made if the need arises. The use of NORMEX is illustrated in Fig. 1, where $-\log b$ is plotted against $-\log (B-b)b$ for the system $In^{3+}-Cl^-$ in 3 M $NaClO_4$ studied by one of us.[14] B is the total concentration of indium and b the concentration of free In^{3+} as measured by an indium amalgam electrode. The points are experimental and the curves have been computed with NORMEX using a model including the formation of $InCl^{2+}$, $InCl_2^+$, and $InCl_3$ with $\log \beta_1 = 2.57 \pm 0.02$, $\log \beta_2 = 3.84 \pm 0.02$, and $\log \beta_3 = 4.2 \pm 0.1$, which compares well with the values obtained by LETAGROP:
$\log \beta_1 = 2.58 \pm 0.01$, $\log \beta_2 = 3.84 \pm 0.01$, $\log \beta_3 = 4.25 \pm 0.02$

A detailed description of NORMEX and examples of how to use it are given in the paper by Ferri and Wahlberg,[12] and anyone interested can write to Dr. Wahlberg at the University of Stockholm for reprints and further information.

Acknowledgements. This work is part of a program supported by the *Swedish Natural Science Research Council.* Docent Derek Lewis revised the English text.

1. Sillén, L. G. *Acta Chem. Scand.* **10** (1956) 186.
2. Sillén, L. G. *Acta Chem. Scand.* **10** (1956) 803.
3. Biedermann, G. and Sillén, L. G. *Acta Chem. Scand.* **10** (1956) 203.
4. Rossotti, F. J. C. and Rossotti, H. S. *Acta Chem. Scand.* **9** (1955) 1166.
5. Rossotti, F. J. C., Rossotti, H. S. and Sillén, L. G. *Acta Chem. Scand.* **10** (1956) 203.
6. Rossotti, F. J. C. and Rossotti, H. S. *The Determination of Stability Constants*, McGraw, New York 1961.
7. Sillén, L. G. *Acta Chem. Scand.* **15** (1961) 1981.
8. Ingri, N. and Sillén, L. G. *Arkiv Kemi* **23** (1965) 97.
9. Sillén, L. G. and Warnqvist, B. *Arkiv Kemi* **31** (1969) 341.
10. Brauner, P., Sillén, L. G. and Whiteker, R. *Arkiv Kemi* **31** (1969) 377.
11. Ingri, N. *Acta Chem. Scand.* **17** (1963) 597.
12. Ferri, D. and Wahlberg, O. *Chem. Commun.*, Univ. Stockholm 1973, No. XII.
13. Warnqvist, B. *Personal communication* 1971.
14. Ferri, D. *Acta Chem. Scand.* **26** (1972) 733.

Received November 2, 1973.

Polymannuronic Acid 5-Epimerase from the Marine Alga *Pelvetia canaliculata* (L.) Dcne. et Thur.

JOHN MADGWICK, ARNE HAUG and BJØRN LARSEN

Institutt for marin biokjemi, N-7034 Trondheim-NTH, Norway

Polymannuronic acid 5-epimerase was first demonstrated as an extracellular product of the bacterium *Azotobacter vinelandii* (Lipmann).[1] Subsequent work dealt with characterisation of the enzymic epimerisation,[2] the nature of the alginate from *A. vinelandii*,[3] and the mechanism of the enzymatic reaction.[4,5] However, although it seemed likely that brown algae must contain a similar epimerase, it was hard to prove because these plants' enzymes are so difficult to extract due to the presence of large amounts of sulphated polysaccharides and phenolic compounds.[6] This communication presents evidence for an algal alginate epimerase and describes the preparation of a soluble enzyme system from the brown alga *P. canaliculata*. An ammonium sulphate fraction was obtained which catalysed the conversion of polymannuronic acid to a mixed polymer containing guluronic acid residues. The epimerisation reaction was revealed by changes in carbazole reactives[7] and with incorporation of tritium into the polyuronide fraction.[4]

