# Secondary Metabolites from the Sponge *Tedania anhelans:* Isolation and Characterization of Two Novel Pyrazole Acids and Other Metabolites

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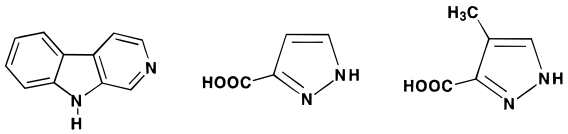
Chemical investigation of the methanol extract of the sponge *Tedania anhelans* yielded the two unusual heteroaromatic acids, pyrazole-3(5)-carboxylic acid (**2**) and 4-methylpyrazole-3(5)-carboxylic acid (**3**), which are reported for the first time as natural products. The other compounds isolated were *p*-hydroxybenzaldehyde, phenylacetamide, 3-phenylpropionic acid, 3-(*p*-hydroxyphenyl)propionic acid, 3-(*p*-methoxyphenyl)propionic acid, $\beta$-carboline (norharman) (**1**), and the four diketopiperazines pro-val, pro-leu, pro-phe, and pro-tyr.

The isolation and identification of tedanolide, a potent antineoplastic agent, from the sponge *Tedania ignis* by Schmitz's group[1] prompted us to investigate in detail the related sponge *Tedania anhelans*[2] (family *Tedaniidae*, order *Poecilosclerida*). A complete report on several components from the sponge *T. ignis* was later made by Cardellina.[3,4] Three important secondary metabolites reported from this family of sponges are 1-methylisoguanosine,[5] a purine nucleoside isolated from *T. digitata*, atisane-3$\beta$,16$\alpha$-diol, a diterpenoid,[6] and tedanolide,[1] a macrolide, from *T. ignis*. We have isolated 12 compounds, including two heteroaromatic acids and $\beta$-carboline (norharman), from the methanol extract of *T. anhelans*.

*T. anhelans* (Lieberkuhn) is a yellow, encrusting fire sponge generally devoid of epizoites and induces necrosis of the tissues of other sponges when they are kept in contact. This sponge, which is common in the intertidal region along the northwestern coast of India, was collected at Okha Gujarat (India). The methanol extract (15 g) of the sponge (2.5 kg) was partitioned between ethyl acetate and water. The organic layer was separated and dried over $Na_2SO_4$ and solvent removed to yield 5 g of ethyl acetate extract. The compounds from this extract were purified by repeated silica gel column chromatography using hexane:ethyl acetate:methanol and hexane:chloroform:methanol gradient systems.

*p*-Hydroxybenzaldehyde, phenylacetamide,[3] phenylpropionic acid, 3-(*p*-hydroxyphenyl)propionic acid, and 3-(*p*-methoxyphenyl)propionic acid were readily identified by the analysis of MS, NMR, and IR data. *p*-Hydroxybenzaldehyde had been previously reported to be produced by the fungus *Ceratocystis clavigera*,[7] associated with blue disease of pine, and phenylacetamide from terrestrial plants[8−12] as well as the fungus *Streptoverticillium olivoreticuli*.[13]

The IR spectrum of **1**, which was quite different from the aromatic acids or other diketopiperazines, showed multiple bands in the region 3150−2650 and 1650−1300 $cm^{-1}$, indicating the presence of aromatic ring and amino groups. EIMS displayed the molecular ion peak at *m/z* 168, and the UV spectrum with absorptions at 235 (31 000), 290 (17 900), and 350 (4600) nm was characteristic of the $\beta$-carboline moiety. Compound **1** was identified as norharman by comparison of its spectral data with those of the fungal product reported in the literature.[14,15]




**1** **2** **3**

The diketopiperazines pro-val, pro-leu, pro-phe, and pro-tyr were also identified as constituents of the sponge. These compounds have all been previously isolated from fungi and marine sponges; pro-val, pro-leu, and pro-phe were reported to be produced by the sponge *T. ignis*[6] and from cultures of the bacterium associated with *T. ignis*,[16] while pro-leu is also a metabolite of fungi[7,17] and microorganisms.[18] The diketopiperazines pro-phe and pro-tyr are predominantly produced by fungi.[17,19−21]

Compound **2** showed UV maxima at 205 (7300) and 260 (5780) nm and IR bands at 3450−2700, 1724, and 1674 $cm^{-1}$ indicative of an aromatic amino acid-type structure. Both EIMS and CIMS revealed its molecular weight to be 112. The $^1H$ NMR spectrum in pyridine-$d_5$ had four signals at $\delta$ 13.04, 12.22 (−OH, −NH) 7.55 (1H, d, $J$ = 7.8 Hz), 5.83 (1H, d, $J$ = 7.8 Hz), while the $^{13}C$ NMR spectrum showed signals at $\delta$ 165.7 (COOH), 142.3 (C-3), 135.2 (C-5), 101.4 (C-4). The elemental composition was calculated to be $C_4H_4N_2O_2$, revealing it to be either an imidazole or pyrazolecarboxylic acid. The former was ruled out from the carbon chemical shifts. The structure of this compound was established as pyrazole-3(5)-carboxylic acid based on vicinal vinyl proton coupling constants.

