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Isolation of 2-L-Amino-3-hydroxy-1-propane Sulphonic Acid from *Polysiphonia fastigiata*

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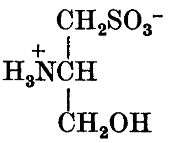
The low-molecular weight internal salts and carbohydrates from the red alga *Polysiphonia fastigiata* have been investigated. A new internal salt, shown to be 2-L-amino-3-hydroxy-1-propane sulphonic acid, and in addition mytilitol, laminitol, mannitol and a glycosidic compound were isolated.

It has recently been shown that taurine and certain N-substituted taurine derivatives [1,2] seem to be common constituents of the red algae. The red alga *Polysiphonia fastigiata*, *Florideae*, order *Ceramiales*, from which dimethyl-β-propiothetin chloride [3] and 2-D-glyceric acid α-D-mannopyranoside [4,5] have previously been isolated, has now been found to contain an internal salt, 2-L-amino-3-hydroxy-1-propane sulphonic acid (I), together with scyllitol, mytilitol, laminitol, mannitol and a new glycoside which is non-reducing and has the chromatographic properties of a disaccharide.



$$\begin{array}{r@{}l} & \mathrm{CH_2SO_3^-} \\ & \,| \\ \overset{+}{\mathrm{H_3N}} & \mathrm{CH} \\ & \,| \\ & \mathrm{CH_2OH} \end{array}$$

I

While scyllitol occurred only in very small quantities the amount of mytilitol found makes it most probable that this compound originates from the alga itself. The red algae *Gelidium cartilagineum* [1] and *Gigartina leptorhynchos* [2] gave mytilitol in very small quantities only and since it has been isolated from the blue mussel *Mytilus edulis* it was supposed that it might come from contaminating invertebrates in the samples extracted.

The isolation of laminitol and mannitol support earlier findings [6] that these substances are common in the red as well as in the brown algae.

The mannitol mother liquors, which contained mannitol and compounds with lower R_F-values, were hydrolysed yielding a mixture containing only traces of reducing sugar. The hydrolysate was acetylated and the acetates subjected to paper chromatography in a methyl sulphoxide-petroleum ether system [7]. The presence of mannitol, laminitol and mytilitol was indicated but only traces of sorbitol and no dulcitol.

The new internal salt can formally be derived from D-cysteic acid by reduction of the carboxyl group and therefore the name D-cysteinolic acid is suggested for the compound. It seemed to travel a little slower than taurine both on a carbon column and on paper chromatograms run in ethyl acetate-acetic acid-water (3:1:1). The compound could be detected on paper chromatograms by spraying with ninhydrin reagent but rather strong heating was required to produce the colour. When the silver nitrate-sodium ethoxide reagent was used the substance first appeared as a lighter spot which darkened only after several hours.

After purification by adsorption on Amberlite resin IR 400 in the OH-state and subsequent elution with dilute acetic acid and recrystallisation, the compound had m. p. 279—282° (decomp.), $[\alpha]_D^{23} + 7°$ (water) and gave an analysis which agreed fairly well with the composition $C_3H_9NO_4S$. A sample was oxidised with sodium periodate in aqueous solution. The mixture was buffered with sodium bicarbonate; potassium iodide and sodium arsenite solutions were added, and the excess arsenite titrated with iodine solution. No sharp endpoint could be detected, indicating that some reaction between iodine and the oxidised compound was occurring. Instead the periodate consumed was determined by the addition of potassium iodide and hydrochloric acid to the oxidation mixture and titration with sodium thiosulphate. A periodate consumption of 1 mole was indicated. One mole of formaldehyde determined as the dimedon complex was liberated.

