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AMINO ACIDS AND LOW-MOLECULAR-WEIGHT CARBOHYDRATES OF SOME MARINE RED ALGAE*

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Key Word Index—Rhodophyta; red algae; free protein and non-protein amino acids; low-MW carbohydrates.

Abstract—Amino acids and low-MW carbohydrates of 18 red algae have been analyzed. Several non-protein amino acids have been identified, including pyrrolidine-2,5-dicarboxylic acid (**3c**) and *N*-methylmethionine sulfoxide (**5**), new natural products, and 13 known compounds, citrulline, β-alanine, γ-aminobutyric acid, baikiain (**1**), pipecolic acid (**2**), domoic acid (**3a**), kainic acid (**3b**), azetidine-2-carboxylic acid (**4**), methionine sulfoxide, taurine, *N*-methyltaurine, *N*,*N*-dimethyltaurine and *N*,*N*,*N*-trimethyltaurine. Sugars present were mainly floridoside, isofloridoside and mannoglyceric acid. Details of the structural elucidation of new compounds are also given.

INTRODUCTION

Although morphological characters are ordinarily considered to be of pre-eminent importance in taxonomy, classical separation of algae into major groups or divisions rests primarily upon two biochemical characters, distinctive pigments and distinctive food reserves. Unfortunately, information on the distribution of other algal products is at this time inadequate to enable chemotaxonomic correlations at the level of lower systematic categories to be made. Thus, we commenced a general survey of the chemical constituents of algae. This may also lead to the discovery of new compounds of potential biomedical value, since crude extracts of several species have been reported [1] to possess pharmacological (antibacterial, antifungal, antiviral, etc.) activities.

In the present paper we report the occurrence of amino acids and low-MW carbohydrates in 18 macroscopic marine algae belonging to the division Rhodophyta, class Florideophyceae.

RESULTS AND DISCUSSION

By a procedure based on ion-exchange resins, aqueous alcoholic extracts of algae were separated into cationic, anionic and neutral fractions. These fractions were each examined by appropriate methods (see Experimental) and individual constituents identified as far as possible.

Amino acid constituents

All species of red algae examined have a similar composition of protein amino acids (Table 1). Thus, all species contain low concentrations of basic amino acids and show a general prevalence of the same compounds (aspartic and glutamic acids, which often make up 50% or more of the total, alanine, glycine and serine). *Lophocladia lallemandi*, a species reputedly having penetrated in

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* Part I in the series "Constituents of Red Algae".

Table 1. Distribution of free protein

Species	Amino acid (mol/100 mol)					
	Asp	Thr	Ser	Asn	Gln	Glu
Nemalionales						
Helminthocladiaceae						
1. *Liagora distenta* (Mert.) C. Ag.	12·7	4·7	6·5	—	—	31·5
Chaetangiaceae						
2. *Scinaia furcellata* (Turn.) Bivona	10·2	3·0	10·1	1·7	0·7	28·3
Gelidiales						
Gelidiaceae						
3. *Gelidium latifolium* (Grev.) Thuret et Bornet	18·7	3·3	11·3	—	4·0	31·6
4. *Pterocladia pinnata* (Huds.) Papenfuss	8·6	7·3	18·6	—	1·8	18·6
Cryptonemiales						
Cryptonemiaceae						
5. *Grateloupia proteus* Kütz.	20·3	7·0	13·0	T	T	27·3
Corallinaceae						
6. *Amphiroa beauvoisii* Lamx.	6·8	8·7	11·0	—	—	12·6
Gigartinales						
Gigartinaceae						
7. *Gigartina acicularis* (Wulf.) Lamx.	19·0	1·3	12·9	3·2	—	17·3
8. *Gigartina teedi* (Roth) Lamx.	26·8	0·7	3·6	3·2	—	30·1
Nemastomaceae						
9. *Schizymenia dubyi* (Chauv.) J. Ag.	17·2	1·6	0·8	—	2·5	39·5
Hypneaceae						
10. *Hypnea musciformis* (Wulf.) Lamx.	7·4	T	10·5	—	—	19·6
Sphaerococcaceae						
11. *Caulacanthus ustulatus* (Mert.) Kütz.	36·2	3·0	3·5	—	—	34·8
12. *Sphaerococcus coronopifolius* (Good. et Wood.) C. Ag.	7·7	3·1	12·3	3·9	—	21·2
Ceramiales						
Ceramiaceae						
13. *Centroceras clavulatum* Mont.	7·8	4·4	1·3	—	7·8	31·6
Rhodomelaceae						
14. *Lophocladia lallemandi* (Mont.) Schmitz	0·8	0·8	0·7	—	—	3·1
15. *Laurencia paniculata* (C. Ag.) J. Ag.	2·5	17·7	5·1	—	—	3·0
16. *Laurencia obtusa* (Huds.) Lamx.	19·5	2·3	6·4	T	1·9	40·6
17. *Alsidium corallinum* C. Ag.	20·5	1·2	5·0	—	—	56·0
18. *Acanthophora najadiformis* (Delile) Papenfuss	31·6	1·2	4·7	—	—	27·1

