Short Communications and Preliminary Notes

Free sulfuric acid in the brown alga, *Desmarestia*

The Pacific Coast waters of North America harbor several representatives of the genus *Desmarestia* that are characterized by an extreme acidity. Since, for this reason, they cause severe discoloration of other algae placed in one container with them, they are usually avoided by collectors; even the *Desmarestias* alone, when bruised, sometimes give a blue color reaction, probably due to the effect of their acid on fucoxanthin. The following table gives some literature data on the acidity of various *Desmarestia*-species, including *D. viridis* which was studied extensively in Europe by H. KYLIN.

TABLE I

Species	*pH value of juice*	*Authors and year*
D. latissima	0.78	WIRTH & RIGG, 1937[10]
D. intermedia	1.13	
Unidentified *D.* species	1.35	
D. viridis	1.8	KYLIN, 1938[4b]
D. aculeata	6.8	
D. munda	1.0	BLINKS, 1951[1]
D. herbacea	2–3	
D. latifrons	5	

In 1953 and 1954 the present author, working with *D. herbacea, D. intermedia, D. ligulata* and *D. munda* found pH values usually varying between 1 and 2. BLINKS' interesting suggestion that there may be a connection between acidity and fleshiness (mainly based on the observation that the delicate species, *D. latifrons*, has a relatively high pH) is not borne out by the fact that *D. viridis* is at the same time decidedly acid and very delicate.

The high acidity exhibited by *Desmarestia* extracts and press juices seems to be almost unique in the plant kingdom, the only comparable example in higher plants being the case of a *Begonia* with cell sap of pH 0.9–1.36[7]. In the animal world, the presence of mineral acids in the digestive juices of certain gastropods is well known; in a recent article, for instance, FISH[3] discusses the possible effects on lake fertility of the excretion of sulfuric acid by the snails *Melanoides* and *Caelatura*. The most outstanding example of the occurrence of free acid, however, remains that of the so-called vanadium cells in Ascidians (WEBB[9]); the acid, in that case, is sulfuric acid.

As to the nature of the acid, in *Desmarestia* there is no unanimity of opinion. KYLIN[4a] noticed that no charring occurred when he concentrated his *Desmarestia*-extracts; since he also was able to prepare Ca-malate from the latter, and obtained a positive color reaction with $FeCl_3$, he concluded that the acid was malic acid (charring should have occurred if it had been sulfuric). On the basis of the assumption that malic acid is the only acid present, he calculated a malic acid content of 3.96%. WIRTH AND RIGG[10], on the other hand, basing their conclusions mostly on the shape of the alkali titration curve of the *Desmarestia* press juice, claimed sulfuric acid to be practically the only one in 3 Pacific Coast species. A reinvestigation of the problem by KYLIN[4c] did not lead to an essential change in the latter's opinion, although this time he reported the presence of a small amount (0.22%) of citric acid in addition to the malic.

The following, modern, methods have been used by the present writer in an attempt to solve the controversy:

(1) *Filter paper chromatography*. This method was applied to ether extracts obtained from press juices and aqueous extracts, as well as to these aqueous fluids themselves. Butanol-formic acid-water mixtures were used most often; for details of techniques we must refer to items 2 and 5 of the list of references. In most extracts, small amounts of malic and citric acid could be demonstrated; traces of succinic acid were present in a few cases. No evidence could be found for the presence of oxalic acid. In all cases, there was a very pronounced spot of a substance with a low R_F value, corresponding with that of sulfate.

