Frankel, E. N. "Chemistry of Autoxidation: Mechanism, Products and Flavor Significant". In *Flavor Chemistry of Fats and Oils*; Min, D. B., Smouse, T. H., Eds.; American Oil Chemists' Society: Champaign, IL, 1985.
Hayashi, T.; Namiki, M. "Formation of Two-Carbon Sugar Fragment at an Early Stage of the Browning Reaction of Sugar with Amine". *Agric. Biol. Chem.* **1980**, *44*, 2575–2580.
Hayashi, T.; Namiki, M. "On the Mechanism of Free Radical Formation during Browning Reaction of Sugars with Amino Compounds". *Agric. Biol. Chem.* **1981**, *45*, 933–939.
Hayashi, T.; Namiki, M. "Role of Sugar Fragmentation in an Early Stage Browning of Amino-Carbonyl Reaction of Sugar with Amino Acids". *Agric. Biol. Chem.* **1986**, *50*, 1965–1970.
Hodge, J. E. "Chemistry of Browning Reactions in Model Systems". *J. Agric. Food Chem.* **1953**, *1*, 928–943.
Hwang, S. S.; Carlin, J. T.; Bao, Y.; Hartman, G. J.; Ho, C.-T. "Characterization of Volatile Compounds Generated from the Reactions of Aldehydes with Ammonium Sulfide". *J. Agric. Food Chem.* **1986**, *34*, 538–542.
Izzo, M. T. "Protein-Lipid Interactions in Model Systems of Extruded Zein and Corn Oil". M.S. Thesis, Rutgers University, New Brunswick, NJ, 1987.
Kato, H.; Kanako, S.; Okitani, A.; Ito, M.; Hayase, F. "Modification of Lysine Residues to Alkyl-Substituted Pyridiniums on Exposure of Proteins to Vaporized Hexanal". *Agric. Biol. Chem.* **1986**, *50*, 1223.
Maga, J. A. "Pyrazines in Flavours". In *Food Flavours, Part A. Introduction*; Morton, I. D., Macleod, A. J., Eds.; Elsevier Scientific: Amsterdam, 1982.
Majlat, P.; Erdos, Z.; Takacs, J. "Calculation and Application of Retention Indices in Programmed Temperature Gas Chromatography". *J. Chromatogr.* **1974**, *91*, 89–103.
Nawar, W. W. "Chemistry of Thermal Oxidation of Lipids". In *Flavor Chemistry of Fats and Oils*; Min, D. B., Smouse, T. H., Eds.; American Oil Chemists' Society: Champaign, IL 1985.
Okitani, A.; Kaneko, S.; Tashiro, Y.; Hayashi, F.; Kato, H. "Polymerization of Proteins and Impairment of Their Amino Acid Residues due to Vaporized Hexanal". In *Amino-Carbonyl Reactions in Food and Biological Systems*; Fujimaki, M., Namiki, M., Kato, H., Eds.; Kodansha: Tokyo, 1986.
Quackenbush, F. W.; Firch, J. G.; Rabourn, W. J., McQuistan, M.; Petold, E. N.; Kargl, T. E. "Analysis of Carotenoids in Corn Grain". *J. Agric. Food Chem.* **1961**, *9*, 132–135.
Snyder, J. M.; Frankel, E. N.; Selke, E. "Capillary Gas Chromatographic Analyses of Headspace Volatiles from Vegetable Oils". *J. Am. Oil Chem. Soc.* **1985**, *62*, 1675–1679.
Weeks, W. W. *Biogeneration of Aromas*; Parliment, T. H., Croteau, R., Eds.; ACS Symposium Series 317; American Chemical Society: Washington, DC, 1986; pp 157–166.

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Isolation of 4-Hydroxy-2-(hydroxymethyl)-5-methyl-3(2*H*)-furanone from Sugar Amino Acid Reaction Mixtures

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From reaction mixtures of glucose with amino acids the compounds extractable by acetonitrile were analyzed. In addition to the well-known 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one (the isomeric **6**), 4-hydroxy-2-(hydroxymethyl)-5-methyl-3(2*H*)-furanone (**5**) was isolated as a further main product for the first time. **5** and **6** react with primary amines (amino acids) forming pyrrolinones of structure **9**, whereas the so-called amino hexose reductones **8** are obtained with secondary amines.

