



Aliphatic pyrrolidine amides from two tropical convolvulaceous species[☆]

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Abstract

Seven aliphatic pyrrolidine amides with branched and linear saturated C₁₅–C₁₉ acyl moieties were detected in vegetative plant organs of *Ipomoea aquatica* and *Merremia quinquefolia* as well as in seeds of *M. quinquefolia* by GC–MS analysis. One of the compounds was isolated from both species and characterized as 1-(14-methylhexadecanoyl)pyrrolidine, a new natural product. The presence of 1-hexadecanoylpyrrolidine and 1-octadecanoylpyrrolidine was confirmed by comparison of their GC–MS data with those of synthesized compounds. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: *Ipomoea aquatica*; *Merremia quinquefolia*; Convolvulaceae; GC–MS; Amides; Alkamides; Pyrrolidine amides; 1-(14-Methylhexadecanoyl)pyrrolidine; 1-Hexadecanoylpyrrolidine; 1-Octadecanoylpyrrolidine

1. Introduction

The Convolvulaceae comprise nearly 2000 predominantly tropical species. A wide variety of low-molecular N-containing secondary metabolites has been found in this family over the years: ergolines (Hofmann & Tschertter, 1960), pyrrolidines (e. g. cuscohygrine) (Evans & Somanabandhu, 1974; Jenett-Siems & Eich, 1994), lipophilic tropanes (Orechhoff & Konowalowa, 1933; Weigl, Kaloga & Eich, 1992) and hydrophilic tropanes (calystegines) (Goldmann et al., 1990), indolizidine and pyrrolizidine alkaloids (Gourley, Heacock, McInnes, Nikolin & Smith, 1969;

Jenett-Siems, Kaloga & Eich, 1993) as well as cyanogenic glycosides (Nahrstedt, Jensen & Wray, 1989). Furthermore, different types of amides were isolated: *N*-feruloyltyramine (Tseng et al., 1986), serotonin-hydroxycinnamic acid conjugates of the ipobscurine-type (Eich, Henn, Kolshorn, Pertz & Schulz, 1989) and lignanamides (Henrici, Kaloga, & Eich, 1994). The present study reports on the detection and isolation of aliphatic pyrrolidine amides from *Ipomoea aquatica* FORSK., the leaves of which are used as a vegetable in Southeast Asia, and *Merremia quinquefolia* (L.) H. HALL. f., a pantropical twiner of American origin.

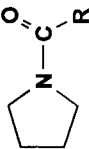
2. Results and discussion

The petrol ether and dichloromethane extracts of the roots and aerial vegetative parts (*I. aquatica*, *M. quinquefolia*) as well as of the seeds (*M. quinquefolia*) were analyzed by GC–MS. Two groups of compounds were characterized as pyrrolidine amides with saturated aliphatic C₁₅–C₁₉ acyl moieties by their mass

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Table 1
GC–MS analysis of the petrol ether and dichloromethane extracts of *Merremia quinquefolia* and *Ipomoea aquatica*

Compound	R =	 $\text{O}=\text{C}-\text{N}(\text{R})$	RI [M] ⁺ , characteristic ions (rel. int.)	<i>Merremia quinquefolia</i>			<i>Ipomoea aquatica</i>		
				aerial parts ^b	roots	seeds	aerial parts ^b	roots	
1, Alkaloid MQ-A ₁	C ₁₄ H ₂₉ branched		2395 295 (1), 126 (19), 113 (100), 98 (7), 70 (8), 55 (7), 43 (6)	+	–	–	–	–	–
2, Alkaloid MQ-A ₂	C ₁₅ H ₃₁ branched		2490 309 (1), 126 (16), 113 (100), 98 (7), 70 (8), 55 (7), 43 (8)	+	–	–	–	–	–
3, 1-Hexadecanoylpyrrolidine (alkaloid MQ-B ₂) ^a	C ₁₅ H ₃₁ linear		2530 309 (2), 126 (21), 113 (100), 98 (7), 70 (9), 55 (8), 43 (9)	+	–	–	+	–	+
4, 1-(14-Methylhexadecanoyl)pyrrolidine (alkaloid MQ-A ₃) ^a	C ₁₆ H ₃₃ branched		2605 323 (2), 126 (17), 113 (100), 98 (6), 70 (8), 55 (7), 43 (7)	+	+	+	+	–	+
5, Alkaloid MQ-A ₄	C ₁₇ H ₃₅ branched		2703 337 (1), 126 (16), 113 (100), 98 (6), 70 (10), 55 (10), 43 (16)	+	–	–	–	–	–
6, 1-Octadecanoylpyrrolidine (alkaloid MQ-B ₄) ^a	C ₁₇ H ₃₅ linear		2742 337 (2), 126 (17), 113 (100), 98 (6), 70 (7), 55 (6), 43 (7)	+	–	–	–	–	–
7, Alkaloid MQ-A ₅	C ₁₈ H ₃₇ branched		2815 351 (1), 126 (16), 113 (100), 98 (6), 70 (7), 55 (5), 43 (5)	+	+	–	+	–	+