These facts are in accordance with the formulation of the internal salt as 2,3-(amino-hydroxy-)-1-propane sulphonic acid, which upon periodate oxidation should give formylmethane sulphonic acid; this would be expected to undergo further oxidation. By analogy with the naturally occurring L-cysteine and L-cysteic acid the structure 2-D-amino-3-hydroxy-1-propane sulphonic acid seemed to be the most probable for the compound, and L-cysteinolic acid was therefore synthesised from L-cysteine. The latter was benzylated, the S-benzyl cysteine esterified with ethanol and the ethyl ester reduced to S-benzyl cysteinol. After removal of the benzyl group the cysteinol was oxidised with bromine in acid aqueous solution. From the reaction product a compound was isolated in 24 % yield based upon S-benzyl-L-cysteine, which was the only crystalline intermediate. It had the same melting point (279—281°) and chromatographic behaviour as the internal salt from the alga, but the opposite optical rotation, $[\alpha]_D^{23}$ —6° (water); the mixed melting point of the two compounds showed a strong depression. As the X-ray diffraction patterns of the natural and synthetic materials were identical it follows that the internal salt from the alga is D-cysteinolic acid and thus configurationally related to the rare D-amino acids. It is also of interest that neither taurine nor its derivatives have been found in amounts sufficient to permit their isolation.

EXPERIMENTAL *

The alga (360 g) was extracted continuously with ether for 3 days and then with methanol for 14 days. The methanol extract was concentrated *in vacuo*, the residual syrup stirred with water and the aqueous solution after filtration passed through Amberlite IR 120 and IR 4B and then concentrated to dryness yielding a syrup (9.9 g). After standing for two months the syrup was dissolved in ethanol-water (50 %) depositing crystals which were removed (Fraction 1). The filtrate was passed through a layer of decolorising carbon, concentrated to dryness, the residue dissolved in water (150 ml) and this solution added to the top of a carbon-Celite column (1:1, 35 × 4.5 cm). The column was first eluted with water (2 000 ml) and then with dilute ethanol the concentration of which was linearly increased (0—30 %, 4 000 ml). Fractions were collected automatically and investigated by paper chromatography using as solvent ethyl acetate-acetic acid-water (3:1:1). As it turned out that the sample had not been completely deionised before being put on the column, the appropriate fractions were combined and filtered through Amberlite IR 120 and IR 4B.

Fraction No. 1 contained a mixture of cyclitols.

Fraction No. 2 contained D-cysteinolic acid in addition to traces of compounds with the R_F-values of glycerol, volemitol and scyllitol.

Fraction No. 3 contained D-cysteinolic acid and mytilitol.

Fraction No. 4 contained laminitol and traces of D-cysteinolic acid.

Fraction No. 5 contained small amounts of mannitol and traces of laminitol.

Fraction No. 6 contained mannitol.

Fraction No. 7 contained mannitol and traces of compounds with lower R_F-values.

The next fractions contained small amounts of several compounds with the chromatographic characteristics of non-reducing disaccharides. Hydrolysis yielded mixtures which on paper chromatograms gave spots for galactose, glucose and glycerol. The following fractions were more closely examined:

Fraction No. 8 gave one principal spot with the R_F-value of α,α-trehalose. A hydrolysed sample gave one strong spot for glucose and two faint spots for galactose and glycerol in a butanol-pyridine-water (3:1:1.5) solvent.

Fraction No. 9 contained several components one of which, with a low R_F-value, predominated. After hydrolysis, a sample run in the butanol solvent gave spots with the R_F-values of galactose and glycerol.

As only traces of reducing sugars could be present, the above fractions were further purified by treatment with Amberlite IR 400 in the OH-state to remove internal salts. These were recovered by elution of the ion exchanger with 5 % acetic acid.

Carbohydrates. After combining the appropriate fractions, purification by chromatography on carbon and recrystallisation gave the following carbohydrate compounds:

Scyllitol (9 mg) from fractions 1 and 2, m. p. 300° (decomp.), acetate m. p. 275—280°;

Mytilitol (150 mg) from fractions 1, 3 and 4, m. p. 266—269°, acetate m. p. 186—188°, undepressed on admixture with mytilitol hexaacetate;

Laminitol (213 mg) from fraction 1 and 4, m. p. 270—274°, acetate m. p. 155—157°, alone or admixed with authentic laminitol;

Mannitol (108 mg) from fractions 5, 6 and 7, m. p. 160—162° alone or admixed with an authentic sample of D-mannitol;

Unknown compound (60 mg) from fraction 8. It was chromatographically similar to α,α-trehalose and gave a strong glucose spot after hydrolysis. After standing for several months the syrup has started to crystallise.

