# Gypsins A−D from *Gypsophila arabica*

Maryan Bruzual De Abreu,[†,||] Fabrizio Dal Piaz,[‡,||] Abeer Temraz,[§] Ammar Bader,[⊥] Nunziatina De Tommasi,[‡] and Alessandra Braca*[,†]

*Dipartimento di Chimica Bioorganica e Biofarmacia, Università di Pisa, Via Bonanno 33, 56126 Pisa, Italy, Dipartimento di Scienze Farmaceutiche, Università di Salerno, Via Ponte Don Melillo, 84084 Fisciano (SA), Italy, Faculty of Pharmacy, Al Azhar University, Nasr-City, 11371 Cairo, Egypt, and Faculty of Pharmacy, Al-Zaytoonah Private University of Jordan, P.O. Box 130, 11733 Amman, Jordan*

*Received December 19, 2007*

Four new cyclopeptides, named gypsins A−D (**1**−**4**), together with one known oleanane saponin, were isolated from the roots of *Gypsophila arabica*. The structures of *cyclo*(-Leu[1]-Pro[2]-Leu[3]-Trp[4]-Pro[5]-Gly[6]-) (**1**), *cyclo*(-Leu[1]-Pro[2]-Tyr[3]-Phe[4]-Pro[5]-Gly[6]-) (**2**), *cyclo*(-Ala[1]-Pro[2]-Tyr[3]-Leu[4]-Leu[5]-Pro[6]-Pro[7]-Ala[8]-) (**3**), and *cyclo*(-Leu[1]-Trp[2]-Pro[3]-Gly[4]-Gly[5]-Ser[6]-Ser[7]-) (**4**) were elucidated by 1D and 2D NMR spectroscopy including 1D-TOCSY, DQF-COSY, 2D-ROESY, HSQC, and HMBC experiments, as well as ESI tandem mass spectrometric fragmentation analysis and chemical evidence.

The family Caryophyllaceae is well known for their saponins of industrial interest,[1] flavonoids,[2] and cyclopeptides:[3] these last compounds showed interesting biological activities including cytotoxic, antiplatelet, antimalarial, immunomodulating, immunosuppressive, $Ca^{2+}$ antagonistic, cyclooxigenase and tyrosinase inhibiting, and estrogen-like activity.[4]

As a part of a continuing investigation of the Caryophyllaceae of Jordan,[5] we carried out the phytochemical study of *Gypsophila arabica* Barkoudah, a glabrous perennial herb, 30−80 cm high, occurring mainly in West Irano-Turanian and Saharo-Arabian territories.[6] A previous screening of the cytotoxic and antimalarial activities of the aqueous extract of the aerial parts showed strong activity in a melanoma assay.[7] No phytochemical study of the plant has been yet reported.

Herein we describe the isolation and structural elucidation of four new cyclopeptides, named gypsins A−D (**1**−**4**), by means of spectroscopy, as well as ESI tandem MS fragmentation analysis and chemical evidence.

## Results and Discussion

The $CHCl_3$−MeOH extract of the roots of *G. arabica* were subjected to Sephadex LH-20 column chromatography, followed by Si gel column and RP-HPLC, to afford four new cyclopeptides, gypsins A−D (**1**−**4**), and one known oleanane saponin.

Gypsin A (**1**) showed an $[M + H]^+$ ion peak at *m*/*z* 664.3723 in the HRESIMS, consistent with the molecular formula $C_{35}H_{49}N_7O_6$ (calcd 663.3744). The peptide nature of **1** was supported by the presence of six amide carbonyl signals between $\delta$ 167.5 and 171.8 and four secondary amide protons ($\delta$ 7.77−8.66) in the $^{13}C$ and $^1H$ NMR spectra, respectively (Table 1). The amino acid composition of the acid hydrolysate of **1** revealed the presence of two Leu, two Pro, one Trp, and one Gly. The absolute configuration of amino acids established by GC analysis indicated that all the chiral amino acids were L. This amino acid composition accounted for 14 of the 15 degrees of unsaturation calculated from the molecular formula, requiring that **1** was a cyclic hexapeptide. The cyclic nature of **1** was also evident by the high degree of chemical shift dispersion observed for amide proton signals resonating between $\delta$ 7.77 and 8.66. Small-ring cyclopeptides tend to show a strong conformational preference in solution, in accordance with their size. This, coupled with the presence of two Pro residues in **1**, likely is the cause of the observed broad dispersion of these signals.[4] Nevertheless, a single stable conformer on the NMR time scale (DMSO-$d_6$) was displayed by the occurrence of well-resolved, sharp $^1H$ and $^{13}C$ NMR signals. The structure of each amino acid moiety was deduced by 1D TOCSY and 2D NMR experiments. The isolated amide proton resonances in an uncrowded region of the $^1H$ NMR spectrum were the starting point for the 1D TOCSY experiment. The results of 1D TOCSY, COSY, and HSQC experiments allowed the sequential assignments of all the proton and carbon resonances to the individual amino acids as reported in Table 1. The amino acid sequence of **1** was deduced by extensive 2D NMR analysis, including HMBC and ROESY measurements and MS techniques. The sequence determination was performed by observing HMBC correlations of the carbonyl carbon of one amino acid residue with the amide and/or α-protons of the neighboring residue. Key correlations, such as $NH_{leu1}$ ($\delta$ 7.85) to $C\alpha_{leu\ 1}$ (48.2 ppm), $CO_{gly6}$ (170.6 ppm), and $C\alpha_{gly6}$ (41.4 ppm), $NH_{trp4}$ ($\delta$ 8.66) to $CO_{leu3}$ (171.2 ppm), $C\alpha_{leu3}$ (49.2 ppm), $CO_{trp4}$ (171.0 ppm), and $C\beta_{trp4}$ (26.5 ppm), $NH_{leu3}$ ($\delta$ 7.77) to $CO_{leu3}$ (171.2 ppm), and $H\beta_{trp4}$ ($\delta$ 3.15) to $C\alpha_{trp4}$ (53.6 ppm), $CO_{trp4}$ (171.0 ppm), $C2_{trp4}$ (123.8 ppm), $C3_{trp4}$ (129.5), and $C3a_{trp4}$ (127.3 ppm) allowed the sequence determination of two structural units, Gly[6]-Leu[1]-Pro[2] and Leu[3]-Trp[4], respectively (Table 1). The presence of two Pro residues that lack amide protons made the determination of the primary structure of **1** difficult to obtain by NMR data alone. Determination of the primary structure of **1** was obtained on the basis of HRESIMS/MS. The daughter ion spectrum of the singly charged ion at *m*/*z* 664.372, corresponding to the $[M + H]^+$ ion of **1**, generated a relatively uninformative and complex pattern of fragment ions, due to the cyclic nature of the fragmented peptide.[8] However, immonium ions produced as a secondary fragmentation (a combination of a y- and a-type cleavage) of the amide bond during low-energy CID provided support for the amino acid composition of **1**.[9] In particular, ions detected at *m*/*z* 159.085, 86.100, 70.067, and 30.035 demonstrated the presence of Trp, Leu, Pro, and Gly residues, respectively. Compound **1** was subjected to partial acid hydrolysis (see Experimental Section) in order to obtain an acyclic species for LC-MS/MS analysis.[10] The resulting chromatogram consisted of two main peaks, the first showing a retention time of 22.6 min, corresponding to the acyclic form of **1** (experimental *m*/*z* 682.382, theoretical *m*/*z* 682.393), and the second showing a retention time of 25.5 min for the unhydrolyzed species. As shown in Figure 2a, fragment ions detected in the tandem mass spectrum of the ion at *m*/*z* 682.382 (all the *m*/*z* values measured for the daughter ions showed an accuracy ranging from 2 to 11 ppm) resulted in a b and y series, permitting assignment of the

* To whom correspondence should be addressed. Tel: +39-050-2219688. Fax: +39-050-2219660. E-mail: braca@farm.unipi.it.
† Università di Pisa.
|| Equally contributing authors.
‡ Università di Salerno.
§ Al Azhar University.
⊥ Al-Zaytoonah University of Jordan.

