1. Peisach, J., Aisen, P. and Blumberg, W. E. *The Biochemistry of Copper*, Academic, New York 1966.
2. Banaszak, L. J., Watson, H. C. and Kendrew, J. C. *J. Mol. Biol.* **12** (1965) 130.
3. Bryce, G. F., Roeske, R. W. and Gurd, F. R. N. *J. Biol. Chem.* **240** (1965) 3837.
4. Österberg, R. *Acta Chem. Scand.* **19** (1965) 1445.
5. Leden, I. Diss., Lund 1943.
6. Sillén, L. G. *Acta Chem. Scand.* **10** (1956) 186.
7. Ingri, N. and Sillén, L. G. *Arkiv Kemi* **23** (1965) 97.
8. Freeman, H. C., Robinsson, G. and Schoone, J. C. *Acta Cryst.* **17** (1964) 719.
9. Blount, J. F., Frazer, K. A., Freeman, H. C., Szymanski, J. T. and Wang, C. H. *Acta Cryst.* **22** (1967) 396.

Received January 24, 1968.

A New Methylated Uronic Acid from Paper Pulp Hydrolysates

EVA R. NELSON, PETER F. NELSON * and OLOF SAMUELSON

Department of Engineering Chemistry, Chalmers Tekniska Högskola, Göteborg, Sweden

Recent examinations of the acidic components of the hydrolysates of paper pulps prepared from birch,[1] eucalyptus and pine [2,3] woods, have shown that 4-*O*-methyl-D-glucuronic acid is the major monouronic acid present. A methylated uronic acid of unknown structure was shown to be an important component of the acids of birch sulphate pulp, whereas it could not be detected in birch hemicellulose. Eucalypt sulphate, pine sulphate, and eucalypt neutral sulphite pulps were also found to contain this acid. All four pulps had been prepared under alkaline conditions and it is noteworthy that no trace of the acid was found in a pine bisulphite pulp or in bisulphite cooking liquors.[4] These results indicate that the new acid was formed by some rearrangement of the 4-*O*-methyl-D-glucuronic acid units in xylan under alkaline cooking conditions.

Results and discussion. As demonstrated by Perry and Hulyalkar,[5] uronic acids can be reduced with sodium borohydride to the corresponding aldonic acids without detectable side reactions. It was expected that the new methylated uronic acid would yield a methylated aldonic acid on reduction and that demethylation of the latter would give a known aldonic acid. Perry and Hulyalkar have tested their method using a variety of uronic acids but no experiments were reported concerning its application to methylated acids. Trial experiments were therefore conducted with 4-*O*-methyl-D-glucuronic acid to determine suitable conditions for the reduction and demethylation reactions.

Reduction of 4-*O*-methyl-D-glucuronic acid with sodium borohydride gave a single methylated aldonic acid in high yield. Demethylation of this compound with hydrobromic acid yielded gulonic acid as expected. Reduction of the unknown acid with sodium borohydride also gave a single acidic product, confirming that the original compound was a uronic acid and not a ketoaldonic acid. The product of the reduction gave no reaction with carbazole, and was assumed to be a methylated aldonic acid. On demethylation with hydrobromic acid it yielded idonic acid, identified by comparison of its chromatographic behaviour with that of an authentic sample. This comparison was made using automated column chromatography on Dowex 1-X8 (Ac^-) in both sodium acetate (Fig. 1) and acetic acid, and by paper chromatography. Further confirmation was obtained by preparation of the tetra-*O*-trimethylsilyl-1,4-lactone and comparison

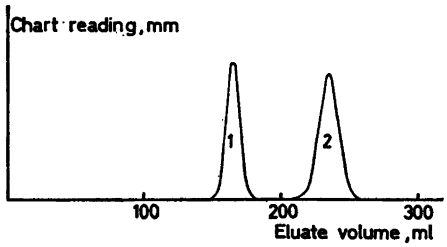

Fig. 1. Separation of 3-*O*-methyl-L-idonic acid (1) from the demethylated acid (2) in 0.05 M sodium acetate on an anion exchange column, 6 × 835 mm.

