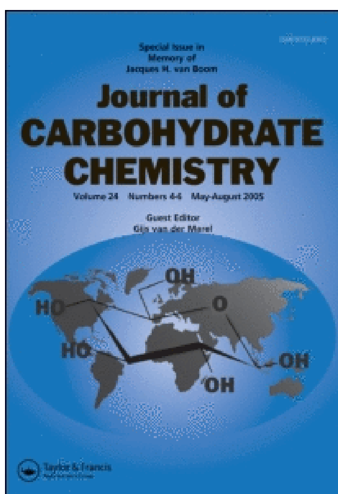
This article was downloaded by:
On: *23 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Carbohydrate Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

Quantitative Determination of Carbohydrates in Solution via a Reduction/Permethylation Technique

Richard F. Helm[ab]; Anthony H. Conner[c]; Raymond A. Young[a]
[a] Department of Forestry, University of Wisconsin-Madison, Madison, Wisconsin [b] Department of Forest Products, Oregon State University, Corvallis, OR, U.S.A. [c] USDA Forest Products Laboratory, Madison, Wisconsin

To cite this Article Helm, Richard F. , Conner, Anthony H. and Young, Raymond A.(1987) 'Quantitative Determination of Carbohydrates in Solution via a Reduction/Permethylation Technique', Journal of Carbohydrate Chemistry, 6: 4, 569 — 586
To link to this Article: DOI: 10.1080/07328308708058886
URL: http://dx.doi.org/10.1080/07328308708058886

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

J. CARBOHYDRATE CHEMISTRY, 6(4), 569-586 (1987)

QUANTITATIVE DETERMINATION OF CARBOHYDRATES IN SOLUTION VIA A REDUCTION/PERMETHYLATION TECHNIQUE

Richard F. Helm,[†*] Anthony H. Conner[‡] and Raymond A. Young[†]

[†]Department of Forestry, University of Wisconsin-Madison, Madison, Wisconsin 53706

[‡]USDA Forest Products Laboratory, Madison, Wisconsin 53705-2398

Downloaded At: 11:48 23 January 2011

Received February 25, 1987 - Final Form June 23, 1987

ABSTRACT

A reduction/permethylation technique has been developed for the quantitative gas chromatographic analysis of anhydrosugars, monosaccharides, and disaccharides. All derivatizations are carried out in the same vial, and transfers are not necessary until recovery of the permethylated derivatives. The reaction scheme is extremely simple and offers improved accuracy over standard GC carbohydrate analysis protocols. Emphasis is placed on quantitative compound recovery, and relative response factors are presented for several carbohydrates. The sodium hydroxide/methyl iodide permethylation reaction has also been shown to be a viable replacement for standard dimsyl anion procedures utilized for methylation analysis of polysaccharides. Application of the reduction/permethylation sequence to the analysis of the hydrolyzate resulting from the dilute sulfuric acid hydrolysis of cellulose demonstrates that reversion products account for a considerable amount of the glucose losses, with levoglucosan, 1,6-anhydro-β-D-glucofuranose, isomaltose, and gentiobiose predominating.

* To whom correspondence should be addressed. Present address: Department of Forest Products, Oregon State University, Corvallis OR 97331-5704 (U.S.A.).

Copyright © 1987 by Marcel Dekker, Inc.

INTRODUCTION

The determination of trace amounts of carbohydrates in complex mixtures can be determined accurately through the use of a derivatization scheme providing either increased sensitivity, for the use of HPLC,[1-5] or volatility, allowing a gas chromatographic analysis.[5-10] While numerous advances have been made in the field of HPLC carbohydrate separations,[11-12] the resolution capabilities of capillary gas chromatography cannot be surpassed. Therefore derivatization procedures aimed at the use of GC will provide the best overall chances of separating and quantitating the carbohydrate(s) of interest in complex mixtures.

