A second extraction with acetone gave atranorin (102 mg, 0·17%) crystallized from chloroform–petrol [1] and identified in a similar manner as the other reported substances. Traces of lecanoric acid were identified by TLC.

This is the first time that the presence of atranorin has been noted in the Roccellaceae.

Acknowledgements—We are greatly indebted to Dr. H. H. Appel, Universidad Santa Maria, Chile, for his kind interest and technical advice and to Dr. G. Follmann, Kassel, Germany, for the authentic samples of lecanoric and roccellic acids and atranorin from Zopf's collection.

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TWO NEW POLYSACCHARIDES FROM *COLPOMENIA SINUOSA*

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(*Revised Received* 14 *February* 1975)

Key Word Index—*Colpomenia sinuosa*; Phaeophyceae; Scytosiphonales; sulphated polysaccharides; glucurono-mannogalactan.

Many brown algal species contain sulphated polysaccharides other than fucoidan. Larsen *et al.* [1] have reported the isolation of a fucose-containing polysaccharide, ascophyllan, from *Ascophyllum nodosum*. This polysaccharide contained 25·2% fucose, 26% xylose, 19·2% sodium glucuronate, 12% ester sulphate, and 12% protein. Another sulphated polymer, glucuronoxylofucan, containing L-fucose (49%), D-xylose (10%), D-glucuronic acid (11%), sulphate (20%), and protein (3·8%) has also been isolated from *Ascophyllum nodosum* [2]. Furthermore, a soluble complex, extracted from *Ascophyllum nodosum* [3], contained fucose, xylose, galactose, traces of mannose, glucuronic, mannuronic and guluronic acids, half-ester sulphate and traces of firmly bound protein. More recently, Abdel-Fattah *et al.* [4] have reported the isolation of sargassan, a sulphated heteropolysaccharide from *Sargassum linifolium*, containing glucuronic acid, galactose, mannose, xylose and fucose in addition to 12·2% SO_4^{2-} and 3·85% protein. Mian and Percival [5] have separated 'fucans' from *Himanthalia lorea*, *Bifucaria bifurcata*, and *Padina pavonia*; these were characterized by their variable proportions of fucose, xylose, glucuronic acid, galactose and sulphate half ester. The present note describes the isolation of two new sulphated polysaccharides from *Colpomenia sinuosa*.

The percentage composition of *Colpomenia sinuosa* was found to be: 24·31% ash, 12·92% crude protein, 11·06% total lipids, 5·51% mannitol, 3·11% laminaran, 19·79% alginic acid and 13·38% acid-extractable polymer [6]. No LM carbohydrates were found in the alcoholic extract of alga after removal of mannitol. Acid hydrolysis of the algal material and PC of the hydrolysate gave mannuronic, guluronic, and glucuronic acids, their lactones, in addition to galactose, glucose, mannose, xylose and fucose. Further chromatographic analysis revealed the sugar components of the isolated alginic acid and the acid-extractable polymer [6]. Mannuronic and guluronic acids were identified as the components of alginic acid, while glucuronic acid, galactose, mannose, xylose and glucose in addition to fucose were found to construct the acid-extractable polymer [6].

The method of Black *et al.* [6], for fucoidan extraction, was modified to fractionate the acid-extractable polymer into water soluble and water insoluble forms, in the ratio of 1:2, respectively.

Complete acid hydrolysis of these polysaccharides afforded the same sugars as in the crude polymer. The water soluble polysaccharide contained relatively higher proportions of galactose (30·77%), mannose (24·02%) and glucuronic acid (23·07%) and less amounts of xylose (14·64%) and fucose (7·50%) in addition to traces of glucose. Thus this polysaccharide is a glucuronomannogalactan with smaller amounts of xylose and fucose. It is a unique polysaccharide for the Phaeophyceae and may be characteristic of the family Scytosiphonaceae. Although other polysaccharides containing these sugars have been isolated from other brown seaweeds [1–5], the proportions have never been of this order. By contrast, the second water-insoluble polysaccharide was found to contain a higher proportion of glucose (41·13%) which may be, partially, due to contamination with cold water-insoluble form of laminaran. Other sugar proportions were mannose (19·32%), glucuronic acid (17·54%) and galactose (22·51%); traces of xylose and fucose were also detected.

The water insoluble polysaccharide showed a high ash content (27·21%) as compared with that of the water soluble product (6·07%). This may be due to the precipitation of many inorganic contaminants, as their insoluble carbonate form, during the neutralization of the algal extract with Na_2CO_3. The sulphate contents (36·30 and 8·72% respectively) were higher than the respective ash contents.

Although the water soluble polysaccharide was deproteinized with trichloroacetic acid, traces of proteins were still present (Folin reagent). Moreover, the product had a brown colour which could not be removed by charcoal treatment. However, it gave negative reaction with ninhydrin. These results collectively suggest the presence of traces of protein linked to the polysaccharide. A similar soluble complex was, however, isolated by Larsen *et al.* [3] from *Ascophyllum nodosum* and found to contain, in addition to the polysaccharide, a trace of firmly bound protein. The water insoluble polysaccharide contained 14·81% protein.

EXPERIMENTAL

Specimen. Colpomenia sinuosa, a seaweed belongs to family Scytosiphonaceae, was collected in March 1974 from Roushdy at Alexandria. After collection, the plants were thoroughly washed, dried and finally milled. The values were calculated on dry basis.