* — Indicates absence and T indicates trace amounts.

recent times from the Red Sea into the Mediterranean through the Suez Canal, is unique among the species examined by its low concentration of acidic amino acids and its considerable accumulation of proline, which parallels the occurrence of azetidine-2-carboxylic acid, a specific inhibitor of its higher homolog [2]. A number of non-protein amino acids, which are more interesting from the taxonomic viewpoint, have also been observed and most of them characterized; the pertinent data are presented in Table 2 and are mentioned below.

Citrulline. Originally isolated from the juice of watermelon [3], citrulline has been subsequently detected by PC in a variety of plant sources. By virtue of its role as an intermediate in the biosynthesis of arginine, it is presumed to be universal in plants. In the present survey, it was detected in the majority of the species examined and sometimes found in high concentration, as in *Grateloupia proteus.*

β-Alanine and γ-aminobutyric acid. These amino acids, which have been reported to be rather common constituents in many plants, are present in very few of the algae examined and always in trace amounts.

L-*Baikiain* (**1**). Isolated from extracts of the Rhodesian teak, *Baikiaea plurijuga* [4], and subsequently from one other legume, *Caesalpinia tinctoria* [5], and, in addition, found in the red seaweeds *Corallina officinalis*, *Gracilaria secundata* and *Pterocladia capillacea* (syn. *P. pinnata*) [6], this imino acid has now been detected, often in small amounts, in many of the algae investigated.

amino acids in some species of red algae

Amino acid (mol/100 mol)*												Total amino acid (mmol/kg dry wt)
Pro	Gly	Ala	Val	Met	Ile	Leu	Tyr	Phe	Lys	His	Arg	
2·5	6·0	15·1	3·0	—	2·0	2·4	T	1·8	1·0	0·6	10·2	7·6
2·0	10·9	23·8	3·4	T	1·8	1·5	T	0·9	T	—	1·7	50·6
1·9	13·1	8·0	0·9	—	T	T	—	1·9	4·1	0·5	0·8	69·6
2·3	6·5	23·8	3·5	1·3	1·8	1·1	T	1·0	2·9	T	0·9	125·0
0·9	8·1	10·7	3·9	T	3·2	1·9	T	0·9	0·8	—	2·0	74·7
7·9	16·2	15·9	8·9	—	4·5	1·0	1·0	5·5	T	T	T	13·6
1·5	8·8	14·3	2·7	—	1·8	1·2	1·6	6·8	0·9	T	6·7	18·6
T	8·7	11·7	3·1	—	2·1	2·4	1·3	6·3	T	T	—	15·1
T	6·8	22·8	1·2	—	0·7	0·8	—	—	4·4	T	1·7	52·1
4·2	24·5	27·1	4·7	—	1·4	0·6	T	T	T	T	T	15·3
1·7	5·0	8·9	2·3	—	0·7	T	—	3·9	T	T	T	96·6
0·9	6·5	29·4	6·5	—	3·1	2·1	T	—	0·5	2·2	0·6	17·3
2·7	8·3	34·4	T	—	T	T	T	T	1·7	—	T	23·5
54·8	8·3	25·6	5·9	—	T	—	T	T	T	T	T	129·8
7·7	37·6	8·1	11·9	—	4·3	0·8	—	—	T	1·3	T	11·9
T	12·1	7·7	2·7	—	1·6	T	T	5·2	T	T	T	71·4
T	7·0	7·4	1·4	—	0·9	T	T	—	0·6	T	T	59·6
3·6	10·0	13·7	2·7	—	1·3	1·8	—	1·8	0·5	T	T	48·1

Its identification was established by isolation from *Pterocladia pinnata*, in which it represents more than half of the free amino acid complement, and comparison was made of its physical properties with those reported in the literature.

L-*Pipecolic acid* (**2**). First isolated from plant extracts simultaneously by Morrison [7] and by Zacharius *et al.* [8] pipecolic acid has been shown to occur widely in plants, particularly in legumes. It co-occurs, in small amounts, with baikiain in 3 of the 18 species examined.