The limiting factor for accurate gas chromatographic analysis of carbohydrates is the formation of a suitably volatile and stable compound in quantitative yield. Trimethylsilylation and trifluroacetylation schemes have been shown to be quite useful for the separation of a wide range of alditols, cyclitols, and disaccharides.[6-8] The procedures do not address the issue of peak multiplicity, and thus a peak results for each anomeric form of a reducing sugar. Complex carbohydrate mixtures are therefore almost impossible to quantitate. Chaves Das Neves and coworkers[9,10] have recently considered the problem of peak multiplicity and have demonstrated that reduction and subsequent permethylation of disaccharide methoximes provides an excellent alternative to the standard GC analysis schemes. The technique has been shown to be suitable for both mono- and disaccharides. However, the procedure requires the concentration and heating of 1M methanolic hydrogen chloride solutions which may give rise to glycosidic bond cleavage or compound degradation.

Permethylated carbohydrate derivatives are more volatile than standard peracetylated and pertrimethylsilylated derivatives, highly stable, of relatively low molecular weight, and the mass spectral fragmentation patterns have been investigated extensively. Thus permethylated carbohydrates are ideally suited

Downloaded At: 11:48 23 January 2011

for GC and GC-MS analysis. Ciuanu and Kerek[13] have described a carbohydrate permethylation technique utilizing powdered sodium hydroxide, dry dimethylsulfoxide (DMSO) and methyl iodide. This technique, with minor modifications, has been coupled with a standard alkaline borodeuteride reduction step which permitted the separation, identification, and quantification of all carbohydrates investigated. Reactions are carried out in the same vial and without transfers. The high stability of carbohydrate methyl ethers allows the preparation of standard mixtures which can be used repeatedly for the generation of response factors.

RESULTS AND DISCUSSION

Downloaded At: 11:48 23 January 2011

Quantitative Aspects of Reduction/Permethylation Analysis.

Reduction. Aqueous alkaline reduction of carbohydrates with sodium borodeuteride places a deuterium label at the C-1 and C-2 carbons of aldoses and ketoses, respectively. While this eliminates the peak multiplicity problem encountered with aldoses, the reduction of a ketose will result in the formation of two diasteromers (i.e., fructose will yield mannitol and glucitol with the deuterium label located at C-2).

Steric factors will play a role in determining the ratio of the ketose reduction products. This can be seen in Table 1 where the reduction products of three fructosyl-containing carbohydrates were quantitatively evaluated via the reduction/permethylation analysis scheme. The sterospecificity is most dramatic with the reduction of turanose, an α-(1,3)-linked ketodisaccharide. Although the mechanism of borodeuteride reductions is quite complicated and not fully understood, attack from the less hindered side (*trans* to the glucopyranosyl substituent) favors the formation of the glucitol form of the mono-D-glucopyranosyl-alditol, nigeritol. A glucopyranosyl group at C-6 does not affect reduction stereospecificity as the mannitol/glucitol ratio for the reduction of palatinose was about that of fructose (Table 1).

TABLE 1

Aqueous Alkaline Borodeuteride Reduction Sterospecificity for Fructosyl-Containing Carbohydrates

	Reduction Product Yields[a]	
Carbohydrate	Mannitol Form	Glucitol Form
Fructose	43.7 ± 0.1	56.3 ± 0.1
Palatinose 6-O-α-D-glucopyranosyl-D-fructose	42.0 ± 0.4	58.0 ± 0.4
Turanose 3-O-α-D-glucopyranosyl-D-fructose	32.0 ± 0.3	68.0 ± 0.3

[a]The yields are based upon the GC analysis of triplicate reduction/permethylations.

Downloaded At: 11:48 23 January 2011

The elimination of the boric acid formed after decomposition of sodium borodeuteride is necessary to avoid the formation of trimethylborate during subsequent permethylation. Acidification (acetic acid) and water evaporation (40 °C) yielded the anhydrous conditions required for removal of boric acid as the volatile trimethylborate (via coevaporation with methanol). Inductively-coupled plasma analysis of the reaction mixture obtained after repeated methanol evaporations revealed that over 99% of the borate originally present was eliminated after two anhydrous methanol evaporations at 40 °C.

Permethylation. The ability to isolate the permethylated derivatives in quantitative yields is of utmost importance in generating accurate information on unknown mixtures. Table 2 shows that several chloroform extractions were required to recover all permethylates.

TABLE 2

Analysis of Permethylated Derivative Recoveries by Extraction with Chloroform

	Percent Recovery of Permethylated Derivatives[a]			
	Number of Chloroform Washes			
Permethylate	Original	1	2	3
Quebrachitol	92.2	7.4	0.4	---
Glucitol	94.8	5.0	0.2	---
Levoglucosan	83.6	14.1	2.0	0.3
Cellobiitol	95.2	4.8	---	---

[a]See Experimental for details. Percentages are based on peak areas relative to an internal standard (18) and are the average of duplicate permethylations.