^a Authentic reference compounds, obtained by isolation or synthesis; all other compounds were characterized by their mass fragments.

^b Aerial parts: stems and leaves.

fragmentation pattern (Table 1), both in *I. aquatica* and *M. quinquefolia*. The compounds classified as alkaloid MQ-A₁–alkaloid MQ-A₅ (**1**, **2**, **4**, **5**, **7**) showed regular differences of approximately 100 units in their retention indices (RI) and of 14 mass units in their molecular ions, indicating a set of homologous compounds. Additionally, two compounds named alkaloid MQ-B₂ and MQ-B₄ (**3**, **6**) were detected, which were, according to their [M]⁺ at *m/z* 309 and 337, isomeric to alkaloid MQ-A₂ (**2**) and alkaloid MQ-A₄ (**5**). Since **3** and **6** had higher retention indices than **2** and **5**, it could be postulated that **3** and **6** have acyl moieties, which are linear or at least less branched than those of **2** and **5**. It could be shown by synthesis and GC–MS analysis of 1-hexadecanoylpyrrolidine and 1-octadecanoylpyrrolidine that these synthetical compounds have retention indices identical with those of **3** and **6**; therefore the alkaloid MQ-B₂ and MQ-B₄ possess a linear acyl moiety.

The tentative identification of **1**, **2**, **4**, **5** and **7** as pyrrolidine amides linked with branched saturated fatty acids could be confirmed by isolation of **4** from the roots of *I. aquatica* and the epigeal vegetative parts of *M. quinquefolia*. Surprisingly, this compound showed a positive reaction with Dragendorff's reagent on TLC. Its structure was elucidated by EIMS, FABMS, HRMS, ¹H NMR, ¹H, ¹H COSY, ¹³C NMR, DEPT and HETCOR. The EIMS showed a molecular ion peak at *m/z* 323, which was confirmed by FABMS (positive mode). The molecular formula was determined as C₂₁H₄₁NO by HRMS. Additionally, the EIMS showed a peak at *m/z* 70, indicative of a pyrrolidine moiety, a base peak at *m/z* 113, caused by McLafferty rearrangement, as well as characteristic peaks at *m/z* 266 [M–C₄H₉], *m/z* 294 [M–C₂H₅] and *m/z* 308 [M–CH₃], indicating the presence of a branched alkyl chain, as depicted in Fig. 1. The ¹³C NMR and DEPT spectra revealed the presence of two methyl groups, one methine group, the carbonyl group of an amide, eight methylene groups and additionally a cluster of methylene groups, which could not be clearly distinguished. In the ¹H NMR and ¹H, ¹H COSY, two methylene groups of the pyrrolidine moiety at δ 1.88 and 1.98 (2 H each, m, 2 × H-3' and H-4') coupled with two methylene groups at δ 3.43 and 3.48 (2 H each, t, 2 × H-2' and H-5'). The differing shifts of H-2'/H-5' as well as H-3'/H-4' and of the corresponding carbons were probably caused by restricted rotation of the N–C bond of the amide moiety due to partial double-bond character, which led to *cis*- and *trans*-location of these protons. A similar phenomenon has been observed e.g. for *N*-formyl-2-methylpiperidine and its methyl group before (LaPlanche & Rogers, 1963). The appearance of one secondary methyl group at δ 0.86 (3 H, d, *J* = 6.5 Hz, H-17) and one primary methyl group at δ 0.88 (3 H, t, *J* = 7.3 Hz, H-16) as

