Journal of Natural Products
Vol. 51, No. 5, pp. 1017-1020, Sep-Oct 1988

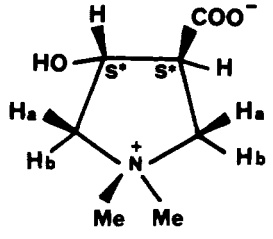
TWO NEW DRAGENDORFF-POSITIVE COMPOUNDS FROM MARINE ALGAE

SEBASTIANO SCIUTO,* ROSA CHILLEMI, RAFFAELE MORRONE,[1] ANGELA PATTI, and MARIO PIATTELLI

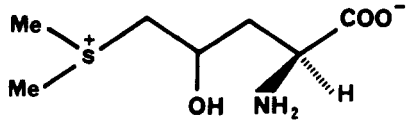
Dipartimento di Scienze Chimiche, Università di Catania, viale A. Doria 6, 95125 Catania, Italy

Dragendorff-positive compounds have been often isolated from or, largely on chromatographic evidence, identified in marine algae. Apart from a few tertiary amino compounds [e.g., hordenine (1) or 4-hydroxy-*N*-methylproline (2)], they are quaternary ammonium compounds, mainly betaines of protein or non-protein amino acids, and, less frequently, tertiary sulfonium derivatives (3–5). In the course of our continuing search for compounds of this class from Mediterranean red algae (4–6), we have isolated, by a combination of ion-exchange and partition chromatography, a betaine from *Grateloupia proteus* Kütz. (Cryptonemiaceae; Cryptonemiales) and a sulfonium inner salt from *Lophocladia lallemandi* (Mont.) Schmitz (Rhodomelaceae; Ceramiales), whose general properties indicated that they were previously unreported compounds. Essentially on the basis of spectral data, they have been assigned the structures of 4-hydroxy-*N*,*N*-dimethylpyrrolidinio-3-carboxylate [**1**] and 5-dimethylsulfonio-4-hydroxy-2-aminovalerate [**2**], respectively.

Isolated from the neutral amino acid fraction of *G. proteus* in a yield of 0.009% of the fresh weight of the alga



1



2

was compound **1**, $[\alpha]^{25}D + 14.4°$, $C_7H_{13}O_3N$ (combustion analysis). Its amphoteric nature was evident from its behavior in electrophoresis and ion-exchange chromatography. The mass spectrum did not show the molecular ion, the ion at highest mass being observed at *m/z* 141 $[M - H_2O]^+$; the spectrum also contained diagnostically important peaks at *m/z* 97 $[M - H_2O - CO_2]^+$, 82 $[M - H_2O - CO_2 - Me]^+$, 58 $[CH_2 = NMe_2]^+$, and 42 $[CH \equiv N - Me]^+$.

The ^{13}C-nmr spectrum (D_2O) of **1** contained a carboxylate resonance at δ 179.65 (shifted to 175.74 at pH 2), two methyls bonded to a positively charged nitrogen atom (57.02) overlapping a methine signal that was shifted to 53.98 after acidification (C-3), two methylene triplets at 70.73 (C-2) and 74.71 (C-5), and a methine at 76.27 (C-4). The ^1H-nmr spectrum, measured in D_2O, displayed well separated and fully analyzable signals after acidification to pH 2. Apart from the resonances from methyls on quaternary nitrogen at δ 3.25 and 3.31, the spectrum consisted of a six-spin system, which could be analyzed as the superimposition of an ABMX and an A′B′X system, with the X part in common [ABMX system: AB part δ 4.03 (H-2_a) and 3.83 (H-2_b), $J_{AB} = 12.0$ Hz; M part δ 3.57 (H-3), $J_{AM} = 8.55$ Hz, $J_{BM} = 8.5$ Hz; X part δ 4.92 (H-4), $J_{XM} = 3.6$ Hz. A′B′X system: A′B′ part δ 3.79 (H-5_a) and 3.62 (H-5_b), $J_{A'B'} = 12.9$ Hz; X part δ 4.92, $J_{A'X} =$

[1]Istituto del C.N.R. per lo Studio delle Sostanze Naturali di Interesse Alimentare e Chimico-Farmaceutico, viale A. Doria 6, 95125 Catania, Italy.