Downloaded At: 11:48 23 January 2011

Sodium acetate, formed after the elimination of boric acid, does not affect the methylation reaction. Yields from the purported technique were identical to those in which standard cation exchange resins were used for simultaneous borodeuteride quenching and sodium ion removal. Sodium acetate is relatively insoluble in DMSO, although some dissolution occurred once methyl iodide was added to the reaction mixture, presumably due to the formation of methyl acetate and sodium iodide.

The standard procedure following isolation of the permethylated samples in the organic phase (in this case chloroform) is to eliminate residual DMSO as well as the polar reaction products with several water washes. This extraction will provide cleaner chromatograms for quantitative analysis. Although water washes will eliminate DMSO very effectively, losses of low molecular weight permethylates will occur as well. This is exemplified by levoglucosan permethylate in Table 3. Four washes of the

TABLE 3

Elimination of DMSO and Levoglucosan Permethylate with Successive Water Washes[a]

Number of Extractions	% DMSO Remaining	% Levoglucosan Remaining
Original	100	100
1	9.8	99.0
2	0.7	87.5
3	trace	75.1
4	---	65.4

[a]The water washes were performed on the permethylated derivatives as obtained from three chloroform extractions (See Experimental for details). The chloroform layer was washed with twice the volume of water for the number of times indicated. The percentages are based on peak areas versus an internal standard (18) and are the average of duplicate permethylations.

Downloaded At: 11:48 23 January 2011

chloroform layer results in complete DMSO removal along with a 35% loss of permethylated levoglucosan. However, the byproducts associated with standard dimsyl reactions are not formed during hydroxide methylation. Therefore a high concentration of contaminants, which would interfere with the GC analysis, are not present, eliminating the need of the water washes. One water wash will eliminate 91.2 % of the original DMSO (see Table 3) without significant loss of permethylated derivatives. This is a reasonable approach to decreasing the rather large DMSO peak, as long as standard solutions from which response factors are generated are treated in the same fashion.

GC Separation Characteristics. Figure 1 and Table 4 depict the separation characteristics of the SP2340 capillary column. The poly(cyanoalkylsiloxane)-based column is highly polar with a temperature limit of 250 °C. Definitive compound identification can be accomplished by the use of GC-MS and selected-ion

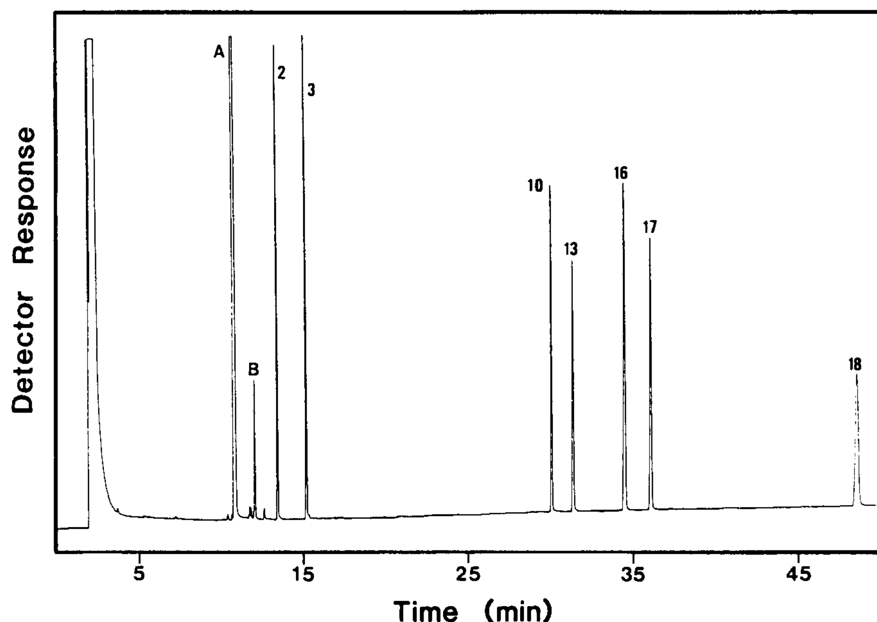


FIG. 1. GC separation of a reduced and permethylated standard carbohydrate mixture on a glass capillary SP2340 column. Temperature programming: 100 °C for 2 min, 5 °C/min to 220 °C, hold for 30 min. A, DMSO; B, ethyl methyl sulfoxide; for other compounds refer to Table 4.

