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ONIUM COMPOUNDS FROM THE RED ALGA *PTEROCLADIA CAPILLACEA*

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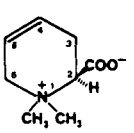
In the course of a continuing study of metabolites from Mediterranean red algae, we have isolated, in addition to several non-protein amino acids (1–4), some Dragendorff-positive compounds (ammonium and sulfonium salts) (5,6). The distribution of quaternary ammonium compounds and their possible taxonomic value have been reviewed recently by Blunden and Gordon (7). The present paper describes the identification of the Dragendorff-positive compounds from the red alga *Pterocladia capillacea* (Gmelin) Bornet (Gelidiaceae, Gelidiales).

The neutral amino acid fraction, isolated by ion-exchange chromatography from an aqueous extract of *P. capillacea*, contained several Dragendorff-positive compounds, two of them having tlc properties that implied their novelty. These two new metabolites have been isolated by a combination of ion-exchange and preparative liquid chromatography.

Compound **1**, whose amphoteric nature was revealed by its behavior on ion-exchange resins as well as on paper electrophoresis, was isolated in a yield of 0.004% of the fresh wt of the alga. Elemental analysis and mass measurements ($[M]^+$ *m/z* 155) established the molecular formula $C_8H_{13}NO_2$. The mass spectrum (70 eV) of the compound displayed major peaks at *m/z* 110 $[M-CO_2-H]^+$, 96, $M-CO_2-CH_3]^+$, 94 [*N*-methylpyridinium ion], 58 $[(CH_3)_2N=CH_2]^+$, and 42 $[CH_3\text{-}N\equiv CH]^+$. The transitions 155–>110 and 96–>94 were supported by *meta*-stable peaks at *m/z* 78.06 and 92.04, respectively. The ^{13}C-nmr spectrum displayed two methyl quartets at 47.12 [^1J (^{14}N, ^{13}C-CH_3) = 3.7 Hz] and 53.56 ppm [^1J (^{14}N, $^{13}CH_3$) = 3.9 Hz], one carboxyl (carboxylate) resonance at 170.63, one α amino acid methine at 70.93 [d, C-2; ^1J (^{14}N, ^{13}C-2) = 3.0 Hz], two olefinic methines at 120.00 (d, C-5) and 125.56 (d, C-4) and two methylene triplets at 26.90 (C-3) and 63.34 [C-6; ^1J (^{14}N, ^{13}C-6) = 3.2 Hz]. In the ^1H-nmr spectrum of compound **1**, determined in D_2O, the methyl groups appeared as two distinct singlets at δ 3.23 and 3.29, the C-2 methine gave a double doublet at δ 3.95 partially obscured by a multiplet at δ 4.05 assigned to the C-6 methylene, and the C-3 protons appeared as a multiplet centered at δ 2.73. Two remaining complex signals centered at δ 5.73 and 6.03 were assigned to C-5 and C-4 methines, respectively, on the basis of decoupling experiments. After acidification to pH 2 (CF_3COOH) the conformationally rigid inner salt **1** was converted into the more flexible trifluoroacetate causing the methyl singlets to collapse in a single peak at δ 3.30. Concomitantly, the C-2 signal, as expected for a proton α to the amino acid function, was shifted downfield to δ 4.38 (dd, J = 6 and 8 Hz).

From these data, the structure of *N,N*-dimethyl-1,2,3,6-tetrahydropyridinio-2-carboxylate [**1**] (baikiain betaine) was assigned to the novel onium salt. Confirmation of the structure and determination of the configuration at the chiral center were obtained through the identity of the chromatographic, spectral, and chiroptical properties of the natural compound with those of a sample of L-baikiain betaine prepared from L-baikiain according to the method of Patchett and Witkop (8). It is relevant to observe that in the alga in question L-baikiain represents more than half of the total fraction of free amino acids (1).

Compound **2** (0.0025% of the fresh wt of the alga) showed mobilities in ion-exchange chromatography and electrophoresis that suggested the presence of both basic and acidic functions.



