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SHORT REPORTS

β-L-ASPARTYLGLYCINE FROM THE RED ALGA *CERAMIUM RUBRUM*

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Key Word Index—*Ceramium rubrum*; Ceramiaceae; red algae; peptides; β-L-aspartylglycine.

INTRODUCTION

Since the pioneering work of Haas and Hill [1], only a limited number of low MW peptides have been isolated from marine algae. These include eisenine (L-pyrrolid-5-one-2-carbonyl-L-glutaminyl-L-alanine) and fastigiatine (L-pyrrolid-5-one-2-carbonyl-L-glutaminyl-L-glutamine) isolated respectively from the brown seaweeds *Eisenia bicyclis* [2] and *Pelvetia fastigiata* [3], L-citrullinyl-L-arginine from the red alga *Grateloupia turuturu* [4], L-arginyl-L-glutamine from the green algae *Enteromorpha linza* and *Ulva pertusa* [5], and a partially characterized pentapeptide of glutamic acid and aspartic acid (or their amides) which has been named analipine after the brown alga, *Analipus japonicus* [6].

We wish to report here the isolation of a further peptide, β-L-aspartylglycine, from the red alga *Ceramium rubrum* (Huds.) C. Ag. (Ceramiaceae).

RESULTS AND DISCUSSION

This peptide was isolated by ion-exchange chromatography followed by recrystallization in a yield of 0.022% of the fr. wt of the alga. The acidic character of the peptide was revealed by its behaviour on ion-exchange resins and during paper electrophoresis (E_{asp} = 1.05 at pH 4.5). From the molecular formula and the result of acid hydrolysis it was evident that the isolated compound was a dipeptide of aspartic acid and glycine. Since in the PMR spectrum (determined in D_2O) addition of acid caused a substantial shift of the signal of the proton α to the amino acid function, it was deduced that aspartic acid was the N-terminal amino acid. This was confirmed by converting the peptide into the DNP-derivative, which gave DNP-aspartic acid and glycine on acid hydrolysis. A positive test with sodium hypochlorite and fuchsin [7] favoured the structure of β-aspartylglycine, but the mp values reported in the literature (see Experimental) were quite different from that of the natural peptide. For direct comparison we prepared both isomeric aspartylglycines according to Buchanan *et al.* [8]. Mp, IR, colour reaction with ninhydrin, and chromatographic and electrophoretic behaviours of β-aspartylglycine were in complete agreement with those of the algal compound.

EXPERIMENTAL

Extraction and isolation. Fresh alga (500 g) was homogenized and extracted with EtOH to a final concn of 70%. The extract was clarified by centrifugation and passage through a column of Dowex 50W-X8 (H^+) (400 ml). The resin was washed thoroughly with H_2O and the amino acids eluted with N NH_4OH. The eluate was concd and applied to a column of Dowex 1-X8 (AcO^-) (300 ml). After washing with H_2O, gradient elution was applied from 0 to 0.5 M HOAc. Fractions containing the peptide, which emerged from the column after aspartic acid and partly overlapped with pyrrolidine-2,4-dicarboxylic acid [9], were pooled and concd to small vol. After standing overnight in the cold, crystals (110 mg) were obtained which, after drying *in vacuo* at 110° for 4 hr, had mp 230–232° (dec.) (lit.: 153° [10], 190–200° [11]; monohydrate 153–156° [11], 163° [8]), $[\alpha]_D^{20}$ −9.6° (*c* 1 in H_2O), +7.7° (*c* 1 in H_2O + 1 mol HCl) and +31° (*c* 1 in 5 N HCl) (lit.: +7.2° (*c* 2.4 in H_2O + 1 mol HCl) [10], +13.9° (*c* 2.6 in H_2O + 1 mol HCl) [11], +12.7° (*c* 2 in H_2O + 1 mol HCl [8]). (Found: C, 38.15; H, 5.27; N, 14.83. Calc. for $C_6H_{10}O_5N_2$: C, 37.89; H, 5.26; N, 14.74%). PMR (D_2O) δ: 3.0 (2H, *d*, *J* = 6 Hz), 4.0 (2H, *s*), 4.14 (1H, *t*, *J* = 6 Hz); after addition of CF_3COOD to pH 2 the triplet at δ 4.14 was shifted to 4.33, while the other signals were not affected significantly.