Downloaded At: 11:48 23 January 2011

monitoring. The alditol moiety, tagged with a deuterium atom at C-1 or C-2 (for aldoses and ketoses, respectively) provides reliable cleavage patterns for distinguishing between linkage positions but not linkage configurations.[14-17] The use of GC-MS is invaluable for the identification of unknown compounds in complex mixtures when proper standards are not readily available.

Response Factors. The generation of accurate response factors is essential for quantitative work. Since the anhydro-sugars, alditols, and mono-D-glucosylalditols have different carbon contents, the respective flame responses will reflect the effective carbon composition. Table 5 summarizes the factors determined in this work for the different carbohydrate types. The

TABLE 4

Relative Retention Time of the Carbohydrates Investigated[a]

Permethlyate	RRT[a]
Xylitol (1)	0.2375 ± 0.0001
Quebrachitol (2)	0.2756 ± 0.0001
Glucitol (3)	0.3109 ± 0.0002
Mannitol (4)	0.3148 ± 0.0002
Levoglucosan (5)	0.4065 ± 0.0002
1,6-anhydro-β-D-glucofuranose (6)[b]	0.4601 ± 0.0002
3-O-α-D-glucopyranosyl-D-mannitol (7)	0.6071 ± 0.0005
Kojibiitol (8)[b]	0.6087 ± 0.0004
Laminaribiitol (9)	0.6142 ± 0.0004
Cellobiitol (10)	0.6167 ± 0.0004
Nigeritol (11)	0.6206 ± 0.0004
Maltitol (12)	0.6288 ± 0.0003
Sophoritol (13)	0.6442 ± 0.0004
Trehalose (14)	0.6472 ± 0.0004
6-O-α-D-glucopyranosyl-D-mannitol (15)	0.7020 ± 0.0004
Isomaltitol (16)	0.7073 ± 0.0003
Gentiobiitol (17)	0.7407 ± 0.0004
Acetyl-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (18)	1.0000

[a]Based on the retention time of 18 (48.71 min). Temperature programming: 100 °C for 2 min, 100 °C to 220 °C at 5 °C/min, hold at 220 °C for 30 min.
[b]Identification based on GC-MS analysis.

relative response factor represents the amount of material required to provide the same peak area as the internal standard (18). The inverse is thus the relative sensitivity of the FID to equal amounts of standard and unknown.[18]

It was found that each isomer, regardless of linkage or configuration, gave the same flame response within experimental

Downloaded At: 11:48 23 January 2011

TABLE 5

Relative Response Factors for Several Types of Permethylated Carbohydrates

Permethylate	RRF1[a]	RRF2[b]	Relative Peak Area[c]
Quebrachitol	0.564	0.767	1.304
Hexitol	0.536	0.795	1.257
Levoglucosan	0.619	0.780	1.282
Trehalose	0.603	0.822	1.216
Mono-D-glucosylalditol	0.675	0.930	1.076
Glucose pentaacetate	1.000	1.000	1.000

[a]Response factor based on the original amount (by weight) of material charged (i.e., hexose or disaccharide).
[b]Response factor based on the theoretical yield (by weight) of the borodeuteride reduced and permethylated carbohydrate.
[c]Relative peak area for each compound on a permethylate weight basis (1/RRF2).

Downloaded At: 11:48 23 January 2011

error. The relative peak area of permethylated hexitols determined in this work coincides relatively well with the theoretical effective carbon response (e.c.r.) value for glucitol permethylate (1.188) as suggested by Sweet et al.[19] That the experimentally determined hexitol response factor is quite similar to the e.c.r. value is strong evidence for the validity of the technique for quantitative determination of carbohydrates in complex mixtures. The equivalence of flame response values for carbohydrate isomers makes the determination of response factors relatively simple. A solution containing the internal standard as well as individual representations of each carbohydrate type to be expected is all that is required. The experimental error encountered during reduction/permethylation analysis quantitation was routinely less than 3%.