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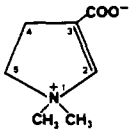

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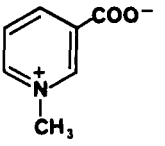
Its mass spectrum (70 ev) exhibited peaks at *m/z* 97 and 82 consistent with consecutive losses of CO_2 and of a methyl group from the parent ion (*m/z* 141) and peaks at *m/z* 71 and 55 representing feasible sequential losses of C_2H_2 and CH_4 from the ion at *m/z* 97. Other major peaks were at *m/z* 58 $[CH_2{=}N(CH_3)_2]^+$ and 42 $[CH{\equiv}N\text{-}CH_3]^+$. The ^{1}H-nmr spectrum of **2**, determined in D_2O, revealed the presence of a dimethylammonium group as a singlet at δ 3.25, a methylene adjacent to a positively charged nitrogen atom at δ 3.95 (t, H-5, J=7.4 Hz), a multiplet at δ 3.01 assigned to the C-4 methylene group and a lowfield olefinic methine at δ 6.50 (s, H-2). After conversion into the hydrochloride, a marked deshielding (0.35 ppm) of H-2 was observed as the result of protonation of the conjugated carboxylate group. Pd-catalyzed hydrogenation of **2** afforded a compound that was chromatographically and spectroscopically (tlc, ms, nmr) indistinguishable from β-stachydrine. From the above evidence, compound **2** was assigned the structure of *N,N*-dimethyl-Δ^2-pyrrolinio-3-carboxylate.

In addition to the two novel betaines, the following Dragendorff-positive compounds were identified and isolated from *P. capillacea*: trigonelline [**3**], homarine [**4**], glycine betaine, β-stachydrine [**5**], and dimethyl-β-propiothetin.

Trigonelline, widely distributed in terrestrial plants as well as in marine invertebrates, has been reported only once from an algal source, *Trichocarpus crinitus*



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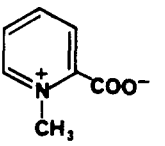

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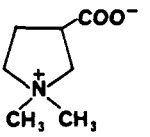

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(9); in this alga homarine co-occurs. It is also found in the green alga *Platymonas subcordiformis* (10); glycine betaine has been reported from several marine algae; β-stachydrine has been identified as the major Dragendorff-positive compound from some species of the family Ceramiaceae (11); dimethyl-β-propiothetin is widely distributed in marine algae.

Examination of different collections of the alga over a two-year period showed that while trigonelline, glycine betaine, and dimethyl-β-propiothetin were consistently present, compound **2** and β-stachydrine could be detected only in the spring samples and baikiain, betaine, and homarine in the summer ones. The origin of this seasonal dependence is not clear at the present time. The co-occurrence of compound **2** and β-stachydrine could be explained by a possible biosynthetic relation between the two metabolites.

The accumulation mechanism of these betaines and their physiological role in the alga is an interesting problem.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mass spectra were obtained on an AEI MS 902 instrument at 70 eV (direct injection). ^{1}H- and ^{13}C-nmr spectra at 80 and 20.1 MHz, respectively, were recorded in D_2O (sodium trimethylsilylpropionate as internal reference) with a Bruker WP-80 instrument. Optical rotations were measured with a Perkin-Elmer 141 polarimeter. Preparative liquid chromatography (preparative lc) was carried out on a Jobin-Yvon MiniPrep LC instrument. Hptlc were run on glass, precoated Si

gel-F_{254} and cellulose-F_{254} plates (Merck). *n*-BuOH-HOAc-H_2O (8:2:2) was the solvent system used to run hptlc as well as preparative lc. Spots on chromatograms were detected by ninhydrin, Dragendorff's reagent, and uv light (254 nm).

PLANT MATERIAL.—*P. capillacea* was harvested in various locations off the coast of Catania. Voucher specimens were deposited in the University Herbarium, Institute of Botany, Catania, Italy.

EXTRACTION AND PURIFICATION.—Fresh alga (1 kg) was homogenized and extracted with 70% aqueous EtOH (3 × 3 liters) under continuous stirring. The combined extracts were concentrated in vacuo, clarified by centrifugation, and applied to a column of Dowex-50W (H^+). After the resin was washed with H_2O, the total amino acid fraction, containing Dragendorff-positive compounds, was eluted with 2 M NH_4OH and the eluate evaporated under reduced pressure. The residue was dissolved in H_2O and then passed successively through columns of Dowex-1 (^-OAc) and Amberlite IRC-50 (H^+) to remove acidic and basic amino acids, respectively. The final aqueous eluate was taken to dryness, and the residue was fractionated by preparative lc on Li-Chroprep Si-60 (25–40 μm); the separation was monitored by hptlc on cellulose and/or Si gel. Several enriched fractions were obtained which, appropriately pooled, were further purified by cc on cellulose powder (Merck); hptlc on cellulose was used to monitor the separation. Fractions containing pure individual compounds were pooled and taken to dryness.

