A New *N*-Carboxyindole Alkaloid from the Marine Sponge *Rhaphisia pallida*

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A new indole alkaloid pallidin (**1**), together with the two known compounds, cyclo(L-pro-L-leu) and 1,3-dimethylxanthine, were isolated from the sponge *Rhaphisia pallida*. The structures of these metabolites were defined by spectroscopic methods.

Substituted diketopiperazine derivatives of several naturally occurring indole alkaloids have been isolated and have exhibited interesting biological activities.[1] For example, barettin, isolated from the marine sponge *Geodia baretti*,[2] showed inhibiting activity on electrically induced contractions of an isolated Guinea pig ileum, and austamide was one of the toxic principles of moldy maize meal.[3] In a study of the sponge *Rhaphisia pallida* Ridley (Halichondriidae), we have isolated the new alkaloid pallidin (**1**), a member of the rare structural class of *N*-carboxyindole alkaloids. Indole-3-carboxylic acid derivatives have been known from red algae[4,5] and brown algae.[6] So far, there is no example of a naturally occurring *N*-carboxyindole alkaloid. However, L-carboxyindole itself and some derivatives have been prepared.[7,8] Pallidin is the first isolation of an *N*-carboxyindole alkaloid from a natural source. Two known compounds, cyclo(L-pro-L-leu) and 1,3-dimethylxanthine, were also obtained from this species.
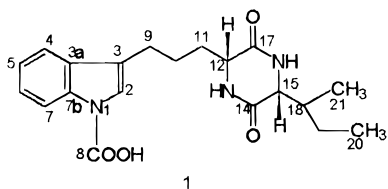
The ethanol extract of *R. pallida* yielded pallidin (**1**), cyclo(L-pro-L-leu), and 1,3-dimethylxanthine by vacuum and flash chromatography.

The ^{13}C-NMR and FABMS (MH$^+$, *m/z* 372) of pallidin (**1**) supported the molecular formula $C_{20}H_{25}N_3O_4$ (mol wt 371). The pattern of ^{1}H-NMR signals in the aromatic proton region indicated an indole moiety, and the four signals at δ 7.34, 7.41, 7.63, and 8.86 ppm were attributed to the four neighboring aromatic protons. The ^{13}C-NMR signal at 166.2 ppm and an IR absorption at 1700 cm^{-1} established the presence of a COOH group. A typical *N*-carboxyindole UV absorption strongly supported this determination.[9] The ^{13}C NMR signal at 131.2 (d) ppm was assigned to C-2 of indole; thus, a part of the structure of **1** must be an *N*-carboxyindole with a substituent at C-3.

The mass spectrum of **1** (Figure 1) gave two important fragments A and B at *m/z* 161 and *m/z* 211, respectively, which obviously originated via cleavage of the
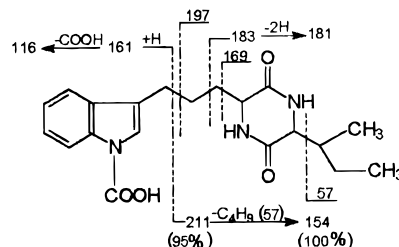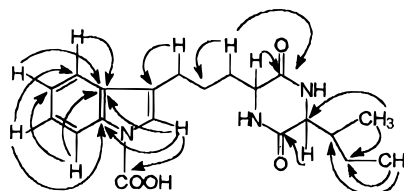
Figure 1. MS fragment ions of **1** (*m/z*).

Figure 2. Key HMBC correlations for **1**.

C_3–C_9 bond. Fragment A at *m/z* 161 represents the *N*-carboxyindole moiety. It lost OH or COOH to display an [A − OH] ion at *m/z* 144 and an [A − COOH] ion at *m/z* 116, respectively. This confirmed that the carboxyl group must be located at the N-1 position. In addition, the position of the carboxyl group was unambiguously confirmed by the HMBC spectrum (Figure 2).

The remaining part of the structure was a diketopiperazine system. IR data for **1** showed NH bands at 3393 and 3310 cm^{-1} and amide carbonyl bands near 1690 and 1644 cm^{-1} (amide I), which together with the absence of an amide II band clearly suggested the existence of a diketopiperazine system.[2,3] The ^{1}H-NMR spectrum showed an exchangeable signal at δ 8.37 ppm which was assigned to two NH protons.