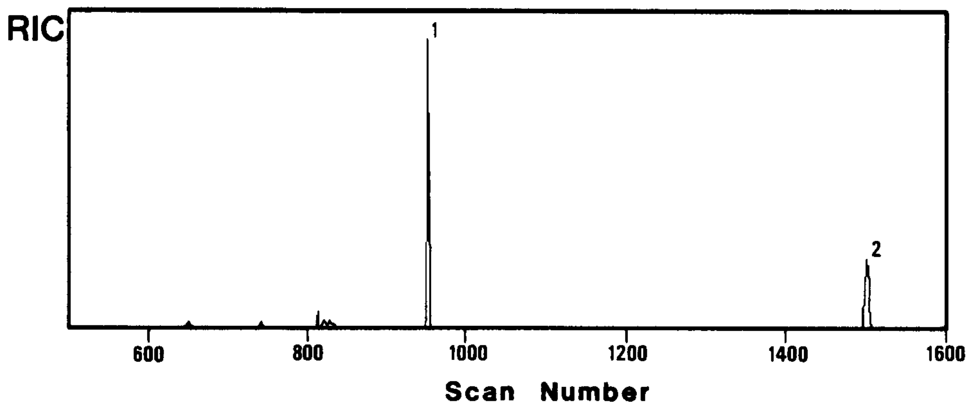


FIG. 2. GC-MS reconstituted total-ion current from the methylation analysis of amylose, employing the powdered sodium hydroxide methylation technique. 1, 1,4,5-tri-O-acetyl-2,3,6-tri-O-methyl-D-glucitol; 2, internal standard (18). Temperature programming: 150 °C for 5 min, 10 °C/min to 230 °C.

Downloaded At: 11:48 23 January 2011

Applications of the Reduction/Permethylation Technique

Methylation Analysis. The methylation reaction for the analysis of polysaccharides invariably involves the use of the dimsyl anion generated from any number of basic reagents.[20] Considerable amount of time and care must be taken in forming the dimsyl anion, which is very labile and provides a considerable amount of byproducts during the methylation reaction.

Substitution of the NaOH methylation scheme purported here in a standard methylation analysis protocol[21] was attempted on amylose and the GC-MS result is shown in Figure 2. The retention time and mass spectrum of the only major peak confirmed the structure as 1,4,5-tri-O-acetyl-2,3,6-tri-O-methyl-D-glucitol. The results from the methylation analysis of pullulan revealed two permethylated alditol acetate peaks with a 62:38 product ratio (2,3,6-Me-Glu : 2,3,4-Me-Glu). This is quite close to the 67:33 ratio calculated from the postulated structure of this (1→6) terminally linked maltotriosyl polysaccharide. Thus the powdered NaOH permethylation technique may be an appropriate substitute for

standard dimsyl schemes when methylating DMSO-soluble polysaccharides, and should be investigated further. The procedure should also be applicable to the Reductive Cleavage method of Gray and coworkers.[22]

Analysis of a Cellulose Hydrolyzate. The results for the analysis of the hydrolyzate resulting from the dilute sulfuric acid hydrolysis of cellulose are shown in Table 6 and Figure 3. The Lobry de Bruyn-Alberda van Ekenstein transformation occurs under acidic conditions,[23] which gives rise to both mannose and fructose. Since the reduction of fructose will lead to both glucitol and mannitol, the mannitol peak represents the total amount of mannose plus approximately one-half (44%, see Table 1) of the fructose formed during cellulose hydrolysis which is not subsequently dehydrated. However, since the rate of fructose dehydration is considerably faster than that of aldoses, the yield of fructose will be insignificant relative to that of mannose.[23-26] It is possible that some of the mannitol permethylate detected could have arisen from the trace hemicelluloses present in almost all celluloses. The concentrations found, however, exceed that to be expected based on the amount of mannose present in the Avicel before acid hydrolysis.