10.1021/np700735a CCC: $40.75 
Published on Web 06/25/2008

**Table 1.** $^{1}H$ and $^{13}C$ NMR Data of Compounds **1** and **2** (DMSO-$d_6$, 600 MHz)[a]

| | | **1** | | | **2** | | |
|---|---|---|---|---|---|---|---|
| | position | $\delta_H$ | $\delta_C$ | HMBC | $\delta_H$ | $\delta_C$ | HMBC |
| Leu[1] | α | 4.42 dt (7.9, 5.0) | 48.2 | C═O, C═OGly[6], C*β*, C*γ* | 4.39 dt (8.3, 5.0) | 48.4 | C*β*, C*γ* |
| | *β* | 1.33 m | 42.5 | | 1.33 br t (5.5) | 42.4 | |
| | | 1.26 m | | | 1.23 m | | |
| | *γ* | 1.42 m | 23.7 | | 1.42 m | 23.8 | |
| | Me*δ* | 0.83 d (6.5) | 23.5 | C*β*, C*γ* | 0.84 d (6.5) | 21.5 | C*β*, C*γ* |
| | | 0.81 d (6.5) | 21.1 | C*β*, C*γ* | 0.81 d (6.5) | 23.2 | C*β*, C*γ* |
| | NH | 7.85 d (7.8) | | C═O, C═OGly[6], Cα, CαGly[6] | 7.83 d (7.7) | | C═O, C═OGly[6], CαGly[6], Cα |
| | C═O | | 167.5 | | | 167.8 | |
| Pro[2] | α | 3.02 br d (8.0) | 59.5 | C═O, C*δ* | 4.03 br d (8.0) | 60.6 | C*β*, C*γ*, C*δ* |
| | *β* | 1.56 m | 29.3 | | 1.99 q (10.0, 6.0) | 31.1 | C═O |
| | | 0.12 m | | | 1.42 m | | |
| | *γ* | 1.21 m | 21.1 | | 1.57 m | 21.0 | |
| | | 1.12 m | | | 1.34 m | | |
| | *δ* | 3.08 ddd (11.0, 10.0, 7.9) | 45.8 | C═O, C*β*, C*γ* | 3.62 br dd (16.0, 7.0) | 46.1 | |
| | | 3.04 br t (9.7) | | | 3.15 br dd (16.0, 9.0) | | |
| | C═O | | 171.8 | | | 170.5 | |
| Leu[3] | α | 4.68 dd (8.0, 6.0) | 49.2 | C═O, C*β* | | | |
| | *β* | 1.60 m | 40.4 | C═O, Cα, C*γ* | | | |
| | | 1.25 br t (5.5) | | | | | |
| | *γ* | 1.52 m | 23.7 | | | | |
| | Me*δ* | 0.83 d (6.5) | 23.5 | | | | |
| | | 0.81 d (6.5) | 21.1 | | | | |
| | NH | 7.77 d (8.0) | | C═O, Cα | | | |
| | C═O | | 171.2 | | | | |
| Tyr[3] | α | | | | 4.73 br dd (9.5, 5.5) | 52.4 | C═OPro[2], C*β* |
| | *β* | | | | 2.79 dd (14.0, 5.5) | 36.0 | C═OPro[2] |
| | | | | | 2.73 dd (14.0, 9.2) | | C═OPro[2] |
| | 1 | | | | | 128.0 | |
| | 2/6 | | | | 7.06 d (8.0) | 130.0 | C*β*, C1, C3/5, C4 |
| | 3/5 | | | | 6.60 d (8.0) | 114.3 | C1, C4 |
| | 4 | | | | | 155.6 | |
| | NH | | | | 7.72 d (8.5) | | C═O, C═OPro[2], Cα, C*β* |
| | C═O | | | | | 170.0 | |
| Trp[4] | α | 4.22 ddd (12.0, 7.5, 4.0) | 53.6 | | | | |
| | *β* | 3.15 dd (14.0, 4.0) | 26.5 | C═O, Cα, C2, C3, C3a | | | |
| | | 2.97 dd (14.0, 12.5) | | C═O, Cα, C2, C3 | | | |
| | 1 (NH) | 10.7 d (2.0) | | | | | |
| | 2 | 7.33 d (2.0) | 123.8 | C-3, C-7a | | | |
| | 3 | | 109.5 | | | | |
| | 3a | | 127.3 | | | | |
| | 4 | 7.36 d (8.0) | 110.9 | | | | |
| | 5 | 6.94 t (8.0) | 118.1 | C3a, C4, C6 | | | |
| | 6 | 7.06 t (8.0) | 121.0 | C4, C5, C7a | | | |
| | 7 | 7.35 d (8.0) | 118.1 | C3a, C5 | | | |
| | 7a | | 137.8 | | | | |
| | NH | 8.66 br s | | C═O, C═OLeu[3], CαLeu[3], Cα, C*β* | | | |
| | C═O | | 171.0 | | | | |
| Phe[4] | α | | | | 4.28 br dd (9.5, 5.5) | 53.3 | C═O, C═OTyr[3], C*β* |
| | *β* | | | | 3.07 br dd (9.5, 6.0) | 36.7 | C═O, Cα, C1, C2/6 |
| | | | | | 2.86 br t (9.0) | | C═O, Cα, C1, C2/6 |
| | 1 | | | | | 135.8 | |
| | 2/6 | | | | 7.33 dd (7.5, 1.5) | 128.4 | C1, C3/5 |
| | 3/5 | | | | 7.25 t (7.5) | 129.0 | C*β* |
| | 4 | | | | 7.29 t (7.5) | 126.9 | |
| | NH | | | | 8.74 br s | | C═O, C═OTyr[3], CαTyr[3], C*β* |
| | C═O | | | | | 169.6 | |
| Pro[5] | α | 4.21 br d (8.0) | 60.8 | C═O, C*β*, C*γ*, C*δ* | 3.19 br dd (9.0, 4.6) | 60.0 | C═O, C*β*, C*γ*, C*δ* |
| | *β* | 2.21 m | 31.5 | C═O, Cα, C*γ* | 1.86 br dd (13.0, 7.5) | 29.9 | C*γ*, C*δ* |
| | | 1.90 m | | | 1.04 m | | C═O |
| | *γ* | 1.76 m | 21.7 | | 1.57 m | 21.3 | |
| | | 1.60 m | | | 1.34 m | | |
| | *δ* | 3.78 m | 46.1 | Cα, C*β*, C*γ* | 3.28 ddd (12.0, 10.0, 8.0) | 45.8 | |
| | | 3.21 m | | | 3.26 br t (9.7) | | |
| | C═O | | 171.5 | | | 170.0 | |
| Gly[6] | α | 4.06 dd (17.0, 4.2) | 41.4 | C═O, C═OPro[5] | 4.06 br d (17.0) | 41.5 | C═O, C═OPro[5] |
| | | 3.30 dd (17.0, 3.6) | | | 3.37 br d (17.0) | | |
| | NH | 8.57 br d (8.0) | | C═O, Cα | 8.60 br d (8.0) | | C═OPro[5], Cα |
| | C═O | | 170.6 | | | 168.0 | |

[a] *J* values are in parentheses and reported in Hz; chemical shifts are given in ppm.

