Chromatographic Separation of Carotenes and Other Chloroplast Pigments on Aluminium Oxide-Containing Paper

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Whereas many of the hydroxylated carotenoids are readily separated by adsorption chromatography on kieselguhr-containing filter paper[1], the less polar carotenes showed too high mobilities to permit a good separation within the latter group of compounds. It is to be expected that a paper with a stronger adsorbent embedded in it might allow the desired separation to be carried out. On our request the filter paper manufacturers Carl Schleicher & Schüll, Dassel, West Germany, very promptly prepared a filter paper (No. 667) which contained approximately 20 % aluminium oxide. Circular chromatography on this paper using petroleum ether as solvent and following the technique previously described [1], gave satisfactory separation of a series of carotenes as seen from Table 1. It was observed that the separations of chloroplast pigments were generally better on this paper than on the kieselguhr-containing (SS No. 287) paper, the zones being narrower on the former. In addition several mixtures of carotenoids from *Viola tricolor* and from *Ranunculus acris* that exhibited similar R_F-values on the kieselguhr paper were readily separated on the aluminium oxide paper since their relative mobilities were different on this adsorbent.

As expected, the activity of the aluminium oxide-containing paper was dependent on its moisture content. The papers were generally heated at 150°C for 15 min. and then immediately used for the chromatographic separation. Activated papers which were left in the air for even short periods gradually lost their activity to a certain extent. The activation mentioned above secured reproduceable R_F-values and gave the paper an activity comparable to that of aluminium oxide, activity grade 2 of Brockmann and Schodder [2]. (R_F × 100 of azobenzene = 80 in petroleum ether containing 20 % benzene). It is recommended, however, always to add a reference compound, such as β-carotene or azobenzene, and to give the mobilities of the compounds in question relative to that of the test substance.

Table 1. R_F-values of carotenes on Schleicher & Schüll Paper No. 667. Activated at 150°C for 15 min. Solvent: Petroleum ether, boiling range 60–80°C.

Compound	R_F-value × 100	
	Petroleum ether	20 % Benzene
α-Carotene[a]	43	66
β-Carotene[b]	38	62
γ-Carotene[c]	5	15
Phytofluene[c]	77	88
ζ-Carotene[c,d]	36	60
Neurosporene[c,d]	15	25
Lycopene[b]	2	8

[a] From *Gigartina stellata* Batt.
[b] Synthetic product from Hoffman-La Roche & Co. Ltd., Switzerland.
[c] Kindly supplied by the Organic Chemistry Laboratories of The Norwegian Technical University, Trondheim.
[d] 2nd isomer [3].

1. Jensen, A. and Liaaen Jensen, S. *Acta Chem. Scand.* **13** (1959) 1863.
2. Brockmann, H. and Schodder, H. *Ber.* **74** (1941) 73.
3. Liaaen Jensen, S., Cohen-Bazire, G., Nakayama, T. O. M. and Stanier, R. Y. *Biochim. et Biophys. Acta* **29** (1958) 477.

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Alkaline Hydrolysis of Glycosidic Linkages

V *. The Action of Alkali on Some Methyl Furanosides

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The aim of our studies on the alkaline hydrolysis of glycosidic linkages has been to illuminate possible reactions of the polysaccharides during the alkaline pulping

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Table 1. Alkaline hydrolysis of some methyl glycosides in 10 % sodium hydroxide at 170°.

Sugar	Configuration	Relationship between OCH_3 and $C_{(2)}-OH$	Furanosides $10^3 \times k^a$	Pyranosides $10^3 \times k^a$
D-Glucose	α	*cis*		1.0
»	β	*trans*	>100	2.5
D-Galactose	α	*cis*	7.8	1.0
»	β	*trans*	28	5.7
D-Mannose	α	*trans*	30	2.8
»	β	*cis*		1.1
D-Xylose	α	*cis*	8.1	1.2
»	β	*trans*	>100	5.8
L-Arabinose	α	*trans*	32	10.0
»	β	*cis*		1.0

a) Rate constants are expressed in Brigg's logarithms and hours.

of wood. Previous communications have dealt with the hydrolysis of various types of pyranosidic linkages. The present investigation is concerned with the alkaline hydrolysis of several methyl furanosides which serve as model substances for the furanosidic residues found in wood hemicelluloses. The results are given in Table 1.

The rate constants for the alkaline hydrolysis of the corresponding pyranosides [1,2] are included in the table for comparison.

It can be seen that the furanosides are considerably more labile than the pyranosides. Furthermore, those furanosides containing the *trans*-configuration about C_1 and C_2 are hydrolysed at a much higher rate than the *cis*-isomers. This fact supports the mechanism which involves elimination of the aglycon group with the subsequent formation of a 1,2-anhydride. Somewhat unexpected is the very high reactivity of the β-gluco- and β-xylofuranosides compared to the other 1,2-*trans*-glycofuranosides studied. The corresponding pyranosidic glycosides of these sugars are the most stable of the hexosides and pentosides studied; this is to be expected because of their greater conformational stability compared to the other pyranosides.

In the case of all *trans*-glycofuranosides, small amounts of neutral products (other than the starting material) were detected in the reaction product. Thus, methyl-β-D-xylopyranoside and levoglucosan were isolated from the products obtained by alkaline treatment of methyl-β-D-xylofuranoside and methyl-β-D-glucofuranoside, respectively. They were formed in small quantities (about 2 %) and it is unknown whether they are reaction products or whether their presence is due to impurities in the non-crystalline starting materials. (It may be noted that small amounts of unidentified, neutral products were formed by alkaline treatments of the other *trans*-furanosides which were crystalline and highly purified).