Downloaded At: 11:48 23 January 2011

The reversion reaction yields both internal and external condensation products. The anhydrosugars, levoglucosan and 1,6-anhydro-β-D-glucofuranose, constituted the major compounds formed. The identification of the anhydrofuranose was via mass spectral analysis with the fragmentation pattern almost identical to that reported by Heyns and Scharmann for the permethylated 1,6-anhydro-β-D-galactofuranose.[27] The anhydroglucofuranose yield was surprisingly high considering the typical amount of the furanose forms of glucose present in aqueous solutions of glucose.[28] Isomaltose and gentiobiose were the most prevalent disaccharide reversion products present in the hydrolyzate, a fact which is consistent with the relative reactivity of carbohydrate

TABLE 6

Yields of Carbohydrates Resulting From the Dilute Sulfuric Acid Hydrolysis of Avicel[a]

Carbohydrate	Yields (percent weight basis)[b]	
	210 °C 2.5 min 1% H_2SO_4	210 °C 5 min 0.5% H_2SO_4
Cellulose Remaining	16.4	21.2
Glucose[c]	37.1	35.9
Mannose[d]	0.77	0.75
Levoglucosan	1.92	2.55
1,6-anhydro-β-D-glucofuranose	0.77	1.03
Kojibiose	0.27	0.22
Sophorose	0.19	0.16
Nigerose	0.30	0.24
Laminaribiose	0.26	0.20
Maltose	0.17	0.11
Cellobiose	0.25	0.19
Isomaltose	1.09	0.91
Gentiobiose	0.73	0.59
Trehalose	trace	trace

[a]3:1 liquid to solid ratio.
[b]Weight basis of the original amount of cellulose charged.
[c]Determined by a glucose oxidase procedure (see Experimental).
[d]Assuming the amount of fructose remaining is insignificant.

Downloaded At: 11:48 23 January 2011

hydroxyl groups. The α-disaccharide linkages were slightly favored for all hydroxyl positions except for the cellobiose/maltose pair, which was due to the formation of cellobiose via both reversion and cellulose hydrolysis. Work is in progress to quantitate the amounts of the major reversion products formed as a function of reaction time, temperature, and acid concentration.

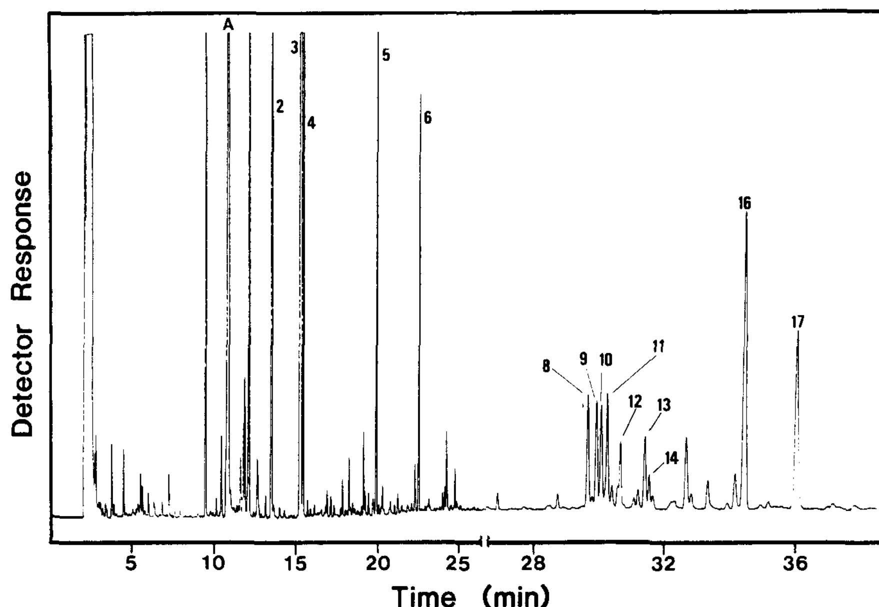


FIG. 3. GC chromatogram resulting from the reduction/permethylation analysis of the dilute sulfuric acid hydrolyzate of cellulose (Avicel). A, DMSO; refer to Table 4 for permethylate compound identification. Hydrolysis conditions: 210 °C, 2.5 min reaction, 1% H_2SO_4, 3:1 liquid-to-solid ratio.

