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N-METHYL-L-ASPARTIC ACID FROM THE RED ALGA *HALOPYTIS INCURVUS*

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In the course of a continuing study of the free amino acids of Mediterranean algae, we observed the presence of an unusual amino acid (ninhydrin-purple) in the acidic amino acid fraction from thalli of the red seaweed *Halopytis incurvus* (Huds.) Batt. This compound, $C_5H_9NO_4$, isolated by ion-exchange chromatography in a yield of 0.00003% fr. wt of the alga, gave positive colour reactions for *N*-alkylamino acids (blue with alkaline nitroprusside in the presence of acetaldehyde and red with *p*-nitrobenzoyl chloride in pyridine). Its ^1H NMR spectrum (D_2O) exhibited a one-proton triplet ($J = 5$ Hz) at δ 3.88 which was shifted to δ 4.42 on acidification to pH 2 (proton α to the amino acid function), a three-proton singlet at δ 2.75 (N—Me) and a two-proton doublet ($J = 5$ Hz) at δ 2.97 (—CH_2—). These results suggested that the isolated compound was *N*-methylaspartic acid. The values of the optical rotation both in water and 6 N HCl were in close agreement with those reported for *N*-methyl-L-aspartic acid [1, 2]. Confirmatory evidence for the identity of the algal amino acid came from the comparison of its physical properties (mp, $[\alpha]$, ^1H NMR) as well as chromatographic behaviour with those of an authentic sample. To our knowledge this is the first reported natural occurrence of *N*-methyl-L-aspartic acid.

EXPERIMENTAL

Plant material. Separate samples of *Halopytis incurvus* were harvested in different localities of the east coast of Sicily (Portopalo, Castelluccio and Pozzillo) and examined individually.

Extraction and isolation. In a typical isolation, the crushed alga (1 kg fr. wt) was extracted ×3 with 70% EtOH and the filtered extract (3 l.) concd *in vacuo* and then passed through a column of Dowex-50W (H^+, 600 ml). After the resin was washed with H_2O, amino acids were eluted with 2 N NH_4OH. The eluate was concd to a small vol. and then applied to a column of Dowex-1 (OAc^-, 1.5 l.). After removal of neutral and basic amino acids by thoroughly washing with H_2O, the acidic amino acids were fractionated with a linear gradient of HOAc from 0 to 0.5 M. *N*-Methylaspartic acid emerged from the column immediately after (and in part overlapped with) aspartic acid. Fractions containing both acidic amino acids were further separated by PC. Recrystallization from EtOH gave colourless crystals (30 mg) which, after drying at 110° for 3 hr, had mp 187°, $[\alpha]_D^{20}$ +14.2° (*c* 1 in H_2O) and +26° (*c* 1 in 6 N HCl). (Found: C, 40.91; H, 6.18; N, 9.61. Calcd for $C_5H_9NO_4$: C, 40.82; H, 6.17; N, 9.52%).

Synthesis of N-*methyl*-L-*aspartic acid.* A reference sample of *N*-methyl-L-aspartic acid was synthesized from *N*-benzyloxycarbonyl-L-aspartic acid following essentially the procedure described by Olsen [3] for the *N*-methylation of monoamino monocarboxylic acids, but using a greater excess of methylating reagent and longer reaction time. In spite of that, reaction was incomplete and after removal of the protective group by hydrogenolysis, *N*-methylaspartic acid was separated from unreacted aspartic acid by ion-exchange chromatography. Mp 187–188° (lit. 190° [4]), $[\alpha]_D$ +15.6 (*c* 1 in H_2O) (lit. −15.4° for the D isomer [1]) and +26.8° (*c* 1 in 6 N HCl) (lit. hemihydrate +25.3° in 6 N HCl [2]).

Chromatographic comparison. The isolated amino acid and authentic *N*-methylaspartic acid had the same chromatographic behaviour in TLC in *n*-BuOH–HOAc–H_2O (12:3:5) and PhOH–H_2O (3:1): R_{asp} 1.35 and 2.4 (cellulose), and 2.8 and 2.4 (Si gel), respectively.

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