Analytical methods. Ash content was determined by heating the sample to constant weight at 800°. Organic N was determined by Kjeldahl's method and multiplied by 6·25. In case of the soluble polysaccharide, protein determination was carried out by the method of Lowry *et al.* [7]. Total lipids were isolated by Soxhlet extraction with $CHCl_3$–MeOH (2:1) for 12 hr. Mannitol was determined by extraction with 85% EtOH for 24 hr [8]. After isolation, mp and mmp were determined. It was also identified chromatographically using *n*-BuOH–HOAc–H_2O (12:3:5) and the Dedonder reagent. After removal of crystalline mannitol, the remaining alcoholic extract was concd under vacuum at 35° and then examined by PC, using standard solvents and spray reagents. Acid hydrolysis of the algal material was carried out with 2 N H_2SO_4 and the sugars were identified by PC in the usual way. Laminaran was determined according to the method of Black *et al.* [9]. After extracting alginic acid with Na_2CO_3 according to the method of Cameron *et al.* [10], it was determined gravimetrically by precipitation in 5% HCl (W/V). The isolated alginic acid was hydrolysed in 2 N H_2SO_4 [11] and the hydrolysis products compared with authentic uronic acids and their lactones by PC.

Isolation of the new sulphated polysaccharides. The dried ground *Colpomenia sinuosa* (50 g) was stirred with HCl (500 ml) at pH 2 and 100° for 3 hr [6]. After filtration, the algal residue was re-extracted in the same way and the two extracts were combined and neutralized with satd. Na_2CO_3 followed by dialysis against H_2O for 48 hr. The dialyzed soln was centrifuged and the residue was washed with EtOH and Et_2O then dried under vacuum at 40°. The resulted dry material was the water insoluble polysaccharide. Supernatant was then percolated through a column of Lewatite S 100 (H^+) resin and the effluent dialyzed against H_2O for 48 hr. Thereafter, the dialyzed soln was concd under vacuum at 40° to about 100 ml and satd. soln of trichloroacetic acid was added to give final concn of 10%. The pptd proteins were centrifuged off and removal of trichloroacetic acid from the supernatant was achieved by extraction with Et_2O (×3). The aq. layer was then separated and dialyzed for 2 days against H_2O. Thereafter the dialyzed soln was concentrated at 40° to half its vol. and treated with 4 vol. of EtOH. The pptd polysaccharide was isolated by centrifugation, washed with EtOH and Et_2O then dried under vacuum at 40°. The resulted dry product was the soluble polysaccharide.

Analysis of isolated polysaccharides. Standard methods were used throughout. Total carbohydrate was determined by the phenol–H_2SO_4 method [12]. Sulphate was determined via barium chloranilate [13].

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Phytochemistry, 1975, Vol. 14, pp. 1868–1870. Pergamon Press. Printed in England.

LACTONE-FORMING ACIDS IN SUCCULENT PLANTS

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(*Received* 26 *February* 1975)

Key Word Index—Angiosperms; succulent plants; isocitric acid; glucaric acid; phorbic acid; quinic acid; distribution.

Plants and sources. Families and species examined are listed in Table 1. Species of the Cactaceae, except for *Echinopsis triumphans*, *Opuntia ficus-indica* and *Pachycereus pringlei*, was supplied by Apotekare Jan G. Bruhn, Department of Pharmacognosy, Faculty of Pharmacy, University of Uppsala, Stockholm, Sweden. Identification was carried out by Dr. Helia Bravo and Dr. Hernando Sánchez-Mejorada, Departamento de Botánica, Instituto de Biologia, Universidad Nacional Autónoma de México, México D.F. *Pachycereus pringlei* was supplied by Dr. A. A. Benson, Scripps Institution of Oceanography, La Jolla, Calif., who was also responsible for the identification of this species. All the other species originate from the Botanical Garden, University of Oslo, Norway. They were identified and partly supplied by the director of the Garden, Dr. Per Sunding.

Previous work. Earlier investigations indicate that hydroxy acids, which can form lactones (lactone-forming acids), are widely distributed within succulent plants. [1–7].

Present work. The present investigation supports earlier findings, as all the plants investigated were found to contain one or more lactone-forming acids (Table 1). The following acids were used as reference compounds: dilactophorbic acid [3], erythronolactone, D-galactaric acid, D-glucaric acid (prepared from calcium D-saccharate by means of Dowex 50 [H^+]), hibiscus acid, homocitric acid lactone, DL-isocitric acid lactone, quinic acid, quinide [5] and shikimic acid.

Table 1. Lactone-forming acids in succulent plants

Plant family and species*	Lactone-forming acids identified	Criteria for identification
Liliaceae		
Aloe vera L.	Isocitric acid	TLC, GLC (OV-17, OV-225)
Amaryllidaceae		
Agave sisalana Perrine	Glucaric acid	TLC
	Isocitric acid	TLC, GLC (OV-17, OV-225)
Crassulaceae		
Echeveria elegans Rose	Isocitric acid	TLC, GLC (OV-17, OV-225), GC–MS
	Phorbic acid	TLC, GLC (OV-17), GC–MS
Kalanchoë longiflora Schltr.	Isocitric acid	TLC, GLC (OV-17, OV-225), GC–MS
Kalanchoë pinnata (Lamk.) Pers. (*Bryophyllum calycinum* Salisb.)	Isocitric acid [11]	TLC, GLC (OV-17, OV-225), GC–MS
Sedum acre L.	Isocitric acid [2]	TLC, GLC (OV-17, OV-225)