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SHORT REPORTS

THE IDENTIFICATION OF 4-HYDROXY-*N*-METHYLPROLINE IN THE RED ALGA *CHONDRIA COERULESCENS*—SPECTRAL INFORMATION

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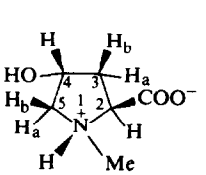
Key Word Index—*Chondria coerulescens*; Rhodomelaceae; red alga; amino acid; (−)-(2*S*,4*R*)-1-methyl-4-hydroxypyrrolidine-2-carboxylic acid; 4-hydroxy-*N*-methylproline.

Abstract—4-Hydroxy-*N*-methylproline, an unusual amino acid which has been reported in only a few species of higher plants, has been identified in the aqueous extract of the red alga *Chondria coerulescens*. Spectral features of this compound are discussed in detail.

In previous work [1–3], we examined the amino acid composition of several species of red algae. In the course of this study, the GC profile of the amino acid fraction from the red alga *Chondria coerulescens* (Rhodomelaceae, Ceramiales) showed the presence of a large amount of an unusual compound (the area of its GC-peak was near 50% of the total area of the peaks). This compound, which was ninhydrin-negative but reacted with Dragendorff's and iodoplatinate reagents, was isolated by ion-exchange chromatography (in a yield of 0.23% dry wt) and identified as (−)-(2*S*,4*R*)-1-methyl-4-hydroxypyrrolidine-2-carboxylic acid (4-hydroxy-*N*-methylproline, **1**) by comparison with a synthetic sample obtained according to Suyama and Kanao [4] by monomethylation of 4-hydroxy-*trans*-L-proline.

Compound **1**, which has been never found in algae, has been previously reported in higher plants, i.e. *Croton gobouga* [5] and *Afrormosia elata* [6]; assignment of the structure was mainly based on its conversion to the corresponding betaine, namely betonicine, and no spectral data were reported. We wish to describe here the spectral features of this amino acid. The mass spectrum (70 eV) shows fragment ions at *m/z* 100 and 82 for sequential losses of carboxyl and water from the parent ion (*m/z* 145) and at *m/z* 42 for Me–$\overset{+}{N}$ ≡ CH. The ^{1}H NMR spectrum of **1** (270 MHz, D_2O) contained, in addition to a three-proton singlet (N–Me) at δ2.96, an ABMX system for H-3a, H-3b (AB part), H-2 (M) and H-4 (X), whose X multiplet was further split by the A′M′X pattern involving H-4, H-5a and H-5b. The chemical shifts (δ scale) and coupling constants (Hz), obtained from this analysis with the aid of extensive double resonance experiments, are:



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(a) for the ABMX system $\delta_{H\text{-}3a}$ 2.43, $\delta_{H\text{-}3b}$ 2.20, $\delta_{H\text{-}2}$ 4.05 and $\delta_{H\text{-}4}$ 4.48 ($J_{3a,\,3b}$ = 13.5 Hz, $J_{3a,\,2}$ = 7.5 Hz, $J_{3b,\,2}$ = 10.5 Hz, $J_{3a,\,4}$ = 1.8 Hz and $J_{3b,\,4}$ = 4.8 Hz); and (b) for the A′M′X system $\delta_{H\text{-}5a}$ 3.10, $\delta_{H\text{-}5b}$ 3.82 ($J_{5a,\,5b}$ = 12.6 Hz, $J_{5a,\,4}$ = 2.1 Hz and $J_{5b,\,4}$ = 4.8 Hz). After addition of CF_3COOH to pH 2, the signal at δ4.05 was shifted to δ4.40 as expected for a proton α to the amino acid function. When TFA was used as solvent, the N–Me resonance split into a doublet (δ3.04, *J* = 5 Hz) arising through coupling with an $\overset{+}{N}$–H proton [7]; the small downfield shift of the N–Me signal in strong acid indicates that, in neutral aqueous solution, **1** exists mainly as an inner salt.

It is to be noted that, while in *trans*-4-hydroxyproline, whose ^{1}H NMR spectrum has been analysed in detail by Abraham *et al.* [8–10], protons at C-5 have very similar chemical shifts, in compound **1** the effect of the *N*-methyl group, deshielding on the *trans* proton and shielding on the *cis* proton [11], introduces a difference of δ0.72 between the chemical shifts of H-5a and H-5b; consequently, the A′B′X pattern in *trans*-4-hydroxyproline is replaced by an A′M′X system in **1**. Moreover, that the molecule of **1** assumes preferentially the conformation in which carboxylate and methyl groups bear a *trans* relationship to each other (anticipated on the basis of steric factors [12]) is evidenced by the chemical shifts of the C-5 protons and the relevant vicinal coupling constants.

The ^{13}C NMR of **1** (20.1 MHz, D_2O) displayed a singlet at δ170.2 (COO^-), two doublets at 69.7 (C-2) and 69.1 (C-4), two triplets at 62.4 (C-5) and 38.7 (C-3) and a quartet at 43.4 (N–Me). The hydrochloride salt of **1** showed a ^{13}C NMR spectrum in which the resonances of C-2 and COO^- were shifted upfield (at δ67.7 and 168.1 respectively) as the result of the protonation of the carboxylate group [13].

In previous work [1, 2] we observed that several red algae of the Rhodomelaceae contained exceptionally high quantities of free proline, differing from other species in the same family, among them *Chondria coerulescens*. It has also been observed [3] that the ability to accumulate this amino acid is independent of the phenological state or

variations in habitat and shows only a partial correlation with taxonomic groupings within the family.