Unknown compound (200 mg) from fraction No 9, m. p. 196—198°, $[\alpha]_D^{23}$ +88° (water, c = 1.8). After a sample had been hydrolysed in 0.2 N HCl at 108° for 14 h a strong spot, indistinguishable from that of galactose, and a rather faint spot with the R_F-value of glycerol were obtained on chromatograms run in butanol-pyridine-water (3:1:1.5). This substance will be further investigated.

The mannitol mother liquors from fractions Nos. 6 and 7 could also contain other hexitols as well as mannitol and on paper chromatograms they gave additional spots with

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* All melting points are corrected.

lower R_F-values. They were hydrolysed in 0.2 N HCl at 100° over night. Paper chromatograms of the hydrolysate showed 2 strong spots with the R_F-values of a hexitol and a C-methyl cyclitol, while only traces of reducing sugars were detectable. A sample of the mixture was acetylated, and the acetates subjected to paper chromatography on Whatman No. 1 filter paper impregnated with methyl sulphoxide, using petroleum ether (b. p. 65—75°) or petroleum ether-*iso*propyl ether (1:2) as the mobile phase. The chromatograms were afterwards soaked with dilute silver nitrate solution (saturated solution-water 1:100), dried and sprayed with 1 N NaOH in ethanol. Mannitol and laminitol were indicated together with traces of sorbitol but no dulcitol.

Internal salts

From fractions Nos. 2, 3 and 4 only, an internal salt was obtained in appreciable amounts (325 mg), m. p. 270—275°. After adsorption on Amberlite IR 400 in the OH-state, elution with 5 % acetic acid and recrystallisation from dilute ethanol it had m. p. 279—282° (decomp.) and $[\alpha]_D^{23}$ + 7° (water, c = 2.0). (Found: C 23.6; H 5.88; N 8.27; S 18.0. Calc. for $C_3H_9NO_4S$: C 23.2; H 5.85; N 9.03; S 20.7.)

Of the combined mother liquors from fractions Nos. 2, 3 and 4, a sample (*ca.* 10 % of the total amount) was oxidised with excess periodate to decompose any D-cysteinolic acid. After de-ionising and evaporating, less than 1 mg of material was obtained. On paper chromatograms several ninhydrin-positive components were detected, none of which, however, could be taurine or N-methyltaurine.

The periodate consumption of the internal salt from fractions Nos. 2, 3 and 4 could not be determined as described previously [2] since the products of oxidation interfered with the titration of excess arsenite with iodine in a sodium bicarbonate buffer. Instead the sample (*ca.* 1 mg) in water (0.5 ml) was oxidised with 0.03 M sodium periodate (1 ml). At intervals potassium iodide solution (20 % in water, 0.5 ml) and 2 N hydrochloric acid (2 ml) were added and the liberated iodine titrated with 0.05 N sodium thiosulphate. After about 20 min the periodate consumed was 1.0 mole and this value rose to about 1.2 in 3 h.

The formation of formaldehyde upon periodate oxidation was 0.9 mole as determined by the method of Reeves [8]. Dimedone compound m.p. 191—193,5° alone or admixed with an authentic sample.

Synthesis of L-cysteinolic acid

S-Benzyl-L-cysteine was prepared following the directions of Hegedüs [9]. In a three-necked 500 ml flask equipped with a stirrer, dry L-cysteine hydrochloride (13 g) was added in portions to liquid ammonia (200 ml) and then sodium was added until a stable blue colour was produced. After the excess of sodium had been removed by the addition of small amounts of ammonium chloride, freshly distilled benzyl chloride (10.5 ml) was added. The ammonia was allowed to evaporate and the flask was evacuated and heated to 50° for 30 min. The residue was extracted with water, the solution filtered and the filtrate after extraction with ether brought to pH 5—6 with hydrochloric acid. After standing in a refrigerator overnight, the S-benzyl-L-cysteine was filtered off and washed with water. Yield 14.9 g (85 % of theory), m. p. 203—206° (decomp.).