sequence Leu[1]-Pro[2]-Leu[3]-Trp[4]-Pro[5]-Gly[6]-OH for the acyclic form of **1**. In particular, the b ion series was almost complete, and its fragment ions were very intense compared to the y series. This suggests a preferred localization of the positive charge on the N-terminal of the linear peptide. Other product ions were detected at *m*/*z* 397.219 and 300.166, corresponding to the internal fragments Leu-Trp-Pro and Leu-Trp, respectively. The expected immonium ions for the individual amino acids were also observed. The geometries of the peptide linkage at the Pro residues were distinguished by analysis of $^{13}C$ NMR data and of the pattern of dipolar coupling in the ROESY spectra. The *cis*/*trans* conformation of proline could be determined on the basis of $\Delta\delta_{\beta\gamma}$ differential values of $^{13}C$ NMR chemical shifts of $C_\beta$ and $C_\gamma$ in proline.[11,12] The NMR data of **1** indicated a *cis* geometry for both Leu[1]−Pro[2] and Trp[4]−Pro[5] peptide bonds. The differential values of the two proline moieties, $\Delta\delta_{C\beta-C\gamma} = 8.2$ and 9.4, respectively, and ROESY correlations between $H\alpha_{Leu1}$/H-$\alpha_{Pro2}$ and $H\alpha_{Trp4}$/$H\alpha_{Pro5}$ were consistent with *cis* geometry for

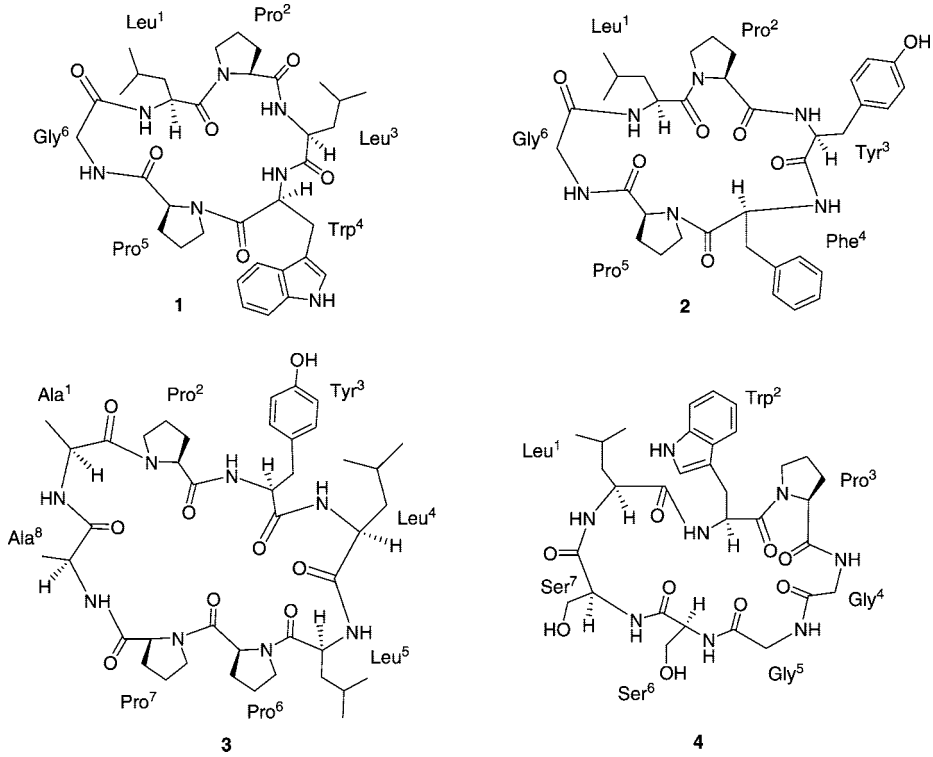


**Figure 1.** Structures of gypsins A−D (**1**−**4**).

both Leu[1]−Pro[2] and Trp[4]−Pro[5] peptide bonds. On the basis of the above data, the structure of gypsin A (**1**) was determined as *cyclo*(-Leu[1]-Pro[2]-Leu[3]-Trp[4]-Pro[5]-Gly[6]-).

Compound **2** showed an $[M + H]^+$ ion peak at *m*/*z* 675.3432 in the HRESIMS spectrum, consistent with the molecular formula $C_{36}H_{46}N_6O_7$ (calcd 674.3428), requiring 17 degrees of unsaturation. The hexapeptide skeleton was suggested from the presence of six amide carbonyl carbons in the $^{13}C$ NMR spectrum (Table 1) between $\delta$ 167.8 and 170.5. In the $^1H$ NMR data of **2** (Table 1), four amide NH protons were observed. Chemical shifts for all of the individual protons of the six amino acid residues of **2** were ascertained from a combination of COSY and 1D TOCSY NMR analyses. The $^{13}C$ NMR chemical shifts of their attached carbons were unambiguously assigned from the HSQC spectrum. The amino acid units were identified as Phe, Tyr, Leu, Gly, and two Pro. These residues accounted for 16 of the 17 degrees of unsaturation, requiring that **2** is a cyclopeptide. Compound **2** was subjected to hydrolysis and derivatization and analyzed by chiral GC, revealing the presence of L-Tyr, L-Phe, L-Leu, and L-Pro. The peptide sequence determination was based on HMBC and ROESY data. In compound **2**, two diagnostic ROESY cross-peaks could be observed, namely, $H_2\alpha$-Gly[6]/NH-Leu[1] and H$\alpha$-Tyr[3]/NH-Phe[4]. In the HMBC spectrum (Table 1) key cross-peaks were observed between $NH_{leu1}$ and $CO_{gly6}$, $C\alpha_{gly6}$, and $C\alpha_{leu1}$; $NH_{gly6}$ and $C\alpha_{gly6}$; $NH_{phe4}$ and $CO_{phe4}$, $CO_{tyr3}$, and $C\alpha_{tyr3}$; $NH_{tyr3}$ and $CO_{tyr3}$, $C\alpha_{tyr3}$, $C\beta_{tyr3}$, and $CO_{pro2}$; and $H\beta_{pro2}$ and $CO_{pro2}$, providing the sequence for the fragments Pro[2]-Tyr[3]-Phe[4] and Gly[6]-Leu[1]. As for **1**, the complete sequence of compound **2** was verified by ESIMS/MS analysis of the acyclic peptide resulting from partial hydrolysis. LC-MS/MS analysis of the product of partial hydrolysis of **2** showed two main peaks corresponding to the acyclic and the cyclic peptide. Fragment ions of the molecular ion of the linear peptide (*m*/*z* 693.351) belonged to the b and y series, resulting in the Leu[1]-Pro[2]-Tyr[3]-Phe[4]-Pro[5]-Gly[6]-OH sequence shown (Figure 2b). The geometry of the proline amide bonds in Pro[2] and Pro[5] were *cis*, as deduced from the $^{13}C$ NMR chemical shifts of the $\beta$- and $\gamma$- positions in the two Pro residues (Pro[2]: $\delta_{C\beta}$ 31.1 and $\delta_{C\gamma}$ 21.0; Pro[5]: $\delta_{C\beta}$ 29.9 and $\delta_{C\gamma}$ 21.3) and by ROESY correlations between $H\alpha_{phe4}$/$H\alpha_{pro5}$. Therefore, the structure of gypsin B (**2**) was determined to be *cyclo*(-Leu[1]-Pro[1]-Tyr[3]-Phe[4]-Pro[5]-Gly[6]-).

