Biosynthetic Relationships Between Sulfonium and N-Methylated Compounds in the Red Alga Vidalia volubilis

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BIOSYNTHETIC RELATIONSHIP BETWEEN SULFONIUM AND *N*-METHYLATED COMPOUNDS IN THE RED ALGA *VIDALIA VOLUBILIS*

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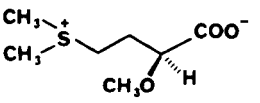
ABSTRACT.—Tracer experiments have been carried out on *Vidalia volubilis*, whose hydrophilic fraction contains (*S*)-4-dimethylsulfonio-2-methoxybutanoate [**1**], (*S*)-*N*-methylaspartic acid [**2**], and 2-amino-5-trimethylammoniopentanoate [**3**], a betaine previously unreported in nature. The results have shown that 4-dimethylsulfonio-2-methoxybutanoate can act as methyl donor in the biosynthesis of *N*-methylaspartic acid but not in that of 2-amino-5-trimethylammoniopentanoate.

In marine red algae methylsulfonium salts frequently occur, often in association with *N*-methyl compounds (1,2). Previous works on *Chondria coerulescens* (Rhodomelaceae) have shown that this alga elaborates 3-dimethylsulfoniopropanoate (dimethyl-β-propiothetin, DMP) and *S*-methylmethionine (SMM), along with 4-hydroxy-*N*-methylproline (HMP) and 4-trimethylammoniobutanoate (γ-aminobutyric acid betaine, GABAB). When the alga was fed with [$^{14}CH_3$]DMP, HMP specifically labeled at the *N*-methyl group was isolated whereas no incorporation of tracer was observed into GABAB. In a parallel experiment, after administration of [$^{14}CH_3$]SMM no radioactivity was detected in either of the *N*-methyl compounds present in the alga (3). From this result the hypothesis that in marine red algae sulfonium compounds can act as methyl donors in transmethylation reactions leading to *N*-methylated compounds has been put forward.

In the present study we report the results obtained with another rhodomelaceous seaweed, *Vidalia volubilis* (L.) J. Ag. (Rhodomelaceae, Ceramiales), which is reported to contain an *S*-methyl compound, (−)-(*S*)-4-dimethylsulfonio-2-methoxybutanoate [**1**] (DMMB) (4). Preliminary re-examination of the hydrophilic fraction of this alga revealed the presence of two *N*-methyl compounds, namely (+)-(*S*)-*N*-methylaspartic acid [**2**] (MAsp) (5), and a betaine previously unreported as natural product. To this compound the structure of 2-amino-5-trimethylammoniopentanoate [**3**] (ATMP) has been assigned on the basis of its spectroscopic features (see Experimental).

To investigate whether also in *V. volubilis* *S*-methyl compounds can serve as methyl



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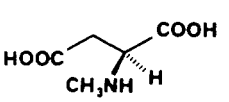

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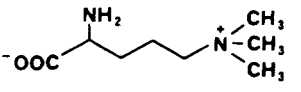

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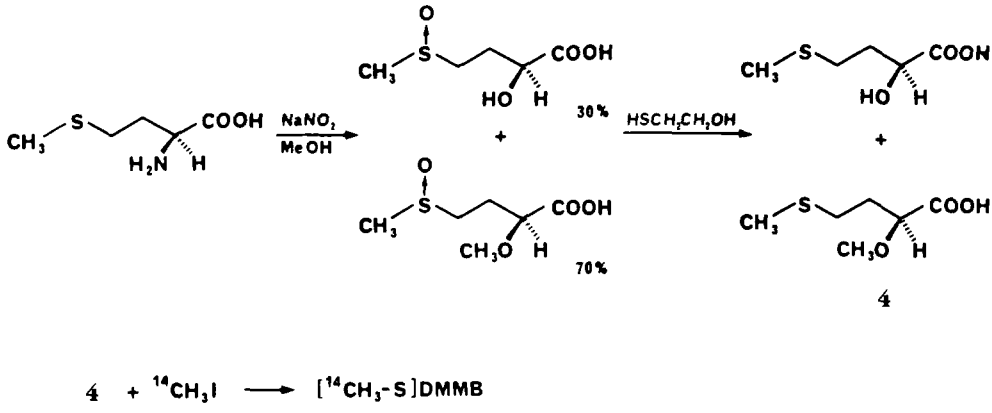