5.8 Hz, $J_{B'X}$ = 2.4 Hz]. In neutral solution the H-3 signal showed an upfield shift of 0.26 ppm, as expected for a proton α to a carboxylic function.

Acetylation of **1** afforded a monoacetate, whose ^{1}H-nmr spectrum (D_2O) exhibited an acetoxymethyl resonance at δ 2.12, while the carbinolic proton appeared at δ 5.65 showing a downfield shift of 0.73 ppm.

On the evidence above, the new natural betaine was assigned structure **1**, in which the relative stereochemistry at C-3 and C-4 was assumed to be as depicted based on the lack of nOe between H-3 and H-4. This assumption was reinforced by the observation that for the *trans* configuration, contrary to the *cis* one, a conformation exists whose coupling constants, calculated using a generalized Karplus equation (7), are closely comparable with those observed.

Compound **2**, $C_7H_{15}O_3NS$ (combustion analysis), reacted with both Dragendorff's reagent and ninhydrin and was isolated from the basic amino-acid fraction of *L. lallemandi* in a yield of 0.01% of the fresh weight of the alga. In the mass spectrum of **2** the molecular ion was not discernible, and fragments were observed at *m/z* 114 $[M-H_2O-C_2H_5S]^+$, 88 $[114-C_2H_2]^+$, 70 $[114-CO_2]^+$, and 62 $[SMe_2]^+$. Transitions 114↦88 and 114↦70 were confirmed by meta-stable peaks at *m/z* 67.93 and 42.98, respectively. The ^{13}C-nmr spectrum (D_2O) contained resonances for two methyls at δ 26.29 and 27.10, two methylenes at 36.64 (C-3) and 51.18 (C-5), two methines at 52.91 (C-2) and 64.89 (C-4), and a carboxylate carbon at 174.42 ppm. The chemical shifts of the methyls suggested that they were embodied in a dimethylsulfonium group, which was confirmed by the formation of dimethylsulfide on treatment of **2** with base. The ^{1}H-nmr spectrum (D_2O) of **2** displayed, in addition to two singlets at δ 2.96 and 2.98 ($-SMe_2$), a doublet of doublets at 3.95 (J = 5.7 and 6.5 Hz) shifted to δ 4.34 on acidification to pH 2 and assigned to the proton α to the amino acid function (H-2). This signal was homonuclearly coupled to a 2H multiplet at 2.23 (H-3), which was, in turn, interrelated with a 1H multiplet at 4.36 (H-4). The last signal was also shown to be coupled with a methylene signal at 3.55 (H-5), thus completing the proton sequence.

Treatment of **2** with MeOH in the presence of HCl afforded 5-dimethylsulfonium-4-methoxy-2-aminovaleric acid methyl ester chloride, $C_9H_{20}O_3NSCl$ (δ 3.36, s, -OMe; δ 3.89, s, -COOMe), whose mass spectrum showed diagnostically important peaks at *m/z* 207, 148, and 87 representing feasible sequential losses of $Me^{35}Cl$, COOMe, and C_2H_5S from the molecule, and also at 116 $[148-MeOH]^+$ and 62 $[SMe_2]^+$.

These data led to structure **2** for the new algal metabolite. According to the Clough-Lutz-Jirgensons (8) rule, the shift of the molecular rotation of **2** to a more positive value from H_2O to acid solution ($[M]_D$ +2.5° in H_2O and +24.3° in 5 N HCl) indicates an *S* configuration at C-2, if the rotatory contribution of the C-4 center is assumed not to be influenced by the variation of the pH. This assumption seems justified because comparison of the ^{1}H-nmr spectrum of **2** before and after addition of DCl did not show any shift of the proton at C-4.