Compound **3** had UV [207 (7320), 262 (5770) nm] and IR [3300−2700, 1730, 1673 $cm^{-1}$] absorptions similar to those of pyrazole-3(5)-carboxylic acid (**2**). EIMS suggested its molecular weight to be 126, 14 units more than compound **2**. $^1H$ NMR in pyridine-$d_5$ showed signals at 13.04, 12.22 (−NH, −OH), 7.33 (1H, s), 1.97 (3H, s), and $^{13}C$ NMR in pyridine-$d_5$ showed signals at 166.2 (COOH), 153.2 (C-3), 137.8 (C-5), 108.8 (s, C-4), and 12.5 ($-CH_3$). On the basis of the carbon chemical shifts of **2** and unsubstituted pyrazine,[22,23] the structure of this compound was assigned as 4-methylpyrazole-

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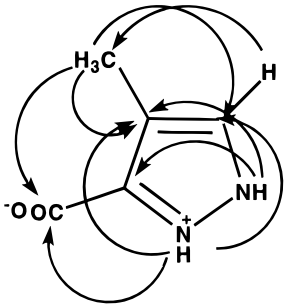


**Figure 1.** Long-range $^1H-^{13}C$ coupling observed in the SINEPT spectra of **3**.

3(5)-carboxylic acid (**3**). The structures were further confirmed by long-range $^1H-^{13}C$ coupling observed in the SINEPT studies as shown in Figure 1.

Compounds **2** and **3** have not been reported as natural products.

## Experimental Section

**General Experimental Procedures.** Melting points were determined on a Laboratory Device's Mel-temp melting point apparatus and are uncorrected. IR spectra were taken on a Nicolet Model 10-MX FTIR instrument. Ultraviolet spectra were obtained using a Hewlett-Packard 8450 A UV−vis spectrophotometer. NMR spectra were recorded on a Bruker 200 spectrometer (200 MHz), with chemical shifts ($\delta$) expressed downfield to TMS. SINEPT studies were performed on a Varian XL-300 instrument using TMS as an internal standard. EIMS were recorded on a Finnigan Mat-312 mass spectrometer, and CIMS were recorded on a VG-70-SE instrument;

**Marine Sponge Material.** *T. anhelans* was collected from the intertidal areas of western Gujarat coast of India during the winter of 1989 and transported to the laboratory in methanol. A specimen of the sponge is on deposit at the National Institute of Oceanography Institute at Dona Paula, Goa, India, under voucher no. NIO 445.

**Extraction of *T. anhelans*.** The sponge (2 kg) was ground and soaked with methanol. After 2 days, the solution was filtered, solvent was removed, and the residue was partitioned between $H_2O$ and EtOAc. The organic fraction was dried, applied to a silica gel column (size 60 × 2 cm), and eluted with hexane−EtOAc (0 to 100%) and MeOH with increasing gradient. Fatty acids and other aliphatic acids eluted first followed by aromatic deaminated acids and their derivatives. Further elution yielded **1** and the fractions containing mixture of cyclic dipeptides and pure aromatic acids **2** and **3**. Cyclic peptides as well as aromatic acids were separated by flash chromatography on silica gel, eluting with a gradient of hexane−$CHCl_3$ and MeOH with increasing polarity. The $\beta$-carboline (**1**) and aromatic acids **2** and **3** were further purified by HPLC to yield 12, 11, and 9 mg, respectively.

***β*-Carboline (1):** mp 196 °C; IR(KBr) $\nu_{max}$ 3115, 2955, 2640, 1625, 1560, 1500 $cm^{-1}$; UV (MeOH) $\lambda_{max}$ 235 (31 000), 290 (17 900), 350 (4600) nm; $^1H$ NMR ($CDCl_3$) $\delta$ 9.43 (1H, br, NH), 8.97 (1H, s, 1-H), 8.45 (1H, d, $J$ = 5 Hz, 3-H), 8.13 (1H, d, $J$ = 7.8 Hz), 7.97 (1H, d, $J$ = 5 Hz, 4-H), 7.55 (2H, bs), 7.30 (1H, m); $^{13}C$ NMR ($CDCl_3$) $\delta$ 140.83 (s), 138.40 (d), 136.07 (s), 133.34 (d), 129.29 (s), 128.75 (d), 121.86 (d), 121.40 (s), 120.20 (d), 114.92 (d), 111.81 (d); EIMS $m/z$ 169 ($MH^+$).

**Pyrazole-3(5)-carboxylic acid (2):** mp 293 °C dec; UV(MeOH) $\lambda_{max}$ 204, 260; IR(KBr) $\nu_{max}$ 3432−2700 (br), 1724, 1674, 1447, 1418, 1383, 1237, 1216, 993, 837, 560, 540, 475 $cm^{-1}$; $^1H$ NMR (pyridine-$d_5$) $\delta$ 13.04 (COOH, br), 12.22 (NH, br), 7.55 (5-H, d, J = 7.75 Hz), 5.83 (4-H, d, $J$ = 7.75 Hz); $^{13}C$ NMR (pyridine-$d_5$) ppm 165.7 (s, C═O), 142.3 (s, C-3), 135.2 (d, C-5), 101.4 (d, C-4); CIMS $m/z$ 113 ($MH^+$).

**4-Methylpyrazole-3(5)-carboxylic acid (3):** mp 299 °C dec; UV(MeOH) $\lambda_{max}$ nm 207 (7319), 262 (5766); IR (KBr) $\nu_{max}$ 3300−2700, 1730, 1673, 1482, 1446, 1425, 1382, 1240, 1205, 1028, 985, 935, 843, 815, 758, 737, 556, 471 $cm^{-1}$; CIMS 127 ($MH^+$); $^1H$ NMR (pyridine-$d_5$) $\delta$ 13.04 (1H, br), 12.22 (1H, br), 7.33 (3 or 5-H, s), 1.97 (3H, s); $^{13}C$ NMR (pyridine-$d_5$) ppm 166.2 (s, C═O), 153.2 (s, C-3), 137.8 (d, C-5), 108.8 (s, C-4), 12.5 (q, $CH_3$); EIMS $m/z$ 126 ($M^+$) (100), 112 (8), 96 (4), 83 (22), 70 (10), 55 (100).

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