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(−)-(*S*)-4-DIMETHYLSULFONIO-2-METHOXYBUTYRATE FROM THE RED ALGA *RYTIPHLOEA TINCTORIA*

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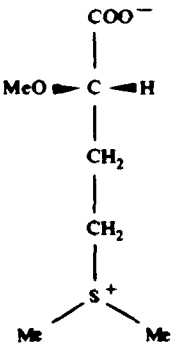
Abstract—An unusual compound (−)-(*S*)-4-dimethylsulfonio-2-methoxybutyrate, was identified in aqueous–ethanolic extracts of the red alga *Rytiphloea tinctoria.*

In the course of our continuing research on the constituents of Mediterranean algae we observed the presence of an unusual compound in the neutral amino acid fraction obtained from aqueous–EtOH extracts of the red alga *Rytiphloea tinctoria* (Clem.) C. Ag. by ion-exchange chromatography. This compound, which did not react with ninhydrin but gave a red-orange colour with Dragendorff's reagent, was isolated from the amino acid fraction by chromatography on a strongly acid cation exchange resin as a levorotatory syrupy liquid. The amphoteric nature of the compound was revealed by its behaviour on ion-exchange resins as well as by paper electrophoresis.

Elemental analysis and mass measurement (M^+ *m/z* 178) established the molecular formula $C_7H_{14}O_3S$. The IR spectrum included two bands at 1590 and 1400 cm^{-1} attributable to a carboxylate function, confirmed by formation of a methyl ester which showed the methyl ester absorption at δ 3.80. Two methyl resonances in the ^{13}C NMR spectrum (20.1 MHz, D_2O) at 23.43 and 23.73 ppm suggested a dimethylsulfonium group, confirmed by formation of dimethylsulfide upon treatment with base. The ^{13}C NMR also indicated the presence of two methylenes at 25.49 (*t*, C-3) and 39.03 (*t*, C-4), one methine at 78.20 (*d*, C-2), one methoxy group at 56.28 (*q*) and one carboxylate carbon at 175.74 ppm. The ^1H NMR (270 MHz), determined in D_2O after addition of CF_3COOD to pH 2, displayed a 1 H double double double doublet at δ 2.16 (J = 8, 8, 8.1, 15 Hz; H_aC–3), a 1 H double double double doublet at 2.37 (J = 3.9, 8, 8, 15 Hz; H_bC–3), two singlets at 2.85 and 2.86 (3 H each, SMe_2), a 2 H multiplet at *ca* 3.36 (H_2C–4) partially obscured by the overlapping resonance of a OMe group at 3.38, and a double doublet at 4.06 (1 H, J = 3.9 and 8.1 Hz; HC–2) assignable to a methine which might be linked to the carboxylate group since in the spectrum taken without added acid it appeared at δ 3.75. The above data established the sequence $-CH_2-CH_2-CH-COO^-$ thus leading to two alternative structures with only one, i.e. **1**, compatible with the observed chemical shifts. The magnetic non-equivalence of the methyl groups attached to the sulfur atom is possibly due to conformational preference of the $-SMe_2$ group with respect to the asymmetric center. Confirmation of structure **1** and proof of the *S*-configuration was obtained by treatment of **1** with hydroiodic acid which gave (−)-(*S*)-4-dimethylsulfonio-2-hydroxybutyrate identical (NMR, [α], TLC) with a synthetic sample prepared from (*S*)-methionine.



1

The survey of a number of Rhodomelaceous algae revealed that the new metabolite also occurs in *Halopitys*

incurvus (Huds.) Batt. and *Vidalia volubilis* (L.) J. Ag., both belonging to the same tribe (Amansieae) as *Rytiphloea tinctoria*. Therefore, the occurrence of **1** seems to possess a certain taxonomic value.

Very few sulfonium compounds have been so far isolated from natural sources, i.e. *S*-methylmethionine which seems to be of wide distribution in higher plants [1] and dimethyl-β-propiothetin (3-dimethylsulfonio-propionate) isolated from the red alga *Polysiphonia fastigiata* [2]. Compound **1**, as dimethyl-β-propiothetin, is possibly biogenetically related to methionine [3].