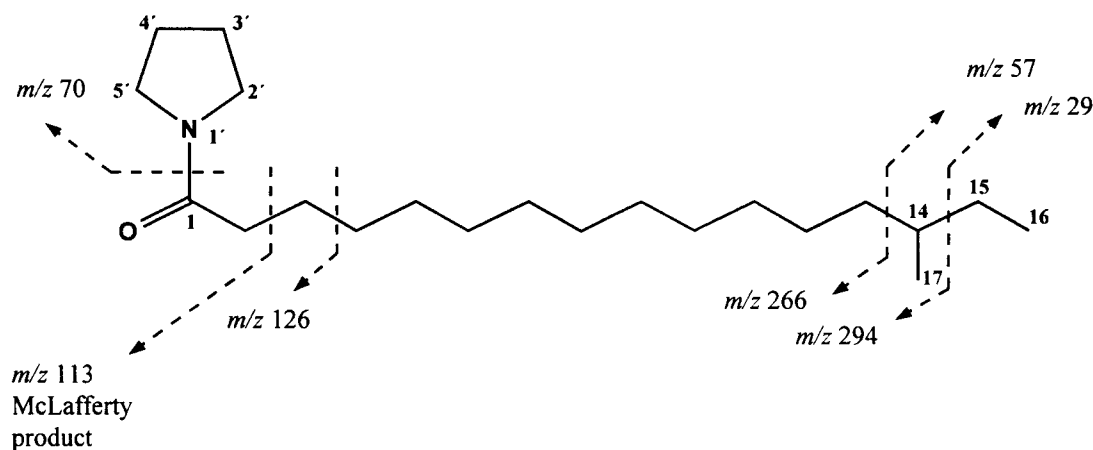


Fig. 1. Structure and EIMS fragmentation pattern of MQ-A₃ (4).

well as the corresponding ^{13}C NMR signals at δ 11.8 (C-16) and 19.6 (C-17) corroborated the branched structure of the acyl moiety. Therefore, **4** was characterized as 1-(14-methylhexadecanoyl)pyrrolidine, a new natural product.

To the best of our knowledge, no pyrrolidine amides with a branched saturated acyl moiety have been described as natural products before. Even linear 1-hexadecanoylpyrrolidine (**3**) and 1-octadecanoylpyrrolidine (**6**) have only been characterized by GC–MS analysis of *Piper amalago* L. (Piperaceae), so far (Achenbach, Fietz, Wörth, Waibel, & Portecop, 1986). However, the occurrence of aliphatic pyrrolidine amides does not seem to be a general feature for convolvulaceous species, since such compounds could not be detected in various other species of the genera *Argyrea*, *Falkia*, *Ipomoea*, *Merremia*, *Odonellia*, *Operculina* and *Turbina* (Mann, 1997; Tofern, 1999).

Furthermore, this is the first report on the occurrence of alkamides in the Convolvulaceae in general. To date, alkamides like isobutylamides, isopentylamides, pyrrolidine amides and piperidine amides have been found in the plant kingdom. The pyrrolidine amides are mainly restricted to the Asteraceae and the Piperaceae (Greger, 1984; Greger, Hofer, & Werner, 1987; Greger, Zdero, & Bohlmann, 1987; Parmar et al., 1997; Singh, Dhar, & Atal, 1971). In contrast to the pyrrolidine amides found in *I. aquatica* and *M. quinquefolia* most of those compounds have olefinic or acetylenic acyl moieties (Greger, 1984). While the secondary metabolism of the families mentioned so far shows no close resemblance to that of the Convolvulaceae, the detection of the pyrrolidine amides conioidine A and B in *Chamaesaracha conioides* (DUN.) BRITT. (Solanaceae) (Chan et al., 1993) is chemotaxonically quite remarkable, since both families belong to the Solanales. In contrast to the compounds described in this paper conioidine A

and B are substituted at C-2' and possess a shorter acyl moiety, though.

