An Unusual Sulfur-Containing Diketopiperazine from the Bermudian Sponge Tedania ignis

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AN UNUSUAL SULFUR-CONTAINING DIKETOPIPERAZINE FROM THE BERMUDIAN SPONGE *TEDANIA IGNIS*[1]

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ABSTRACT.—A new sulfur-containing diketopiperazine, cyclo(L-Pro-L-thioPro) [**1**], was isolated from extracts of the Bermudian sponge *Tedania ignis*. The structure was determined through spectral analyses and confirmed by synthesis.

In the course of our investigation of the minor aromatic metabolites in extracts of the sponge *Tedania ignis* Duchassaing and Michelotti (Demospongiae) (1,2), we encountered numerous fractions containing diketopiperazines. Schmitz *et al.* (3) had previously reported several such constituents of *T. ignis*, and our laboratory has recently shown that the diketopiperazines reported by Schmitz's group are actually produced by an associated bacterium (4). Herein we report the structure of a novel sulfur-containing cyclic dipeptide from extracts of Bermudian *T. ignis*.

The collection, extraction, and fractionation of *T. ignis* have been described (2). Fractionation of both the CCl_4- and $CHCl_3$-soluble fractions of the organic extract by a series of gel permeation chromatographies (BioBeads S-X4 and Sephadex LH-20) gave mixtures of diketopiperazines which were resolved by centrifugal countercurrent chromatography [$CHCl_3$-MeOH-H_2O (25:34:20), ascending]. In addition to diketopiperazines reported earlier by Schmitz *et al.* (3), we found cyclo(L-Pro-L-Phe) and a new compound. Cyclo(L-Pro-L-Phe) was readily identified by spectral comparisons with literature data (5).

Accurate mass measurements (eims) provided the molecular formula $C_9H_{12}N_2O_2S$ for the new compound. The ^{13}C-nmr spectrum contained resonances for two amide carbonyl groups and seven sp^3 carbons (δ 23–63); the molecule had to be tricyclic. The absence of N-H stretching absorption in the ir and the lack of exchangeable protons in the ^{1}H nmr indicated that both amides were tertiary. The ^{1}H-nmr spectrum consisted of a series of resonances between 1.5 and 4.8 ppm which were well resolved at 500 MHz. The chemical shifts and multiplicities of certain signals (C/H-6,7,8; see Table 1) were characteristic of a proline fragment (1,2,5); this assignment was confirmed by ^{1}H-^{1}H COSY analyses. The proline and two tertiary amide residues left a C_3H_5S fragment and two sites of unsaturation to be assigned. An isolated methylene group (δ 4.75 and 4.50, ea 1 H, d, J = 10 Hz) had to be placed between a nitrogen and the sulfur; the azathioacetal carbon resonated at 48.5 ppm. The remaining elements comprised an ABX system. This new compound had to be **1**, a diketopiperazine constructed from the common amino acid proline and the relatively rare 3-thioproline.

The absolute stereochemistry was defined as *S,S* by synthesis from the com-

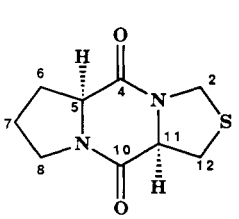

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TABLE 1. Nmr Assignments for Cyclo(L-Pro-L-thioPro) [**1**].[a]

Position	^{13}C	^{1}H
2	48.5	4.75,4.50,ea 1H,d,10
4	165.8[b]	—
5	60.5	4.20,1H,t,8
6	27.8	2.15,2.35,ea 1H,m
7	23.2	1.95,2.05,ea 1H,m
8	45.3	3.55,2H,m
10	164.2[b]	—
11	62.8	4.45,1H,t,7
12	32.5	3.45,3.35,ea 1H,dd,12,7

[a]Recorded in $CDCl_3$; assignments based on ^{1}H-^{1}H COSY and ^{1}H-^{13}C HETCOR experiments. ^{13}C-nmr entries are δ; ^{1}H-nmr entries are δ, multiplicity, *J* in Hz.