DiMe ester hydrochloride. (MeOH–HCl): δ (D_2O) 3.13 (2H, *d*, *J* = 5 Hz), 3.72 and 3.83 (3H each, *s*), 4.0 (2H, *s*), 4.5 (1H, *t*, *J* = 5 Hz). MS *m/e* 186 (M^+ − MeOH), 159 (M^+ − COOMe), 158 (M^+ − HCOOMe), 127 (M^+ − MeOH − COOMe), 70 (M^+ − COOMe − $NHCH_2COOMe$).

Acid hydrolysis. Peptide (2 mg) was refluxed with 6N HCl (1 ml) for 1 hr. HCl was removed *in vacuo* and amino acids converted by standard procedure [12] into N-trifluoroacetyl *n*-butyl esters. Gas-chromatographic analysis (1.5% OV-17 on 80–100 mesh H.P. Chromosorb G) showed the presence of aspartic acid and glycine in equimolar amounts.

DNP-*derivative.* Dinitrophenyl derivative of the peptide was prepared by the method of Sanger [13]. Its hydrolysis products on refluxing with 6 H HCl for 1 hr were identified by TLC as DNP-aspartic acid and glycine.

Synthesis of peptide. Synthetic samples of both L-aspartylglycines were obtained according to Buchanan *et al.* [8]. Dried *in vacuo* β-L-aspartylglycine had mp 230–232° and $[\alpha]_D^{20}$ +29° (*c* 1 in 5 N HCl).

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DIALLYL DI-, TRI- AND TETRASULPHIDE FROM *ADENOCALYMMA ALLIACEAE*

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Key Word Index—*Adenocalymma alliaceae*; Bignoniaceae; diallyl disulphide; diallyl trisulphide; diallyl tetrasulphide; 1-octen-3-ol.

Abstract—The essential oil from flowers and leaves of *Adenocalymma alliaceae* (Bignoniaceae) consists almost entirely of diallyl di-, tri-and tetra-sulphide, a mixture formerly encountered within the *Allium* genus but never before convincingly recognized within the class of dicotyledonous angiosperms.

INTRODUCTION

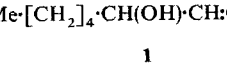
In 1911, Peckolt [1] drew attention to the garlic odour of the leaves of the Brazilian species *Adenocalymma sagotii* Bur. et K. Sch. ('Cipó d'alko'), Bignoniaceae, used by the native population in combating infections. We now report the results of a study by GC–MS of the odoriferous principles in fresh leaves and flowers of *A. alliaceae* Mart.

RESULTS AND DISCUSSION

When concentrated ethereal extracts of steam distillates of fresh leaves of *A. alliaceae* were subjected to GLC, a remarkably simple pattern appeared, essentially consisting of four peaks, numbered 1–4 in the order of increasing retention time; the relative peak heights were *ca* 1:5:5:1. In the flower distillate, only peaks nos. 2–4, in an unchanged ratio, were prominent.

The MS characteristics of the compounds, represented by peaks nos. 1–4, are summarized in Table 1. From these data their structures can be unequivocally established as 1-octen-3-ol (**1**), diallyl disulphide (**2**), diallyl trisulphide (**3**), and diallyl tetrasulphide (**4**). The eight most prominent peaks in the MS of fraction no. 1 (Table 1) are identical with those reported for 1-octen-3-ol [3], a widespread alcohol within the plant kingdom. More interest is associated with the sulphur constituents (**2**)–(**4**). About 85 years ago, Semmler [4] established the presence of **2**, **3**, and, tentatively, **4** in garlic oil (*Allium sativum*). The finding of **2** and **3** in garlic has been repeatedly confirmed and extended to other species of the genus *Allium* [5, 6]. The unambiguous recognition of diallyl di-, tri-, and tetra-sulphide (**2**)–(**4**) in volatiles from a dicotyledon, however, appears to be without precedent. The alleged occurrence of **2** in asafoetida, an oleoresin from *Ferula* species [7], we consider doubtful in view of the detailed studies recently performed on asafoetida volatiles in the Danish laboratory and elsewhere [8, and refs. therein]. Again, the suggested presence of **2** in volatiles from *Descurainia sophia* (Cruciferae) [9] rests on unconvincing evidence.

In *Allium* sp., **2** and **3** derive from allicin (**5**) which, in its turn, results from enzymic fission of alliin (**6**) [6, and refs. therein]. It would be of considerable interest to know whether an identical chemical apparatus is operating within dicotyledonous taxa. Studies towards this goal are in progress.



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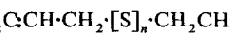

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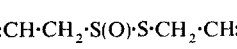
3, $n = 3$
4, $n = 4$



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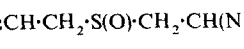

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