3. Experimental

3.1. General

^1H NMR and ^{13}C NMR spectra were recorded on a Bruker DPX 400 spectrometer with TMS as internal standard. EIMS, HRMS and FABMS spectra were obtained with Varian MAT CH₇A, Finnigan MAT 711 and Finnigan MAT CH₅DF spectrometers, respectively. TLC and prep. TLC: precoated silica gel plates 60 F₂₅₄ (0.2 mm); Et₂O–petrol (3:1) or cyclohexane–CHCl₃–MeOH (5:4.5:0.5) (solvent systems I and II), detection with Dragendorff's reagent.

3.2. Plant material

Aerial vegetative parts and roots were obtained from plants cultivated in the greenhouse of the Institut für Pharmazie (Pharmazeutische Biologie), Freie Universität Berlin, Germany, where voucher specimens are deposited. The plants were grown from seeds collected near Don Muang/Thailand (*I. aquatica*) and near Guayaquil/Ecuador (*M. quinquefolia*).

3.3. Extraction for GC–MS analysis

Ground dried plant material (50 g) was extracted with MeOH (3 × 400 ml) at room temperature under protection against daylight. After evaporation the residue was dissolved in 2% aq. tartaric acid (250 ml) and successively extracted with petrol (3 × 150 ml) and CH₂Cl₂ (3 × 150 ml). After evaporation in vacuo these extracts were used for the pyrrolidine amide analysis.

3.4. GC–MS analysis

A GC equipped with a 30 m $\times$ 0.32 mm fused-silica capillary column (DB-1) was used. Conditions: injector 250°C; split ratio 1:20; temp. programme 70–300°C, 6°C min⁻¹; carrier gas He 0.5 bar. The capillary column was directly coupled to the quadrupole mass spectrometer Finnigan MAT 4515. EI mass spectra were recorded at 40 eV. Retention indices (RI): Kovats indices were calculated with reference to a set of coinjected *n*-alkanes (C₉–C₂₈) (Kovats, 1958).

3.5. Synthesis of the pyrrolidine amides 3 and 6

2.85 g pyrrolidine and 5.6 g hexadecanoyl chloride as well as 2.85 g pyrrolidine and 6.0 g octadecanoyl chloride were dissolved in 5 ml pyridine, respectively, and left for 3 h at room temperature. Then the solution was added to 50 ml 10% aq. HCl and extracted with EtOAc (5 $\times$ 25 ml). The organic layer was dried with Na₂SO₄ and evaporated in vacuo; after crystallization from a MeOH–H₂O mixture 4.5 g of **3** and 4.7 g of **6** were obtained, resp.

3.6. 1-Hexadecanoylpyrrolidine (**3**)

¹H NMR (400 MHz, CDCl₃): δ 0.91 (3 H, t, J = 6.7 Hz, CH₃), 1.28 (24 H, m, 12 $\times$ CH₂), 1.66 (2 H, m, 2 $\times$ H-3), 1.88 (2 H, m, 2 $\times$ H-3'/2 $\times$ H-4'), 1.96 (2 H, m, 2 $\times$ H-3'/2 $\times$ H-4'), 2.27 (2 H, t, J = 7.7 Hz, 2 $\times$ H-2), 3.43 (2 H, t, J = 6.7 Hz, 2 $\times$ H-2'/2 $\times$ H-5'), 3.49 (2 H, t, J = 6.9 Hz, 2 $\times$ H-2'/2 $\times$ H-5').

