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6-AMINO-6-CARBOXY-2-TRIMETHYLAMMONIOHEXANOATE FROM THE RED ALGA *SCHOTTERA NICAEENSIS*

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ABSTRACT.—A new metabolite, 6-amino-6-carboxy-2-trimethylammoniohexanoate has been isolated from the red alga *Schottera nicaeensis*, and its structure was determined by spectral and chemical methods. Its distribution in about fifty species of Rhodophyceae has been investigated.

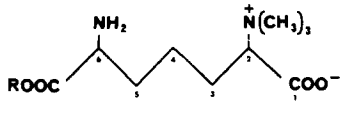
Dragendorff-positive compounds isolated from or detected in red algae include glycine betaine (1), β-alanine betaine (2), γ-butyrobetaine (3), proline betaine (stachydrine) (4), 4-(2-dimethylaminoethyl)phenol (hordenine) (5), picolinic acid betaine (homarine) (6), nicotinic acid betaine (trigonelline) (6), trimethyltaurine (7), choline sulphate (8), 3-dimethylsulphoniopropionate (9), and 4-dimethylsulphonio-2-methoxybutyrate (10).

In a search for compounds of this type in Mediterranean red algae, we have now found that *Schottera nicaeensis* (Duby) Shott. [syn. *Petroglossum nicaeense* (Lamouroux ex Duby) Guiry et Hollenberg] (Gigartinales) accumulates a highly polar metabolite that reacts with both Dragendorff's reagent (orange) and ninhydrin (purple). Ion-exchange chromatography of the EtOH extract of the red alga and further purification by hplc afforded chromatographically pure **1** as an off-white, hygroscopic powder, $[\alpha]^{25}D$ −6.4. On account of its salt-like characteristics, the mass spectrum was uninformative, and the molecular formula was established as $C_{10}H_{20}O_4N_2$ by combustion analysis.

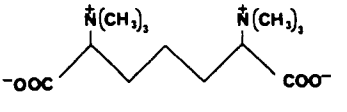
The ^{13}C-nmr spectrum contained two carboxyl (carboxylate) resonances at 172.45 and 170.78 ppm, two α-amino acid methines at 76.45 (d, C-2) and at 53.01 (d, C-6), three methylene triplets at 29.63 (C-3), 26.05 (C-5), and 21.37 (C-4), and a three-methyl quartet at 52.29 [$-N(CH_3)_3$]. In the ^1H nmr, determined at 250 MHz in D_2O, the C-4 methylene protons appeared as a 2-H multiplet at δ 1.49, while those of the C-3 and C-5 methylenes gave rise to a complex 4H signal centred at δ 2.01. A 9H singlet at δ 3.19 was assigned to the $-N(CH_3)_3$ group. The remaining signals were a 1H double doublet at δ 3.74 (J=10 and 3.7 Hz) and a 1H triplet at δ 4.00 (J=6.2 Hz), which were assigned respectively to the C-2 and C-6 methines. This assignment was based on the shift at δ 4.05 of the first signal after protonation of the carboxylate function and comparison with the spectrum of 2,6-trimethylammonio-heptanedioate (**3**) (see Experimental section).

Esterification of **1** with MeOH in the presence of HCl afforded the monomethyl-ester **2** (δ 3.78, singlet, 3H, $-OCH_3$); comparison of its ^1H-nmr spectrum (see Experimental section) with that of the original compound showed that the C-7 carboxylic group, rather than the C-1 carboxylate group, had been esterified, because in the latter case a downfield shift for the C-2 methine resonance would have been expected.

The mass spectrum of **2**, which showed a small molecular ion at *m/z* 246 confirming the molecular formula of **1**, could be interpreted taking into account that betaines show fragmentation patterns from their pyrolysis product(s) in the gas phase (11), the most



1 R=H
2 R=CH_3



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important pyrolytic process involving an intermolecular transfer of a methyl group from the quaternary nitrogen to the carboxylate function. Accordingly, the mass spectrum of **2** displayed diagnostically important peaks from the corresponding transalkylated compound at *m/z* 215 (M^+-OCH_3), 187 (M^+-$COOCH_3$), 156 (M^+-OCH_3-$COOCH_3$), 142 [187-$HN(CH_3)_2$], 128 (M^+-2$COOCH_3$), 84 [CH_2=CH−CH=$\overset{+}{N}(CH_3)_2$], and 58 [CH_2=$\overset{+}{N}(CH_3)_2$].