L-*Domoic acid* (3a). Isolated by Daigo [9] from *Chondria armata*, it has been now found in one other species of the family Rhodomelaceae, *Alsidium corallinum.*

L-*Kainic acid* (3b). Previously isolated from *Digenea simplex* [10] (Rhodomelaceae; order Ceramiales), this imino acid also occurs in *Centroceras clavulatum* (Ceramiaceae; order Ceramiales).

Pyrrolidine-2,5-*dicarboxylic acid* (3c). This new compound, mp 340–345° dec., $[\alpha]_D$ −112°, has been isolated from *Schizymenia dubyi.* Owing to its strong acidity (in paper electrophoresis it still migrates as anion at pHs down to 1·9), it was not retained by the cation-exchange column and was therefore found in the anionic fraction, from which it was obtained by recrystallization from EtOH. 3c gave on paper color reactions characteristic of many imino acids (yellow with ninhydrin and blue with isatin). Assignment of the structure was based on the following considerations. Elemental analysis established the empirical formula as $C_6H_9O_4N$ and this was shown to be the molecular formula by chemical ionization

Table 2. The distribution of non-

Species	Compound (mmol/kg dry wt)							
	Cit	β-Ala	γ-AbA	Baik	Pip	Dom	Kai	PDC
1. *Liagora distenta*	0·1	—	—	—	—	—	—	—
2. *Scinaia furcellata*	0·5	—	T	0·7	—	—	—	—
3. *Gelidium latifolium*	—	T	—	0·9	—	—	—	—
4. *Pterocladia pinnata*	0·4	—	—	118·8	1·3	—	—	—
5. *Grateloupia proteus*	19·7	—	—	—	—	—	—	—
6. *Amphiroa beauvoisii*	—	—	—	0·9	0·5	—	—	—
7. *Gigartina acicularis*	8·4	—	—	0·3	—	—	—	—
8. *Gigartina teedi*	0·2	—	—	0·3	—	—	—	—
9. *Schizymenia dubyi*	1·1	—	—	0·6	—	—	—	26·9
10. *Hypnea musciformis*	—	—	—	—	—	—	—	—
11. *Caulacanthus ustulatus*	—	—	—	1·5	—	—	—	—
12. *Sphaerococcus coronopifolius*	0·4	—	—	—	—	—	—	—
13. *Centroceras clavulatum*	0·3	—	—	0·3	0·6	—	1·5	—
14. *Lophocladia lallemandi*	0·1	—	—	0·2	—	—	—	—
15. *Laurencia paniculata*	—	—	—	—	—	—	—	—
16. *Laurencia obtusa*	11·7	T	—	—	—	—	—	—
17. *Alsidium corallinum*	2·6	—	—	0·2	—	1·6	—	—
18. *Acanthophora najadiformis*	3·7	—	—	—	—	—	—	—

Key: Cit = citrulline; β-Ala = β-alanine; γ-AbA = γ-aminobutyric acid; Baik = L-baikiain; Pip = L-pipecolic acid; acid; MetO = L-methionine sulfoxide; Me-MetO = L-*N*-Methylmethionine sulfoxide; Tau = taurine; Me-Tau = *N*-methyl-

mass spectrometry (*m/e* 160; MH^+). It exhibited adsorption bands at 1675 (unionized COOH) and 1565 cm^{-1} (COO^-) in the IR spectrum. The NMR spectrum in D_2O displayed two complex signals at δ 2·17 (4H, H_2C-3 and H_2C-4) and 4·31 (2H, HC-2 and HC-5). These data and the MS fragmentation pattern were consistent with the formulation of **3c** as pyrrolidine-2,5-dicarboxylic acid. Confirmatory evidence was obtained from aromatization with selenium of the dimethyl ester of **3c** to 2,5-dicarbomethoxypyrrole. The high negative molecular rotation in H_2O ($[M]_D$ $-178{\cdot}2°$), which is not far from twofold that of L-proline ($[M]_D$ $-99{\cdot}2°$), and the shift to a more

Table 3. The distribution of low-molecular-weight carbohydrates in some red algae

Species	Sugar (mmol/kg dry wt)		
	Flo	Isoflo	MGA
1. *Liagora distenta*	26·1	—	0·15
2. *Scinaia furcellata*	39·6	9·9	0·25
3. *Gelidium latifolium*	30·5	T	—
4. *Pterocladia pinnata*	20·2	1·6	2·03
5. *Grateloupia proteus*	19·0	1·6	—
6. *Amphiroa beauvoisii*	12·7	—	0·03
7. *Gigartina acicularis*	88·3	18·2	0·08
8. *Gigartina teedi*	8·3	10·3	—
9. *Schizymenia dubyi*	1·2	5·1	—
10. *Hypnea musciformis*	55·0	—	0·15
11. *Caulacanthus ustulatus*	75·6	—	—
12. *Sphaerococcus coronopifolius*	8·3	—	0·39
13. *Centroceras clavulatum*	1·2	—	22·82
14. *Lophocladia lallemandi*	—	—	21·85
15. *Laurencia paniculata*	78·0	37·2	0·52
16. *Laurencia obtusa*	81·6	—	2·76
17. *Alsidium corallinum*	29·7	3·2	11·89
18. *Acanthophora najadiformis*	79·6	—	2·13

