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THETINES AND BETAINES OF THE RED ALGA *DIGENEA SIMPLEX*

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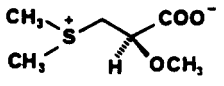
ABSTRACT.—From the aqueous extract of the red alga *Digenea simplex*, seven Dragendorff-positive compounds have been isolated and their structures determined by spectral and chemical methods. The taxonomic significance of their occurrence and possible biosynthetic relationships between some of these compounds are also discussed. Two are new metabolites that have been assigned the structures (*R*)-3-dimethylsulfonio-2-methoxypropanoate [**1**] and (*S*)-2-acetamido-5-trimethylammoniopentanoate [**2**].

Several Dragendorff-positive compounds, quaternary ammonium and tertiary sulfonium derivatives (1,2), have been isolated from algae. In the course of our continuing search for metabolites of this class from Mediterranean red algae we have examined the amino acid fraction from aqueous extracts of the red alga *Digenea simplex* (Wulf) C. Ag. (Rhodomelaceae, Ceramiales). By a combination of ion-exchange and preparative liquid chromatography, two previously unreported compounds, (+)-(*R*)-3-dimethylsulfonio-2-methoxypropanoate [**1**] and (+)-(*S*)-2-acetamido-5-trimethylammoniopentanoate [**2**], have been isolated in addition to the known thetine (2-dimethylsulfonio-ethanoate), (−)-(*S*)-4-dimethylsulfonio-2-methoxybutanoate, glycine betaine, γ-aminobutyric acid betaine (4-trimethylammoniobutanoate), and L-carnitine [(−)-(*R*)-3-hydroxy-4-trimethylammoniobutanoate].

Compound **1**, $C_6H_{12}O_3S$ (combustion analysis), was isolated in 0.001% yield of the fresh wt of the alga. Its mass spectrum showed the molecular ion at *m/z* 164 and additional fragmentations at 132 and 71, representing feasible sequential losses of MeOH and C_2H_5S from the molecule. Fragments at *m/z* 62 $[Me_2S]^+$, 58 $[CH_2{=}CH{-}OMe]^+$ and 47 $[CH_2{=}SH]^+$ were also observed.

The ^{13}C-nmr spectrum of **1** contained resonances for two methyls of a dimethylsulfonium group at δ 28.68 and 29.25, and, in addition, signals for a methylene and a methine at δ 49.55 and 80.21, respectively, and a methoxy group at δ 60.59. The spectrum was completed by the resonance of the carboxylate carbon at δ 181.36, a value decidedly downfield (ca. 5 ppm) in comparison to a "normal" carboxylate carbon of an α-methoxy or α-amino acid; the β effect of the dimethylsulfonium group explains this shift. Apart from two singlets at δ 2.93 ($-SMe_2$) and 3.38 (-OMe), the ^{1}H-nmr spectrum consisted of a three-spin ABX system, with the AB part at δ 3.56 (H_a-3) and 3.67 (H_b-3) (J_{AB} = 13.8 Hz) and the X part at 4.09 (H-2; J_{AX} = 8.1 Hz and J_{BX} = 3.9 Hz). From the above data, the structure of 3-dimethylsulfonio-2-methoxypropanoate was deduced for the new algal metabolite. Confirmation of the structure and proof of the *R* configuration



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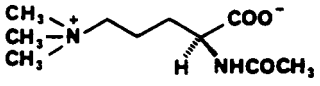

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came from demethylation (hydriodic acid) to (+)-(*R*)-3-dimethylsulfonio-2-hydroxypropanoate, identified by comparison of its chromatographic, spectral and optical properties with those of an authentic sample prepared from *S*-methyl-L-cysteine. Treatment of this latter compound with nitrous acid, which is known to react with α-amino acids to give the corresponding hydroxy acids with retention of configuration at C-2 (3), afforded (2*R*)-2-hydroxy-3-(methylsulfinyl)propanoic acid as a mixture of two epimers at the sulfur atom being formed on account of the concurrent oxidation at sulfur. The conversion of the thioether function of *S*-methyl-L-cysteine into the relevant sulfoxide, in this reaction, was in agreement with our previous work in which the thioether function of methionine had been converted into sulfoxide by treatment with nitrous acid (4). Reduction of the crude epimeric mixture with 2-mercaptoethanol gave (*R*)-2-hydroxy-3-methylthiopropanoic acid, which upon methylation with MeI (5) followed by ion-exchange chromatography led to (+)-(*R*)-dimethylsulfonio-2-hydroxypropanoate.