Since *Chondria coerulescens* contains large amounts of 4-hydroxy-*N*-methylproline, it is possible that other Rhodomelaceous species which do not show high levels of proline might accumulate metabolites biogenetically related to this amino acid. Further work is needed to support this hypothesis.

EXPERIMENTAL

Plant material. Samples of *C. coerulescens* were harvested in different stations of the coast of Catania. The frozen alga was transferred to the laboratory and immediately freeze-dried. Voucher specimens are retained in the University Herbarium (Istituto di Botanica, Università di Catania).

Extraction and isolation. Dry alga (corresponding to 500 g fr. wt) was homogenized and extracted with 70% aq. EtOH (1 l. × 3). The extracts were pooled, clarified by centrifugation, concd *in vacuo* and filtered through an Amicon CF50 Ultrafilter. The filtrate was then applied to a column of Dowex 50 W (H^+) and, after the resin was washed with H_2O, the amino acid fraction containing **1** was eluted with 2 N NH_4OH; the eluate was taken to dryness and the residue dissolved in H_2O. The soln was then passed successively through columns of Dowex-1 (^-OAc) and Amberlite IRC-50 (H^+) to remove acidic and basic amino acids respectively. The aq. eluate, concd to a small vol., was fractionated on a column of Dowex 50 W (H^+; 3 × 95 cm) with a linear gradient of HCl from 0 to 1 N (2 l.). The separation was monitored by GC and TLC. GC analyses were achieved, after derivatization of amino acids into *N*-trifluoroacetyl *n*-butyl esters, using a glass column packed with EGA [14, 15]. The R_t relative to Ala for compound **1** was 1.55. TLC analyses were run on precoated Si gel plates, using the following solvent systems: (1) *n*-BuOH–HOAc–H_2O (12:3:5), R_f 0.23; and (2) *n*-PrOH–NH_4OH (7:3), R_f 0.42. Ninhydrin, iodoplatinate and Dragendorff's reagent were used as chromogenic sprays. Fractions containing **1** hydrochloride were pooled and taken to dryness. Recrystallization from EtOH gave **1** hydrochloride as colourless crystals which, after drying at 110° for 2 hr, had mp 183–184° (dec.) (lit. 181–183° [6]), $[\alpha]_D^{25}$ −58.9° (H_2O; *c* 1.2) [lit. −55° (MeOH; *c* 2.3) [6]]. (Found: C, 39.5; H, 6.8; N, 7.6; Cl, 20.0. Calc. for $C_6H_{11}NO_3$.HCl: C, 39.6; H, 6.7; N, 7.7; Cl, 19.6%.) IR ν_{max}^{KBr} cm^{-1}: 3160 (–OH) and 1720 (>C=O). The hydrochloride of **1**, dissolved in H_2O, was then charged on a column of Dowex 50 W (H^+) and HCl was removed by washing with H_2O. The column was then eluted with 2 N NH_4OH and the eluate was taken to dryness to give 200 mg **1**. After crystallization from MeOH, **1** had mp 238° (dec.) [lit. 238–240° (dec.) [6]; 242° [5]], $[\alpha]_D^{25}$ −82.7° (H_2O; *c* 1.1) (lit. −86.6° (H_2O; *c* 1.5) [6], −84.9° (H_2O; *c* 4.9) [5]). EIMS (probe) 70 eV, *m/z* (rel. int.): 145 $[M]^+$ (3.1), 100 $[M - {}^+_-COOH]^+$ (100), 82 $[M - COOH - H_2O]^+$ (46.3), 42 $Me - \overset{+}{N} \equiv CH$ (24.1).

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REFERENCES

1. Impellizzeri, G., Mangiafico, S., Oriente, G., Piattelli, M., Sciuto, S., Fattorusso, E., Magno, S., Santacroce, C. and Sica, D. (1975) *Phytochemistry* **14**, 1549.
2. Impellizzeri, G., Mangiafico, S., Piattelli, M., Sciuto, S., Fattorusso, E., Santacroce, C. and Sica, D. (1977) *Biochem. Syst. Ecol.* **5**, 77.
3. Sciuto, S., Piatteli, M., Chillemi, R., Cormaci, M. and Furnari, G. (1980) *Phytochemistry* **19**, 2751.
4. Suyama, T. and Kanao, S. (1965) *Yakugaku Zasshi* **85**, 284.
5. Goodson, J. A. and Clewer, H. W. B. (1919) *J. Chem. Soc.* 923.
6. Morgan, J. W. W. (1964) *Chem. Ind. (London)* 542.
7. Ma, J. C. N. and Warnhoff, E. W. (1965) *Can. J. Chem.* **43**, 1849.
8. Abraham, R. J. and McLauchlan, K. A. (1962) *Mol. Phys.* **5**, 195.
9. Abraham, R. J. and McLauchlan, K. A. (1962) *Mol. Phys.* **5**, 513.
10. Abraham, R. J. and Thomas, W. A. (1964) *J. Chem. Soc.* 3739.
11. Andreatta, R. H., Nair, V. and Robertson, A. V. (1967) *Aust. J. Chem.* **20**, 2701.
12. Becconsall, J. K., Jones, R. A. Y. and McKenna, J. (1965) *J. Chem. Soc.* 1726.
13. Horsley, W. J. and Sternlicht, H. (1968) *J. Am. Chem. Soc.* **90**, 3738.
14. Roach, D. and Gehrke, C. W. (1969) *J. Chromatogr.* **44**, 269.
15. Roach, D. and Gehrke, C. W. (1969) *J. Chromatogr.* **43**, 303.