Key: Flo = floridoside; Isoflo = isofloridoside; MGA = mannoglyceric acid.

protein amino acids in red algae

Compound (mmol/kg dry wt)							Unidentified ninhydrin-positive compounds
A2C	MetO	Me-MetO	Tau	Me-Tau	Me_2-Tau	Me_3-Tau	
—	0·5	—	3·0	4·2	—	—	A
—	1·5	—	T	—	2·2	—	B
—	0·2	3·9	8·0	T	109·0	—	—
—	—	—	63·6	12·6	100·0	3·4	—
—	7·3	2·5	9·0	—	—	—	—
—	4·5	—	2·3	—	8·1	—	—
—	0·9	—	3·8	—	—	—	—
—	0·4	—	4·1	—	—	—	—
—	—	—	4·5	—	—	—	—
—	T	—	2·0	—	—	—	C
—	—	—	2·0	—	—	—	—
—	2·0	—	3·4	—	—	—	—
—	2·2	37·6	T	—	—	—	—
25·0	—	—	—	—	—	—	B
—	—	—	3·0	T	4·9	1·3	—
—	—	—	4·7	—	7·1	—	—
—	2·8	—	T	—	—	—	—
—	1·7	—	T	—	—	—	D, E

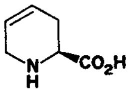
Dom = L-domoic acid; Kai = L-kainic acid; PDC = pyrrolidine-2,5-dicarboxylic acid; A2C = L-azetidine-2-carboxylic taurine; Me_2-Tau = *N,N*-dimethyltaurine; Me_3-Tau = *N,N,N*-trimethyltaurine.

positive value when the rotation was measured in 5 N HCl point to the *L,L*-configuration for the isolated material.

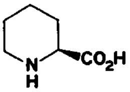
L-*Azetidine-2-carboxylic acid* (**4**). Discovered in species of the Liliaceae [11–14] and in members of the Agavaceae and Amaryllidaceae [15], this acid has more recently been found in seedlings of *Delonix regia* [16], in some other legumes [5] and in exceedingly small amounts, in sugar beet [17]. We have now isolated it from *L. lallemandi* and this is, to our knowledge, the first reported occurrence in lower plants.

L-*Methionine sulfoxide*. This rather common natural amino acid has been now identified in several red algae and isolated from *Amphiroa beauvoisii*. The specific optical rotation is in agreement with the literature value for the isomer having the *R*-configuration at the asymmetric sulfur atom [18], and this rules out the possibility that it could be an artifact from the non-enzymatic oxidation of methionine.

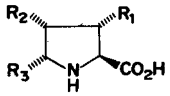
N-*Methyl*-L-*methionine sulfoxide* (**5**). This substance was isolated as the major component from the amino acid complex of *C. clavulatum* and



(1)



(2)

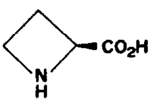


(3a) $R_1 = CH_2CO_2H$; $R_2 = CMe{=}CH{-}CH{=}CH{\cdot}CHMeCO_2H$

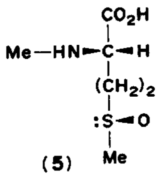
$R_3 = H$

(3b) $R_1 = CH_2CO_2H$; $R_2 = CMe{=}CH_2$; $R_3 = H$

(3c) $R_1 = R_2 = H$; $R_3 = CO_2H$



(4)



(5)

identified as a minor constituent of two other species. The structure of this amino acid, $C_6H_{13}O_3NS$, mp 224–227°, $[\alpha]_D^{20}$ −33·6°, followed from the IR (ν_{max}^{KBr} 1040 cm^{-1}, S=O) and NMR spectrum in CF_3COOD, which displayed signals at δ 2·89 (2H, *m*, H_2C-3), 2·95 (3H, *s*, S-Me), 3·10 (3H, *s*, N-Me), 3·44 (2H, *m*, H_2C-4) and 4·42 (1H, *t*, HC-2). On reduction with HI, **5** gave *N*-methyl-L-methionine, $C_6H_{13}O_2NS$, mp 234–236°, $[\alpha]_D^{20}$ +18·2°, whose physical properties matched that reported in the literature for the D isomer. Considering that *N*-methylation of L-amino acids is known to be accompanied by a more positive shift in the specific rotation [19], the value observed for the isolated sulfoxide agreed with that expected for the isomer having the *R*-configuration at the asymmetric sulfur atom and this shows **5** not to be an artifact.