* Present address: Australian Paper Manufacturers Ltd., Melbourne.

Table 1. Chromatographic data.

Compound	D_v on Dowex 1-X8 (Ac^-)			R_{GL}	Retention time (min) of TMS ether on QF 1 170° 30 ml/min
	0.05 M NaAc	0.5 M HAc	1 M HAc		
4-*O*-Methyl-D-glucuronic acid	12.1		17.6	0.66	—
3-*O*-Methyl-L-gulonic acid	9.5	11.8		1.28	6.3
Demethylated 3-*O*-methyl-L-gulonic acid	15.1	14.6		0.42	7.1
Gulonic acid	15.0	14.6		0.42	7.2
4-*O*-Methyl-L-iduronic acid	13.4		11.5	0.79	—
3-*O*-Methyl-L-idonic acid	9.1	13.0		1.92	6.1
Demethylated 3-*O*-methyl-L-idonic acid	13.1		6.1	0.69	8.4 *
Idonic acid	13.0		6.0	0.69	8.4 *
Gluconic acid	14.1	13.6	6.2	1.00 0.20	6.4, 6.05

* Retention time on DC 560-EGSP-Z at 185° was 10.2 min. On NPGS at 165° 6.9 min.
R_{GL} is migration on paper chromatograms relative to gluconolactone (1.00) in ethyl acetate-acetic acid-water (100:13:10).[8]

of its retention time with that of an authentic sample on three different stationary phases. As may be seen from Table 1, the D_v values [6] and retention times of the demethylated acid and its trimethylsilyl derivative showed excellent agreement with those of the reference compounds.

Measurement of the mass spectrum of the trimethylsilyl derivative of the demethylated acid showed conclusively that the compound was a 1,4-lactone of an aldohexonic acid. The spectrum was significantly different from those of the corresponding derivatives of galactonic, gluconic, gulonic, mannonic, and talonic acids, but was identical in all respects with that of idonic acid. The remote possibility that idonic acid may have been formed by epimerization of gluconic acid under the conditions of the demethylation was excluded by showing that gluconic acid is stable under these conditions.

These results demonstrate that the product of reduction of the original unknown acid was a methylated idonic acid, but give no information about the position of the methyl group. It was shown, however, that the reduction product of the new uronic acid could be produced in low yield by treating 3-*O*-methylgulonic acid with dilute sodium hydroxide at 100°. Since a shift in the position of the methyl group under these conditions is very unlikely, the reduction product was almost certainly 3-*O*-methylidonic acid. The question was finally settled by examination of the mass spectrum of the trimethylsilyl derivative of the lactone of the methylated idonic acid. This showed conclusively that the methyl group was attached to the oxygen atom at carbon 3, so that the original uronic acid must have been 4-*O*-methyliduronic acid.

The assignment of the L-configuration to the new uronic acid follows from its positive rotation, which is somewhat higher than that of L-iduronic acid. This configuration is to be expected if the compound is formed by epimerization at C_5 of 4-*O*-methyl-D-glucuronic acid units in xylan. Since the latter units are bound glycosidically in the xylan, such an epimerization is equivalent to a C_2 epimerization of the corresponding aldonic acid. As noted above, partial epimerization of 3-*O*-methyl-L-gulonic to 3-*O*-methyl-L-idonic acid can be effected under relatively mild conditions, and the same is true of epimerization of gulonic to idonic acid.

Idonic acid for reference purposes was prepared by reduction of 5-ketogluconic acid using essentially the method of Hamilton and Smith.[7] It was also prepared by

reduction of 2-ketogulonic acid, a reaction which does not appear to have been described hitherto. In the latter case, the idonic acid is, of course, accompanied by gulonic acid, and may be separated from the latter by ion-exchange chromatography in either acetic acid or sodium acetate.