SCHEME 1

sources in the biosynthesis of *N*-methyl compounds, [$^{14}CH_3$-*S*]DMMB was needed as a presumptive precursor, and it was synthesized as illustrated in Scheme 1. L-Methionine in MeOH was reacted with nitrous acid to give 2-methoxy-4-(methylsulfinyl)butanoic acid and lesser amounts of 2-hydroxy-4-(methylsulfinyl)butanoic acid. The conversion of the thioether function of methionine into the relevant sulfoxide, in this reaction, was not surprising considering previous work in which thioethers had been converted into sulfoxides by treatment with various nitrogen oxides (6,7). The crude mixture of the two sulfoxides was reduced with 2-mercaptoethanol to yield 2-methoxy-4-methylthiobutanoic acid [**4**] and 2-hydroxy-4-methylthiobutanoic acid, which were separated by cc. Pure **4** was methylated with [^{14}C]methyl iodide to give [$^{14}CH_3$-*S*]-DMMB·HI, from which the inner salt was obtained by ion-exchange chromatography.

After experiments to determine the conditions for optimal incorporation of tracer into the *N*-metabolites, [$^{14}CH_3$-*S*]DMMB was administered to freshly collected thalluses of *V. volubilis* in sterile sea-water, using the incubation apparatus described in previous work (3). At the end of the incubation period DMMB, MAsp, and ATMP were isolated, purified by chromatography, and checked for chemical purity by high performance tlc. The absence of contaminating radioactive material was confirmed by 2D tlc and subsequent autoradiography.

After isolated compounds were quantified by ^{1}H-nmr spectroscopy, their radioactivity was determined by liquid scintillation counting (3).

Incorporation of label into MAsp, but not ATMP, was observed (Table 1). Degra-

TABLE 1. Distribution of Radioactivity in Different *N*-Methyl Compounds from *Vidalia volubilis* Supplied with 4-[$^{14}CH_3$]dimethylsulfonio-2-methoxybutanoate ([$^{14}CH_3$S]DMMB).[a]

Metabolite	Administered [$^{14}CH_3$S]DMMB[b] (103.15 μCi/mM)		
	Inc. %	Sp. Inc. %	Total wt (mg)
4-Dimethylsulfonio-2-methoxybutanoate	2.50	0.368	110.2
N-Methylaspartic acid	0.039	0.023	22.8
2-amino-5-trimethylammoniopentanoate	n.i.[c]	n.i.	5.1

[a]Inc. % = % absolute incorporation, Sp. Inc. % = % specific incorporation. Total wt is the total amount of metabolite isolated from 40 g (fresh wt) of alga after feeding experiment.

[b]Total activity 9.4 μCi.

[c]n.i. = no incorporation.

dation of labeled MAsp via exhaustive methylation followed by Hofmann degradation indicated that essentially all the ^{14}C was located at the methyl group.

Moreover, as a control of the efficiency of the isolation and purification procedure, in a separate experiment [$^{14}CH_3$-*S*]DMMB was added to an aqueous EtOH extract of the alga and the extract subjected to the same isolation procedure as in the feeding experiment. No radioactivity was observed in either MAsp or ATMP.

These data and the previous observations on *C. coerulescens* indicate that methylsulfonium salts occurring in marine algae can act as methyl sources in the biosynthesis of *N*-methylated compounds. Furthermore, the fact that following administration of a certain radioactive *S*-Me compound only some of the *N*-Me metabolites incorporate the label indicates that in the red algae examined transmethylation has a considerable donor-acceptor specificity.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^1H- and ^{13}C-nmr spectra were recorded at 250 and 62.9 MHz, respectively, on a Bruker AC-250 instrument. Unless otherwise stated, D_2O was used as solvent and sodium 3-trimethylsilyl-2,2,3,3,-d_4-propanoate (TSP) as internal reference. Mass spectra were obtained on an AEI MS 902 instrument. Optical rotations were measured on a Perkin-Elmer 141 polarimeter. High performance tlc (hptlc) was run on glass-precoated HPTLC Si gel-F_{254} or cellulose plates (Merck) using the following solvent systems: (a) *n*-BuOH–HOAc–H_2O (12:3:5), (b) *n*-hexane–EtOAc–HOAc (70:30:1), (c) *n*-PrOH–HOAc–H_2O (12:3:5), and (d) EtOH– 30%NH_4OH (7:3). Ninhydrin, Dragendorff's, or iodoplatinate reagent (8) was used for spot detection. Autoradiograms were prepared by exposing the tlc plates (Si gel) to Kodak X-OMAT AR-2 film for 2 weeks. Radioactivity measurements were carried out with a Beckmann LS-1801 liquid scintillation counter in Ready Safe scintillation cocktail (Beckmann).