The interpretation of the ^{1}H–^{1}H COSY and ^{1}H–^{13}C COSY NMR spectra established the presence of the following three subunits: −HC=CHCH=CH−, −CH$_2$-CH$_2$CH$_2$CH(N)−, and −CH(N)CH(CH$_3$)CH$_2$(CH$_3$). The connectivities of these subunits were deduced by analyzing the cross-peaks observed in the HMBC spectrum and by comparing the NMR data with those of the known compound austamide.[3]

In the MS of **1**, fragment B at *m/z* 211 (94%) represented the diketopiperazine system. It lost a CH$_2$ unit to give a conspicuous peak at *m/z* 197 and further lost two CH$_2$ to give *m/z* 169. The latter lost CH$_3$ to give a prominent peak at *m/z* 154 (Figure 1), which suggested that the second part of the structure must be a dipeptide moiety, cyclo(norvaline-isoleucine).
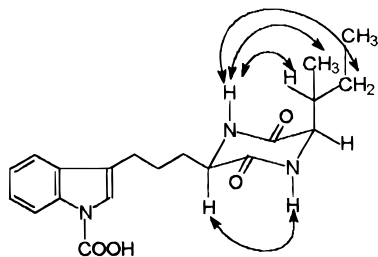
Figure 3. Expression of major NOESY correlation in **1**.

NOESY experiments (Figure 3) were employed to assign the relative configuration for **1**. These suggested the relative configuration of C-12 and C-15 should be the same as those reported for prolyl-2-(1′,1′-dimethyl-allyl)tryptophyldiketopiperazine.[3] However, the configuration of C-18 could not be assigned.

The ^{1}H- and ^{13}C-NMR chemical shifts and the IR and mass spectrum of compound **2** revealed that it is a cyclodipeptide. Comparison of its spectral data with those reported in the literature,[10] identified compound **2** as cyclo(L-pro-L-leu). This compound had previously been isolated from fungi[11] and plants.[12]

The mp and UV, ^{1}H- and ^{13}C-NMR, and mass spectral data of compound **3** were consistent with those of 1,3-dimethylxanthine.[13]

Experimental Section

General Experimental Procedures. Mps were determined on a X$_4$ apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX-600 instrument (for compound **1**) and Jeol FX-90Q instrument (for compounds **2** and **3**). A Nicolet 5DX FT-IR spectrometer and a Perkin-Elmer 240C automatic elemental analyzer were used. Mass spectra were measured with a ZAB-HF-3F mass spectrometer. Preparative HPLC was carried out by using a μ-Porasil SiO$_2$ column with UV detection.

Extraction and Isolation. The sponge *R. pallida* was collected off the Bay of Lingshui, Hainan Island, at a depth of 3−5 m. The voucher specimen (no. 93-9) is preserved in the Research Centre of Organic Natural Products, Zhongshan University.

The sun-dried specimen (1.3 kg) was immersed in EtOH at rt. The combined extracts were evaporated *in vacuo*. The residue was partitioned between EtOAc−H$_2$O three times. The combined EtOAc extracts (5.7 g) were chromatographed on Si gel, eluting with EtOAc−petroleum ether with gradually increasing amounts of EtOAc.

The fraction eluted with 65% EtOAc in petroleum ether was further subjected to flash chromatography on high performance silica, eluting with increasing amounts of MeOH in CH$_2$Cl$_2$. The fraction eluted with 3% MeOH−CH$_2$Cl$_2$ gave crude cyclo(L-pro-L-leu) (60 mg), which was further purified by crystallization from Me$_2$CO−petroleum ether (1:1) to give hexagonal crystals. The fraction eluted with 6% MeOH−CH$_2$Cl$_2$ yield a solid. It was subjected to preparative TLC, using Me$_2$CO−petroleum ether−glacial HOAc (2:3:0.03) as solvents, and then was purified by recrystallization from MeOH to afford colorless crystals of **1** (15 mg).

The fraction eluted with 80% EtOAc in petroleum ether was further separated by high performance silica flash chromatography, eluting with CHCl$_3$−n-BuOH (85:

15) to give a colorless solid. The solid was purified by HPLC (μ-Porasil SiO$_2$ column, UV detector, at 271 nm) using glyme−n-hexane (1:1) as eluent to yield colorless needles of 1,3-dimethylxanthine (10 mg).