Downloaded At: 11:48 23 January 2011

EXPERIMENTAL

Materials. All aqueous solutions were prepared with degassed Milli-Q water (Millipore; Bedford, MA). Unless otherwise specified, an *in vacuo* desiccation implies that the sample was stored over phosphorous pentoxide, under a 500 μm vacuum. Evaporations were conducted on a 12-port analytical evaporator (Organomation; Northborough, MA) under a stream of filtered nitrogen. The carbohydrates used were available from the major chemical supply houses and used without further purification, with the exception of quebrachitol (1-L-2-O-methyl-*chiro*-inositol), which was the kind gift of Prof. L. Anderson, Department of Biochemistry, University of Wisconsin-Madison.

HPLC grade DMSO was dried by vacuum distillation over calcium hydride (450 μm, 50 °C), and stored sealed under high-purity argon in an argon-purged desiccator containing phosphorous pentoxide. Powdered sodium hydroxide was prepared by grinding pellets (98%) with mortar and pestal in an anhydrous glove box. The fraction that passed a 65-mesh screen was used for all subsequent permethylations and was stored in sealed vials under nitrogen. Sodium borodeuteride (98%) and methyl iodide (99%) were obtained from Aldrich Chemical Co. (Milwaukee, WI) and were used as received.

Downloaded At: 11:48 23 January 2011

Reduction. Aliquots (1-2 mL) of standard and unknown carbohydrate solutions (containing 2-5 mg/mL total carbohydrate) were injected into septa-sealed reaction vials which contained 10 mmoles of sodium borodeuteride per meq carbonyl and continously swept with nitrogen gas. The reaction was left unstirred for 4 h at room temperature.

Acetone (200 μL) was added to quench excess reductant, and the reaction was acidified with the addition of 100 μL acetic acid. Absolute methanol (2 mL) was added and the reaction vial contents was evaporated to dryness (35-40 °C). Three additional methanol evaporations (2 mL each) followed, after which the reduced sample was placed in a desiccator (*in vacuo*) until methylation.

Methylation. The procedure was that of Ciucanu and Kerek[13] with minor modifications. The alditol sample was dissolved in 500 μL DMSO in a stirred, argon-flushed, thermostated (20-23 °C) reaction vial for 30 minutes. Powdered sodium hydroxide (25 mg) was added and the mixture was stirred for 15 min. Methyl iodide (70 μL) was injected into the vial, and the reaction continued for 20 min. The amount of reagent used was based on 3.3 meq of sodium hydroxide/2.7 mL of DMSO/5.5 meq of methyl iodide/meq hydroxyl group.

The reaction was quenched with 2 of mL water and stirred until the reaction mixture was clear. The sample was quantitatively transferred to a separatory funnel containing 5 mL of water and 2 mL of chloroform. This original extraction was followed by two additonal chloroform washes (2 mL). A standard solution of 18 was added to the total combined organic layer which was hence dried (sodium sulfate), filtered, and ready for analysis. Samples may be evaporated if necessary but must not be taken to a syrup as this leads to the loss of some of the more volatile permethylated alditols.

Downloaded At: 11:48 23 January 2011

Instrumental Analysis. Gas chromatography was performed on a Hewlett Packard HP 5880 instrument operating in the split mode (20:1) with helium as the carrier gas. A SP2340 fused silica capillary column (0.20mm X 30m; Supelco, Bellefonte, PA) was used for all separations. The injection port and detector (FID) temperatures were kept at 250 °C. Column pressure was maintained at 15 psi., and the hydrogen and air flow rates were 30 mL/min and 430 mL/min, respectively. Several oven temperature programs were used with a 2-minute initial hold at 100 °C followed by a 5 °C/min ramp to 220 °C providing the best overall separation of the cellulose hydrolyzate.

GC-MS was performed on a Finnigan 4510 instrument operating with an INCOS data collection system. A SP2340 column (0.20mm X 60m) was used for all separations with temperature programming as described previously for GC. The scan range was typically 70 - 500 m/z.