[b]Assignments may be interchanged.

mercially available L-amino acids. Using the procedures of Itoh *et al.* (6) and Nitecki *et al.* (7), the methyl ester of proline was coupled with the *t*-BOC derivative of 3-thioproline. The protecting groups were then removed under hydrolytic conditions, and the second amide bond was formed. This approach is known to proceed without racemization. The synthetic material, $[\alpha]D -112.8°$, was identical in all respects with the natural product, $[\alpha]D -114.3°$. Compound **1** was, therefore, cyclo(L-Pro-L-thioPro).

While **1**, a novel compound, was inactive in all our in-house bioassays (brine shrimp cytotoxicity, phytotoxicity, plant growth regulatory, antimicrobial, and insecticidal), other thiazolidine-carboxylic acid (3-thioproline) derived diketopiperazines have been prepared and found to have mild to strong neurosedative effects (8).

EXPERIMENTAL

ISOLATION.—The collection, extraction, and early fractionation steps have been described elsewhere (2). Compound **1** was isolated from the CCl_4 and $CHCl_3$ solubles by a sequence of gel permeation chromatographies: BioBeads S-X4 (fraction 7), Sephadex LH-20 (fractions 1 and 2), and a second Sephadex LH-20 (fraction 3). Final purification employed centrifugal countercurrent chromatography [$CHCl_3$-MeOH-H_2O (25:34:20), ascending].

CHARACTERIZATION.—Yield 4.8 mg (from 86 g crude extract); $[\alpha]^{23}D -114.3°$ (*c* = 0.14, $CHCl_3$); ν max ($CDCl_3$) 1659, 1423 cm^{-1}; ms *m/z* (%) $[M]^+$ 212.0648 (100) ($C_9H_{12}N_2O_2S$ requires 212.0620), 138 (17), 124 (20), 87 (15); ^{1}H and ^{13}C nmr see Table 1.

SYNTHESIS OF CYCLO(L-PRO-L-THIOPRO) [**1**].—To 0.133 g (1.0 mM) of L-thioproline was added 0.2735 g (1.1 mM) BOC-ON, 10 ml of 15% aqueous Me_2CO, and 200 μl triethylamine. The mixture was stirred for 2 h at room temperature. The Me_2CO was evaporated, and the remaining aqueous phase was washed with EtOAc. The aqueous layer was then acidified with 1 M HCl and extracted with CH_2Cl_2. Evaporation of the CH_2Cl_2 phase gave the crude product, 0.1792 g (64%).

The BOC-thioproline (0.1792 g) was dissolved in 10 ml CH_2Cl_2 and 140 μl triethylamine. L-Proline methyl ester hydrochloride (0.1648 g, 1.0 mM) was added along with 0.1920 g (1 mM) *N*-ethyl-*N'*-(3-dimethyl-aminopropyl)-carbodiimide hydrochloride. The mixture was stirred overnight at −5°. The solution was washed sequentially with equal volumes of H_2O, 1 N citric acid, 5% $NaHCO_3$, and H_2O. The organic layer, which contained the crude product, was evaporated to dryness.

The crude *t*-BOC-dipeptide methyl ester was dissolved in 10 ml of HCO_2H and stirred at room temperature for 2 h. The HCO_2H was removed in vacuo, and the dipeptide ester formate was dissolved in 5 ml toluene and 25 ml *sec*-butanol. The mixture was refluxed for 2 h and the solvents removed by rotary evaporation.

The crude diketopiperazine was purified on a Sephadex LH-20 column (158 × 2.0 cm) using MeOH-MeCN (4:1). Evaporation of fraction 5 gave a white crystalline solid (63%) whose spectral data matched that of the natural product: $[\alpha]^{23}D -112.8°$ (*c* = 0.19, $CHCl_3$); mp 168–170°.

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