The above data led to structure **1** for the new algal metabolite. Definite proof was obtained by exhaustive methylation of the amino group in non-epimerizing conditions according to the procedure reported by Patchett and Witkop (12). The permethylated compound was chromatographically and spectroscopically (^{1}H nmr) indistinguishable from a sample of 2,6-trimethylammonioheptanedioate obtained by methylation of 2,6-diaminopimelic acid. Because the semisynthetic sample was optically inactive, it was deduced that the two chiral centers in **1** have opposite configuration.

The new metabolite also occurs in *Gastroclonium clavatum* (Roth) Ardiss. (Rhodymeniales) and *Liagora distenta* (Mert.) c. Ag. (Nemalionales), while it was not detected in a number of other species of red algae (about fifty) representative of all the seven orders belonging to the Florideae (see Table 1). This restricted distribution indicates a possible taxonomic value. It is to be also noted that the closely related laminine (*N,N,N*-trimethyllysine), originally isolated from *Laminaria angustata* (13) and widespread in brown algae, until now has not been found in red algae. If one considers that 2,6-diamino pimelic acid in a number of organisms is the biosynthetic precursor of lysine, compound **1** could bear the same relationship to laminine, and its accumulation

TABLE 1. Red Algae Analyzed for the Presence of Compound **1**

Nemalionales
- Helminthocladiaceae: *Liagora distenta, Liagora viscida, Nemalion helminthoides.*
- Chaetangiaceae: *Scinaia forcellata.*

Gelidiales
- Gelidiaceae: *Gelidium latifolium, Pterocladia pinnata.*

Gigartinales
- Nemastomaceae: *Nemastoma dichotoma, Schizymenia dubyi* (and its tetrasporophyte *Haematocelis rubens*).
- Plocamiaceae: *Plocamium cartilagineum.*
- Sphaerococcaceae: *Caulacanthus ustulatus, Sphaerococcus coronopifolius.*
- Hypneaceae: *Hypnea musciformis.*
- Phyllophoraceae: *Gymnogongrus griffithsiae, Schottera nicaeensis, Phyllophora nervosa.*
- Gigartinaceae: *Gigartina acicularis, Gigartina teedi.*

Rhodymeniales
- Rhodymeniaceae: *Botryocladia botryoides.*
- Champiaceae: *Gastroclonium clavatum, Lomentaria articulata.*

Cryptonemiales
- Gloiosiphoniaceae: *Schimmelmannia schousboei, Thuretella schousboei.*
- Peyssonneliaceae: *Peyssonnelia rosa-marina, Peyssonnelia rubra, Peyssonnelia squamaria.*
- Corallinaceae: *Amphiroa beauvoisii, Amphiroa rigida, Corallina elongata, Corallina granifera, Goniolithon byssoides, Jania rubens.*
- Cryptonemiaceae: *Grateloupia proteus, Grateloupia filicina, Halymenia floresia.*

Bonnemaisoniales
- Bonnemaisoniaceae: *Falkenbergia rufolanosa.*

Ceramiales
- Ceramiaceae: *Centroceras clavatum, Ceramium ciliatum, Ceramium rubrum, Spyridia filamentosa, Wrangelia penicillata.*
- Delesseriaceae: *Nitophyllum punctatum.*
- Rhodomelaceae: *Acantophora najadiformis, Alsidium corallinum, Alsidium helminthochorton, Chondria coerulescens, Chondria dasyphylla, Digenea simplex, Halopitys incurvus, Laurencia obtusa, Laurencia paniculata, Laurencia undulata, Lophocladia lallemandii, Polysiphonia subulata, Pterosiphonia pennata, Rytiphloea tinctoria, Vidalia volubilis.*

in red algae could be the consequence of the lack of the enzymic system required for decarboxylation.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mass spectra were obtained on an AEI MS 902 instrument at 70 eV (direct injection). ^{1}H-nmr spectra were recorded in D_2O (sodium trimethylsilylpropionate as internal reference) at 250 MHz with a Bruker WM-250 instrument and at 80 MHz with a Bruker WP-80 instrument. ^{13}C-nmr spectra were determined at 20.1 MHz on a Bruker WP-80 instrument. Optical rotations were measured with a Perkin-Elmer 141 polarimeter. Tlc was carried out on glass precoated cellulose and silica gel plates (Merck). Spots were detected by spraying with ninhydrin or Dragendorff's reagent (14). Preparative liquid chromatography (preparative lc) was carried out on a Jobin-Yvon MiniPrep LC instrument.