Taurine and N-*methyltaurines.* In 1955, during an investigation of the sugar components of the red alga *Furcellaria fastigiata*, Lindberg [20] isolated *N*,*N*-dimethyltaurine. This substance as well as *N*-methyltaurine and taurine itself were subsequently found in *Ptilota pectinata*, *Porphyra umbilicalis* and *Gelidium cartilagineum* [21]. Their occurrence in other red seaweeds was detected in the present survey and, in addition, the fully methylated derivative (2-trimethylammoniumethanesulfonate or taurobetaine) has been isolated from *P. pinnata* and identified in *Laurencia paniculata*. This appears to be the first report of this compound as plant product; it had been previously found in Nature as a constituent of marine invertebrates (sponges [22, 23] and gorgonians [24]).

Unidentified ninhydrin-positive compounds. Besides the above mentioned non-protein amino acids, some unknown ninhydrin-positive compounds were detected by automated amino acid analysis (AAA) and/or 2-D TLC. *Compound A* was present in the cationic fraction of *Liagora distenta* and in AAA was eluted between serine and glutamic acid. Analysis of the free amino acids of *Scinaia furcellata* and *L. lallemandi* showed a new component (*compound B*) that came off the column before methionine sulfoxide. In the elutogram of the amino acids from *Hypnea musciformis* a ninhydrin-positive peak (*compound C*) appeared after the valine peak. Moreover, at least two ninhydrin reacting components (*compounds D* and *E*) were observed in the anionic fraction from *Acanthophora najadiformis*. Chemical identification of these substances awaits the isolation of adequate amounts.

Low-molecular-weight carbohydrates

Low-molecular-weight carbohydrates in both neutral and anionic fraction were identified and determined by GLC after silanization. The results (Table 3) show that a relatively simple sugar pattern occurs in most of the 18 species surveyed. Floridoside (2-*O*-α-D-galactopyranosylglycerol), already frequently reported in algae [25–30], is invariably present, with the sole exception of *L. lallemandi*, and frequently a major sugar. Isofloridoside (1-*O*-α-D-galactopyranosylglycerol), originally found in *Porphyra umbilicalis* of the class Bangiophyceae and supposed to be characteristic for this taxon [31], has been subsequently discovered also in the Florideophyceae [32]. Our survey shows that this sugar is rather less common than floridoside, being present, sometimes in concentration exceeding that of the latter, in about 50% of the present sample. Mannoglyceric acid (2-*O*-α-D-mannopyranosyl-D-glyceric acid), first discovered in the red alga *Polysiphonia fastigiata* [33], was subsequently found in more than trace amounts in members of the order Ceramiales, especially in the family Rhodomelaceae, and also in the Gigartinales and Cryptonemiales [27, 28, 34, 35]. Our results suggest that this sugar may be distributed throughout the class Florideophyceae. In addition to floridoside, isofloridoside and mannoglyceric acid, the major sugars, several oligosaccharides were often present in trace amounts and were not further investigated. *C. clavulatum* was exceptional in that at least 12 components were present in substantial concentrations in the neutral fraction and that floridoside constituted only 5% of the total.

EXPERIMENTAL

Plant material. Red algae were collected from the littoral zone of the east coast of Sicily, in several stations between Taormina and Capo Passero. They were transported to the laboratory on ice and carefully freed from foreign macroscopic marine flora and fauna.

Chromatography. TLC was run on precoated Merck cellulose or Si gel plates. Preparative PC was carried out on Whatman 3MM paper. The following solvent systems were used: 1. *n*-BuOH–HOAc–H_2O (12:3:5). 2. PhOH–H_2O (3:1).

3. *n*-PrOH–conc NH_4OH–H_2O (20:1:4). 4. *n*-PrOH–conc NH_4OH–H_2O (20:2:3). Solvents 1 and 2 were used in that order to develop 2-D TLC. Chromogenic reagents were: (a) 0·1% ninhydrin in acetone; (b) Ehrlich's reagent (2% *p*-dimethylaminobenzaldehyde in a 1:4 mixture of conc HCl and acetone); (c) 0·2% isatin in acetone; (d) 1% $Ce(SO_4)_2$ in 2 N H_2SO_4; (e) 0·1 N $AgNO_3$ and conc NH_4OH (1:1). GLC analyses were performed on 2 m × 2·2 mm i.d. stainless steel columns packed with 2·5% SE-30 on Chromosorb W (80–100 mesh) operating isothermally at 200°. Flow rate was 50 ml N_2/min.