Compound **3** showed an $[M + H]^+$ ion peak at *m*/*z* 823.4755 in the HRESIMS spectrum, indicating the molecular formula $C_{42}H_{62}N_8O_9$ (calcd 822.4640). The octapeptide skeleton was suggested from the presence of eight carbonyl carbons in the $^{13}C$ NMR spectrum (Table 2) between $\delta$ 170.0 and 173.3. The greater number of methylene carbons (12) and unsaturation number (16) suggested additional proline units compared to **1** and **2**. Eight amide carbonyls, one aromatic ring, and three proline moieties accounted for 15 degrees of unsaturation, leaving one for the cyclic structure of **3**. Inspection of 1D and 2D NMR data permitted assignments of some substructures in compound **3**. HMBC correlations were observed between $NH_{ala1}$ and $CO_{ala8}$ and $C\alpha_{ala8}$; $NH_{ala8}$ and $C\alpha_{ala8}$ and $CO_{pro7}$; $H\alpha_{ala8}$ and $CO_{ala8}$, showing the sequence Pro[7]-Ala[8]-Ala[1]; and between $NH_{tyr3}$ and $CO_{tyr3}$; $H\alpha_{tyr3}$ and $CO_{tyr3}$ and $C\beta_{tyr3}$; $C1_{tyr3}$ and $NH_{leu4}$, $CO_{leu4}$, and $C\alpha_{leu4}$; $H\alpha_{leu4}$ and $CO_{leu4}$, $CO_{tyr3}$, and $C\beta_{leu4}$; $NH_{leu5}$ and $CO_{leu5}$ and $CO_{leu4}$; $H\alpha_{leu5}$ and $CO_{leu5}$, $C\beta_{leu5}$, and $C\gamma_{leu5}$, showing the sequence Tyr[3]-Leu[4]-Leu[5] (Table 2). Two proline moieties were also recognized. The Tyr[3] aromatic system was elucidated on the basis of both COSY and HMBC correlations. An HMBC correlation between the amide proton of Tyr ($\delta$ 8.03) and the carbonyl carbon of Pro[2] ($\delta$ 170.0) connected Pro[2] and Tyr[3], allowing the formation of the larger partial structure Pro[7]-Ala[8]-Ala[1]-Pro[2]-Tyr[3]-Leu[4]-Leu[5]. 1D TOCSY, COSY, and HMBC correlations led to the construction of the Pro[6] and Pro[7] units; however these lacked HMBC correlations to the carbonyl carbons of neighboring residues. Nevertheless, the sequence of **3** must be as shown in order to complete its cyclic structure. This sequence was supported by ESIMS/MS. As before, immonium ions were detected at *m*/*z* 44.055, 70.061, 86.089, and 136.074 corresponding to Ala, Pro, Leu, and Tyr residues, respectively. The complete sequence of **3** was also obtained by LC-MS/MS analysis of the acyclic peptide that resulted from partial hydrolysis (*m*/*z* 841.486). The product ion spectrum generated (Figure 2c) revealed ions of the b and y fragment series, indicating Ala[1]-Pro[2]-Tyr[3]-Leu[4]-Leu[5]-Pro[6]-Pro[7]-Ala[8]-OH. Analysis of **3** by chiral GC indicated all residues to possess the L-configuration. Differential values of $\Delta\delta_{C\beta-C\gamma}$ were

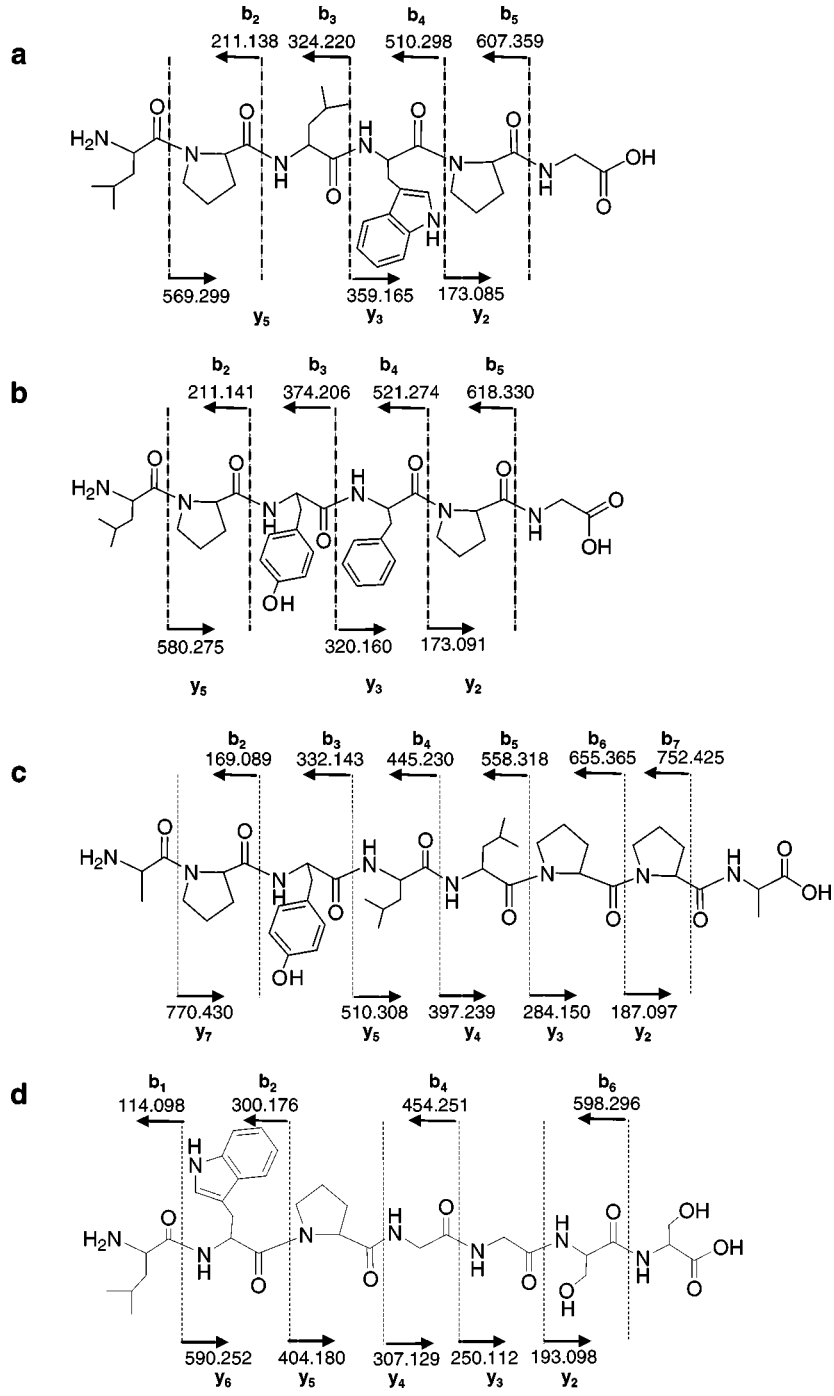


**Figure 2.** ESIMS fragment analysis of the acyclic forms of gypsins A (a), B (b), C (c), and D (d).

calculated for Pro[7] (8.7 ppm), indicating a *cis* geometry. The *cis*/*trans* geometry of Pro[2] and Pro[6] could not be characterized due to the absence of informative ROEs and diagnostic $\Delta\delta_{C\beta-C\gamma}$ of these units. The structure of **3**, gypsin C, was thus assigned as *cyclo*(-Ala[1]-Pro[2]-Tyr[3]-Leu[4]-Leu[5]-Pro[6]-Pro[7]-Ala[8]-).

