)). HR-EI-MS: 457.3400 ($C_{25}H_{47}NO_6^+$; calc. 457.3403). EI-MS: *Fig. 1*.

Phlomisteriod (=*4-[(3β,22*E*)-Stigmasta-5,22-dien-3-yloxy]phenol*; **2**). Amorphous soild. M.p. 196–198°. $[\alpha]_D^{20} = -51.5$ ($c = 0.28$, $CHCl_3$/MeOH). IR ($CHCl_3$): 3428, 1037. ^{1}H-NMR (500 MHz, $CDCl_3$): 0.83 (*s*, Me(19)); 0.70 (*s*, Me(18)); 0.99 (*d*, *J* = 6.5, Me(21)); 0.84 (*t*, *J* = 8, Me(29)); 0.81 (*d*, *J* = 8, Me(26)); 0.97 (*d*, *J* = 8, Me(27)); 5.03 (*dd*, *J* = 5.2, 7.5, H–C(23)); 5.12 (*dd*, *J* = 15.2, 7.5, H–C(22)); 5.22 (br. *s*, H–C(6)); 6.50 (*d*, *J* = 8.43, H–C(2′), H–C(3′), H–C(5′), H–C(6′)); 7.24 (*s*, OH). ^{13}C-NMR (500 MHz, $CDCl_3$): 156.5 (C(4′)); 142.5 (C(5)); 142.4 (C(1′)); 135.1 (C(2′)); 135.1 (C(3′)); 135.1 (C(5′); 135.1(C(6′); 132.4 (C(6)); 119.7 (C(23)); 119.6 (C(22)); 77.3 (C(3)); 56.2 (C(17)); 51.1 (C(9)); 51.0 (C(14)); 48.0 (C(13)); 42.7 (C(24)); 41.7 (C(12)); 39.4 (C(4)); 39.3 (C(10)); 36.8 (C(1)); 35.6 (C(20)); 33.0 (C(25)); 32.5 (C(7)); 30.0 (C(2)), 30.0 (C(8)); 28.1 (C(16)); 28.0 (C(28)); 23.3 (C(15)); 20.8 (C(27)); 20.6 (C(11)); 19.9 (C(19)); 19.6 (C(26)); 18.1 (C(21)); 15.4 (C(29)); 12.6 (C(18)). HR-EI-MS: 504.3967 ($C_{35}H_{52}O_2^+$; calc. 504.3967).

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