Compound **2**, $C_{10}H_{20}N_2O_3$ (combustion analysis), was isolated in a yield of 0.0015% of the fresh wt of the alga. Its mass spectrum contained a molecular ion at *m/z* 216 and other diagnostically important peaks at *m/z* 157 $[M - Me - CONH_2]^+$, 99 $[O{=}C{=}CH - NHCOMe]^+$, 85 $[Me_2N - CH_2 - CH{=}CH_2]^+$, 70 $[NH_2{=}CH - CH_2 - CH{=}CH_2]^+$, 58 $[Me_2N{=}CH_2]^+$, and 42 $[MeN{\equiv}CH]^+$. The ^{13}C-nmr spectrum of **2** displayed a carboxylate resonance at δ 176.62, a methine signal at 57.23 (C-2), two methylene triplets at 31.22 (C-3) and 22.13 (C-4), a methylene bound to a positively charged nitrogen at 68.82 (C-5), and a signal for three methyl groups at 55.78. The acetamide group gave resonances at δ 24.84 (*Me*-CO-NH-) and 181.04 (Me-*CO*-NH-). The ^{1}H-nmr spectrum showed, in addition to two singlets at δ 2.02 (*Me*-CONH-) and 3.09 (-NMe_3), a doublet of doublets at 4.17 (J = 3.0 and 6.9 Hz) assigned to the proton α to the amide function. A four-proton multiplet at δ 1.81 ($W/2$ = 20.7 Hz) attributable to the methylenes at C-3 and C-4 and a doublet of doublets at 3.33 (J = 5.4 and 10.2 Hz) for the protons on C-5 completed the spectrum. These data indicated the structure of 2-acetamido-5-trimethylammoniopentanoate for **2**. The chiral center was assigned the *S* configuration on the basis of the comparison of the optical rotation of **2** with that of an authentic sample prepared from α-*N*-acetyl-L-ornithine, according to Patchett and Witkop (6).

Thetine, (*S*)-4-dimethylsulfonio-2-methoxybutanoate, glycine betaine, γ-aminobutyric acid betaine, and L-carnitine have been also isolated and their identities ascertained by comparison of their spectral, chemical, and optical properties with those of authentic samples.

Compound **1** is a new addition to the meager list of algal sulfonium inner salts, which includes 3-dimethylsulfoniopropanoate (1), 4-dimethylsulfonio-2-methoxybutanoate (7), and 5-dimethylsulfonio-4-hydroxy-2-aminopentanoate (8). While the first compound occurs in a number of marine algae, the other two have only been found in species of red algae belonging to the family Rhodomelaceae (2) and therefore seem to be valid taxonomic markers. Compound **2** is the *N*-acetyl derivative of ornithine betaine, recently identified as a metabolite of the red alga *Vidalia volubilis* (Rhodomelaceae) (4). Thetine, the sulfur analogue of glycine betaine, has been suggested to play a role in biological transmethylations in animals (9) but had never been isolated before from a natural source. Glycine betaine and γ-aminobutyric acid betaine have been frequently detected in brown and red seaweeds (1,2). Finally, the occurrence in an alga of L-carnitine, a well known animal metabolite never previously isolated from plants, appears

noteworthy also in consideration of its relatively high concentration in the alga (0.01% fresh wt).

Glycine betaine is known to be a catabolite of carnitine in *Pseudomonas* sp. (10), while γ-aminobutyric acid betaine is a biosynthetic precursor of carnitine in rats (11), and it cannot be excluded that similar metabolic relationships exist in the alga. On the other hand, compound **2** could be biosynthetically related to carnitine, into which it could be converted, via γ-aminobutyric acid betaine, through deacylation, α-oxidation, and decarboxylation.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H- and ^{13}C-nmr spectra were recorded in D_2O at 250 and 62.9 MHz, respectively, on a Bruker AC-250 instrument, using sodium 3-trimethylsilyl-2,2,3,3-d_4-propanoate (TSP) as internal reference; chemical shifts are given in ppm. Mass spectra were obtained on a Fisons ZAB 2SE instrument. Optical rotations were measured on a Perkin-Elmer 141 polarimeter. Tlc and hptlc were run on glass precoated Si gel or cellulose plates (Merck) using the following solvent systems: (a) *n*-BuOH–HOAc–H_2O (12:3:5), (b) *n*-PrOH–HOAc–H_2O (12:3:5), (c) EtOH-30% NH_4OH (7:3). Spots were detected by ninhydrin or Dragendorff's reagents. Separations by preparative liquid chromatography (plc) were carried out on LiChroprep Si-60 (Merck).