The HRESIMS of compound **4** ($C_{32}H_{44}N_8O_9$) showed a $[M + H]^+$ ion peak at *m*/*z* 685.3334 (calcd 684.3231). The $^1H$ and $^{13}C$ NMR spectra (Table 3) contained resonances that were characteristic of a peptide. GC amino acids analysis of **4** showed the presence of two Ser, two Gly, one Leu, one Pro, and one Trp all in the L-configuration. Taken together these data require **4** to be a cyclic peptide. Analysis of 1D TOCSY and COSY spectra revealed the proton connectivity within each amino acid resonance, and the corresponding carbon resonances were elucidated on the basis of the HSQC spectrum. The configuration of Pro[3] was defined by analysis of the chemical shift of C$\beta$ and C$\gamma$ carbons at $\delta$ 31.0 and 21.5, respectively, in agreement with a *cis* proline. The gross structure including the sequence of the amino acids was assembled on the basis of HMBC correlations. HMBC correlations between each carbonyl carbon and neighboring amide NH and/or α-protons indicated the partial sequence Gly[4]-Gly[5]-Ser[6]-Ser[7]-Leu[1]-Trp[2]. Key correlations were observed between $NH_{leu1}$ and $CO_{ser7}$ and $CO_{leu1}$; $H\alpha_{leu1}$ and $CO_{ser7}$; $NH_{trp2}$ and $C\alpha_{leu1}$ and $CO_{trp2}$; $NH_{gly4}$ and $C\alpha_{gly4}$ and $CO_{gly4}$; $H\alpha_{gly4}$ and $CO_{gly4}$; $NH_{gly5}$ and $C\alpha_{gly5}$ and $C\alpha_{gly4}$; $H\alpha_{gly5}$ and $CO_{gly4}$ and $CO_{gly5}$; $NH_{ser6}$ and $CO_{ser6}$ and $C\beta_{ser6}$; $H\beta_{ser6}$ and $CO_{gly5}$ and $CO_{ser6}$; $NH_{ser7}$ and $CO_{ser6}$ and $CO_{ser7}$ (Table 3). The Pro[3] residue gave no diagnostic HMBC correlations. Compound **4** was subjected to partial acid hydrolysis, followed by LC-MS/MS analysis of the reaction products, in order to elucidate its amino acid sequence. The resulting chromatogram consisted of three main

**Table 2.** $^1$H and $^{13}$C NMR Data Compound **3** (DMSO-$d_6$, 600 MHz)[a]

| | position | $\delta_H$ | $\delta_C$ | HMBC |
|---|---|---|---|---|
| Ala[1] | α | 4.34 dq (7.0, 6.5) | 48.7 | C=O, C*β* |
| | *β* | 1.37 d (6.5) | 18.9 | C=O |
| | NH | 7.92 d (6.8) | | C=O, C=OAla[8], CαAla[8] |
| | C=O | | 173.1 | |
| Pro[2] | α | 4.05 t (9.0) | 63.0 | C=O, C*β*, C*δ* |
| | *β* | 1.90 m | 28.0 | C*δ* |
| | | 0.60 m | | |
| | *γ* | 1.61 m | 25.9 | |
| | | 1.53 m | | |
| | *δ* | 3.70 br t (8.6) | 48.1 | Cα, C*β* |
| | | 3.45 m | | |
| | C=O | | 170.0 | |
| Tyr[3] | α | 4.46 br dd (9.5, 5.5) | 56.4 | C=O, C*β*, C1 |
| | *β* | 3.01 m | 36.2 | C1, C2 |
| | | 2.82 m | | C1, C2 |
| | 1 | | 128.0 | |
| | 2/6 | 7.03 d (8.0) | 129.0 | C1, C4 |
| | 3/5 | 6.69 d (8.0) | 115.9 | C1, C4 |
| | 6 | | 156.2 | |
| | NH | 8.03 d (9.0) | | C=O, *C*=OPro[2] |
| | C=O | | 170.2 | |
| Leu[4] | α | 4.00 dt (9.2, 5.0) | 54.0 | C=O, C=OTyr3, C*β* |
| | *β* | 1.50 m | 41.1 | C*γ*, C*δ* |
| | | 1.38 m | | |
| | *γ* | 1.65 m | 24.7 | Cα, C*δ* |
| | Me*δ* | 0.86 d (6.6) | 23.6 | C*β* |
| | | 0.83 d (6.6) | 22.4 | C*β* |
| | NH | 7.96 d (7.5) | | C=O, Cα |
| | C=O | | 172.4 | |
| Leu[5] | α | 4.33 ddd (12.0, 9.2, 4.0) | 52.0 | C=O, C*β*, C*γ* |
| | *β* | 1.59 m | 40.2 | C=O, C*δ* |
| | | 1.43 m | | C=O, C*δ* |
| | *γ* | 1.49 m | 25.0 | |
| | Me*δ* | 0.87 d (6.0) | 23.6 | C*β* |
| | | 0.81 d (6.0) | 21.6 | C*β* |
| | NH | 7.80 d (9.2) | | C=O, C=OLeu[4] |
| | C=O | | 172.0 | |
| Pro[6b] | α | 4.11 t (8.0) | 60.3 | C=O, C*β*, C*δ* |
| | *β* | 1.90 br dd (11.9, 8.0) | 29.0 | |
| | | 1.33 m | | |
| | *γ* | 1.68 m | 26.0 | |
| | | 1.60 m | | |
| | *δ* | 3.60 ddd (11.9, 10.0, 8.0) | 47.2 | C*β* |
| | | 3.56 br t (9.9) | | |
| | C=O | | 171.0 | |
| Pro[7b] | α | 4.40 br d (8.0) | 62.0 | C=O, C*γ*, C*δ* |
| | *β* | 2.07 br dd (12.0, 8.0) | 30.5 | C=O, C*δ* |
| | | 1.56 m | | |
| | *γ* | 1.35 m | 21.8 | |
| | | 0.74 m | | |
| | *δ* | 3.52 ddd (11.9, 10.0, 8.0) | 47.2 | Cα |
| | | 3.44 m | | |
| | C=O | | 171.5 | |
| Ala[8] | α | 3.90 dq (7.0, 6.5) | 50.9 | C=O, C=OPro[7] |
| | *β* | 1.25 d (6.5) | 19.7 | C=O |
| | NH | 7.97 d (7.0) | | C=OPro[7], Cα |
| | C=O | | 173.3 | |

[a] *J* values are in parentheses and reported in Hz; chemical shifts are given in ppm. [b] Assignments may be interchangeable.

peaks: two of these (retention times 14.6 and 16.2 min, respectively) showed the same *m/z* value (703.332), consistent with that expected for the acyclic peptide (703.341), whereas the third one was the unreacted cyclic peptide. The presence of two different species having the same *m/z* value suggested that partial hydrolysis of **4** generated two linear peptides, resulting from two ring-opening sites. Nevertheless, analyses of the MS/MS spectra generated by the fragmentation of the two isomeric ions demonstrated that the differences between the two peptides involve their entire sequence. This result clearly indicated that two peptides showing the same amino acidic composition but a different primary structure were present. In their cyclic form they coeluted in $C_{18}$ HPLC, but it was possible to describe the amino acid sequence of both these compounds by the LC-MS/MS analysis of the linear products. The apparent molecular ratio between the two species following the hydrolysis was about 1:3, but a different efficiency of the reaction for the two peptides cannot be excluded. CID fragmentation of the pseudomolecular ion (*m/z* 703.332) of the peptide eluted at 14.6 min (**4**) generated a spectrum mainly composed of the b and y series of ions shown in Figure 2d. Analysis of this spectrum confirmed the Leu[1]-Trp[2]-Pro[3]-Gly[4]-Gly[5]-Ser[6]-Ser[7]-OH sequence for compound **4**. In contrast to peptides **1**−**3**, the tandem MS of **4** showed an almost complete y series and some less intense b ions, demonstrating that in this case the positive charge was more stabilized on the C-terminus of the linear peptide. Internal ions were observed at *m/z* 299.140, 212.110, and 155.085, corresponding to the fragments Pro[3]-Gly[4]-Gly[5]-Ser[6], Pro[3]-Gly[4]-Gly[5], and Pro[3]-Gly[4], respectively. Thus, gypsin D (**4**) was defined as *cyclo*(-Leu[1]-Trp[2]-Pro[3]-Gly[4]-Gly[5]-Ser[6]-Ser[7]-).