Materials and methods: P. canaliculata was harvested from Lade and Flakk, Trondheimsfjord, on 18.5.1973 and 19.8.1973, respectively, and deep-frozen at -15°C. A brei of *P. canaliculata* was made in water at 0°C, (800 ml/100 g wet wt.), using an Ultra-Turrax® blendor. The material remaining after filtration with gauze (*ca.* 0.5 mm, 4 layers) was discarded. Centrifugation, (7000 *g*, 10 min, 4°C), gave a pellet which was resuspended in iced water and resedimented. An acetone powder, (500 mg/100 g wet algae), was prepared from the washed pellet,[8] and subsequently extracted, (4 h, 20°C), with a solution containing phosphate buffer, (pH 7.8, 0.05 M), NaCl, (0.2 M), and dithiothreitol (10^{-4} M). Solubles were separated by centrifugation, treated with ammonium sulphate at 0°C, and the precipitates between 40 % and 90 % saturation combined and dialysed against tris(hydroxymethyl)ami-

nomethane/HCl buffer (pH 8.3, 0.012 M, at 0°C, 1 h). Enzymatic activity was detected using a carbazole assay,[7] and by the incorporation of tritium.[4] The amount of tritium incorporation was estimated by liquid scintillation counting as described previously.[4] Protein was determined according to Lowry *et al.*[9]

Results. Table 1 shows the changes in carbazole colour of polymannuronic acid after treatment over an extended reaction time. In the absence of borate, mannuronic acid gives a low extinction in the carbazole reaction, and the increases observed must be due to the transformation of mannuronic acid residues into guluronic acid residues, having a much higher extinction in the absence of borate.[2,7] In the presence of borate, the two monomers have approximately the same extinctions. The ratio of these extinctions, which gives the changing proportion of mannuronic to guluronic acid, decreased significantly within 90 min. The exact amount of conversion to guluronic acid is unknown because the enzyme extract contained considerable polysaccharide, including alginic acid of undetermined uronic acid composition. The epimerisation of polymannuronic to a mixed, guluronic acid containing polymer was also checked independently on these samples using electrophoretic separation on paper,[10] of the hydrolysed polymer. Visual examination of the paper strips showed the appearance of guluronic acid after 5 h incubation.

Table 1. Assay of epimerase activity in *P. canaliculata* using the carbazole reaction.

Incubation time (h)	Carbazole reactives (extinction at 530 nm)		Ratio With borate/ without borate
	Without borate	With borate	
0	0.58	0.72	1.24
0.5	0.64	0.80	1.25
1.5	0.72	0.74	1.03
4.5	0.96	0.70	0.73
18.0	1.36	0.61	0.45

Incubation mixture: Enzyme solution, 1.5 mg/ml protein (albumin equivalents); TRIS-HCl, pH 8.3, 12 mM; $CaCl_2$, 2.6 mM; Polymannuronic acid 7 mM; NaCl 150 mM; Dithiothreitol, 1.24 mM; 20°C. (*P. canaliculata* harvested 18.5.73).

In a separate experiment, the *P. canaliculata* enzyme system, (harvested 19.8.73), was assayed in tritiated water (total activity approximately 20 mCi).[4] The reaction was terminated with acid, (0.1 N, HCl), and the whole reaction mixture dialysed against distilled water. Total counts were measured on redissolved precipitates, and also on the individual uronic acids after hydrolysis and ion-exchange chromatography. Table 2 shows that of the activity found in the incubation mixture at the start of the experiment, only an insignificant amount was present in the uronic acids. After 5 h incubation a significant proportion of the activity was recovered in the uronic acid fraction with about four times more in the guluronic as in the mannuronic acid fraction. Compared with the results obtained for the *Azotobacter* epimerase, as discussed in some detail in a previous publication,[4] the present work indicates the presence of polymannuronic acid epimerase in *Pelvetia canaliculata*. The fact that relatively more activity was found in the mannuronic acid, than was found in *Azotobacter* experiments, might mean that there was a higher degree of reversibility for the algal enzyme.

Table 2. Incorporation of tritium by uronic acids during enzymic epimerisation of polymannuronic acid.

Time (h)	Activity, (counts per minute)		
	Total	Guluronic acid	Mannuronic acid
0	14840	5	33
5	49630	2539	672

Incubation mixture: Enzyme solution, 1.2 mg/ml protein (albumin equivalents); Collidine, pH 7.0, 25 mM; $CaCl_2$, 2.6 mM; Polymannuronic acid, 0.7 mM; Tritium, 2 mCi/ml; 20°C. (*P. canaliculata* harvested 19.8.73).