[^{14}C]MeI, purchased from NEN Radiochemicals, had specific activity of 6.2 mCi/mM. The reported specific activity for [^{14}C]MeI is as given by the supplier, and the purity of this compound was not checked prior to use. When required, it was diluted with cold material to the desired specific activity.

PLANT MATERIAL.—Thalluses of *V. volubilis* were collected near Catania, Sicily. Voucher specimens are kept at the University Herbarium, Institute of Botany, Catania.

ISOLATION OF 2-AMINO-5-TRIMETHYLAMMONIOPENTANOATE [**3**] FROM *VIDALIA VOLUBILIS*.—Alga (200 g fresh wt) was extracted with 70% aqueous EtOH, and 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 subfractions was performed according to a procedure reported previously (9). (−)-(*S*)-4-Dimethylsulfonio-2-methoxybutanoate and (+)-(*S*)-*N*-methylaspartic acid were found in neutral and acidic fractions, respectively. A careful examination of the basic fraction revealed the presence of a constituent previously unreported in nature (0.0125% of fresh wt). This constituent was isolated by preparative tlc (Si gel, solvent c, R_f 0.09). The ^{13}C-nmr spectrum of this compound displayed a carboxyl (carboxylate) resonance at δ 177.15 (C-1), an α-amino acid methine at 57.02 (C-2), two methylene signals at 21.65 and 30.15 (C-4 and C-3 respectively), a methylene bound to a positively charged nitrogen at 68.49 (C-5), and a three-methyl signal at 55.88 (Me_3N-, $^1J[^{14}N, ^{13}CH_3] = 4.1$ Hz). In the ^1H-nmr spectrum resonances were seen at δ 1.94 (m, $W_{1/2} = 15.8$ Hz, H-3 and H-4), 3.10 (s, MeN-), 3.35 (t, $J = 7.6$ Hz, H-5), and 3.68 (t, $J = 5.4$ Hz, shifted to 3.99 on acidification to pH 2, H-2). From these data the structure of 2-amino-5-trimethylammoniopentanoate has been assigned to the new compound. In agreement with what is known for betaines (10), the mass spectrum did not show the molecular ion and displayed fragments deriving from pyrolytic products, particularly those originated by an intermolecular Me transfer from the quaternary nitrogen to the carboxylate function. Thus, diagnostically important peaks were observed at *m/z* (%) 142 (4.19) $[M-MeOH]^+$, 115 (3.49) $[M-COOMe]^+$, 84 (27.90) $[Me_2N{=}CH{-}CH{=}CH_2]^+$, 70 (27.85) $[CH_2{=}CH{-}CH_2{-}CH{=}NH_2]^+$, 59 (20.90) $[Me_3N]^+$, 58 (100) $[Me_2N{=}CH_2]^+$, and 42 (37.21) $[Me{-}N{\equiv}CH]^+$.

LABELED PRECURSOR.—Synthesis of (−)-(*S*)-4-[$^{14}CH_3$]dimethylsulfonio-2-methoxybutanoate ([$^{14}CH_3$-*S*]-DMMB) was performed as follows. L-Methionine (500 mg) was dissolved in a mixture of MeOH (390 ml) and HOAc (35.3 ml), and to the ice-cooled solution 65 ml of 3 N $NaNO_2$ in MeOH was added dropwise and without stirring. After keeping at room temperature for 1 h, the solution was concentrated and applied to a column of Dowex-50W (H^+). The resin was eluted with H_2O, and the eluate taken to dryness to give 260 mg of a 7:3 mixture of 2-methoxy-4-(methylsulfinyl)butanoic acid (^1H-nmr δ 2.69, s, MeSO-; 3.40, s, -OMe; H-2 split in two triplets at δ 4.08 and 4.11, $J = 4.50$ Hz and $J = 4.45$ Hz re-

