Phytochemistry, 1977, Vol. 16, pp. 1601–1602 Pergamon Press. Printed in England.

PYRROLIDINE-2,4-DICARBOXYLIC ACID, A NEW NATURALLY OCCURRING IMINO ACID

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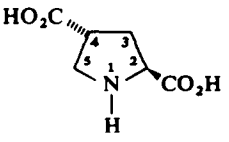
(*Received* 5 *April* 1977)

Key Word Index—*Chondria coerulescens*; *C. dasiphylla*; *Ceramium rubrum*; Rhodomelaceae; Ceramiaceae; red algae; non-protein imino acid; pyrrolidine-2,4-dicarboxylic acid.

INTRODUCTION

In the course of a study of the free amino acids in algae [1], *Chondria coerulescens* (Crouan) Falk. was found to contain a substance which apparently differed from known amino acids. The present paper reports its isolation and identification.



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RESULTS AND DISCUSSION

The new imino acid was isolated by ion-exchange chromatography in a yield of 0.012% of the fresh weight of alga. On paper it gave colour reactions characteristic of many imino acids (blue with alkaline nitroprusside in the presence of acetaldehyde, yellow with ninhydrin and blue with isatin) [2]. The acidic character of the compound was revealed by its behaviour on ion-exchange resins as well as by paper electrophoresis (E_{asp} = 1.07 at pH 4.5). The MS displayed a small M^+ at *m/e* 159 ($C_6H_9NO_4$) and major peaks at *m/e* 114 (M^+—CO_2H) and 68 (M^+—CO_2H—HCO_2H). Me esterification gave a product whose MS showed a M^+ at *m/e* 187 and intense fragments at *m/e* 128 and 68, consistent with consecutive losses of CO_2Me and HCO_2Me respectively. Additional ions representing feasible losses of Me, MeOH and (CO_2Me + MeOH) were present at *m/e* 172, 155 and 96. All the evidence so far indicated that the unknown compound was a pyrrolidine-dicarboxylic acid. Further structural information was obtained from the PMR spectrum of the free imino acid, determined in D_2O, which showed a 1H triplet at δ 4.33 (J = 8 Hz, H2) coupled to a 2H multiplet at δ 2.52 (H3) and two further complex multiplets centered at δ 3.35 (1H) and 3.57 (2H) which were assigned to 4- and 5-Hs, respectively. From this it was concluded that the new compound is pyrrolidine-2,4-dicarboxylic acid (**1**). Confirmatory evidence came from aromatization with Se of the diMe ester of **1** to 2,4-dicarbomethoxypyrrole.

According to the Clough–Lutz–Jirgensons rule [3–5], the shift of the molecular rotation of **1** to a more positive value from water to acid solution ($[M]_D$ − 73.1° in H_2O and − 47.2° in 5N HCl) indicates a *S*-configuration at C-2, if the rotatory contribution of the C-4 centre is assumed not to be influenced by the variation of the pH. This assumption seems justified since comparison of the PMR spectrum in D_2O with that after addition of 1 mol proportion of HCl gives $\Delta\delta$ values ($\Delta\delta = \delta^{H_2O} - \delta^{HCl}$) of −0.32 and −0.13 ppm for the 2- and 4-hydrogens respectively, suggestive that the main ionization change was at 2-CO_2H. The configuration at C-4 can be assigned tentatively as *S* on the basis of the rule of the optical superposition [6] (*S*-proline, $[M]_D$ − 99.2°; *S*-β-proline, $[M]_D$ + 21.3° [7]).

A survey of a number of Florideophyceae (4 Nemalionales, 7 Cryptonemiales, 2 Gelidiales, 11 Gigartinales, 3 Rhodymeniales, 1 Bonnemaisoniales, 18 Ceramiales) representative of all the 7 orders into which this class is usually divided, revealed that the new imino acid also occurs in two further species of the Ceramiales, namely *Chondria dasiphylla* (Wood.) Falk. (Rhodomelaceae) and *Ceramium rubrum* (Huds.) C. Ag. (Ceramiaceae).

Other acidic imino acids until now isolated from algae include kainic and allokainic acids from *Digenea simplex* (Rhodomelaceae) [8], and domoic acid from *Chondria armata* (Rhodomelaceae) [9]. Kainic acid also occurs in *Centroceras clavulatum* (Ceramiaceae) and domoic acid in *Alsidium corallinum* (Rhodomelaceae) [10]. Pyrrolidine-2,5-dicarboxylic acid from *Schizymenia dubyi* (Nemastomaceae; order Gigartinales) [10] provides the only exception in occurring out of the order Ceramiales. From these limited data it appears that this type of compound possesses a certain chemotaxonomic value.

EXPERIMENTAL

Chondria coerulescens was collected in shallow water near Catania. *C. dasiphylla* and *Ceramium rubrum* were respectively harvested at Porto Palo and Capo Mulini, Sicily.