EXPERIMENTAL

Extraction and isolation. Fresh alga (500 g) was homogenized and extrd with EtOH to a final concn of 70%. The extract, clarified by centrifugation, was 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 2 M NH_4OH. The eluate was concd to a small vol. and then 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 concd and applied to a column of Dowex-50W (H^+; 3 × 85 cm) which was then eluted with a linear gradient of HCl from 0 to 0.5 M. The separation was monitored by TLC (cellulose, *n*-BuOH–HOAc–H_2O, 12:3:5, R_f 0.45; Si gel, PhOH–H_2O, 3:1, R_f 0.76; Dragendorff's reagent as chromogenic spray). Fractions containing **1**, which emerged from the column immediately after proline, were pooled and evapd to dryness. The residue was redissolved in H_2O and passed through a column of Dowex-1 (OAc^-) to remove chloride ions. The eluate was taken to dryness giving 500 mg of **1** as a colourless syrupy liquid, $[\alpha]_D^{25}$ −26.6° (*c* 1 in H_2O) (found: C 47.2, H 7.89; S 18.1. $C_7H_{14}O_3S$ requires C 47.17; H 7.91; S 17.98%); by paper electrophoresis it had the following mobilities relative to lysine: 0.21 in pyridine–HCO_2H pH 4.5 and 0.51 in HCO_2H pH 2.4.

Methyl ester chloride. (MeOH–HCl): δ (60 MHz, D_2O) 4.15 (1 H, *dd*, J = 4.5 and 7.5 Hz); 3.80 (3 H, *s*); 3.41 (3 H, *s*); 3.39 (2 H, *m*); 2.90 (6 H, *s*); 2.27 (2 H, *m*). MS *m/z* 178 (M^+ − MeCl), 146 (M^+ − MeCl–MeOH), 119 (M^+ − MeCl–COOMe), 71 (C_4H_7O), 61 (C_2H_5S), 52 ($Me^{37}Cl$), 50 ($Me^{35}Cl$).

Treatment of **1** *with base.* A soln of **1** (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).

Synthesis of (−)-(S)-4-*dimethylsulfonio*-2-*hydroxybutyrate.* Methylation of methionine with MeI in HOAc–HCO_2H according to ref. [4] gave (3-amino-3-carboxypropyl)dimethylsulfonium iodide (84%), $[\alpha]_D^{25}$ +16.8° (*c* 1 in H_2O). ^1H NMR (60 MHz, D_2O) δ 3.83 (1 H, *t*, J = 6 Hz), 3.45 (2 H, *m*), 2.92 (6 H, *s*), 2.25 (2 H, *m*); ^{13}C NMR (20.1 MHz, D_2O) δ 172.04 (C-1), 52.76 (C-2), 39.65 (C-4), 25.09 (C-3), 25.09 (2C, SMe_2). This compound was converted into the sulfate by absorption on a weakly acid cation exchange resin, followed by elution with H_2SO_4. The sulfate was treated with HNO_2 according to ref. [5] to give (−)-(*S*)-4-dimethylsulfonio-2-hydroxybutyrate (78%), isolated by absorption on and elution (2 N NH_4OH) from Dowex-50W (H^+); $[\alpha]_D^{25}$ −18.4° (*c* 1 in H_2O). ^1H NMR (60 MHz, D_2O) δ 4.30 (1 H, *dd*, J = 5 and 7 Hz), 3.43 (2 H, *t*, J = 7.5 Hz), 2.92 (6 H, *s*), 2.25 (2 H, *m*); ^{13}C NMR (20.1 MHz, D_2O) δ 179.30 (C-1), 70.38 (C-2), 40.25 (C-4), 24.89 (C-3), 23.25 2C, SMe_2). TLC: cellulose, *n*-BuOH–HOAc H_2O (12:3:5, R_f 0.36); Si gel, PhOH–H_2O (3:1, R_f 0.67).

Treatment of **1** *with* HI *to produce* (−)-(S)-4-*dimethylsulfonio*-2-*hydroxybutyrate.* A soln of **1** (75 mg) in 57% HI was refluxed for 3 hr and then taken to dryness. The residue was dissolved in H_2O and applied to a column of Dowex-50W (H^+). After washing with H_2O, the column was eluted with 2 M NH_4OH. The alkaline eluate was evapd to dryness giving a syrupy residue (37 mg) which had physical properties (NMR, $[\alpha]_D^{25}$, TLC) identical with those of a synthetic sample of (−)-(*S*)-4-dimethylsulfonio-2-hydroxybutyrate.

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