spectively, due to the presence of the two diastereomers) and 2-hydroxy-4-(methylsulfinyl)butanoic acid (^{1}H-nmr δ 2.70, s, MeSO-; two triplets at δ 4.41, J = 4.55 Hz, and 4.44, J = 4.50 Hz, for H-2 of the two diastereomers). The crude mixture dissolved in H_2O (100 ml) was treated with 2-mercaptoethanol (5 ml), and the solution was kept at 80° for 4 h. After concentration in vacuo the reaction mixture was applied to a column of Dowex-1 (OH^-) which was washed with H_2O. Elution with 2 N HOAc gave a mixture of 2-methoxy-4-methylthiobutanoic acid [**4**] and lesser amounts of 2-hydroxy-4-methylthiobutanoic acid, which were separated by plc (LiChroprep Si-60 40–63 μm, solvent b). Fractions containing pure **4** (hptlc, Si gel, solvent a, R_f 0.80; solvent b, R_f 0.33) were pooled, taken to a small volume, and freeze-dried to give 75 mg of a yellowish oil, $[\alpha]^{25}D$ −35° (c = 0.79, H_2O). The ^{1}H-nmr spectrum of **4** showed a complex multiplet at δ 2.04 ($W_{1/2}$ = 37.5 Hz, H-3) partially overlapped to a singlet at δ 2.08 (s, MeS-). Other resonances were observed at δ 2.59 (t, J = 7.35 Hz, H-4), 3.38 (s, -OMe), and 4.05 (dd, J = 4.5 and 7.8 Hz, H-2). The ^{13}C-nmr spectrum displayed signals at δ 17.06 (MeS-), 31.65 (C-3), 34.31 (C-4), 58.28 (-OMe), 81.52 (C-2), and 179.32 (C-1). The eims showed peaks at m/z (%) 164 (32.7) $[M]^+$, 146 (16.4) $[M-H_2O]^+$, 119 (6.7) $[M-COOH]^+$, 116 (2.76) $[M-MeSH]^+$, 103 (17.45) $[M-61]^+$, 90 (24.7) $[M-MeS\text{-}CH{=}CH_2]^+$, 75 (22.5) $[90-Me]^+$, 71 (20.0) $[CH_2{=}CH\text{-}CH{=}OMe]^+$, and 61 (100) $[MeS{=}CH_2]^+$. Methylation of **4** (50 mg) using [^{14}C]MeI (80 μl, specific activity 178.51 μCi/mM) in a mixture of HCOOH and HOAc, according to Toennies and Kolb (11), gave [$^{14}CH_3$-*S*]DMMB.HI, from which the inner salt was obtained by absorption on Dowex-50 W (H^+) followed by elution with 2 N NH_4OH. Treatment of [$^{14}CH_3$-*S*]DMMB with an aqueous solution of $H_2PtCl_6{\cdot}6H_2O$ and recrystallization from EtOH/H_2O to constant specific activity (103.15 μCi/mM) gave 67.3 mg of a pale orange product which decomposed between 112° and 120° depending on the rate of heating. From the platinichloride, [$^{14}CH_3$-*S*]DMMB was recovered by ion-exchange chromatography on Dowex-50W (H^+).