Pallidin (1) was obtained as colorless crystals (MeOH): mp 124−125 °C; $[\alpha]^{25}_D$ −52.4° (*c* 0.040, MeOH); UV (EtOH) λ max nm (log ϵ) 281 (3.90), 272 (3.88), 252 (4.08), 228 (4.11), 212 (4.49); IR (KBr) ν max 3393 (NH), 3310 (NH), 2913, 2870, 1700 (C=O, acid), 1690 (C=O, amide), 1644 (C=O, amide), 1581, 1525, 1447, 1307, 1194, 1032, 751 cm^{-1}; ^{1}H NMR (pyridine-d_5, 600 MHz) δ 12.96 (1 H, s, H-8), 8.86 (1 H, d, J = 7.5 Hz H-7), 8.73 (2 H, s, exchangeable, NH), 8.50 (1 H, d, J = 2.6 Hz, H-2), 7.63 (1 H, d, J = 7.6 Hz, H-4), 7.41 (1 H, t, J = 7.5 Hz, H-5), 7.34 (1 H, t, J = 7.6 Hz, H-6), 4.16 (1 H, dd, J = 8.3 and 7.8 Hz, H-12), 4.11 (1 H, d, J = 2.9 Hz, H-15), 3.61 (1 H, dt, J = 11.4 and 8.0 Hz, H-9a), 3.45 (1 H, ddd, J = 11.4, 8.5, 3.2 Hz, H-9b), 2.45 (1 H, m, H-18), 2.27 (1 H, m, H-11a), 2.11 (1 H, m, H-11b), 1.73 (1 H, m, H-19a), 1.69 (1 H, m, H-10a), 1.61 (1 H, m, H-10b), 1.54 (1 H, m, H-19b), 1.20 (3 H, d, J = 7.1 Hz, H-21), 0.92 (3 H, t, J = 7.3 Hz, H-20); ^{13}C NMR (pyridine-d_5, 125.8 MHz) δ 169.1 (s, C-17), 166.2 (d, C-8), 164.3 (s, C-14), 136.2 (s, C-7a), 131.2 (d, C-2), 126.0 (s, C-3a), 121.5 (d, C-6), 102.5 (d, C-5 and C-7), 111.0 (d, C-4), 108.2 (s, C-3), 58.9 (d, C-15), 57.6 (d, C-12), 43.7 (t, C-9), 34.5 (d, C-18), 27.2 (t, C-11), 23.2 (t, C-19), 21.1 (t, C-10), 13.9 (q, C-21), 10.9 (q, C-20); EIMS (70 eV) m/z [M]$^+$ 371, 211 (93), 197 (13), 181, 169, 161 (29), 154 (100), 144 (8), 116 (9), 107 (27), 77 (30), 69 (25).

Cyclo(L-pro-L-leu) was obtained as colorless hexagonal crystals (MeOH); mp 160−162 °C; IR (KBr) ν max 3261, 2952, 2875, 1668, 1638, 1471, 1432, 1300, 1158, 1130, 917, 709, 668, 641 cm^{-1}; ^{1}H-NMR (CDCl$_3$) δ 4.12 (1 H, t, H-2), 2.35 and 2.14 (2 H, m, H-3), 2.03 and 1.90 (2 H, m, H-4), 3.57 and 3.54 (2 H, ddt H-5), 4.02 (1 H, dd, H-2′), 2.06 and 1.53 (2 H, m, H-3′), 1.75 (1 H, m, H-4′), 1.01 (3 H, d, H-5′), 0.96 (3 H, d, H-6′); ^{13}C-NMR (CDCl$_3$) δ 170.2 (s, C-1), 59.0 (d, C-2), 28.1 (t, C-3), 23.2 (t, C-4), 45.5 (t, C-5), 166.2 (s, C-1′), 53.4 (d, C-2′), 28.1 (t, C-3′), 24.7 (d, C-4′), 22.7 (q, C-5′), 21.2 (q, C-6′); EIMS m/z [M]$^+$ 210 (2.5), 209 (1.2), 195 (10.1), 167 (17.3), 154 (100), 139 (18.0), 125 (39.7), 112 (5.0), 96 (20.3), 86 (90.0), 70 (99.4), 55 (52.2).

1,3-Dimethylxanthine was obtained as colorless needles (pyridine−MeOH 1:5): mp 251−252 °C; UV (MeOH) λ max (log ϵ) 271 (3.95), 208 (4.16); ^{1}H-NMR (CDCl$_3$) δ 8.24 (1 H), 3.52 (3 H) ppm; ^{13}C-NMR δ 154.8 (s, C-2), 151.4 (s, C-4), 107.3 (s, C-4a), 139.8 (d, C-6), 148.1 (s, C-7a), 29.4 (q, C-1), 27.4 (q, C-3); EIMS m/z [M]$^+$ 180, 165 (74), 14 (10), 132 (14), 114 (89), 92 (100), 70 (71), 58 (79).

References and Notes

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