S-Benzyl-L-cysteine ethyl ester. S-Benzyl-L-cysteine (14.9 g) was refluxed for 3 h in dry ethanol (300 ml) containing sulphuric acid (10 ml). After cooling on ice and neutralisation with a cooled solution of potassium carbonate (15 g) in water (20 ml) the mixture was filtered with Celite and the filtrate concentrated to dryness. The residue was covered with ether and 2 N sodium hydroxide solution (100 ml) added with cooling and shaking. The ether extract was dried over potassium carbonate and after filtration evaporated to dryness yielding an oil (9.6 g).

From the aqueous alkaline solution benzyl cysteine (4.2 g) was recovered by neutralisation with hydrochloric acid.

S-Benzyl-L-cysteinol was prepared by a method analogous to that used by Karrer and Aman [10] for the synthesis of S-propyl-L-cysteinol. A solution of S-benzyl-L-cysteine ethyl ester (9.6 g) in dry ether (80 ml) was slowly added to a solution of lithium aluminium hydride (3 g) in dry ether (150 ml) contained in a three-necked flask with a stirrer and a condenser. After the mixture had been stirred for an additional 30 min, excess hydride was decomposed by the addition of ethyl acetate (20 ml) in ether (20 ml) and a mixture of water (6 ml), ethanol (10 ml) and ether (20 ml). After standing overnight the ether solution was filtered by suction from the precipitate, dried over potassium carbonate and concentrated to dryness. Crude S-benzyl-L-cysteinol was obtained as an oil (8.1 g).

L-*Cysteinol.* The debenzylation of S-benzyl-L-cysteinol was carried out by the method of du Vigneaud. Sodium was added to a solution of crude S-benzyl-L-cysteinol (8.1 g) in liquid ammonia (*ca.* 200 ml) until a stable blue colour appeared. The mixture was allowed to stand for 30 min and then neutralised by the addition of ammonium sulphate (7 g). After most of the ammonia had evaporated the flask was evacuated and kept at 50° for 15 min. The residue was extracted with dry ethanol (150 ml), the solution was filtered and the filtrate concentrated. The residual syrup was dissolved in water (100 ml) and the solution after extraction with ether concentrated under reduced pressure yielding a syrup (5.5 g). This amount exceeds the theoretical yield by 1.1 g, probably due to insufficient drying. Paper chromatograms of the product run in ethyl acetate-acetic acid-water (3:1:1) gave only one strong and a few very faint spot when sprayed with ninhydrin reagent.

L-*Cysteinolic acid.* While the oxidation of cystine to cysteic acid with bromine in hydrochloric acid solution [11] proceeds smoothly (80—90 % yield), the oxidation of L-cysteinol under the same conditions gave an inferior result. Preliminary experiments which were followed by paper chromatography indicated that the oxidation took several different routes, yielding mainly two compounds behaving as internal salts, one of which proved to be the enantiomorph of the internal salt from the alga. The other component did not crystallise readily and seemed to be unstable upon prolonged storage. It was not further investigated.

Probably hydrolytic reactions took place also, since if the solution after saturation with bromine at 35° was heated to boiling for a short time and cooled, more bromine was then rapidly absorbed.

To a solution of crude L-cysteinol (2.7 g) in 1 N hydrochloric acid (50 ml) bromine was added, with stirring, at such a rate that it was immediately consumed and the temperature did not rise above 35°. After 8.8 g of bromine had been added the uptake of bromine was very slow and the mixture was left over night at room temperature. More bromine

Table 1. Spacing values.