High-voltage electrophoresis. Electrophoresis was carried out on Whatman 3MM paper using a flat-plate watercooled apparatus and a potential difference of 50 V/cm for a period of 45 min. Buffer solutions at pH 1·9, 2·4, 4·5, and 6·8 were used.

Extraction and fractionation. Alga (100 g fr. material) was macerated and extracted with cold 70% EtOH (3 × 400 ml). The combined extracts were concentrated, clarified by centrifugation, dialyzed (Amicon CF50A ultrafilter) and passed successively through columns of Dowex-50W (50–100 mesh, × 2, H^+) and Dowex-1 (200–400 mesh, × 8, OH^-). The final eluate was taken to dryness, giving the "neutral fraction". The "cationic fraction" was obtained by elution of the cation-exchange column with 2 N NH_4OH, while the "anionic fraction" was recovered from the anion-exchange resin by elution with 0·5 N HCOOH. The eluates were separately taken to dryness, and stored until required.

Routine examination of fractions. (a) *Neutral fraction.* An aliquot (10^{-1}) of this fraction was trimethylsilylated according to Sweeley *et al.* [36] and analyzed by GLC. A second portion was examined by TLC (Si gel; solvent 2; visualization with I_2 vapor) for *N,N,N*-trimethyltaurine. (b) *Anionic fraction.* A portion of the anionic fraction, after acetylation with Ac_2O-pyridine for 2 hr at 80° and successive treatment with CH_2N_2, was used for GLC. A second portion was analyzed in solvents 1–3 and using different chromogenic reagents. Taurine and *N*-methyltaurine were visualized with ninhydrin, *N,N*-dimethyltaurine with I_2 vapor. (c) *Cationic fraction.* Individual amino acid constituents present in this fraction were resolved by 2-D TLC, high-voltage electrophoresis and AAA.

L-*Baikiain* (**1**) *and* L-*pipecolic acid* (**2**). The amino acid fraction from *Pterocladia pinnata* (1 kg fr. alga) dissolved in H_2O (200 ml) was freed from basic and acidic amino acids by passing successively through columns of Amberlite IRC-50 (20–50 mesh, H^+) and Dowex-3 (20–50 mesh, OH^-). The final eluate was evaporated to a small vol. and then applied to a column of Dowex-50W (2·5 × 70 cm, H^+) which was eluted with HCl (4·5 l.) gradually increasing from 0·1 to 0·4 N. Eluate was collected in 30 ml fractions. Fractions 67–102 were pooled and taken to dryness to afford L-baikiain–HCl, wherefrom the free imino acid was obtained by resin (Dowex-3) treatment. Recrystallized from EtOH, **1** (2·6 g) had mp 274–275°, $[\alpha]_D^{20}$ −201° (*ca* 1 in H_2O). Lit [4] mp 274°, $[\alpha]_D^{20}$ −201·6°. IR spectrum of the isolated material (as hydrochloride) was identical with that reported in literature [37]. Catalytic reduction with 10% Pd/C in EtOH (3 hr at laboratory temp and pres) furnished L-pipecolic acid.

Fractions 103–150 were taken to dryness and the residue chromatographed on Whatman 3MM paper in solvent 1. The appropriate band, which reacted with ninhydrin giving a blue color, was excised and eluted with H_2O. The extract was concentrated to a product subsequently recrystallized from an Me_2CO–MeOH mixture to give a crystalline compound (35 mg), mp 272°, $[\alpha]_D^{20}$ −25·2° (*ca* 1 in H_2O), which had physical properties identical with those of L-pipecolic acid [38].

L-*Domoic acid* (**3a**). The cationic fraction from *Alsidium corallinum* (2·2 kg fr. wt) was dissolved in H_2O (500 ml) and applied to a column of Dowex-1 (3·5 × 40 cm, 200–400 mesh, $MeCOO^-$). Basic and neutral amino acids were washed through with H_2O and the acidic amino acids were then displaced with HOAc (8 l.) increasing the concentration from 0 to 2·5 N. **3a** emerged from the column after aspartic and glutamic acids and was present in fractions (50 ml) 136–143, which were combined and taken to dryness. The residue was finally recrystallized from aq. EtOH to give domoic acid (235 mg), mp 215° dec., $[\alpha]_D^{20}$ −108° (*ca* 1 in H_2O). Lit. [39] mp 217° dec., $[\alpha]_D^{12}$ −109·6° (Found: C, 51·90; H, 7·21; N, 4·05. Calc. for $C_{15}H_{21}O_6N$–$2H_2O$: C, 51·87; H, 7·25; N, 4·03%).