1. Haug, A. and Larsen, B. *Biochim. Biophys. Acta* **192** (1969) 557.
2. Haug, A. and Larsen, B. *Carbohyd. Res.* **17** (1971) 297.

3. Larsen, B. and Haug, A. *Carbohyd. Res.* **17** (1971) 287.
4. Larsen, B. and Haug, A. *Carbohyd. Res.* **20** (1971) 225.
5. Larsen, B. and Haug, A. Paper read at Seventh International Seaweed Symposium, Sapporo, Japan, August 8th, (1971) 491.
6. Johnston, C. S. and Davies, J. M. Paper read at Sixth International Seaweed Symposium, Santiago, Spain, September 9th, (1968) 501.
7. Knutson, C. A. and Jeanes, A. *Anal. Biochem.* **24** (1968) 470.
8. Madgwick, J., Haug, A. and Larsen, B. *Acta Chem. Scand.* **27** (1973) 711.
9. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. *J. Biol. Chem.* **193** (1951) 265.
10. Haug, A. and Larsen, B. *Acta Chem. Scand.* **15** (1961) 1395.

Received October 8, 1973.

Alkylation Reactions of the Tellurocyanate Ion

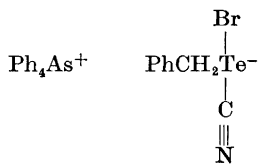
TOR AUSTAD, STEINAR ESPERÅS and JON SONGSTAD

Chemical Institute, University of Bergen, N-5000 Bergen, Norway

This paper reports a study on the reaction between a primary alkyl halide and the tellurocyanate ion in acetonitrile. Methyl iodide and methyl tosylate were first used as substrates, but because of the obnoxious odour of the product(s) from these reactions a primary alkyl halide of higher molecular weight was chosen. Benzyl bromide was found to be ideal for several reasons. The product obtained was a crystalline compound, sufficiently stable for elemental analysis and only slightly malodourous. Furthermore, the reaction was rapid and the experimental difficulties due to the reaction between the tellurocyanate ion and traces of oxygen and moisture [1] were thus negligible. Although the rate of the reaction is high at room temperature, a kinetic study was possible and the rate of the reaction was determined. As the rates of the reactions between benzyl bromide and the selenocyanate ion and between benzyl bromide and the thiocyanate ion could be determined as well, a picture of the relative nucleophilicity of the tellurocyanate ion could be obtained.

Benzyl halides have been known for a long time to form exclusively benzyl thiocyanate [2] and benzyl selenocyanate [3] when they react with ionic thiocyanate and selenocyanate, respectively. From the reaction between equivalent amounts of benzyl bromide and tetraphenylarsonium tellurocyanate in acetonitrile, a slightly yellowish crystalline compound was obtained in high yield. This compound was insoluble in diethyl ether while readily soluble in acetonitrile and acetone. An IR spectrum of the product did not show a peak due to the tellurocyanate ion at 2081 cm^{-1}.[1] As the characteristic peaks of the tetraphenylarsonium ion were present, the product from the reaction necessarily had to be a salt.

The product decomposed slowly in water and protic solvents with the formation of elemental tellurium. The usual qualitative tests for nitrogen and bromine were positive. Elemental analysis suggested the product to be considered as an adduct between tetraphenylarsonium bromide and benzyl tellurocyanate, tetraphenylarsonium bromocyanobenzyltellurate(II).



The compound, crystallized from acetonitrile, is nearly colourless. The prism-shaped crystals are extended along the a axis. Unit cell parameters were calculated from 89 high-order reflections read from Weissenberg $0kl$ and $h0l$ films, employing Ni-filtered Cu$K\alpha$ radiation. Refinement by a least squares program gave final values of $a = 9.471(4)$ Å, $b = 26.345(9)$ Å, $c = 12.899(5)$ Å and $\beta = 114.63(4)°$. There are four formula units per unit cell. (Density, found by flotation 1.60; calc. 1.61 g/cm^3.) From systematic absences, $h0l$ for $l = 2n+1$ and $0k0$ for $k = 2n+1$, the space group is $C_{2h} - P2_1/c$.