Internal salt from the alga (D-cysteinolic acid)		Synthetic L-cysteinolic acid	
d (Å)	I/I_1	d (Å)	I/I_1
9.35	100	9.27	100
5.07	5	5.06	10
4.69	70	4.69	85
4.22	10	4.23	15
4.13	50	4.13	70
3.91	35	3.90	50
3.65	25	3.65	35
3.35	15	3.35	15
3.26	10	3.26	10
3.16	70	3.16	30
3.13	15	3.13	25
2.73	35	2.73	35
2.51	5	2.51	10
2.31	10	2.31	10
2.25	5	2.25	10
1.95	5	1.95	10
1.88	5	1.88	10

(0.25 g) was then quickly consumed. After the reaction mixture had been heated to boiling for a few minutes and cooled to room temperature, bromine (0.5 g) was added until no more was absorbed.

After concentration *in vacuo*, dilution with water and de-ionisation with Amberlite IR 120 and IR 4B the reaction mixture was taken to dryness yielding an amorphous mixture (2.7 g). This was separated on a carbon-Celite column (1:1; 35 × 4.5 cm) using water as eluant. A fraction was obtained which on paper chromatograms gave a spot indistinguishable from that of the internal salt from the alga. Concentration and crystallisation from dilute ethanol gave a product with m. p. 265—275° (decomp.). The yield was 1.16 g or 24 % calculated from the S-benzyl-L-cysteine utilised. Further recrystallisation yielded a pure product m. p. 279—281° (decomp.), $[\alpha]_D^{23}$ $-6°$ (water, $c = 2.0$). Mixed m. p. with the internal salt from the alga (m. p. 279—282°) 245—260°.

X-Ray diffraction. The X-ray diffraction patterns for the cysteinolic acid from the alga and for synthetic L-cysteinolic acid (Table 1) were determined on a "Norelco X-ray Diffraction Apparatus with High Angle Goniometer" (Philips Co).

(Cu $K\alpha$) = 1.5418 Å. The samples for examination were finely ground, suspended in ethanol and spread in a thin layer on glass plates and the ethanol allowed to evaporate.

The discrepancies between the relative intensities (I/I_1) obtained from the two samples may be due to slightly different orientation of the crystals.

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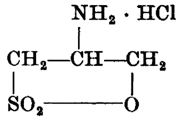
Added in proof: Recently the synthesis and oxidation of cysteinol has been described by J. C. Crawhall, D. F. Elliott and K. C. Hooper *(J. Chem. Soc.* **1956** 4066). These authors have found that oxidation of cysteinol hydrochloride with bromine water yields cysteic acid and occasionally the sultone II. No cysteinolic acid was produced.



II.

With a view to these findings, L-cysteic and L-cysteinolic acid were compared. A sample of L-cysteic acid decomposed at ca 275° on rapid heating, whereas L-cysteinolic acid melted at 279—281° before any considerable decomposition took place. The mixed samples rapidly decomposed at 260—270°. On a paper chromatogram run in a butanol-pyridine-water mixture (2:3:1.5) mixture the R_F values were 0.17 and 0.35 for L-cysteic and L-cysteinolic acid respectively.

REFERENCES

1. Lindberg, B. *Acta Chem. Scand.* **9** (1955) 1323.
2. Wickberg, B. *Acta Chem. Scand.* **10** (1956) 1097.
3. Challenger, F. and Simpson, Margaret I. *J. Chem. Soc.* **1948** 1591.
4. Colin, H. and Augier, J. *Compt. rend.* **209** (1939) 1450.
5. Bouveng, H., Lindberg, B. and Wickberg, B. *Acta Chem. Scand.* **9** (1955) 807.
6. Lindberg, B. *Acta Chem. Scand.* **9** (1955) 1097.
7. *To be published.*
8. Reeves, R. E. *J. Am. Chem. Soc.* **63** (1941) 1476.
9. Hegedüs, B. *Helv. Chim. Acta* **31** (1948) 737.
10. Karrer, P. and Aman, G. *Helv. Chim. Acta* **33** (1950) 305.
11. *Org. Synthesis* Coll. Vol. **3** (1955) 226.

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