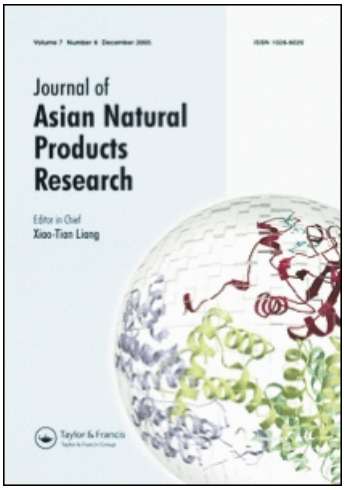

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Structural determination of crotamides A and B, the new amides from *Croton sparsiflorus*

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ORIGINAL ARTICLE

Structural determination of crotamides A and B, the new amides from *Croton sparsiflorus*

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Two new amides crotamides A and B, have been isolated from the *n*-hexane soluble fraction of *Croton sparsiflorus* in addition to salisomide and *N*-(4-hydroxyphenethyl)-octacosanamide reported for the first time from this species. Their structures were assigned from spectral data including 1D and 2D NMR spectroscopic data.

Keywords: *Croton sparsiflorus*; Euphorbiaceae; amides; crotamide A; crotamide B

1. Introduction

The family Euphorbiaceae comprises 300 genera, of which 24 have been found so far in Pakistan [1]. One of these is the *Croton* genus, which comprises about 1300 species growing as trees, shrubs, and herbs in tropical and subtropical regions of both hemispheres [2]. One of the species is *Croton sparsiflorus* (syn. *C. bonplandianus*), which is a woody shrub growing in sandy clay soil in Asia and South America [1]. In Pakistan, it grows in Punjab and Sind provinces [1]. It is used as a potent antihypertensive agent [3–5] and causes sharp fall in blood pressure [6]. The literature survey revealed that a number of alkaloids have so far been reported from this plant [7–12]. The chemotaxonomic and ethnopharmacological importance of the genus *Croton* prompted us to carry out further phytochemical studies on *C. sparsiflorus*. As a result, we herein report the isolation and structural elucidation of two new amides named as crotamides A (**1**) and B (**2**), along with salisomide (**3**) and *N*-(4-hydroxyphenethyl)-octacosanamide (**4**), reported for the first time from this species (Figure 1).

2. Results and discussion

The 80% ethanolic extract of *C. sparsiflorus* (whole plant) was divided into *n*-hexane, dichloromethane, ethyl acetate, *n*-butanol, and water-soluble fractions. A series of column chromatography techniques applied to the *n*-hexane-soluble fraction resulted in the isolation of crotamides A and B along with salisomide and *N*-(4-hydroxyphenethyl)-octacosanamide.

Crotamide A (**1**) was obtained as a white amorphous powder, mp 96–97°C. The UV spectrum showed λ_{max} at 258 nm, while the IR spectrum showed the presence of secondary amides (3313 and 1660 cm^{-1}) and aromatic moiety (1600–1400 cm^{-1}).

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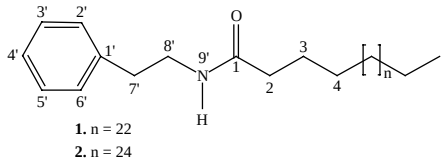

Figure 1. Structures of crotamides A (**1**) and B (**2**).

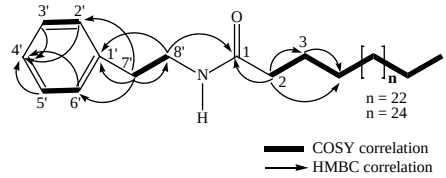

Figure 2. $^{1}H-^{1}H$ COSY and HMBC correlations of crotamides A (**1**) and B (**2**).