Preliminary experiments on the alkaline hydrolysis of methyl-α- and -β-D-glucopyranosyl uronic acid have also been conducted. These substances, which were non-crystalline and incompletely characterised, are hydrolysed by alkali at even a higher rate than the furanosides.

Hamilton and co-workers [3] have recently studied the alkaline pulping of coniferous wood. These woods contain arabino-glucurono-xylans and they showed that the L-arabinofuranose residues were rather resistant, whereas the 4-*O*-methyl-D-glucopyranosyluronic acid residues were easily cleaved during the pulping operation. It is not known whether the L-arabinofuranosidic linkages in the polysaccharide have the α- or β-configuration. Only an α-arabinofuranoside was studied in the present investigation and it was found to be rather labile; the β-anomer would be expected to be more stable. The 4-*O*-methyl-D-glucopyranosyl uronic acid residue is known to be α-linked, and the corresponding model substance was found to be quite labile.

However, it should be stressed, that the reactivities of the arabinoglucuronoxylan and the model substances are not strictly comparable, for the reactivity of the furanosidic residues is undoubtedly influenced by its position in the polysaccharide molecule.

Experimental. All melting points are corrected. Distillations were carried out under reduced pressure. Paper chromatography was conducted on Whatman No. 1 filter paper using the following solvent systems:

A. Butanol-pyridine-water, 6 : 2 : 3

B. Butanone, saturated with water.

Substances. The D-xylo- and D-galacto-furanosides were prepared according to Augestad and Berner.[4] Methyl-α-D-mannofuranoside was prepared according to Haworth *et al*[6]. Methyl-α-L-arabinofuranoside was prepared as described for the corresponding D-form by Wright and Khorana[5]. It had m.p. 45–48° and $[\alpha]_D^{20}$ — 124°(water) in good agreement with reported values.

Methyl-β-D-glucofuranoside was prepared by a Fisher synthesis, following the technique devised by Augestad and Berner[4]. Glucose (60 g) was dissolved by stirring in 0.55 % methanolic hydrogen chloride; the solution was kept at room temperature overnight. The acid was removed by treatment with lead carbonate and the solution concentrated to a syrup. Paper chromatographic analysis of this syrup in solvent system B revealed the presence of four components: glucose; a mixture of α- and β-pyranosides, migrating as a single component; and the α- and β-furanosides, which were well separated. Part of the syrup (37 g) was added to the top of a cellulose column (6.4 × 130 cm) which was then eluted with solvent system B. Methyl β-D-glucofuranoside, the faster of the two anomers, was eluted between 18 000–30 000 ml. Concentration of this fraction gave methyl β-D-glucofuranoside as a chromatographically-pure syrup showing $[\alpha]_D^{20}$ −54° in water. Comparison with the reported value $[\alpha]_D$ −77°) indicated that the substance was incompletely dried.

Reaction of the glycosides with alkali was carried out as previously described[2]. In the case of the uronides, the following procedure was employed. After alkaline hydrolysis the reaction mixture was neutralised with hydrochloric acid and concentrated to dryness. The residue was dissolved in water and an aliquot concentrated and subjected to methoxyl analysis.

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1. Dryselius, E., Lindberg, B. and Theander, O. *Acta Chem. Scand.* **12** (1958) 340.
2. Janson, J. and Lindberg, B. *Acta Chem. Scand.* **13** (1959) 138.
3. Hamilton, J. K., Partlow, E. V. and Thompson, N. S. *Tappi* **14** (1958) 803.
4. Augestad, I. and Berner, E. *Acta Chem. Scand.* **8** (1954) 251.
5. Haworth, W. N., Hirst, E. L. and Webb, J. I. *J. Chem. Soc.* **1930**, 651.
6. Wright, R. S. and Khorana, H. G. *J. Am. Chem. Soc.* **80** (1958) 1994.

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Confirmation of the Structure of Ketomanoyl Oxide

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Recent work in this laboratory has shown that hinokiol, hinokione[1] and totarolone[2] all have an oxygen function at C(3) (steroid numbering). The original assignment[3] of the keto group of ketomanoyl oxide to the 2-position has recently been questioned[4]*.

In connection with mass-spectrometric work on related substances[5] the mass-spectra of the tetra-deuterated and non-deuterated dihydroketomanoyl oxide were recorded (Figs. 1 and 2). A comparison of the two spectra shows clearly that 4 hydrogen atoms have been replaced by deuterium, since the peaks at *m/e* 307 (*M* + 1), 291 (*M*-15), and 277 (*M*-29) in the spectrum of dihydroketomanoyl oxide (Fig. 2) are found 4 mass units higher, at *m/e* 311, 295, and 281, in the spectrum of deuterated dihydroketomanoyl oxide (Fig. 1). These peaks are probably due to ions obtained from the molecule after addition of a proton (307 and 311), removal of a methyl group (291 and 295) and an ethyl group (277 and 281), respectively. The result shows that position 2, originally assigned to the oxo

* During the preparation of this manuscript a communication has appeared in which the same approach led to similar results. Grant, P.K. and Hodges, R. *Chem. & Ind. London* **1960** 1300.