MATERIALS.—Thalli of *D. simplex* were harvested at Portopalo, Sicily. Voucher specimens are kept in the Herbarium of the Institute of Botany, University of Catania. Glycine betaine and L-carnitine were purchased from Fluka. 4-Dimethylsulfonio-2-methoxybutanoate and γ-aminobutyric acid betaine were isolated from natural sources (2). Thetine was synthesized according to du Vigneaud *et al.* (9); (Si gel, solvent b, R_f 0.20; cellulose, solvent b, R_f 0.83). Spectroscopic data of this compound, not previously reported, are given here: ^{1}H nmr δ 2.87 (s, -SMe_2), 4.13 (s, -CH_2-); ^{13}C nmr δ 27.72 (-SMe_2), 51.85 (-CH_2-), 171.38 (-COO^-); eims (70 eV, 28°) *m/z* (%) $[M]^+$ 120 (28.4), $[M-CH_2=S]^+$ 74 (29.0), $[Me_2S]^+$ 62 (95.8), $[MeS=CH_2]^+$ 61 (100), $[CH_2=SH]^+$ 47 (87.6), $[SH_3]^+$ 35 (92.3).

EXTRACTION AND ISOLATION.—Fresh alga (1 kg) was carefully freed from macroscopic epibionts and then extracted three times with 30% aqueous MeOH. From the pooled extracts the total amino acid fraction was isolated by absorption on Dowex-50W (H^+) and elution with 2 N NH_4OH. Separation of this fraction into basic, neutral, and acidic amino acid subfractions was performed according to a previously reported procedure (12). Plc (LiChroprep Si-60, 25–40 μm, solvent b) of the neutral amino acid subfraction afforded pure L-carnitine (100.7 mg; Si gel, solvent b, R_f 0.34; solvent a, R_f 0.07; cellulose, solvent a, R_f 0.59) and two fractions A and B which needed further separation. Cc of fraction A on Dowex-50W (H^+; linear gradient of HCl from 0 to 2 N) afforded pure thetine (66.7 mg; Si gel, solvent b, R_f 0.20) and glycine betaine (55.4 mg; Si gel, solvent b, R_f 0.39, solvent c, R_f 0.52). Flash chromatography (Si gel, 40–63 μm, solvent b) of fraction B gave two main subfractions, B1 and B2, which were each subjected to preparative tlc (Si gel, solvent c). Compounds **1** (10 mg; Si gel, solvent c, R_f 0.62) and 4-dimethylsulfonio-2-methoxybutanoate (15 mg; Si gel, solvent c, R_f 0.47; solvent a, R_f 0.11) were isolated from subfraction B1, whereas subfraction B2 afforded pure **2** (15 mg; Si gel, solvent c, R_f 0.40).

The basic amino acid fraction was subjected to plc (LiChroprep Si-60, 25–40 μm, solvent a), and fractions containing pure γ-aminobutyric acid betaine (8 mg; Si gel, solvent a, R_f 0.04; cellulose, solvent b, R_f 0.83) were pooled and taken to dryness.

The purity of each metabolite was checked by hptlc in different solvent systems and the identity of known compounds confirmed by comparison of their chromatographic, spectroscopic, and optical properties with those of authentic samples.

(+)-(R)-3-*Dimethylsulfonio-2-methoxypropanoate* [**1**].—Compound **1** (Si gel, solvent b, R_f 0.29; cellulose, solvent b, R_f 0.77): $[\alpha]^{25}D$ +33° (c = 0.5 in H_2O); eims (70 eV, 163°) *m/z* (%) 164 (2.6), 132 (11.2), 116 (5.9), 105 (14.4), 86 (17.5), 72 (12.5), 71 (13.7), 62 (100), 61 (55.0), 58 (42.5), 47 (90.0), 35 (29.4). *Anal.* calcd for $C_6H_{12}O_3S$, C 43.88, H 7.36, S 19.52%; found C 43.81, H 7.42, S 19.44%.

TREATMENT OF **1** WITH HI.—A solution of **1** (6 mg) in 57% HI was refluxed for 3 h and then taken to dryness. The residue, dissolved in H_2O, was applied to a column of Dowex-50W (H^+) and the resin eluted with 2 N NH_4OH. The alkaline eluate was evaporated to dryness, giving a yellowish oil residue which had nmr, tlc, and optical properties identical with those of a synthetic sample of (+)-(*R*)-3-dimethylsulfonio-2-hydroxypropanoate prepared as described below.

SYNTHESIS OF (+)-(*R*)-3-DIMETHYLSULFONIO-2-HYDROXYPROPANOATE.—*S*-methyl-L-cysteine (Fluka; 500 mg) was dissolved in a mixture of H_2O (400 ml) and HOAc (36 ml) and to the solution 66 ml of 3 N $NaNO_2$ was added dropwise without stirring at 0°. The mixture reaction was kept for 2 h at room temperature and