(2) *Total carbon analysis.* An aqueous extract of dry *D. munda* powder was somewhat purified by precipitating high-molecular material with 80% ethanol; after elimination of the latter substance, and addition of water, an aqueous fluid of pH 1.5 was obtained. 0.7 ml portions of this fluid were frozen at —20° C and subjected to total carbon analysis according to the VAN SLYKE-FOLCH method. The values found for the $BaCO_3$ produced were 76.4 and 74.5 mg, corresponding with about 13 mg of malic acid, or 18.57 g of malic acid per liter. However, it can be shown both experimentally and by calculation, that such a malic acid solution would give a pH of 2.5 instead of 1.5. In other words: even on the basis of the extremely unrealistic assumption that all the carbonaceous material in the extract is malic acid, only 10% of the hydrogen ions can be accounted for! For citric acid, a similar figure is found. Conclusion: another, stronger, acid must be present.

(3) *Enzymic methods.* Malic acid was determined by means of NOSSAL's elegant method of bacterial decarboxylation[6a,b]. It was always present, but in quantities never exceeding 0.1% of the dry weight. The presence of fumaric acid was doubtful. Attempts were made to determine succinic acid with the aid of succinodehydrogenase as described in UMBREIT, BURRIS AND STAUFFER[8]. However, the amounts present were so low that it was felt that the limit of applicability had been reached.

(4) *Titration curves.* Following WIRTH AND RIGG'S example, alkali-titration curves were drawn for *Desmarestia* extracts (for *D. munda* extract, see Fig. 1). It can be seen that the bend in the curve occurs at a pH of about 2.5; since malic acid has a pK_{a1} of about 3.4, it follows that it cannot be held responsible for the phenomena observed.

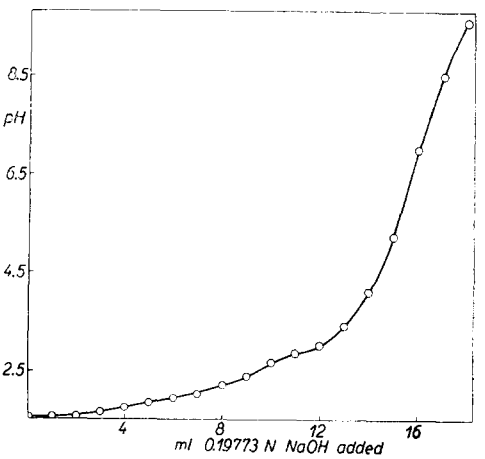

Fig. 1. Alkali-titration curve of *D. munda* extract.

(5) *Balance sheet for the main ions.* Since the spot with the low R_F value mentioned under method 1 (chromatography) could be due to the presence of (neutral) sulfate as well as to that of sulfuric acid, it was decided to draw up a balance sheet for the main ions. The value for the H-ion concentration follows directly from the pH value. Na and K were determined by means of flame spectrophotometry, the other ions by means of other conventional methods. Table II shows that the balance is far from perfect; however, this is a phenomenon frequently encountered even in the most careful analysis of, for instance, soil extracts. The table does show clearly that the sulfate ion is the most abundant one in the *Desmarestia* extract. On the other hand it has to be realized that the statement that *Desmarestia* produces free sulfuric acid is a little misleading, and should be read critically: the hydrogen ions form only a small proportion of the cations counterbalancing the anions.

TABLE II

IONS IN 1 ml OF *Desmarestia* EXTRACT

		Cations		*Anions*	
		mg ions	*m equiv.*	*mg ions*	*m equiv.*
H	0.025	0.025	SO_4	0.150	0.300
K	0.026	0.026	Cl	0.119	0.119
Na	0.135	0.135	PO_4	0.016	0.048
NH_4	0.009	0.009	NO_3	0.005	0.005
Ca	0.042	0.084			
Mg	0.071	0.142	Total		0.472
Total		0.421			

It has thus been shown by various independent methods that among the anions available to match the free hydrogen ions, sulfate ions are the most important ones; organic ions play a minor role.

The mechanism through which acid accumulated in high concentration in *Desmarestia* cells, and the possible function of the acid in cellular metabolism, must for the time being remain objects of speculation.