**Table 3.** $^1$H and $^{13}$C NMR Data Compound **4** (DMSO-$d_6$, 600 MHz)[a]

| | position | $\delta_H$ | $\delta_C$ | HMBC |
|---|---|---|---|---|
| Leu[1] | α | 4.51 ddd (11.7, 8.5, 4.2) | 50.5 | COSer[7], C*β*, C*γ* |
| | *β* | 1.63 m | 41.0 | |
| | | 1.43 m | | |
| | *γ* | 1.52 m | 24.7 | |
| | Me*δ* | 0.83 d (6.5) | 23.1 | C*β*, C*γ* |
| | | 0.86 d (6.5) | 22.7 | C*β*, C*γ* |
| | NH | 7.74 d (8.0) | | C=O, C=OSer[7] |
| | C=O | | 172.0 | |
| Trp[2] | α | 4.26 ddd (12.5, 7.5, 4.0) | 54.0 | C=O, C2 |
| | *β* | 3.18 dd (14.0, 4.0) | 26.8 | C=O, C2, C3 |
| | | 3.01 dd (14.0, 12.5) | | |
| | 1 (NH) | 10.5 d (1.8) | | |
| | 2 | 7.35 d (1.8) | 124.0 | C3, C7a |
| | 3 | | 109.8 | |
| | 3a | | 127.6 | |
| | 4 | 7.37 d (7.8) | 111.0 | |
| | 5 | 6.95 t (7.8) | 118.0 | C3a, C4, C6 |
| | 6 | 7.05 t (7.8) | 121.2 | C4, C5 |
| | 7 | 7.35 d (7.8) | 118.3 | C3a, C5 |
| | 7a | | 138.0 | |
| | NH | 8.70 d (7.6) | | C=O, CαLeu[1], Cα |
| | C=O | | 171.2 | |
| Pro[3] | α | 4.22 br d (8.0) | 61.0 | C=O, C*β*, C*δ* |
| | *β* | 2.18 br dd (12.9, 6.6) | 31.0 | C=O, Cα, C*δ* |
| | | 1.89 m | | |
| | *γ* | 1.60 m | 21.5 | |
| | | 1.48 m | | |
| | *δ* | 3.67 ddd (11.5, 10.2, 8.0) | 47.0 | Cα, C*β* |
| | | 3.44 br d (10.0) | | |
| | C=O | | 171.3 | |
| Gly[4] | α | 4.28 dd (17.0, 4.0) | 43.0 | C=O |
| | | 3.51 dd (17.0, 3.5) | | |
| | NH | 8.50 br d (8.0) | | C=O, Cα |
| | C=O | | 171.2 | |
| Gly[5] | α | 4.34 dd (16.5, 4.0) | 42.8 | C=O, C=OGly[4] |
| | | 3.92 dd (16.5, 3.0) | | |
| | NH | 8.53 br d (8.0) | | Cα, CαGly[4] |
| | C=O | | 172.0 | |
| Ser[6] | α | 4.23 m | 55.2 | C=O, C*β* |
| | *β* | 3.74 dd (11.0, 6.0) | 61.0 | C=O, C=OGly[5] |
| | | 3.60 dd (11.0, 5.5) | | |
| | NH | 8.03 d (8.1) | | C=O, C*β* |
| | C=O | | 171.0 | |
| Ser[7] | α | 4.05 m | 56.3 | C=O, C*β* |
| | *β* | 3.69 dd (11.0, 6.0) | 60.5 | |
| | | 3.52 dd (11.0, 5.5) | | |
| | NH | 8.05 d (8.0) | | C=O, C=OSer[6] |
| | C=O | | 171.3 | |

[a] *J* values are in parentheses and reported in Hz; chemical shifts are given in ppm.

The b and y series ion present in the tandem MS of the other component suggested a Leu[1]-Ser[2]-Ser[3]-Gly[4]-Pro[5]-Gly[6]-Trp[7]-OH sequence for this minor constituent; however insufficient evidence for connectivity by NMR was available to confirm this sequence.

The known oleanane saponin was identified as 23-*O*-*β*-D-glucopyranosyl gypsogenic acid 28-*O*-*β*-D-glucopyranosyl(1→2)-*β*-D-glucopyranosyl-(1→6)-[*β*-D-glucopyranosyl-(1→3)]-*β*-D-glu-

copyranoside, or vaccaroside C, by detailed NMR and MS analyses and comparison with literature data.[13]

Close to 100 cyclopeptides have been isolated from species of Caryophyllaceae composed almost exclusively of amino acid residues.[4] Like other members of this family,[14,15] gypsins A−D are all cyclic homodetic peptides. A comparison of gypsins A−D with cyclopeptides isolated from other Caryophyllaceae spp. showed that gypsin B (**2**) is an isomer of drymarin B isolated from *Drymaria diandra*, differing only in the amino acid sequence.[16] Moreover, many other compounds having both proline residues and aromatic amino acids have been described, but interesting biological activities were reported in only a few cases.[17–22] Cyclopeptides have been reported in the three subfamilies of Caryophyllaceae (Paronychioideae, Alsinoideae, Caryophylloideae). Thus, cyclopeptides, as characteristic components of Caryophyllaceae spp., may be useful as taxonomic markers in this family. This is the first report of cyclopeptides in the genus *Gypsophila*.

## Experimental Section

**General Experimental Procedures.** Optical rotations were measured on a Perkin-Elmer 241 polarimeter equipped with a sodium lamp (589 nm) and a 1 dm microcell. NMR experiments were performed on a Bruker DRX-600 spectrometer at 300 K. All 2D NMR spectra were acquired in DMSO-$d_6$ in the phase-sensitive mode with the transmitter set at the solvent resonance and TPPI (time proportional phase increment) used to achieve frequency discrimination in the $\omega_1$ dimension. The standard pulse sequence and phase cycling were used for DQF-COSY, TOCSY, HSQC, HMBC, and ROESY experiments. The NMR data were processed on a Silicon Graphic Indigo2 workstation using UXNMR software. HRMS were achieved using a nanoLC-MS/MS system, with a nanoAcquity UPLC module and a Q-TOF premiere spectrometer equipped with a nanoelectrospray ion source (Waters-Milford, MA), and provided by a lock-mass apparatus to perform a real-time calibration correction. Chromatographic separations of cyclic and partially hydrolyzed peptides were performed on a capillary BEH $C_{18}$ column (0.075 × 100 mm, 1.7 $\mu$m Waters) using aqueous 1% formic acid (A) and $CH_3CN$ containing 1% formic acid (B) as mobile phases. Peptides were eluted by means of linear gradient from 5% to 50% of B in 25 min and a 300 nL/min flow rate. Capillary ion source voltage was set at 2.5 kV, cone voltage at 35 V, and extractor voltage at 3 V. Peptide fragmentation was achieved using argon as collision gas and a collision cell energy of 25 eV. Mass spectra were acquired in a *m/z* range from 300 to 1500, and MS/MS spectra in a 25 to 900 range. Mass calibration was performed using a mixture of angiotensin and insulin as external standard and [Glu]-fibrinopeptide B human as lock mass standard. Column chromatography was performed over Sephadex LH-20 and Si gel. HPLC separations were conducted on a Shimadzu LC-8A series pumping system equipped with a Waters R401 refractive index detector and Shimadzu injector on a $C_{18}$ $\mu$-Bondapak column (30 cm × 7.8 mm, 10 $\mu$m Waters, flow rate 2.0 mL min$^{-1}$). GC analyses were performed using a Dani GC 1000 instrument on a L-CP-Chirasil-Val column (0.32 mm × 25 m).