FEEDING AND ISOLATION TECHNIQUES.—The incubation apparatus described in a previous work (3) was used. Alga (40 g), transferred to the laboratory in thermostatted sea-water immediately after collection, was freed from extraneous macroscopic organisms by hand sorting, rinsed with sea-water (sterilized by ultrafiltration on 0.22 μm membrane filter), and put into the holder of the incubation apparatus which was immersed in sterile sea-water. The experimental conditions were as follows: temperature 16°, photoperiod 12:12, incubation time 24 h. ^{14}C-Labeled precursor (16.2 mg), dissolved in a small amount of H_2O, was added to the incubation liquid. After the incubation had been quenched, the plant was thoroughly rinsed with sea-water and blotted with filter paper. The alga was extracted with 70% aqueous EtOH, and the total amino acid fraction, isolated by ion-exchange chromatography on Dowex-50W (H^+), was loaded onto a column of Dowex-1 ($^-$OAc). Elution with H_2O gave a mixture of neutral and basic amino acids (fraction A), the acidic amino acids (fraction B) being eluted with 2 N HOAc. Fraction A was further separated into two subfractions containing neutral (A1) and basic amino acids (A2) by cc on Amberlite IRC-50 (H^+) and elution with H_2O and 2 N NH_4OH, respectively. Subfraction A1 was chromatographed on Dowex-50W (H^+; linear gradient of HCl from 0.5 to 1.7 N) to give [$^{14}CH_3$]DMMB·HCl (hptlc, Si gel, solvent a, R_f 0.11; solvent d, R_f 0.40). ATMP was recovered from subfraction A2 by preparative tlc (Si gel, solvent c). Fraction B by cc on Dowex-1 ($^-$OAc; linear gradient of HOAC from 0 to 0.5 N) afforded [$^{14}CH_3$]MAsp (hptlc, Si gel, solvent c, R_f 0.35; cellulose, solvent a, R_f 0.40). Radiochemical purity was checked by 2D tlc (solvent a followed by d) and subsequent autoradiography. A single spot was always observed, corresponding to the metabolite by coloration (revealed by a spray reagent). For quantitative determination, a solution in D_2O of each metabolite was added with a known amount of TSP and the ^{1}H-nmr spectrum recorded. From the ratio of the area of the TSP peak to that of *S*- or *N*-Me resonance, the amount of the isolated compound was easily determined. An aliquot of the solution was then counted in liquid scintillation cocktail (3).

CONTROL EXPERIMENT.—To check the validity of the separation procedure described above, another batch of [$^{14}CH_3$-*S*]DMMB (12.3 mg; specific activity 110.70 μCi/mM) was synthesized and added to the aqueous EtOH extract of the alga (40 g fresh wt). The resulting mixture was processed by the purification protocol described above; isolated compounds **2** and **3** were free of radioactivity.

DEGRADATION OF METABOLIC METHYLASPARTIC ACID.—Radioactive MAsp (12.5 mg) dissolved in MeOH was permethylated with MeI and Ag_2O (12). The corresponding betaine has been reported to undergo Hofmann degradation in very mild conditions (13). Therefore, after removal of the insoluble material by filtration, the reaction mixture was added with 40% aqueous NaOH at room temperature and the evolved Me_3N trapped in CF_3COOH. After the amine was quantified by ^{1}H nmr against a known amount of added TSP, the solution was taken to pH 7 and its radioactivity determined by liquid scintillation counting (3). MAsp isolated after administration of [$^{14}CH_3$-*S*]DMMB had specific activity 23.7 nCi/mM, and [^{14}C]Me_3N obtained following alkali degradation contained essentially the total label (specific activity 20.6 nCi/mM).

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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. R. Chillemi, A. Patti, R. Morrone, M. Piattelli, and S. Sciuto, *J. Nat. Prod.*, **53**, 87 (1990).
4. S. Sciuto, M. Piattelli, and R. Chillemi, *Phytochemistry*, **21**, 227 (1982).
5. S. Sciuto, M. Piattelli, and R. Chillemi, *Phytochemistry*, **18**, 1058 (1979).
6. L. Horner and F. Hübenett, *Ann.*, **579**, 193 (1953).
7. C.C. Addison and J.C. Sheldon, *J. Chem. Soc.*, 2705 (1956).
8. I.M. Hais and K. Macek, "Paper Chromatography, A Comprehensive Treatise," 3rd ed., Academic Press, New York, 1963, pp. 800–801.
9. G. Impellizzeri, S. Mangiafico, M. Piattelli, S. Sciuto, E. Fattorusso, C. Santacroce, and D. Sica, *Biochem. Syst. Ecol.*, **5**, 77 (1977).
10. K. Undheim and T. Laerum, *Acta Chem. Scand.*, **27**, 589 (1973).
11. G. Toennies and J.J. Kolb, *J. Am. Chem. Soc.*, **67**, 849 (1945).
12. A.A. Patchett and B. Witkop, *J. Am. Chem. Soc.*, **79**, 185 (1957).
13. J.P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," John Wiley and Sons, New York, 1961, p. 2766.

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