6. Henson, I. E. and Wheeler, C. T. (1977) *J. Exp. Botany* **28**, 205.
7. Van Staden, J. and Brown, N. A. C. (1977) *Physiol. Plant.* **39**, 266.

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D-HOMOCYSTEIC ACID FROM *PALMARIA PALMATA**

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D-Homocysteic acid was isolated from the red seaweed *Palmaria palmata*. Although amino sulphonic acids are commonly found in aqueous extracts of red algae [1], D- or L-homocysteic acid has not previously been reported from any plant source. Amino acid analyses of hot 80% ethanolic extracts of *Palmaria palmata* showed three major free amino acids, glutamic acid, proline and a ninhydrin-positive compound that eluted from the long column of an automatic amino acid analyser well before aspartic acid. High-voltage paper electrophoresis showed this uncharacterized compound to be negatively charged at pH 6.5 with an electrophoretic mobility similar to that of aspartic acid. The analytical data for the isolated compound is consistent with homocysteic acid. The optical rotation, however, was negative at 589 nm indicating a mixture of 78% D-homocysteic acid and 22% of the L-isomer. It is uncertain if the observed proportions of the optical isomers occurred in the plant or if they resulted from racemization during the isolation procedures. However, when subjected to similar conditions, a pure sample of synthetic D-homocysteic acid showed some racemization resulting in 7% conversion to the L-isomer. Nevertheless, some L-isomer could occur in the plant since homocysteic acid is probably formed from L-methionine. A negative circular dichroism spectrum (peak at 215 nm) of the natural product compared to that of L-homocysteic acid confirmed that the compound isolated from *Palmaria palmata* was largely in the D-configuration. An IR spectrum (KBr pellet) of this material was identical to that of DL-homocysteic acid prepared by performic oxidation [2] of DL-homocystine. The 60 MHz ^{1}H NMR spectrum was identical to one previously reported for homocysteic acid [3], while the 20 MHz ^{13}C NMR spectrum contained the following characteristic resonances [4] and ^{13}C–H spin–spin couplings (4): C-1, δ_c 173.64; C-2, δ_c 54.25, J_{CH} = 147.2 Hz; C-3, δ_c 27.92, $J_{CH} \simeq$ 134 Hz; C-4, δ 49.31, J_{CH} = 136.2 Hz.

The concentration of homocysteic acid in the plant was found to be variable and dependent on the availability of nitrogen. Plants collected in early spring or taken from cultures supplied with nitrogen in excess contained 3.5 mg/g (dry wt) of homocysteic acid. In plants deprived of a source of nitrogen, the concentration of homocysteic acid fell to a minimum of 0.7 mg/g. Homocysteic acid was found in similar concentrations in tetrasporophytes and male plants collected at the same time of year from various locations in Nova Scotia.

EXPERIMENTAL

1 kg batch of *Palmaria palmata*, which was visually free of epiphytes, was soaked in 80% EtOH containing 1 M NH_4OH for 3 days at room temp. The extract was taken to dryness and the residue redissolved in 50 ml H_2O. After filtration through charcoal, the volume of the clear filtrate was reduced to *ca* 15 ml and left overnight in the refrigerator to allow inorganic salts to crystallize. Dowex 50 × 8, H^+ form (100 g) was then added to the mother liquors which were diluted with H_2O until all but the most acidic amino acids were adsorbed. This process was monitored by paper electrophoresis. Subsequently the soln (800 ml) containing the unknown amino acid was concd to *ca* 10 ml and applied to a column of QAE-Sephadex (2.5 × 45 cm) equilibrated with Py acetate buffer soln (0.5 M with respect to Py) at pH 5.0. Fractions were monitored by paper electrophoresis and those containing the unknown acidic amino acid, which was well separated from small amounts of glutamic and aspartic acids, were combined and concd by evapn. The pyridinium salt of the amino acid was transferred to a small column (1 × 2 cm) of Dowex 50 × 8, H^+ form to convert it to the free acid. The eluate was evapd to a clear syrup from which the acidic amino acid formed rosettes of needle shaped crystals (120 mg), which after recrystallizing twice from aq. EtOH had mp 270° (decomp.), $[\alpha]_D^{25}$ −10° (*c* 5 in N HCl). (Found: C, 26.09; H, 4.90; O, 43.65; N, 7.84; S, 17.51. Calc. for $C_4H_9O_5NS$: C, 26.22; H, 4.95; O, 43.67; N, 7.62; S, 17.50%).

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REFERENCES

1. Ito, K., Miyazawa, K. and Matsumoto, F. (1977) *J. Fac. Fish. Anim. Husb.* **16**, 77.
2. Hirs, C. H. W. (1967) *Methods Enzymol.* **11**, 59.
3. Luchi, P. and de Marco, C. (1972) *Analyt. Biochem.* **45**, 236.
4. Voelter, W., Jung, G., Breitmaier, E. and Bayer, E. (1971) *Z. Naturforsch. Teil B* **26**, 213.