**Plant Material.** The roots of *G. arabica* were collected during blooming season in Al-Jubaiha, Amman, Jordan, on July 2003 and were identified by Prof. Barakat Abu-Irmaileh, Department of Plant Protection, Faculty of Agriculture, University of Jordan, Amman, Jordan. A voucher specimen (No. 2497/1) is deposited at the Herbarium Horti Botanici Pisani, Pisa, Italy.

**Extraction and Isolation.** The dried, powdered roots of *G. arabica* (275 g) were defatted with *n*-hexane and successively extracted for 48 h with $CHCl_3$, $CHCl_3$−MeOH (9:1), and MeOH, by exhaustive maceration (3 × 2 L), to give 0.95, 2.1, and 24.4 g of the respective residues. The $CHCl_3$−MeOH extract was chromatographed over Sephadex LH-20 (3 × 30 cm, flow rate of 0.8 mL/min), collecting fractions of 10 mL that were grouped into 10 major fractions (1−10). Fraction 3 (40 mg) was purified over Si gel (1 × 20 cm) with $CHCl_3$−MeOH (4:1), collecting fractions of 3 mL, followed by RP-HPLC over $C_{18}$ $\mu$-Bondapak (30 cm × 7.8 mm, flow rate 2.0 mL min$^{-1}$) with MeOH−$H_2O$ (7:3) as the eluent to yield compound **3** (1.5 mg, $t_R$ = 10 min). Fractions 5 (150 mg) and 7 (113 mg) were separately subjected to Si gel chromatography (2 × 20 cm), eluting with $CHCl_3$ followed by increasing concentrations of MeOH (between 1% and 100%) in $CHCl_3$. Fractions of 7 mL were collected and grouped into five groups (1−5). Group 2 coming from fraction 5 (30 mg) was purified by RP-HPLC over $C_{18}$ $\mu$-Bondapak (30 cm × 7.8 mm, flow rate 2.0 mL min$^{-1}$) with MeOH−$H_2O$ (7:3) as eluent to give compounds **2** (5.0 mg, $t_R$ = 9 min) and **1** (5.8 mg, $t_R$ = 12 min). Group 4 coming from fraction 7 was subjected to RP-HPLC over $C_{18}$ $\mu$-Bondapak (30 cm × 7.8 mm, flow rate 2.0 mL min$^{-1}$) with MeOH−$H_2O$ (55:45) as eluent to give compound **4** (2.5 mg, $t_R$ = 14 min) as a mixture with another cyclopeptide in minor concentration. The MeOH extract was partitioned between *n*-BuOH and $H_2O$ to afford an *n*-BuOH (3.3 g) that was submitted to Sephadex LH-20 (3 × 30 cm, flow rate of 0.8 mL/min) using MeOH as eluent, collecting fractions of 8 mL that were grouped into five major fractions (1−5) by TLC on Si 60 $F_{254}$ gel-coated glass sheets with *n*-BuOH−HOAc−$H_2O$ (60:15:25) and $CHCl_3$−MeOH−$H_2O$ (40:9:1). Fraction 3 (316 mg) was purified by preliminary SPE followed by RP-HPLC over $C_{18}$ $\mu$-Bondapak (30 cm × 7.8 mm, flow rate 2.0 mL min$^{-1}$) with MeOH−$H_2O$ (3:2) to afford pure vaccaroside C (2.0 mg, $t_R$ = 45 min).

**Gypsin A (1):** amorphous powder; $[\alpha]^{25}_D$ −11 (*c* 0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 245 (4.12), 281 (3.94), 290 (3.05) nm; $^1$H and $^{13}$C NMR, see Table 1; HRESIMS *m/z* 664.3723 $[M + H]^+$ (calcd for $C_{35}H_{49}N_7O_6$, 663.3744); ESIMS/MS (parent ion 664.372) *m/z* 636.432 $[M - CO + H]^+$, 567.373 $[M - Pro + H]^+$, 551.333 $[M - Leu + H]^+$, 549.324 $[M - H_2O - Pro + H]^+$, 533.355 $[M - H_2O - Leu + H]^+$, 478.331 $[M - Trp + H]^+$, 454.285 $[M - (Leu - Pro) + H]^+$, 397.253 $[M - (Leu\text{-}Pro\text{-}Gly) + H]^+$, 365.245 $[M - (Trp\text{-}Leu) + H]^+$, 300.197 $[M - (Leu\text{-}Pro\text{-}Gly) + H]^+$, 268.194 $[M - (Trp\text{-}Leu\text{-}Pro) + H]^+$, 159.085 (tryptophan immonium ion), 86.100 (leucine immonium ion), 70.067 (proline immonium ion), 30.035 (glycine immonium ion).

**Gypsin B (2):** amorphous powder; $[\alpha]^{25}_D$ −10 (*c* 0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 280 (3.89); $^1$H and $^{13}$C NMR, see Table 1; HRESIMS *m/z* 675.3432 $[M + H]^+$ (calcd for $C_{36}H_{46}N_6O_7$, 674.3428); ESIMS/MS (parent ion 675.343) *m/z* 647.333 $[M - CO + H]^+$, 578.345 $[M - Pro + H]^+$, 562.302 $[M - Leu + H]^+$, 560.305 $[M - H_2O - Pro + H]^+$, 544.322 $[M - H_2O - Leu + H]^+$, 528.317 $[M - Phe + H]^+$, 521.263 $[M - (Pro\text{-}Gly) + H]^+$, 465.245 $[M - (Leu\text{-}Pro) + H]^+$, 408.223 $[M - (Leu\text{-}Pro\text{-}Gly) + H]^+$, 365.248 $[M - (Phe\text{-}Tyr) + H]^+$, 261.145 $[M - (Leu\text{-}Pro\text{-}Gly\text{-}Phe) + H]^+$, 136.072 (tyrosine immonium ion), 120.077 (phenylalanine immonium ion), 86.101 (leucine immonium ion), 70.069 (proline immonium ion), 30.033 (glycine immonium ion).

**Gypsin C (3):** amorphous powder; $[\alpha]^{25}_D$ −27 (*c* 0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 235 sh (3.85), 282 (4.02); $^1$H and $^{13}$C NMR, see Table 2; HRESIMS *m/z* 823.4755 $[M + H]^+$ (calcd for $C_{42}H_{62}N_8O_9$, 822.4640); ESIMS/MS (parent ion 823.475) *m/z* 795.438 $[M - CO + H]^+$, 752.441 $[M - Ala + H]^+$, 726.425 $[M - Pro + H]^+$, 710.353 $[M - Leu + H]^+$, 692.364 $[M - H_2O - Leu + H]^+$, 660.334 $[M - Tyr + H]^+$, 655.265 $[M - (Pro\text{-}Ala) + H]^+$, 642.313 $[M - H_2O - Tyr + H]^+$, 629.297 $[M - Pro + H]^+$, 597.283 $[M - Leu + H]^+$, 558.302 $[M - (Pro\text{-}Ala) + H]^+$, 500.231 $[M - (Pro\text{-}Ala\text{-}Leu) + H]^+$, 445.225 $[M - (Pro_2\text{-}Ala\text{-}Leu) + H]^+$, 332.144 $[M - (Pro_2\text{-}Ala\text{-}Leu_2) + H]^+$, 266.142 $[M - (Leu\text{-}Pro\text{-}Tyr\text{-}Ala) + H]^+$, 136.074 (tyrosine immonium ion), 86.089 (leucine immonium ion), 70.061 (proline immonium ion), 44.055 (alanine immonium ion).