then evaporated under reduced pressure. The residue was applied to a column of Dowex-50W (H^+) and the crude reaction product, 2-hydroxy-3-(methylsulfinyl)propanoic acid (MeSO-, δ 2.70), was recovered by elution with H_2O. The eluate was concentrated under vacuum and treated with 2-mercaptoethanol (4) to give 2-hydroxy-3-methylthiopropanoic acid, which was purified by ion-exchange on Dowex-1 ($MeCOO^-$) [^{1}H nmr δ 2.02 (s, MeS-), 2.62 (dd, J = 6.7 and 13.9 Hz, H_a-3), 2.75 (dd, J = 4.3 and 13.9 Hz, H_b-3), 4.18 (dd, J = 4.3 and 6.7 Hz, H-2); ^{13}C nmr δ 16.49 (MeS-), 39.33 (C-3), 71.75 (C-2), 176.17 (-COOH)]. Methylation of 2-hydroxy-3-methylthiopropanoic acid with MeI (5) afforded compound **1** as the hydriodide. The relevant inner salt, (+)-(*R*)-3-dimethylsulfonio-2-hydroxypropanoate, was recovered by exchange on Dowex-50W (H^+) and elution with 2 N NH_4OH [tlc Si gel, solvent a, R_f 0.13; solvent c, R_f 0.22; $[\alpha]^{25}D$ +20° (c = 0.70 in H_2O); ^{1}H nmr δ 2.96 and 2.98 (Me_2S-), 3.60 (dd, J = 7.3 and 13.4 Hz, H_a-3), 3.70 (dd, J = 4.3 and 13.4 Hz, H_b-3), 4.49 (dd, J = 4.3 and 7.3 Hz, H-2); ^{13}C nmr δ 28.80 and 28.97 (-SMe_2), 51.40 (C-3), 70.68 (C-2), 179.27 (-COO^-)].

(+)-(S)-2-Acetamido-5-trimethylammoniopentanoate [**2**].—Compound **2** (Si gel, solvent b, R_f 0.1; cellulose, solvent a, R_f 0.58, solvent b, R_f 0.74): $[\alpha]^{25}D$ +16° (c = 0.43 in H_2O); eims (70 eV, 30°) *m/z* (rel. int. %) 216 (0.5), 157 (0.6), 115 (5.6), 112 (2.9), 99 (1.8), 85 (7.8), 70 (10.5), 59 (94.1), 58 (100), 45 (84.9), 42 (62.1). *Anal.* calcd for $C_{10}H_{20}N_2O_3$, C 55.53, H 9.32, N 12.95%; found C 55.48, H 9.38, N 12.99%.

SYNTHESIS OF COMPOUND **2**.—Methylation of α-*N*-acetyl-L-ornithine (Sigma, 25 mg) with MeI in non-epimerizing conditions, according to the procedure of Patchett and Witkop (6), yielded 2-acetamido-5-trimethylammoniopentanoate as the hydriodide. The crude compound, dissolved in H_2O, was applied to a column of Dowex-50W (H^+), which was washed with H_2O and then eluted with 2 N NH_4OH. Evaporation of the eluate afforded compound **2**, which was further purified by plc (LiChroprep Si-60, 25–40 μm; solvent b) and then freeze-dried (27 mg): $[\alpha]^{25}D$ +16° (c = 0.54 in H_2O).

LITERATURE CITED

1. G. Blunden and S.M. Gordon, *Prog. Phycol. Res.*, **4**, 39 (1986).
2. S. Sciuto, R. Chillemi, R. Morrone, A. Patti, and M. Piattelli, *Biochem. Syst. Ecol.*, **17**, 5 (1989).
3. V. Zappia, C.R. Zydek-Cwick, and F. Schlenk, *J. Biol. Chem.*, **244**, 4499 (1969).
4. A. Patti, R. Morrone, R. Chillemi, M. Piattelli, and S. Sciuto, *J. Nat. Prod.*, **55**, 53 (1992).
5. G. Toennies and J.J. Kolb, *J. Am. Chem. Soc.*, **67**, 849 (1945).
6. A.A. Patchett and B. Witkop, *J. Am. Chem. Soc.*, **79**, 185 (1957).
7. S. Sciuto, M. Piattelli, and R. Chillemi, *Phytochemistry*, **21**, 227 (1982).
8. S. Sciuto, R. Chillemi, R. Morrone, A. Patti, and M. Piattelli, *J. Nat. Prod.*, **51**, 1017 (1988).
9. V. du Vigneaud, A.W. Moyer, and J.P. Chandler, *J. Biol. Chem.*, **174**, 477 (1948).
10. J. Miura-Fraboni and S. Englard, *FEMS Microbiol. Lett.*, **18**, 113 (1983).
11. T. Bohmer, *Biochim. Biophys. Acta*, **343**, 551 (1974).
12. G. Impellizzeri, S. Mangiafico, M. Piattelli, S. Sciuto, E. Fattorusso, C. Santacroce, and D. Sica, *Biochem. Syst. Ecol.*, **5**, 77 (1977).

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