L-α-*Kainic acid* (**3b**). The amino acid fraction from *Centroceras clavulatum* (1 kg fr. alga) dissolved in H_2O was percolated through a column of Amberlite IRC-50 (H^+) to remove basic amino acids and the eluate was applied to a column of Dowex-1 (3·5 × 40 cm, $MeCOO^-$), which was thoroughly washed with H_2O. The eluate was set aside for the isolation of *N*-methylmethionine sulfoxide (*vide infra*). The anion-exchange column was then eluted with a linear gradient of HOAc (4 l.) from 0 to 0·2 N and fractions (30 ml) were collected. Fractions 71–87 were taken to dryness and the residue recrystallized from EtOH. The isolated compound (39 mg), mp 250° dec., $[\alpha]_D^{20}$ −15° (*ca* 1 in H_2O), was chromatographically and spectroscopically (IR, NMR) identical with L-α-kainic acid.

Pyrrolidine-2,5-*dicarboxylic acid* (**3c**). The anionic fraction from *Schizymenia dubyi* (2 kg fr. alga) was recrystallized 2× from EtOH to give pure **3c** (580 mg), mp 340–345°, $[\alpha]_D^{20}$ −112° (*ca* 1 in H_2O) and −88·1° (*ca* 1 in 5 N HCl) (Found: C, 45·00; H, 5·74; N, 8·72. $C_6H_9NO_4$ requires: C, 45·28; H, 5·70; N, 8·80%). MS: *m/e* 160 (M + 1), 142 (−H_2O), 114 (−HCO_2H), 96 (−H_2O–HCO_2H) and 68 (−$2HCO_2H$).

Dimethyl ester (MeOH–HCl): *m/e* 187 (M^+), 128 (M^+–CO_2Me), 68 (M^+–HCO_2Me–CO_2Me); δ ($CDCl_3$) 2·06 (4H, *m*, H_2C-3 and H_2C-4), 3·72 (6H, *s*, OMe), 4·00 (4H, *m*, HC-2 and HC-5). This dimethyl 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. Residue was purified by SiO_2 PLC (C_6H_6–Et_2O 7:3). Band R_f 0·5, after elution with Et_2O, afforded 2,5-dicarbomethoxypyrrole (12 mg), identified by comparison with an authentic sample.

L-*Azetidine*-2-*carboxylic acid* (**4**). The cationic fraction from *Lophocladia lallemandi* (1 kg fresh alga) dissolved in H_2O was passed successively through columns of Amberlite IRC-50 and Dowex-3 to remove basic and acidic amino acids, respectively. Final eluate was concentrated to a small volume and applied to a column of Dowex-50 (3 × 75 cm, H^+) which was eluted with HCl (8 l.), gradually increasing from 0 to 0·4 N. Eluate was collected in 50-ml fractions. Fractions 91–130 were combined and taken to dryness. From the residue, **4** (454 mg) was isolated by PC in solvent 4 and recrystallized from aq. EtOH. Physical properties (mp, IR, $[\alpha]_D$) were found to be identical with those of an authentic sample.

N-*Methyl*-L-*methionine sulfoxide* (**5**). The eluate from the Dowex-1 column (*vide supra*, isolation of kainic acid) was evaporated to dryness and the residue subjected to preparative PC in solvent 3. The major band (ninhydrin as spray reagent) was extracted with H_2O and concentrated to dryness. Residue was recrystallized from aq. EtOH to give colorless crystals of **5** (530 mg), mp 224–227°, $[\alpha]_D^{20}$ −33·6° (*ca* 1 in H_2O) (Found: C, 40·05; H, 7·31; N, 7·92; S, 17·62. $C_6H_{13}O_3NS$ requires: C, 40·22; H, 7·31; N, 7·82; S, 17·86%).

Reduction of **5** *with HI.* A soln of **5** (85 mg) in 57% HI (15 ml) was allowed to stand at room temp. for 3 hr and then diluted with H_2O (150 ml) and applied to a column of Dowex-3 (OH^-). The column was eluted with H_2O, the eluate taken to dryness and the residue recrystallized from aq. EtOH to yield *N-methyl-*L*-methionine* (53 mg), mp 234–236° dec., $[\alpha]_D^{20}$ +18·2° (*ca* 1 in H_2O). Lit. [40] for the enantiomer, mp 236–238°, $[\alpha]_D$ −18·6° (Found: C, 44·29; H, 8·11; N, 8·38; S, 19·52. $C_6H_{13}O_2NS$ requires: C, 44·16; H, 8·03; N, 8·58; S, 19·61%).

