

method was applied without difficulty to *n*-butyl mercaptan in a naphtha ("Skellysolve") which was free from unsaturated hydrocarbons. In the presence of the latter, however, the method failed. Hofmann and Sand⁷ were the first to discover that mercuric chloride liberates hydrochloric acid when shaken with an unsaturated hydrocarbon of the olefin series. Several cubic centimeters of alkali was required to neutralize the acid when a 1% mercuric chloride solution was shaken for three minutes with a 1% solution of amylene in benzene.

Summary

1. The iodimetric method for the estimation of mercaptans has been applied to benzene solutions.

2. Two acidimetric methods for the estimation of mercaptans are given: in one the hydriodic acid formed in the iodimetric oxidation is titrated; in the second method, the hydrochloric acid liberated by the action of mercuric chloride on the mercaptan is estimated.

⁷ Hofmann and Sand, *Ber.*, **33**, 1349 (1900).

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THE PROPERTIES OF *d*-MANNURONIC ACID LACTONE

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Since the publication of our paper on the isolation and identification of *d*-mannuronic acid lactone from *Macrocystis pyrifera*,¹ the lactone has been obtained in this Laboratory by hydrolysis of alginic acid from *Fucus serratus* and *Laminaria saccharina*.² As a result of our further work, we have discovered the mutarotation of the lactone which escaped our observation because of the small amount of the substance available in our earlier experiments.

The lactone was isolated through the cinchonine salt as previously described.¹ The melting point and optical rotation of the cinchonine salt from *Fucus serratus* and *Laminaria saccharina* agreed with the values reported for the salt from *Macrocystis pyrifera*.³ Under different conditions of hydrolysis, however, Schmidt and Vocke,⁴ Miwa⁵ and Bird and Haas⁶ have obtained salts with different properties.

¹ Nelson and Cretcher, *THIS JOURNAL*, **52**, 2130 (1930).

² We are indebted to Dr. E. J. Allen, Director of the Marine Biological Station, Plymouth, England, for his kindness in supplying us with these algae.

³ Cretcher and Nelson, *Science*, **67**, 537 (1928); Nelson and Cretcher, *THIS JOURNAL*, **51**, 1914 (1929).

⁴ Schmidt and Vocke, *Ber.*, **59**, 1585 (1926).

⁵ Miwa, *J. Chem. Soc. Japan*, **51**, 738 (1930).

⁶ Bird and Haas, *Biochem. J.*, **25**, 403 (1931).

TABLE I
 CINCHONINE SALTS OF URONIC ACIDS FROM ALGIN

		M. p., °C.	$[\alpha]_D$
Nelson and Cretcher	<i>Macrocystis pyrifera</i>	152	+113.6°
	<i>Fucus serratus</i>	153	+114.3°
	<i>Laminaria saccharina</i>	152	+111.2°
	From crystalline mannuronic lactone	154	+113.3°
Schmidt and Vocke	<i>Fucus serratus</i>	204	
Miwa	<i>Ulopteryx pinnatifida</i> and other algae	174	+140.3°
Bird and Haas	<i>Laminaria</i>	195-197	+112.8°
		161	+154°

These discrepancies seem to indicate the presence of more than one uronic acid in alginic acid⁷ but we believe it is more probable that they are due to differences in degree of hydrolysis. The presence of glucuronic acid has been suggested^{4,8} but has never been established. We have prepared cinchonine mannuronate from crystalline mannuronic lactone and have found that the melting point and specific rotation of this preparation closely check these properties of the cinchonine salt obtained by us after hydrolysis of alginic acid.

The preparation of the cinchonine salt of mannuronic acid after hydrolysis of alginic acid is a matter of considerable trouble. This salt is not a suitable derivative for the characterization of mannuronic acid. The cinchonidine and brucine salts are more easily crystallized and their properties are described in the experimental part.