When summer samples of *P. capillacea* were extracted, fractions from preparative lc that contained homarine and compound **1** were chromatographed on a column of Dowex-50W (H^+; 1 × 100 cm) using a linear gradient of HCl from 0.5 to 2 N (1.2 liters). The separation was monitored by hptlc on cellulose, and fractions containing pure **1** or homarine (both in hydrochloride form) were pooled and evaporated in vacuo. The residues were dissolved in H_2O and charged on columns of Dowex-50W (H^+); after washing with H_2O, the betaines were recovered by elution with 2 M NH_4OH, and the eluates were taken to dryness.

Compound **1**.—Compound **1** (Si gel, R_f 0.17; cellulose, R_f 0.50) was recrystallized from iPrOH, mp 184–186°, $[\alpha]^{25}D$ −103° (c = 1.6, H_2O); ms *m/z* (%) 155 (41.3), 110 (100), 96 (57.4), 94 (44.3), 84 (21.7), 82 (27.0), 58 (69.6), 44 (47.8), 42 (95.7). *Anal.* calcd for $C_8H_{13}NO_2$: C, 61.90; H, 8.45; N, 9.03. Found: C, 61.86; H, 8.47; N, 9.08.

Compound **2**.—Compound **2** (Si gel, R_f 0.08; cellulose, R_f 0.27) was dissolved in H_2O, and the solution was freeze-dried to give an off-white hygroscopic powder, ms *m/z* (%) 141 (14.3), 97 (25.4), 96 (16.3), 83 (49.7), 82 (61.1), 71 (27.8), 58 (55.6), 57 (48.4), 55 (30.9), 44 (100), 42 (42.9). *Anal.* calcd for $C_7H_{11}NO_2$: C, 59.54; H, 7.86; N, 9.93. Found: C, 59.45; H, 7.90; N, 9.98.

Trigonelline [**3**].—Trigonelline [**3**] (Si gel, R_f 0.09; cellulose, R_f 0.32). This compound (0.0095% fresh wt of the alga) gave ms and ^{1}H-nmr spectra identical to those reported in the literature (12,13). Its ^{13}C-nmr spectrum displayed signals at δ 167.06 (s, COO^-), 147.27 (d, C-4), 146.95 (d, C-2), 146.57 (d, C-6), 145.63 (s, C-3), 128.56 (d, C-5), 48.88 (q, N-CH_3). The signals of C-2, C-6, and N-CH_3 showed an additional splitting arising through coupling of these nuclei with that of the quaternary nitrogen.

Homarine [**4**].—(Si gel, R_f 0.16; cellulose, R_f 0.40) The compound (0.0075% fresh wt of the alga) was identified on the basis of its ms and ^{1}H-nmr spectra (14,15). The ^{13}C-nmr spectrum contained resonances at δ 166.00 (COO^-), 147.20 (d, C-4), 146.06 (d, C-6), 145.04 (s, C-2), 127.83 (d, C-3), 126.80 (d, C-5), 47.24 (q, N-CH_3). The resonances of C-2, C-6, and N-CH_3 showed coupling with the quaternary nitrogen nucleus.

β-*Stachydrine* [**5**].—(Si gel, R_f 0.11; cellulose, 0.35) This compound was isolated in a yield of 0.0062% fresh wt of the alga. It was identified by comparison of ^{1}H- and ^{13}C-nmr spectra of its hydrochloride with those reported in the literature (16). Ms *m/z* (%) $[M]^+$ 143 (1.7), $[M-CH_2]^+$ 129 (1.5), $[M-CH_4-H]^+$ 126 (2.0), $[M-CO_2-H]^+$ 98 (2.2), [98−2H] 96 (4.4), [98−CH_4] 82 (2.8), $[CH_2=N(CH_3)_2]^+$ 58 (100), 44 (21.9), $[CH\equiv N\text{-}CH_3]^+$ 42 (25.6).