**Gypsin D (4):** amorphous powder; $[\alpha]^{25}_D$ −18 (*c* 0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 272 sh (3.55), 281 (4.22), 290 (3.96); $^1$H and $^{13}$C NMR, see Table 3; HRESIMS *m/z* 685.3334 $[M + H]^+$ (calcd for $C_{32}H_{44}N_8O_9$, 684.3231); ESIMS/MS (parent ion 685.333) *m/z* 657.315 $[M - CO + H]^+$, 588.286 $[M - Pro + H]^+$, 572.263 $[M - Leu + H]^+$, 570.265 $[M - H_2O - Pro + H]^+$, 554.256 $[M - H_2O - Leu + H]^+$, 531.266 $[M - (Pro\text{-}Gly) + H]^+$, 499.252 $[M - Trp + H]^+$, 483.283 $[M - H_2O - (Leu\text{-}Ser) + H]^+$, 481.163 $[M - H_2O - Trp + H]^+$, 474.265 $[M - (Pro\text{-}Gly) + H]^+$, 386.174 $[M - (Trp\text{-}Leu) + H]^+$, 368.162 $[M - H_2O - (Trp\text{-}Leu) + H]^+$, 299.144 $[M - (Trp\text{-}Leu\text{-}Ser) + H]^+$, 211.137 $[M - (Trp\text{-}Leu\text{-}Ser) + H]^+$, 159.077 (tryptophan immonium ion), 86.097 (leucine immonium ion), 70.058 (proline immonium ion), 60.039 (serine immoniu ion), 30.037 (glycine immonium ion).

**Vaccaroside C:** amorphous powder; ESIMS *m/z* 1319 $[M + Na]^+$, 1157 $[M + Na - 162]^+$, 995 $[M + Na - 162 - 162]^+$, 833 $[M + Na - 162 - 162 - 162]^+$, 671 $[M + Na - 162 - 162 - 162 - 162]^+$.

**Partial Hydrolysis.** Compounds **1**−**4** were subjected to partial hydrolysis to obtain linear peptides: approximately 200 pmol of each molecule was incubated with 100 $\mu$L of 1.2 M HCl at 90 °C for 1 h.

The hydrolysis was stopped by diluting with 3 volumes of cold $H_2O$, and the peptide mixture was analyzed by nanoLC-MS/MS.

**Absolute Configuration of Amino Acids.** A solution of each compound (**1**−**4**, 2.0 mg) in 6 N HCl (1 mL) was heated at 110 °C in a stoppered reaction vial for 24 h. After cooling, the solution was evaporated under a stream of $N_2$. The hydrolysate was dissolved in an anhydrous solution of 3 N HCl in 2-propanol and heated at 110 °C for 30 min. After drying the solution, the residue was dissolved in $CH_2Cl_2$ (0.5 mL), and 0.5 mL trifluoracetic anhydride was added. The mixture was kept in a screw-capped vial at 110 °C for 20 min, the reagents were evaporated, and the mixture was analyzed on an L-CP-Chirasil-Val column (0.32 mm × 25 m). Temperatures of the injector and detector were 200 °C for both. A temperature gradient system was used for the oven, starting at 50−130 at 3 °C/min and 130−190 at 10 °C/min. Comparison of $t_R$ (min) values with those of standard amino acids (Sigma Aldrich) was used: D,L-Ala (10.6, 11.6), D,L-Leu (18.1, 19.2), D,L-Phe (27.1, 27.9), D,L-Pro (18.0, 18.2), D,L-Ser (17.8, 18.3), D,L-Trp (34.2, 36.4), D,L-Tyr (31.7, 31.9).

**Acknowledgment.** The authors are grateful to Prof. B. Abu-Irmaileh, Department of Plant Protection, Faculty of Agriculture, University of Jordan, Amman, Jordan, for collecting and identifying the plant material.

**Supporting Information Available:** This material is available free of charge via the Internet at http://pubs.acs.org.

## References and Notes

(1) Liu, Z.; Li, D.; Owen, N. L.; Grant, D. M.; Cates, R. G.; Jia, Z. *J. Nat. Prod.* **1995**, *58*, 1632–1635.
(2) Ding, Z.; Zhou, J.; Tan, N. *Planta Med.* **1999**, *65*, 578–579.
(3) Zhao, Y-R.; Zhou, J.; Wang, X-K.; Wu, H-M.; Huang, X-L.; Zou, C. *Phytochemistry* **1997**, *46*, 709–714.
(4) Tan, N-H.; Zhou, J. *Chem. Rev.* **2006**, *106*, 840–895.
(5) Braca, A.; Bader, A.; Siciliano, T.; De Tommasi, N. *Magn. Reson. Chem.* **2008**, *46*, 88–93.
(6) Zohary, M. *Flora Palaestina*, Part I; The Israel Academy of Sciences and Humanities: Jerusalem, 1966; p 100.
(7) Sathiyamoorthy, P.; Lugasi-Evgi, H.; Schlesinger, P.; Kedar, I.; Gopas, J.; Pollack, Y.; Golan-Goldhirsh, A. *Pharm. Biol.* **1999**, *37*, 188–195.
(8) Ngoka, L. C. M.; Gross, M. L. *J. Am. Chem. Soc. Mass Spectrom.* **1999**, *10*, 732–746.
(9) Ishikawa, K.; Niwa, Y.; Oishi, K.; Aoi, S.; Takeuchi, T.; Wakayama, S. *Biomed. Environ. Mass Spectrom.* **1990**, *19*, 395–399.
(10) Randazzo, A.; Dal Piaz, F.; Orrù, S.; Debitus, C.; Roussakis, C.; Pucci, P.; Gomez Paloma, L. *Eur. J. Org. Chem.* **1998**, *1*, 2659–2665.
(11) Siemon, I. Z.; Wieland, T.; Pook, K. H. *Angew. Chem., Int. Ed. Engl.* **1975**, *88*, 712–714.
(12) McDonald, L. A.; Foster, M. P.; Phillips, D. R.; Ireland, C. M. *J. Org. Chem.* **1992**, *57*, 4616–4624.
(13) Koike, K.; Jia, Z.; Nikaido, T. *Phytochemistry* **1998**, *47*, 1343–1349.
(14) Yun, Y. S.; Morita, H.; Takeya, K.; Itokawa, H. *J. Nat. Prod.* **1997**, *60*, 216–218.
(15) Wang, Y. C.; Tan, N. H.; Zhou, J.; Wu, H. M. *Phytochemistry* **1998**, *49*, 1453–1456.
(16) Ding, Z.; Zhou, J.; Tan, N.; Teng, R. *Planta Med.* **2000**, *66*, 386–388.
(17) Morita, H.; Kayashita, T.; Kobata, H.; Gonda, A.; Takeya, K.; Itokawa, H. *Tetrahedron* **1994**, *50*, 6797–6804.
(18) Auvin-Guette, C.; Baraguey, C.; Blond, A.; Xavier, H. S.; Pousset, J. L.; Bodo, B. *Tetrahedron* **1999**, *55*, 11495–11510.
(19) Hsieh, P. W.; Chang, F. R.; Wu, C. C.; Wu, K. W.; Li, C. M.; Wang, W. Y.; Gu, L. C.; Wu, Y. C. *Helv. Chim. Acta* **2004**, *87*, 57–66.
(20) Hsieh, P. W.; Chang, F. R.; Wu, C. C.; Wu, K. W.; Li, C. M.; Chen, S. L.; Wu, Y. C. *J. Nat. Prod.* **2004**, *67*, 1522–1527.
(21) Morita, H.; Kayashita, T.; Shimomura, M.; Takeya, K.; Itokawa, H. *J. Nat. Prod.* **1996**, *59*, 280–282.
(22) Mu, Q.; Tang, W. D.; Liu, R. W.; Li, C. M.; Lou, L. G.; Sun, H. D.; Hu, C. Q. *Planta Med.* **2003**, *69*, 826–830.

NP700735A