3.7. 1-Octadecanoylpyrrolidine (**6**)

¹H NMR (400 MHz, CDCl₃): δ 0.90 (3 H, t, J = 6.8 Hz, CH₃), 1.28 (28 H, m, 14 $\times$ CH₂), 1.66 (2 H, m, 2 $\times$ H-3), 1.88 (2 H, m, 2 $\times$ H-3'/2 $\times$ H-4'), 1.96 (2 H, m, 2 $\times$ H-3'/2 $\times$ H-4'), 2.27 (2 H, t, J = 7.6 Hz, 2 $\times$ H-2), 3.43 (2 H, t, J = 6.8 Hz, 2 $\times$ H-2'/2 $\times$ H-5'), 3.48 (2 H, t, J = 6.8 Hz, 2 $\times$ H-2'/2 $\times$ H-5').

3.8. Isolation of MQ-A₃ (**4**) from *I. aquatica*

Dried ground roots (102 g) were extracted with 3.2 l MeOH at room temperature under protection against daylight. After evaporation the residue was dissolved in 0.4 l 2% aq. tartaric acid and extracted with 0.9 l petrol and 0.9 l CH₂Cl₂, successively. These two extracts were combined and evaporated; the residue (4.5 g) was chromatographed on a silica gel column with cyclohexane–CHCl₃ (5:5) and cyclohexane–CHCl₃–MeOH–32% aq. NH₃ (50:45:5:0.2). As preliminary TLC analysis had shown, the addition of ammonia to the eluent improved the separation of **4** from other lipophilic constituents. The combined frs

containing **4** were further purified twice by prep. TLC ((1) solvent system I (developed twice); (2) solvent system II; elution of the zone containing **4** with CHCl₃–acetone (9:1), respectively) to give **4** (13 mg), which was similarly obtained from epigeal vegetative parts of *M. quinquefolia*, as well.

3.9. 1-(14-Methylhexadecanoyl)pyrrolidine (**4**) (=MQ-A₃)

R_f 0.22 (I), 0.50 (II). $[\alpha]_D^{20} + 5^\circ$ (CHCl₃, c 0.4). IR (KBr) ν_{max} (cm⁻¹): 1628, 3438. EIMS 70 eV, m/z (rel. int. %): 323 (5), 308 (1), 294 (2), 266 (1), 126 (15), 113 (100), 70 (11), 55 (15), 43 (33). (+)-FABMS m/z : 324 [M + H]⁺. HRMS 80 eV, m/z : 323.3189 (C₂₁H₄₁NO, calc. 323.3188), 308.2955 (C₂₀H₃₈NO, calc. 308.2953), 294.2798 (C₁₉H₃₆NO, calc. 294.2797), 266.2484 (C₁₇H₃₂NO, calc. 266.2484), 126.0919 (C₇H₁₂NO, calc. 126.0919), 113.0841 (C₆H₁₁NO, calc. 113.0841). ¹H NMR (400 MHz, CDCl₃): δ 0.86 (3 H, d, J = 6.5 Hz, 3 $\times$ H-17), 0.88 (3 H, t, J = 7.3 Hz, 3 $\times$ H-16), 1.27 (23 H, m, CH + 11 $\times$ CH₂), 1.66 (2 H, m, 2 $\times$ H-3), 1.88 (2 H, m, 2 $\times$ H-3'/2 $\times$ H-4'), 1.98 (2 H, m, 2 $\times$ H-3'/2 $\times$ H-4'), 2.27 (2 H, t, J = 7.5 Hz, 2 $\times$ H-2), 3.43 (2 H, t, J = 6.8 Hz, 2 $\times$ H-2'/2 $\times$ H-5'), 3.48 (2 H, t, J = 6.9 Hz, 2 $\times$ H-2'/2 $\times$ H-5'). ¹³C NMR (100.6 MHz, CDCl₃): δ 11.8 (q, C-16), 19.6 (q, C-17), 24.8 (t, C-3'/C-4'), 25.4 (t, C-3), 26.6 (t, C-3'/C-4'), 27.5 (t, C-15), 29.9–30.0 (t, C-4–C-12), 34.8 (d, C-14), 35.3 (t, C-2), 37.0 (t, C-13), 46.0 (t, C-2'/C-5'), 47.0 (t, C-2'/C-5'), 172.3 (s, C-1).

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