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1 L. R. BLINKS, *Physiology and Biochemistry of Algae*, Chapter 14 in *Manual of Phycology*, ed. by G. M. SMITH, Waltham, Mass., 1951.
2 F. CRAMER, *Papierchromatographie*, 2. Aufl., Weinheim, 1953.
3 G. R. FISH, *Nature*, 175 (1955) 733.
4 H. KYLIN, a. *Z. Physiol. Chem.*, 197 (1931) 7.
b. *Planta*, 27 (1938) 645.
c. *Forh. Kungl. Fysiogr. Sallsk. Lund*, 14 (1944) 226.
5 J. W. LUGG AND B. T. OVERELL, *Australian J. Sci. Research, A* 1 (1948) 98.
6 P. M. NOSSAL, a. *Biochem. J.*, 49 (1951) 407.
b. *Ibid.*, 50 (1952) 349.
7 E. F. SMITH AND A. J. QUIRK, *Phytopathol.*, 16 (1926) 491.
8 W. W. UMBREIT, R. H. BURRIS AND J. F. STAUFFER, *Manometric Techniques and Tissue Metabolism*, 2nd ed., Burgess Pub. Co, Minneapolis, 1949.
9 D. A. WEBB, *J. Exptl. Biol.*, 16 (1939) 499.
10 H. E. WIRTH AND G. B. RIGG, *Am. J. Bot.*, 24 (1937) 68.

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Addendum. After the completion of the work reported here, an article by MIWA (T. MIWA, *Proc. 7th Pac. Sci. Congr.*, 5 (1953) 78) was brought to my attention. This worker investigated the 3 Japanese species *D. ligulata*, *D. viridis* and *D. tabacoides*. The first two were examined in the dried state only, whereas *D. tabacoides* could be fully studied in fresh material. MIWA's conclusions are in complete agreement with those of the present writer: the results of tests for malic acid were only faintly positive, whereas free sulfuric acid could be demonstrated in abundance. A sulfate content of 25.0% is claimed for the dry fronds of *D. tabacoides*.

The synthesis of glycerides in liver homogenates

Considerable progress has been made during the last few years in the elucidation of the mechanism of phospholipid synthesis[1,2,3]. On the other hand, very little information is available on the biosynthesis of simple glyceride esters. BORGSTRÖM[4] showed that lipase catalyzes the synthesis of triglycerides from diglycerides and fatty acids. However, it is doubtful whether this reaction is of significance in the synthesis of glycerides under physiological conditions in organs other than the digestive tract. KORNBERG AND PRICER[1] demonstrated the formation of an ester linkage with higher fatty acids by an enzyme obtained from guinea pig liver. This enzyme catalyzes the transfer of acyl groups from acyl-CoA compounds to glycerophosphate. However, it did not react with glycerol as an acyl acceptor.

The ability of most rat tissues to incorporate 1-^{14}C-palmitate into triglycerides as well as into phospholipids was demonstrated by JEDEIKIN AND WEINHOUSE[5]. These authors came to the conclusion that triglyceride synthesis is brought about by a mechanism entirely different from that of phospholipid synthesis, since the former proceeded to the same extent in the absence as well as in the presence of respiration, while phospholipid synthesis was completely dependent upon the active respiration of the tissue. They assumed that glyceride synthesis may not involve acyl-coenzyme A formation.

In the present investigation, the incorporation of 1-^{14}C-palmitate into non-phospholipid glycerides by rat liver homogenates was examined. Homogenates were prepared in 3 volumes of buffer (9 parts of 0.154 *M* KCl and 1 part of Tris buffer 0.5 *M*, pH 7.5). Cell fragments and nuclei were removed by low speed centrifugation and the homogenate was freed of most of the fat by filtering through a small pad of cotton wool. To 2 ml of the homogenates 3 μM of potassium 1-^{14}C palmitate (40,000 cts./min), 20 micromoles of K-phosphate buffer (pH 7.5), 10 μM of $MgCl_2$ and ATP as indicated were added and the volume made up to 3 ml. The mixture was incubated