Methylation Analysis. The polysaccharide (2.5 mg) was dissolved in 500 μL DMSO in an argon-purged reaction vial. Sodium hydroxide (25 mg) was added, and the solution was mixed for 30 min. Methyl iodide (70 μL) was injected into the vial and the reaction was continued for 40 minutes. Tha material was worked up and further derivatized according to the methylation analysis sequence of Harris et al.[21]

Cellulose Hydrolyzate Analysis. Avicel (microcrystalline cellulose, $\overline{DP}_w$=197) was added to 3 mm o.d. glass tubes (sealed at one end, 70 mg/tube) which were charged with dilute sulfuric acid to bring about a liquid-to-solid ratio of 3/1, and heat sealed under an inert atmosphere. The hydrolyzed samples were quantitatively recovered, filtered, and the aqueous fraction neutralized to pH 7 (sodium hydroxide). The neutralized hydrolyzate was hence diluted to a known volume, from which an aliquot was taken for glucose analysis (Beckmann Glucose Analyzer, glucose oxidase method). The remaining hydrolyzate was spiked with quebrachitol, freeze-dried, resuspended in methanol, filtered, evaporated to dryness, and desiccated. The resulting material was resuspended in water from which an aliquot was taken and submitted to the reduction/permethylation sequence described previously.

Downloaded At: 11:48 23 January 2011

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Roger C. Pettersen and Dr. James L. Minor of the Forest Products Laboratory for performance of the GC-MS and use of the gas chromatograph, respectively. R.F.H. was funded through the USDA Special Grants Program, the College of Agricultural and Life Sciences, and the Graduate School, University of Wisconsin-Madison.

REFERENCES

1. M. D'Amboise, D. Noël, and T. Hanai, Carbohydr. Res., 79, 1 (1980).

2. P.F. Daniel, D.F. De Feudis, I.T. Lott, and R. H. McCluer, Carbohydr. Res., 97, 161 (1981).

3. C.A. White, S.W. Vass, J.F. Kennedy, and D.G. Large, J. Chromatogr., 264, 99 (1983).

4. E. Coles, V.N. Reinhold, and S.A. Carr, Carbohydr. Res., 139, 1 (1985).

5. K. Robards and M. Whitelaw, J. Chromatogr., 373, 81 (1986).

6. Z.L. Nikolov and P.J. Reilly, J. Chromatogr., 254, 157 (1983).

7 P. Englmaier, Carbohydr. Res., 144, 177 (1985).

8. J.-R. Neeser, Carbohydr. Res., 138, 189 (1985).

9. H.J. Chaves Das Neves, A.M.V. Riscado, and H. Frank, Carbohydr. Res., 152, 1 (1986).

10. H.J. Chaves Das Neves and A.M.V. Riscado, J. Chromatogr., 367, 135 (1986).

11. R.D. Rocklin and C.A. Pohl, J. Liq. Chromatogr., 6, 1577 (1983).

12. T. Okada and T. Kuwamoto, Anal. Chem., 58, 1375 (1986).

13. I. Ciucanu and F. Kerek, Carbohydr. Res., 131, 209 (1984).

14. N.K. Kochetkov and O.S Chizhov, Tetrahedron, 21, 2029 (1965).

15. N.K. Kochetkov and O.S. Chizhov, Adv. Carbohydr. Chem., 21, 39 (1966).

16. J. Kärkkäinen, Carbohydr. Res., 14, 27 (1970).

17. J. Lönngren and S. Svenson, Adv. Carbohydr. Chem. Biochem., 29, 41 (1974).

18. J.T. Scanlon and D.E. Willis, J. Chromatogr. Sci., 23, 333 (1985).

19. D.P. Sweet, R.H. Shapiro, and P. Albersheim, Carbohydr. Res., 40, 217 (1975).

20. A.B. Blakeney and B.A. Stone, Carbohydr. Res., 140, 319 (1985).

21. P.J. Harris, R.J. Henry, A.B. Blakeney, and B.A. Stone, Carbohydr. Res., 127, 59 (1984).

22. D. Rolf and G. Gray, Carbohydr. Res., 152, 343 (1986).

23. T.P Mawhinney, M.A. Madson, and M.S. Feather, Carbohydr. Res., 86, 147 (1980).

Downloaded At: 11:48 23 January 2011

24. M.S. Feather and J. Harris, Adv. Carbohydr. Chem. Biochem., 28, 161 (1973).

25. G. Bonn, J. Carbohydr. Chem., 4, 405 (1985).

26. B.F.M. Kuster and L. Tebbins, Carbohydr. Res., 54, 159 (1977).

27. K. Heyns and H. Scharmann, Carbohydr. Res., 1, 371 (1966).

28. C. Williams and A. Allerhand, Carbohydr. Res., 56, 173 (1977).

Downloaded At: 11:48 23 January 2011