The molecular formula was deduced as $C_{36}H_{65}NO$ by HR-EI-MS, which showed an $[M]^+$ peak at *m/z* 527.5055. The downfield signals of ^{1}H NMR spectrum of compound **1** afforded a multiplet of mono-substituted benzene ring (4H multiplets at δ 7.29, 7.16, and 1H multiplet at δ 7.24). A methylene triplet at δ 3.50 ($J = 6.7$ Hz) showed its connectivity with nitrogen and another methylene triplet at δ 2.79 ($J = 6.7$ Hz) in COSY spectrum. The high-frequency region was a characteristic of a long-chain hydrocarbon part, whereas a methylene proton at δ 2.09 ($J = 7.4$ Hz) indicated its connectivity with the carbonyl moiety. The presence of a terminal methyl group was revealed by a triplet at δ 0.86 ($J = 6.7$ Hz). ^{13}C NMR and DEPT spectra showed a carbonyl carbon signal at δ 173.0 and signals of mono-substituted benzene being observed at δ 139.0, 128.8, 128.6, and 126.5 [13,14]. The chemical shift value of carbonyl at δ 173.0 and a correlation of H-8′ at δ 3.50 with carbonyl carbon provided evidence for the presence of carbonyl group as an amide function. The EI-MS showed an $[M]^+$ peak at *m/z* 527, a fragment at *m/z* 498 due to the loss of terminal ethyl group, and diagnostic fragments at *m/z* 105, 91, and 77 were due to the presence of phenylethyl, benzyl, and phenyl moieties, respectively. In HMBC experiments, H-8′ (δ 3.50) showed ^{2}J correlation with C-7′ (δ 35.7) and ^{3}J correlation with amide carbonyl carbon (δ 173.0) and C-1′ (δ 139.0) revealing its attachment with methylene group. The H-7′ (δ 2.79) showed ^{2}J correlations with C-8′ (δ 40.5) and C-1′ (δ 139.0), as well as ^{3}J correlations with both C-2′ and C-6′ (δ 128.8). The remaining HMBC correlations illustrated in Figure 2 along with COSY correlations were in complete agreement with the assigned structure of crotamide A (**1**) as *N*-(2-phenylethyl)-octacosanamide (Figure 1).

Crotamide B (**2**) was obtained as a white amorphous powder, with a melting point of 100°C. The UV and IR spectra were very similar to those of compound **1**. The HR-EI-MS showed a molecular ion peak at *m/z* 555.5370, consistent with the molecular formula $C_{38}H_{69}NO$. Compound **2**, therefore, differed from compound **1** in having two additional methylene groups, which were found in the hydrocarbon chain, as the EI-MS showed similar diagnostic fragments for phenylethyl and benzyl moieties as observed in the case of compound **1**. The ^{1}H and ^{13}C NMR spectra also showed common features to those of compound **1**, allowing to assign the structure of crotamide B (**2**) as *N*-(2-phenylethyl)-triacontanamide (Figure 1).

Compounds **3** and **4** were identified as salisomide and *N*-(4-hydroxyphenethyl)-octacosanamide by comparison of physical and spectral data with those reported in literature [15,16].

3. Experimental

3.1 General experiment procedures

Column chromatography was carried out using silica gel (230–400 mesh, E. Merck, Darmstadt, Germany). TLC was performed with precoated silica gel G-25-UV_{254} plates (E. Merck) and detection was done at 254 and 366 nm and by spraying ceric sulfate in 10% H_2SO_4 (heating). The UV spectra were recorded on a Hitachi

UV-3200 spectrophotometer, while the IR spectra were recorded as KBr pellet on a Jasco 302-A spectrometer. Mass spectra (EI and HR-EI-MS) were measured in an electron impact mode on Finnigan MAT 12 or MAT 312 spectrometers and ions were given in *m/z* (%). Melting points were determined on a Gallenkemp apparatus and were uncorrected. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX-400 spectrometer in deuterated solvents. The 2D NMR spectra were recorded on a Bruker AMX 400 NMR spectrometer. Chemical shifts were in ppm (δ), relative to tetramethylsilane as an internal standard, and scalar coupling was reported in Hertz.

3.2 Plant material

The whole plant of *C. sparsiflorus* Morong (18 kg) was collected from Karachi and identified by Prof. Dr Surraiya Khatoon, the Plant Taxonomist, Department of Botany, University of Karachi, where a voucher specimen has been deposited in the herbarium.