Pyrrolidine-2,4-dicarboxylic acid. Freshly collected *C. coerulescens* (1 kg) was homogenized and extracted 3 × with EtOH–H_2O (7:3, 2 l. each time). The combined extracts were clarified by centrifugation and applied to a column of Dowex-50W (50–100 mesh, × 2, H^+). After washing the column with H_2O, the amino acids were eluted with 2 M NH_4OH and the eluate evapd to dryness. The residue was redissolved in H_2O and applied to a strongly basic ion-exchange resin (Dowex-1, 200–400 mesh, × 8, AcO^-). The latter was washed with H_2O and then eluted with a

linear gradient of HOAc from 0 to 0.4 M. The separation was monitored by TLC on cellulose plates using the following solvent systems: (A) *n*-BuOH–HOAc–H_2O (12:3:5) and (B) PhOH–H_2O (3:1). **1**, which emerged from the column immediately after aspartic acid, had R_f 0.28 in solvent A and 0.43 in solvent B. The appropriate fractions were pooled and evapd *in vacuo*. The residue was recrystallized from MeOH–H_2O giving 120 mg of **1**, mp 223–225°, $[\alpha]_D^{20}$ − 46.0° (*c* 1 in H_2O) and −29.7° (*c* 1 in 5N HCl).

DiMe ester hydrochloride. (MeOH–HCl): δ (D_2O) 4.7 (1H, *t*, J = 8 Hz, H2), 3.9 and 3.8 (3H each, *s*, 2- and 4-CO_2Me), 3.7 (1H, *m*, H5), 3.45 (1H, *m*, H4) and 2.65 (2H, *m*, H3). This diMe ester (50 mg) was heated with Se (250 mg) at 250° for 30 min. After cooling, the mixture was extracted with EtOAc and the extract taken to dryness. The residue was purified by PLC giving 2,4-dicarbomethoxypyrrole, identified by comparison with an authentic sample.

Amino acid distribution. Samples of fresh algae were macerated and extracted with 70% EtOH and amino acid fractions separated using Dowex-50W columns. The individual components were resolved by 2-D TLC in solvents A and B and on paper electrophoresis (pH 4.5).

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Phytochemistry, 1977, Vol. 16, pp 1602–1603. Pergamon Press. Printed in England.

TYRAMINE FROM *THEOBROMA CACAO*

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(*Received* 18 *April* 1977)

Key Word Index—*Theobroma cacao*; Sterculiaceae; migraine headaches; tyramine.

Tyramine [1] (4-hydroxyphenethylamine) and phenethylamine [2] have been implicated as agents in foods (especially cheese and cocoa-based food stuffs) which trigger the onslaught of severe migraine headaches ('dietary migraines') in certain individuals. Recent reports have noted that tyramine is absent from cocoa based products and have thus implicated phenethylamine as the principal culprit in these cases [2–4]. This note reports the isolation and identification of tyramine from *Theobroma cacao* beans (seeds) and cocoa based products. To our knowledge, this is the first time tyramine has been identified as a naturally occurring constituent of cacao.

Plants. Unroasted seeds of *Theobroma cacao* (unfermented or, fermented) were supplied by Dr. Victor C. Quensel of the Cocoa Research Unit, University of the West Indies, Trinidad. 'Raw' and 'roasted' fermented cacao beams were also supplied by Hershey Foods Corporation, Hershey, PA 17033. Powdered confectionary cocoa (Hershey) was purchased locally.

Previous work. The alkaloids dopamine and salsolinol have been detected in powdered confectionary cocoa. [5] Tyramine is a logical precursor of these compounds.

Present work. TLC [6–8] and HPLC [8] (high performance liquid chromatography with electrochemical detection) and combinations thereof were utilized to identify and quantitate levels of tyramine in various cacao and cocoa samples. EtOAc–acetone extractions of the basified (pH = 10.3) acidic extract (0.1 M $HClO_4$) of pulverized cacao samples were subjected to analysis (see Experimental). The method of standard addition was used to quantitate the tyramine. The tyramine concentrations found in *Theobroma cacao* were as follows (μg/g): unfermented-unroasted, 3.9 ± 0.1; fermented-unroasted, 11.5 ± 0.1; Hershey's 'raw', 3.4 ± 0.1; Hershey's 'roasted' 3.6 ± 0.1; Hershey's powdered, 8.3 ± 0.4. This is the first report of the isolation and identification of tyramine from cacao sources.

EXPERIMENTAL

Pulverised cacao or cocoa samples (1 g), either freshly ground or defatted with petrol; were prepared for analysis by reciprocal shaking (for 15 min) with 10 ml of 0.1 M $HClO_4$. 2 ml aliquots of the supernatants were basified with conc NH_4OH (to pH = 10.3) and satd with NaCl. The solns were extracted with 3 × 4 ml vols of EtOAc–Me_2CO (2:1). The dried (2 g Na_2SO_4)