N,N' DIPHENETHYLUREA, A METABOLITE FROM THE MARINE ASCIDIAN *DIDEMNUM TERNATANUM*

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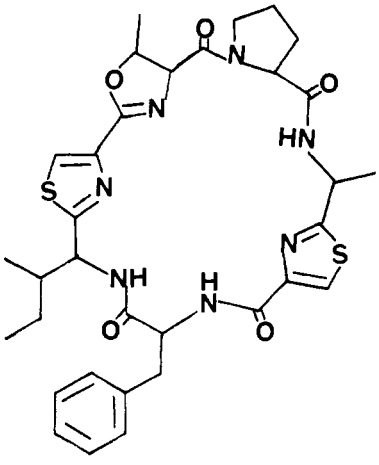
The didemnid ascidians represent a select group of marine chordates which harbor procaryotic algal symbionts (1). Although it has been well documented that unicellular algae occur as endosymbionts in a wide variety of marine organisms (2), their impact on the secondary metabolism of their host organisms remains ill-defined. This is particularly true in cases where the symbiont is a procaryotic alga.

As part of our investigation into the molecular basis of marine symbiosis (3), we have undertaken a systematic study of the secondary metabolites of didemnid ascidians from the Western Caroline Islands. We have previously reported the isolation of cyclic peptides, e.g., ulicyclamide (**1**) from *Lissoclinum patella* (4), which suggested that these symbiotic pairs are capable of *de novo* amino acid synthesis. This is particularly interesting in view of earlier reports that the nitrogen-fixing procaryotes *Lyngbya majuscula* (5) and *Microcystis aeroginosa* (6) produce cyclic peptides.

We now report the isolation of *N,N'-diphenethylurea* (**2**), apparently a product of phenylalanine metabolism, from the didemnid *Didemnum ternatanum*. This provides further evidence for the existence of amino acid metabolism by such symbiotic pairs. Typically, chromatography of an organic extract of the frozen ascidian yielded crystalline **2**, mp 135° (lit. 138–141°) (7), as the only secondary metabolite (28% of organic oil). The structure of **2** was confirmed by



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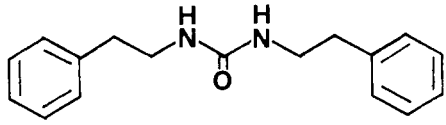

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synthesis from phenethylamine and phosgene.

This represents the first isolation of **2** from a marine organism. Interestingly, its first isolation from a natural source was from a *Streptomyces* sp. (7). The compound was found to be a weak depressant lacking acute toxicity.

EXPERIMENTAL[1]

COLLECTION AND EXTRACTION OF *Didemnum ternatanum*.—Colonies of *D. ternatanum* were collected by snorkeling (−2 to −4 m) at Urukthapal island (7°18′ N, 120°27′, 30″ E) in the Western Carolines. The material was frozen immediately and maintained frozen until extraction. Three different extraction procedures were employed:

1. Frozen tunicate (455 g) was homogenized in methanol (1 liter) in a Waring blender. The resulting suspension was filtered through Whatman #1 paper. The solvent was evaporated to give a watery residue which was partitioned between water and methylene chloride. The methylene chloride layer was evaporated to give the organic soluble material (3.7 g).
2. Frozen tunicate (100 g) was homogenized in methylene chloride (0.5 liter) in a Waring blender. The suspension was filtered through Whatman #1 PS phase separating paper, and the methylene chloride was evaporated to give the organic soluble material (0.62 g).
3. Freeze-dried tunicate (3g) was homogenized in methylene chloride (0.25 liter) and treated according to method 2 to give the organic soluble material (0.064 g).

Tlc inspection of the extracts indicated that all were similar in composition.

[1]The ir spectra were recorded on a Beckman 620 MX spectrophotometer. The uv spectra were recorded on a Cary 15 spectrophotometer. Electron impact mass spectra were recorded on an AEI-MS-902 mass spectrometer. ^{1}H nmr spectra were recorded on a Perkin Elmer R-24B 60 MHz spectrometer and ^{13}C nmr spectra on a Bruker WP-60 MHz Fourier transform spectrometer. Melting points were measured on a Thomas Hoover apparatus and are uncorrected.

ISOLATION OF *N,N'*-DIPHENETHYLUREA.—Column chromatography of the organic soluble material (2.7 g) first on Sephadex LH-20 (methylene chloride/methanol, 3:1), then on silica gel (ethyl acetate) gave *N,N'*-diphenethylurea (0.76 g), colorless crystals from ethanol, mp 135°; ir (CH_2Cl_2) 3450, 3380, 3040, 2940, 2880, 1670, 1650 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 2.70 (t, 2H, J=7 Hz), 3.30 (dt, 2H, J=7,7 Hz), 5.0 (brt, 1H, J=7 Hz), 7.11 (s, 5H); ^{13}C nmr ($CDCl_3$) δ 158.6 (s), 139.3 (s), 128.8 (d), 128.5 (d), 126.3 (d), 41.7 (t), 36.6 (t); eims, m/z 268 $(M)^+$, 177 $(M-C_7H_7)^+$, 147 $(M-C_8H_{11}N)^+$, 91 $(C_7H_7)^+$; hrms, Obs: 268.1574, $C_{17}H_{20}N_2O$ requires 268.1575.

SYNTHESIS OF *N,N'*-DIPHENETHYLUREA.—Phenethylamine (Eastman, 9.5 g, 10 ml) was dissolved in methylene chloride (100 ml) in a 500 ml 2-necked round-bottom flask equipped with reflux condenser and addition funnel. The addition funnel was charged with phosgene (12% solution in benzene, 10 ml) and methylene chloride (50 ml). The contents were added dropwise to the stirred phenethylamine solution over a 2 h period. Faster addition results in formation of significant amounts of monoacylated product. After addition was completed, stirring was continued for 24 h. The reaction, when washed with water (3 X 100 ml), dried over magnesium sulfate and evaporated gave crude product. Recrystallization from ethanol gave white crystals (2.35 g), mp 135°, identical in every respect (ir, nmr, ms) to the natural product.

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LITERATURE CITED

1. R. A. Lewin and N. Withers, *Nature (London)*, **256**, 735 (1975).
2. W. E. Vernberg, "Symbiosis in the Sea," University of South Carolina Press, 1974.
3. C. Ireland and P. J. Scheuer, *Science*, **205**, 922–23 (1979).
4. C. Ireland and P. J. Scheuer, *J. Am. Chem. Soc.*, **102**, 5688–91 (1980).
5. R. E. Moore, *BioScience*, **27**, 797–802 (1977).
6. P. R. Gorham, *Can. Vet. J.*, **1**, 235 (1960).
7. Y. Iwai, A. Hirano, J. Awaya, S. Matsuo and S. Ōmura, *J. Antibiotics*, **31**, 375–6 (1978).