Glycine betaine.—Glycine betaine (0.0056% fresh wt of the alga) was identified by comparison of its chromatographic and spectroscopic properties (tlc, ms, nmr) with those of an authentic sample (Si gel, R_f 0.14; cellulose, R_f 0.55).

Dimethyl-β-propiothetin.—Dimethyl-β-propiothetin (Si gel, R_f 0.12; cellulose, R_f 0.45) was isolated in small amounts (0.0004% fresh wt of the alga) and was identified on the basis of ^{1}H-nmr spectrum of its hydrochloride (16). Ms *m/z* (%) $[M]^+$ 134 (4.0), $[M-CH_2]^+$ 120 (26.0), $[M-2CH_2]^+$ 106 (32.3), $[M-(CH_3)_2S]^+$ 72 (56.2), $[(CH_3)_2S]^+$ 62 (25.1), $[CH_2=S-CH_3]^+$ 61 (90.5), $[C_3H_3O]^+$ 55 (30.7), $[CH_3S]^+$ 47 (100).

SYNTHESIS OF L-BAIKIAIN BETAINE [**1**].—L-Baikiain (200 mg) was exhaustively methylated with MeI in non-epimerizing conditions according to Patchett and Witkop (8). Crude betaine was treated with 6 N HCl at 110° for 2 h. The

mixture was taken to dryness, and the residue, dissolved in H_2O, was charged on a column of Dowex-50W (H^+); after washing with H_2O, the betaine was recovered by elution with 2 M NH_4OH. The eluate was evaporated, and recrystallization from iPrOH gave 65 mg of baikiain betaine.

REDUCTION OF **2** TO GIVE β-STACHYDRINE.—To a solution containing 20 mg of **2** in 50% aqueous EtOH 2 mg of 5% Pd on charcoal were added, and the mixture was stirred overnight at room temperature under an atmosphere of H_2. The suspension was then filtered and taken to dryness. The residue, dissolved in H_2O, was purified by preparative pc (*n*-BuOH-HOAc-H_2O, 8:2:2) yielding 13.7 mg of β-stachydrine.

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LITERATURE CITED

1. G. Impellizzeri, S. Mangiafico, G. Oriente, M. Piattelli, S. Sciuto, E. Fattorusso, S. Magno, C. Santacroce, and D. Sica, *Phytochemistry*, **14**, 1549 (1975).
2. G. Impellizzeri, M. Piattelli, S. Sciuto, and E. Fattorusso, *Phytochemistry*, **16**, 1601 (1977).
3. S. Sciuto, M. Piattelli, and R. Chillemi, *Phytochemistry*, **18**, 1058 (1979).
4. S. Sciuto, R. Chillemi, M. Piattelli, and G. Impellizzeri, *Phytochemistry*, **22**, 2311 (1983).
5. S. Sciuto, M. Piattelli, and R. Chillemi, *Phytochemistry*, **21**, 227 (1982).
6. S. Sciuto, R. Chillemi, and M. Piattelli, *J. Nat. Prod.*, **48**, 602 (1985).
7. G. Blunden and S.M. Gordon, *Prog. Phycol. Res.*, **4**, 39 (1986).
8. A.A. Patchett and B. Witkop, *J. Am. Chem. Soc.*, **79**, 185 (1957).
9. K. Yabe, I. Tsujino, and T. Saito, *Hokkaido Daigaku Suisangakubu Kenkyu Iho*, **16**, 273 (1966).
10. D.M.J. Dickson and G.O. Kirst, *Planta*, **167**, 536 (1986).
11. G. Blunden, S.M. Gordon, W.F.H. McLean, and M.D. Guiry, *Bot. Mar.*, **25**, 563 (1982).
12. E. Larsen, H. Egsgaard, and H. Holmen, *Org. Mass Spectrom.*, **13**, 417 (1978).
13. S. Ghosal and S.K. Dutta, *Phytochemistry*, **10**, 195 (1971).
14. T. Groenneberg, *Chem. Scr.*, **2**, 17 (1972).
15. H. Quast and E. Schmitt, *Liebigs Ann. Chem.*, **732**, 64 (1970).
16. G. Blunden, S.M. Gordon, T.A. Crabb, O.G. Roch, M.G. Rowan, and B. Wood, *Magn. Reson. Chem.*, **24**, 965 (1986).

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