Experimental. Reduction and demethylation of 4-O-methyl-D-glucuronic acid. 4-*O*-Methyl-D-glucuronic acid (10 mg) was dissolved in water and the lactone split with sodium hydroxide at pH 8 for 5 h at room temperature. Sodium borohydride (50 mg) was added and the solution allowed to stand at room temperature for one hour, after which it was placed in the refrigerator overnight. The solution was then treated with Dowex 50 (H^+) (2 g) until effervescence had ceased, when the resin and solution were transferred to a column containing the same resin and the reaction product eluted with water. Evaporation under vacuum below 35°C, followed by five distillations with methanol to remove boric acid, gave a syrup (9 mg). This material gave no reaction with carbazole, and was shown by paper and column chromatography to contain one major component (3-*O*-methyl-L-gulonic acid).

3-*O*-Methyl-L-gulonic acid (5 mg) was placed in a sealed glass tube with 37 % hydrobromic acid (2 ml). The tube was heated in a boiling water bath for 35 min. After cooling, the contents of the tube were applied to a column of Dowex 1-X8 (Ac^-) and eluted with 30 % acetic acid. Evaporation of the acetic acid under vacuum gave a mixture of gulonic acid, unchanged 3-*O*-methylgulonic acid and a small amount of an unidentified compound. The gulonic acid constituted about 40—50 % of the total, the remainder being mainly 3-*O*-methylgulonic acid.

The identity of the gulonic acid was established by automated column chromatography on Dowex 1-X8 (Ac^-) in both 0.05 M sodium acetate and 0.5 M acetic acid, by paper chromatography and by gas chromatography of the trimethylsilyl ether (see Table 1).

Reduction and demethylation of the unknown uronic acid. The unknown uronic acid (12 mg) was reduced after lactone splitting in the same manner as described above for 4-*O*-methyl-D-glucuronic acid. The acidic reduction products again consisted almost entirely of one compound as shown by chromatograms. The demethylation was carried out in the same manner as for 3-*O*-methyl-L-gulonic acid and the product was also obtained in a 50 % yield. It was identified as idonic acid by column and paper chromatography and by gas chromatography and mass spectrometry of the trimethylsilyl ether (see Table 1).

Formation of 3-O-methyl-L-idonic acid from 3-O-methyl-L-gulonic acid. 3-*O*-Methyl-L-gulonic acid (5 mg) was heated with 1.5 M sodium hydroxide (0.5 ml) for 3 h under reflux. The resulting solution was treated with Dowex 50-X8 (H^+) to remove sodium ions, and the product was eluted with water. Evaporation under vacuum below 35° gave a syrup which was shown to contain 3-*O*-methyl-L-idonic acid by paper chromatography, by automated column chromatography in both 0.5 M acetic acid and 0.05 M sodium acetate and by gas chromatography of the trimethylsilyl ether.

Preparation of idonic acid from 2-ketogulonic acid. 2-Ketogulonic acid was reduced with sodium borohydride under the conditions described above for reduction of 4-*O*-methyl-D-glucuronic acid. The resulting mixture of idonic and gulonic acids was separated by ion-exchange chromatography using 0.05 M sodium acetate as eluant. The two acids were formed in about equal amount.

The authors express their sincere thanks to Civiling. Göran Petersson for carrying out the mass spectrometric measurements, and to *1959 Års Fond för Teknisk och Skoglig Forskning samt Utbildning* and *Australian Paper Manufacturers Ltd.* for financial support.

1. Goel, K. and Samuelson, O. *Svensk Papperstid.* **70** (1967) 1.
2. Nelson, P. F. and Samuelson, O. *Svensk Papperstid.* **71** (1968). *In press.*
3. Nelson, P. F. *Svensk Papperstid.* **71** (1968). *In press.*
4. Pettersson, S. and Samuelson, O. *Svensk Papperstid.* **70** (1967) 462.
5. Perry, M. B. and Hulyalkar, R. K. *Can. J. Biochem.* **43** (1965) 573.
6. Samuelson, O. *Ion Exchange Separations in Analytical Chemistry*, Almqvist and Wiksell, Stockholm; Wiley, New York 1963.
7. Hamilton, J. K. and Smith, F. *J. Am. Chem. Soc.* **76** (1954) 3543.
8. Richtzenhain, H. and Moilanen, M. *Acta Chem. Scand.* **8** (1954) 704.

Received January 10, 1968.