Compound **1** is structurally related to two other algal metabolites, i.e., β-stachydrine (β-proline betaine) (9) and its 2,3-dehydro derivative (5), isolated from *Griffithsia flosculosa* and *Pterocladia capillacea*, respectively. Although all these compounds seem to be derived from β-proline, this imino acid has not been found in nature so far.

Besides compound **2**, only two sulfonium salts are known as algal metabolites. One of them, 3-dimethylsulfoniopropionate, originally isolated from the red alga *Polysiphonia fastigiata* (10), has been successively found in a number of marine algae (3), particularly in the Chlorophyceae (11,12). The other one,

4-dimethyl-sulfonio-2-methoxybutyrate, isolated for the first time from the red alga *Rytiphloea tinctoria* (6), has been also found in *Halopitys incurvus* and *Vidalia volubilis*, all belonging to the family Rhodomelaceae. Therefore, the taxonomic relevance of these sulfonium compounds should be further investigated.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ei mass spectra were obtained on an AEI MS 902 instrument at 70 eV (direct injection). ^{1}H- and ^{13}C-nmr spectra were run on an AC-250 Bruker instrument and were recorded in D_2O (without or with added CF_3COOD to pH 2) at 250 and 62.9 MHz, respectively, using trimethylsilylpropionate as internal reference. Optical rotations were measured with a Perkin-Elmer 141 polarimeter. Hptlc were run on glass precoated Si gel-F_{254} and cellulose-F_{254} plates (Merck). The following solvent systems were used: a, *n*-PrOH-HOAc-H_2O (4:1:1); b, *n*-BuOH-HOAc-H_2O (12:3:5); c, phenol-H_2O (3:1).

PLANT MATERIAL.—Thalli of *G. proteus* and *L. lallemandi* were harvested at S.M. La Scala and Acicastello, Sicily, respectively. Voucher specimens were deposited in the Herbarium of the Institute of Botany, Catania, Sicily.

EXTRACTION AND PURIFICATION OF **1**.—Freshly collected *G. proteus* (1 kg) was homogenized and extracted × 3 with 30% aqueous MeOH (2 liters each time). 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 amino acid fraction was eluted with 2 N NH_4OH; the eluate was taken to dryness and the residue dissolved in H_2O. The solution was 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 concentrated and further fractionated by preparative adsorption chromatography (LiChroprep Si-60 25–40 μm; solvent 2). The separation was monitored on hptlc (Si gel, solvent a, R_f 0.21; solvent c, R_f 0.50; cellulose, solvent b, R_f 0.40); fractions containing pure **1** were pooled and taken to dryness. The residue, dissolved in a small amount of H_2O, was freeze-dried, giving 91 mg of **1** as an off-white, hygroscopic powder: $[\alpha]^{25}D$ +14.42° (c = 0.34 in H_2O). *Anal.* calcd for $C_7H_{13}O_3N$: C 52.88, N 8.81, H 8.24%, found C 52.69, N 8.85, H 8.32; ms *m/z* (%) 141 (4.8), 97 (6.2), 96 (10.0), 82 (25.0), 58 (100), 44 (37.0), 42 (51.8).

ACETYLATION OF **1**.—Compound **1** was treated with fivefold molar excess acetyl chloride at room temperature for 2 h. After this time, the solution was taken to dryness under vacuum, and the residue contained chromatographically pure **1** monoacetate (Si gel, solvent c, R_f 0.65).