3.3 Extraction and isolation

The freshly collected whole plant material of *C. sparsiflorus* (18 kg) was shade dried, cut into small pieces, and extracted with 80% ethanol (3 × 20 liter, 10 days). The combined ethanolic extract was evaporated under reduced pressure to yield a residue (300 g), which was divided into *n*-hexane (50 g), CH_2Cl_2 (10 g), EtOAc (6 g), *n*-BuOH (14 g), and water-soluble sub-fractions (220 g). The *n*-hexane-soluble sub-fraction was subjected to column chromatography over silica gel eluting with *n*-hexane–CH_2Cl_2, CH_2Cl_2, and CH_2Cl_2–EtOAc in an increasing order of polarity. The fraction (*n*-hexane:CH_2Cl_2 (1.0:1.0) (20 mg)) was rechromatographed over silica gel successively by eluting with *n*-hexane:CH_2Cl_2 (6.0:4.0 and 5.5:4.5) to obtain compounds **1** (9 mg) and **2** (8 mg), respectively. The fraction obtained by elution of the original column with CH_2Cl_2:EtOAc (8.0:2.0) (17 mg) was also a binary mixture. It was rechromatographed over silica gel eluting with CH_2Cl_2:EtOAc (8.2:1.8 and 7.8:2.2) to afford compounds **3** (6 mg) and **4** (9 mg), respectively.

3.3.1 *Crotamide A (**1**)*

White amorphous powder, mp 96–97°C; UV $\lambda_{max}^{CHCl_3}$ nm: 258 (2.8); IR υ_{max} (KBr) cm^{-1}: 3313 (sec. NH), 1660 (amide CO), 1600, 1505 (aromatic moiety); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.29 (2H, m, H-2′, 6′), 7.16 (2H, m, H-3′, 5′), 7.24 (1H, m, H-4′), 3.50 (2H, t, $J = 6.7$ Hz, H-8′), 2.79 (2H, t, $J = 6.7$ Hz, H-7′), 2.09 (2H, t, $J = 7.4$ Hz, H-2), 1.56 (2H, m, H-3), 1.23 (48H, br, $24 \times CH_2$), 0.86 (3H, t, $J = 6.7$ Hz, CH_2CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 173.0 (C-1), 139.0 (C-1′), 128.8 (C-2′, 6′), 128.6 (C-3′, 5′), 126.5 (C-4′), 40.5 (C-8′), 35.7 (C-7′), 36.9 (C-2), 25.7 (C-3), 29.3–31.9 (C-4-C-26), 22.7 (CH_2CH_3), 14.1 (CH_2CH_3). EI-MS (70 e/v) (rel.int %): 527 (18), 498 (27), 407 (20), 163 (60), 105 (17), 104 (100), 91 (23), 71 (36), 57 (68). HR-EI-MS *m/z* 527.5055 (calcd for $C_{36}H_{65}ON$, 527.5066).

3.3.2 *Crotamide B (**2**)*

White amorphous powder, mp 100°C; UV $\lambda_{max}^{CHCl_3}$ nm: 259 (2.9); IR υ_{max} (KBr) cm^{-1}: 3307 (sec. NH), 1658 (amide CO), 1600, 1505 (aromatic moiety); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.31 (2H, m, H-2′, 6′), 7.20 (2H, m, H-3′, 5′), 7.25 (1H, m, H-4′), 3.52 (2H, t, $J = 6.8$ Hz, H-8′), 2.81 (2H, t, $J = 6.8$ Hz, H-7′), 2.13 (2H, t, $J = 7.6$ Hz, H-2), 1.52 (2H, m, H-3), 1.21 (52H, br, $26 \times CH_2$), 0.87 (3H, t, $J = 6.6$ Hz, CH_2CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 173.2 (C-1), 138.8 (C-1′), 128.7 (C-2′, 6′), 128.6 (C-3′, 5′), 126.8 (C-4′), 40.7 (C-8′), 35.4 (C-7′), 36.8 (C-2), 25.8 (C-3), 29.4-31.8 (C-4-C-28), 22.5

($C-CH_2CH_3$), 14.2 (CH_2CH_3). EI-MS (70 e/v) (rel.int %): 555 (11), 527 (34), 436 (22), 407 (12), 195 (12), 163 (43), 105 (11), 104 (100), 91 (29), 71 (45), 57 (84). HR-EI-MS *m/z* 555.5370 (calcd for $C_{38}H_{69}ON$, 555.5379).

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