The separation of the barium salts of the products of hydrolysis of alginic acid by fractional precipitation with alcohol is a convenient method of isolating mannuronic acid. Schoeffel and Link⁹ have recently applied this method in their study of the preparation of mannuronic lactone.¹⁰

Experimental Part

Preparation of *d*-Mannuronic Lactone.—Hydrolysis of alginic acid from *Macrocystis pyrifera*, *Fucus serratus* and *Laminaria saccharina* with 80% sulfuric acid and the subsequent steps in the preparation of the lactone were accomplished with few changes from our previously described procedure.^{1,3} Among these was the method of removing sul-

⁷ It has been established that alginic acid is a uronic acid polymer exclusively.³

⁸ Atsuki and Tomodo, *J. Soc. Chem. Ind. (Japan)*, **29**, 509 (1926).

⁹ Schoeffel and Link, *J. Biol. Chem.*, **95**, 213 (1932).

¹⁰ Schoeffel and Link state that they obtained a "pure" barium salt after precipitation of polymers with alcohol. The barium content of their preparation was between 26 and 27% (calcd. 26.26%). Without an intimation that their salt was not pure barium mannuronate it may be stated that within the limits of analysis given the presence of a considerable amount of polymers is possible. Conclusions as to the purity of the barium salt may better be based on determination of its reducing power. It has been shown¹ that the lactone is abnormal in its reaction with iodine in alkaline solution (Willstätter-Schudell), but recent determinations in this Laboratory show that it is possible to obtain satisfactory estimations of the lactone with Benedict's solution.

furic acid after hydrolysis. In order to reduce decomposition of the uronic acid, it was found advisable to remove most (90%) of the inorganic acid with barium hydroxide at room temperature and the remainder with an excess of barium carbonate from the hot solution. The solution was concentrated and cooled to remove the more insoluble barium salts and the cinchonine salt was prepared from the filtrate. At times the cinchonine salt was difficult to crystallize. Several extractions of the aqueous solution with chloroform were found to be indispensable. Then the solution was evaporated to a thick sirup and allowed to crystallize in a cooler. The solid mass of salt was broken up and washed with 95% alcohol and filtered on a Büchner funnel.

The crystallization of the lactone from glacial acetic acid was sometimes attended with difficulty. Frequently a gummy substance precipitated while the acetic acid extract of the lactone was cooling. In these cases the crystalline lactone was obtained by decanting the mother liquor and concentrating to a small volume in a vacuum desiccator containing sodium hydroxide. The maximum yield of lactone recovered from the cinchonine salt was 35% of the theoretical. Improvements in the process, particularly the use of other alkaloids, will be investigated in this Laboratory.

The pure lactone is white, without the pink tinge of color usually apparent in the product from the first crystallization. The lactone is deposited from glacial acetic acid in dense clusters at the border of which may be seen, under a high power lens, the projecting ends of crystals of not easily recognizable form. The lactone obtained from *Macrocystis pyrifera*, *Laminaria saccharina* and *Fucus serratus*, after one recrystallization from glacial acetic acid, possessed the same melting point, 139°, which did not change on three further recrystallizations, and (within the limit of experimental error) the same maximum rotation

$$[\alpha]_D^{22} + 93.8^\circ \text{ (} \textit{Macrocystis pyrifera} \text{)}$$

$$[\alpha]_D^{20} + 93.8^\circ \text{ (} \textit{Laminaria saccharina} \text{)}$$

$$[\alpha]_D^{20} + 93.6^\circ \text{ (} \textit{Fucus serratus} \text{)}$$

In all cases the mutarotation was of the character described in the following paragraph.

Mutarotation of *d*-Mannuronic Acid Lactone.—The lactone obtained from *Macrocystis pyrifera* was recrystallized four times from glacial acetic acid as follows. The lactone was dissolved in the minimum amount of solvent heated in a boiling water-bath. The solution was allowed to cool until there was a slight precipitate, quickly filtered on a vacuum filter and allowed to crystallize overnight. After the final crystallization, the lactone was washed several times with absolute alcohol and anhydrous ether and dried. The following table shows the rotation of 0.1892 g. of the recrystallized lactone in 10.08 cc. of aqueous solution. A 1-dm. tube was used. The first recorded specific rotation (three minutes after solution) was $[\alpha]_D^{22} + 79.9^\circ$. The rotation increased rapidly to the maximum, $[\alpha]_D^{22} + 93.8^\circ$, twenty-eight minutes after solution and then slowly decreased. The stoppered tube (with a drop of toluene on the surface of the solution) was allowed to stand at room temperature for 864 hours, at which time the rotation was not constant.