L-*Methionine sulfoxide.* The total amino acid fraction from *Grateloupia proteus* (1 kg fr. alga) in H_2O was passed through a column of Dowex-3, which was washed with H_2O to remove neutral amino acids and then eluted with 0·5 N HCOOH. The eluate, which contained dicarboxylic amino acids and L-methionine sulfoxide, was evaporated to dryness. The residue was dissolved in H_2O and the soln. run through a column of Dowex-1 ($MeCOO^-$); L-methionine sulfoxide was washed with H_2O. Eluate was taken to dryness and the residue further purified by PC in solvent 3 (200 mg). Recrystallization from aqueous EtOH eventually yielded the pure compound, mp 220–225°, $[\alpha]_D^{20}$ −70·1°. Lit. [41] mp 238–239°, $[\alpha]_D^{26}$ −71·6°.

Floridoside. Isolated from the neutral fraction of *P. pinnata* by column chromatography on carbon-Celite according to the method of Lindberg [20]. Recrystallization from EtOH yielded pure floridoside (1·15 g from 1 kg fresh alga), which on acidic hydrolysis gave glycerol and D-galactose in equimolar amounts; mp 128°, $[\alpha]_D^{20}$ +162° (*ca* 2 in H_2O) (lit. [20] mp 127–128°, $[\alpha]_D^{20}$ +163°). Acetate, mp 101° (lit. [20] 100–101°).

N,N,N-*Trimethyltaurine.* The eluate collected until the floridoside breakthrough (see above) was taken to dryness and the residue recrystallized from aqueous EtOH to yield *N,N,N*-trimethyltaurine (130 mg), mp 335°, whose physical properties were identical with those of an authentic sample prepared according to Lindberg [21].

Isofloridoside. Isolated from the neutral fraction of *Gigartina teedi* by a procedure similar to that used for floridoside. The yield (98 mg from 1 kg fr. alga) was low, since the compound did not separate well from floridoside on the carbon column. Recrystallized from EtOH, isofloridoside had $[\alpha]_D^{20}$ +150° (*ca* 1 in H_2O). Lit. [31] $[\alpha]_D^{20}$ +152°. On acid hydrolysis it gave only glycerol and galactose. The mp of our sample (121–123°) was near to that (121°) recorded [42] for the isomorphous mixture of 1-*O*-α-D-galactopyranosyl-D-glycerol and 1-*O*-α-D-galactopyranosyl-L-glycerol. The pure isomers have mp 131·5 and 150° respectively [42].

Mannoglyceric acid. The anionic fraction from *C. clavulatum* (1 kg fr. alga) was dissolved in H_2O (100 ml), the soln. neutralized with $NaHCO_3$ and evaporated to dryness. The residue was extracted 3× with hot 90% MeOH and the combined extracts taken to dryness. The residue was recrystallized repeatedly from aq. MeOH to give sodium mannoglycerate (662 mg), mp 264°, $[\alpha]_D^{20}$ +104·3° (*ca* 2 in H_2O) (lit. [43] mp 265°, $[\alpha]_D$ +106°), wherefrom free mannoglyceric acid (sirupy, $[\alpha]_D^{20}$ +91° (*ca* 2 in H_2O), lit. [43] $[\alpha]_D$ +93°) was regenerated after removal of the sodium by means of a cation exchange resin. Acid hydrolysis yielded mannose and glyceric acid. Acetylation (Ac_2O–C_5H_5N) followed by methylation (CH_2N_2) afforded a penta-acetate methyl ester which after recrystallization from EtOAc–*n*-pentane mixture had mp 91°, $[\alpha]_D^{20}$ +72° (*ca* 1 in MeOH). Lit. [43] mp 91° $[\alpha]_D$ + 73·1°.

Quantitation. (a) *Floridoside and isofloridoside.* They were determined by GLC of their TMS derivatives, using integrated areas of peaks (retention times relative to TMS-sucrose, floridoside 0·23, isofloridoside 0·26). (b) *Amino acids.* They were estimated by AAA using a Beckman analyzer model 116. Glutamine and asparagine were determined by measuring the increase in parent amino acids following hydrolysis (1 N HCl/3 hr in N_2) [44]. Non-protein amino acids poorly resolved from other peaks on the AAA were determined by weight of preparative PC. (c) *Taurine and methylated taurines.* Quantitation was made by weight of preparative TLC fractions (silica gel; solvent 2). (d) *Mannoglyceric acid.* It was determined by GLC of its fully acetylated methyl ester, using integrated areas of peaks (retention time relative to octa-*O*-acetylsucrose 0·18).

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