EXTRACTION AND PURIFICATION OF **2**.—*L. lallemandi* (1 kg fresh alga) was extracted and amino acid fraction isolated as described above for *G. proteus*. From this fraction, acidic amino acids were removed on column of Dowex-1 ($^-$OAc), and the aqueous eluate, concentrated to a small volume, was applied to a column of Amberlite IRC-50 (H^+). After the resin was washed with H_2O, basic amino-acid fraction containing compound **2** was recovered by elution with 2 N NH_4OH. The eluate was taken to dryness in vacuo and the residue subjected to preparative adsorption chromatography (LiChroprep Si-60 25–40 μm; solvent b). The separation was monitored by hptlc (cellulose, solvent b, R_f 0.32; Si gel, solvent b, R_f 0.07; solvent c, R_f 0.12), and the fractions containing pure **2** were pooled and taken to dryness. The residue was then dissolved in H_2O and freeze-dried to give 110 mg of **2** as an off-white, hygroscopic powder. $[\alpha]^{25}D$ +1.3° (c = 0.5 in H_2O) and $[\alpha]^{25}D$ +12.6° (c = 0.5 in 5 N HCl). *Anal.* calcd for $C_7H_{15}O_3NS$: C 43.56, H 7.83, N 7.26, S 16.61%; found C 43.37, H 7.98, N 7.25, S 16.41); in paper electrophoresis (50 V/cm) at pH 4.5 it had E_{Lys} 1.03. Ms *m/z* (%) 114 (5.0), 100 (28.9), 88 (3.0), 87 (13.2), 86 (11.1), 82 (11.8), 70 (10.7), 62 (100), 61 (32.5), 47 (59.3), 45 (52.5), 44 (12.8), 35 (32.14).

TREATMENT OF **2** WITH BASE.—A solution of **2** (30 mg) in 2 M NaOH (5 ml) was heated in a vial fitted with a PTFE-lined screw cap at 90° for 15 min. After cooling, dimethylsulfide was identified in the head space by ms (*m/z* 62).

TREATMENT OF **2** WITH MeOH/HCl.—Compound **2** was treated with 3% HCl in MeOH at room temperature for 12 h. The solution was then taken to dryness in vacuo giving crude 5-dimethylsulfonium-4-methoxy-2-aminovaleric acid methyl ester chloride: Si-gel, solvent b, R_f 0.06; cellulose, solvent b, R_f 0.43; E_{Lys} 1.26 at pH 4.5. Ms *m/z* (%) 207 (0.62), 148 (4.27), 147 (2.96), 116 (7.23), 87 (10.35), 62 (5.57), 61 (6.52), 55 (8.77), 52 (31.9), 50 (100).

ACKNOWLEDGMENTS

Our thanks are due to Prof. G. Furnari (Institute of Botany, University of Catania, Italy) for the identification of plant material. This work was in part supported by a grant from Ministero Pubblica Istruzione, Rome.

LITERATURE CITED

1. K.C. Güven, A. Bora, and G. Sunam, *Phytochemistry*, **9**, 1893 (1970).

2. S. Sciuto, R. Chillemi, M. Piattelli, and G. Impellizzeri, *Phytochemistry*, **22**, 2311 (1983).
3. G. Blunden and S.M. Gordon, *Prog. Phycol. Res.*, **4**, 39 (1986).
4. S. Sciuto, R. Chillemi, and M. Piattelli, *J. Nat. Prod.*, **48**, 602 (1985).
5. S. Sciuto, R. Chillemi, and M. Piattelli, *J. Nat. Prod.*, **51**, 322 (1988).
6. S. Sciuto, M. Piattelli, and R. Chillemi, *Phytochemistry*, **21**, 227 (1982).
7. C.A.G. Haasnoot, F.A.A.M. De Leeuw, H.P.M. De Leeuw, and C. Altona, *Biopolymers*, **20**, 1211 (1981).
8. O. Lutz and B. Jirgensons, *Chem. Ber.*, **63B**, 448 (1930).
9. G. Blunden, S.M. Gordon, W.F.H. McLean, and G.R. Keysell, *Phytochemistry*, **22**, 293 (1983).
10. F. Challenger and M.I. Simpson, *J. Chem. Soc.*, 1591 (1948).
11. F. Challenger, R. Bywood, P. Thomas, and B.J. Haywood, *Arch. Biochim. Biophys.*, **69**, 514 (1957).
12. K.W. Glombitza, *Planta Med.*, **10**, 210 (1970).

Received 14 March 1988