PLANT MATERIAL.—*S. nicaeensis*, *G. clavatum*, and *L. distenta* were collected respectively near Castelluccio, Acicastello, and S. Maria La Scala, Sicily. Voucher specimens were deposited in the Herbarium of the Institute of Botany, Catania, Italy.

EXTRACTION AND PURIFICATION.—*S. nicaeensis* (1 kg, fresh alga) was homogenized and extracted three times with 70% aqueous EtOH at room temperature under continuous stirring. The pooled extracts were concentrated in vacuo, clarified by centrifugation, and then applied to a column of Dowex-50W (H^+). After the resin was washed with H_2O, the total amino acid fraction containing **1** was eluted with 2M NH_4OH. The eluate was taken to dryness and the residue dissolved in H_2O. The solution was passed successively through columns of Dowex-1 ($^-$OAc) and Amberlite IRC-50 (H^+) to remove acid and basic amino acids, respectively. The final eluate was concentrated to a small volume and applied to a column of Dowex-50W (H^+, 3 × 100 cm), which was eluted with a linear gradient of HCl from 0.5 to 1.8 M (7 liters) to remove most of the neutral amino acids. After washing with H_2O until the eluate was neutral, compound **1** was eluted with 2M NH_4OH. Crude **1** was further purified by preparative lc (LiChroprep Si-60 25-40μ; *n*-BuOH-HOAc-H_2O, 12:3:5). The separation was monitored by tlc (cellulose, *n*-BuOH-HOAc-H_2O, 12:3:5, Rf 0.16, phenol-H_2O, 3:1, Rf 0.32; Si-gel, *n*-BuOH-HOAc-H_2O, 12:3:5, Rf 0.04), and the fractions containing pure **1** were pooled and taken to dryness. The residue was dissolved in H_2O, and the solution freeze-dried to give 105 mg of chromatographically pure **1** as an off-white, hygroscopic powder. *Anal.* calcd for $C_{10}H_{20}N_2O_4$: C, 51.72; H, 8.62; N, 12.07. Found: C, 51.60; H, 8.70; N, 12.10. $[\alpha]^{25}D$ −6.4 (c 1.6, H_2O).

Samples of *G. clavatum* (100 g) and *L. distenta* (200 g) were extracted as above to give respectively 15 and 5 mg of **1**.

ESTERIFICATION OF **1** TO GIVE **2**.—Compound **1** was dissolved in a 3% solution of HCl in MeOH and the solution kept overnight at room temperature. After evaporation to dryness, the residue contained chromatographically pure **2** (SiO_2, phenol-H_2O, 3:1, Rf 0.50, brown color with ninhydrin and orange with Dragendorff's reagent); ^{1}H nmr (80 MHz, D_2O) δ 1.49 (2H, m, H-4), 2.00 (4H, m, H-3 and H-5), 3.19 [9H, s, $N(CH_3)_3$], 3.78 (3H, s, OCH_3), 3.80 (1H, partially obscured dd, H-2), 4.13 (1H, t, J=6.2, H-6); ms *m/z* (%) 246 (1.6), 215 (2.7), 187 (2.3), 186 (2.8), 156 (13.8), 142 (37.7), 128 (16.2), 96 (27.5), 84 (38.3), 70 (34.7), 58 (77.8), 42 (100).

EXHAUSTIVE METHYLATION OF **1** TO GIVE **3**.—Compound **1** was exhaustively methylated in non-epimerizing conditions following essentially the procedure reported for the methylation of hydroxyprolines (12). Chromatographically pure, optically inactive 2,6-trimethylammonioheptanedioate (**3**) (39 mg) was obtained starting from 60 mg of **1** (cellulose, *n*-BuOH-HOAc-H_2O, 12:3:5, Rf 0.24; SiO_2, phenol-H_2O, 3:1, Rf 0.52). ^{1}H nmr (80 MHz, D_2O) δ 1.48 (2H, m, H-4), 2.00 (4H, m, H-3 and H-5), 3.19 (18H, s, $N(CH_3)_3$), 3.67 (2H, dd, J=5 and 9 Hz, H-2 and H-6).

A reference sample of 2,6-trimethylammonioheptanedioate was obtained by methylation in the same conditions of commercially available 2,6-diaminopimelic acid (mixture of optical isomers of unspecified composition).

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