TABLE II
MUTAROTATION OF *d*-MANNURONIC LACTONE

Hours	α_D	$[\alpha]_D$	Hours	α_D	$[\alpha]_D$
0.05	+1.50	+79.9	24	1.74	92.7
.13	1.65	87.9	168	1.66	88.4
.22	1.72	91.6	336	1.58	84.2
.30	1.74	92.7	504	1.44	76.7
.38	1.75	93.2	672	1.30	69.3
.47	1.76	93.8	864	1.16	61.8

The rapid initial increase is probably due to attainment of equilibrium between the α and β forms of the aldehyde and the subsequent decrease is due to the formation of mannuronic acid. It was found by titration that the solution contained 39.1% acid and 60.9% lactone when the specific rotation was $+61.8^\circ$. Using these data for an approximate calculation of the equilibrated rotation of *d*-mannuronic acid gives $[\alpha]_D +12.0^\circ$. It may be noted that this value is close to the equilibrium rotation of *d*-mannose, $[\alpha]_D +14.6^\circ$.

The equilibrium ratio of acid and lactone was determined by heating solutions of the lactone at 80° . The percentages of acid and lactone, respectively, were 59.6 and 40.4 after 19.5 hours at 80° . Longer heating did not change this ratio appreciably, but caused a decrease of the optical rotation. Decomposition was evident after heating for thirty-five hours, when the solution became yellow.

Cinchonine Mannuronate.—0.110 g. of mannuronic lactone was dissolved in 20 cc. of 50% alcohol and heated with a slight excess of cinchonine in a water-bath at 80° for two hours. The solution was evaporated under reduced pressure to remove alcohol. Water was added and the solution was filtered to remove a small amount of cinchonine, extracted twice with chloroform and evaporated under reduced pressure to a thick sirup. The salt crystallized before cooling. It was dissolved in 80% alcohol and crystallized by evaporation in a desiccator under reduced pressure to a small volume. After washing with cold 80% alcohol, absolute alcohol and ether, it was dried over phosphorus pentoxide under reduced pressure. 0.106 g. of pure white salt was obtained. The salt crystallizes in sheaves of slender rectangular plates and when dry is quite insoluble in cold water. The melting point was 154° , with decomposition. 0.0285 g. in 3.017 cc. of aqueous solution gave an observed rotation of $+1.07^\circ$ in a 1-dm. tube; $[\alpha]_D^{24} +113.3^\circ$.

Cinchonidine Mannuronate.—Prepared in the same manner as the cinchonine salt and crystallized twice from 95% alcohol. After washing with absolute alcohol and ether, it was dried in a vacuum over phosphorus pentoxide. From 0.090 g. of lactone 0.140 g. of pure white salt was obtained. The compound crystallizes in small clusters of radiating needles and melts with decomposition at 154° .

0.0393 g. in 3.017 cc. of aqueous solution gave an observed rotation of -1.02° in a 1-dm. tube; $[\alpha]_D^{24} -78.3^\circ$.

Anal. (Micro Dumas) Calcd. for $C_{25}H_{32}O_8N_2$: N, 5.73. Found: N, 5.42, 5.43.

Brucine Mannuronate.—Prepared in the same manner as the cinchonine salt and crystallized twice from 80% alcohol. 0.070 g. of buff colored salt was obtained from 0.080 g. of lactone. The compound crystallizes in irregular masses of small needles and melts at 147° with decomposition. 0.0352 g. in 3.017 cc. of aqueous solution gave an observed optical rotation of -0.27° in a 1-dm. tube; $[\alpha]_D^{24} -23.1^\circ$.

Anal. (Micro Dumas) Calcd. for $C_{33}H_{36}O_{11}N_2$: N, 4.76. Found: N, 4.42, 4.47.

Summary

d-Mannuronic lactone has been isolated for the first time from the alginic acid of *Laminaria saccharina* and *Fucus serratus*. Data on the mutarotation of the lactone are presented and an approximate value for the rotation of the equilibrated mixture of α - and β -forms of the free acid has been calculated. The preparation and properties of the cinchonine and the more easily crystallized brucine and cinchonidine salts of *d*-mannuronic acid are described.

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