When pentoses are heated with amino acids, proteins, or salts of simple amines in an almost neutral aqueous solution, a comparatively large amount of 4-hydroxy-5-methyl-3(2*H*)-furanone (**1**) is formed (Severin and Seilmeier, 1967). Under suitable conditions, **1** can be obtained in yields of up to 30% (Peer et al., 1968). This compound is also detectable in foodstuff, e.g. beef broth (Tonsbeek et al., 1968). Dihydrofuranone **1** includes a very reactive methylene group and condenses readily with sugar degradation products containing carbonyl functions to colored compounds of general structure **2** (Ledl and Severin, 1978). Thus, substances **2a** and **2b** could be isolated from a mixture of xylose and glycine (Severin and Krönig, 1973). In these compounds the exocyclic methyl group is sufficiently activated in order to react with a second carbonyl component. Thus, the scheme **1** → **2** → **3** generally reflects browning reactions of pentoses under the conditions of the Maillard reaction (Figure 1).

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In a similar way 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (**4**, furaneol) is formed from methyl pentoses (Hodge, 1963). This substance **4** is a very important aroma compound and is available also as a synthetic product (Büchi et al., 1973) (Figure 2).

Because of these results it could be expected that a dihydrofuranone of structure **5** was formed from hexoses. Some years ago already we heated glucose and fructose with amino acids or methylammonium acetate in aqueous solution and analyzed reaction products extractable by ethyl acetate or methylene chloride. The main component we isolated (Severin and Seilmeier, 1968) was not the required compound **5** but an isomeric crystalline substance, which according to Mills et al. (1970) had to be regarded as 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one (**6**). However, substances with structure **5** have been described erroneously in the literature. With a GC–MS system, Tatum et al. (1967) separated and identified volatile products formed during the storage of orange powder. On this occasion, they assigned structure **5** to one of the compounds. But as has been shown by further investigations, this component was again pyranone **6**. Other

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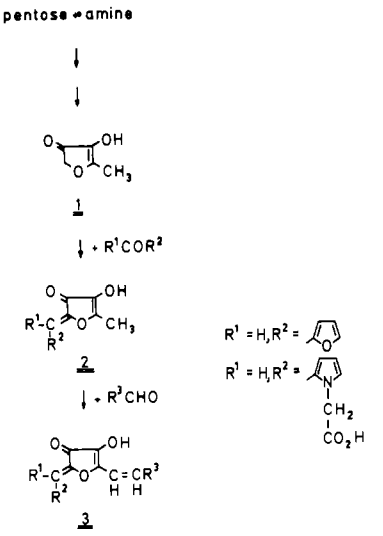

Figure 1.

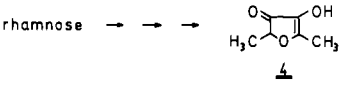

Figure 2.

reports on an alleged proof of furanone **5** are also questionable (Pisarnitskii et al., 1979; Fisher et al., 1983).

EXPERIMENTAL SECTION

1. Isolation of Compound 5 from a Glycine/Glucose Reaction Mixture. Equimolar amounts (0.05 mol) of glycine and glucose were dissolved in 50 mL of water and the resultant mixtures heated for 1 h at pH 7 (phosphate buffer) under reflux. After cooling, the reaction mixture was extracted three times with 100 mL of acetonitrile/ethyl acetate (1:1, v/v). The organic solvents were removed under vacuum at <50 °C. Preparative thin-layer chromatography of the residue was performed on silica gel plates (Merck 5717; 2 mm, 20 × 20 cm; developing solvent ethyl acetate). A band with R_f 0.70–0.75 was separated from plates and silica gel extracted with methanol. Chromatographic purification was repeated on silica gel thin-layer plates (Merck 5744; 0.5 mm, 20 × 20 cm; developing solvent ethyl acetate). Furanone **5** could be isolated in the UV-absorbing band (254 nm) with R_f 0.6. After evaporation of the elution solvent and cooling, compound **5** was obtained as a crystalline substance. Spectral data (^{1}H NMR, MS, IR, UV) were identical with those obtained from a synthetic substance (Ledl, 1979). Silylation (in pyridine with chlorotrimethylsilane and 1,1,1,3,3,3-hexamethyldisilazane) allowed gas chromatographic identification. Gas chromatography [25-m quartz capillary column (Permaphase 25 m × 0.25 (i.d.)) coated with dimethylsilicone; temperature program 100–260 °C, 6 °C/min]: R_t for silylated **5**, 14.55. Yield of substance **5** relative to glucose: 0.05%. MS of silylated **5**: 360 (55, M$^+$), 345 (70), 271 (53), 183 (21), 147 (37), 75 (19), 73 (100).

From a reaction mixture of proline and glucose we also succeeded in isolating compound **5**, yield 0.04%.

2. Reaction of 5 and 6 with Piperidine. Portions (50 mg) of **5** or **6** (van den Ouweland and Peer, 1970) and 50 mg of piperidinium acetate in 5 mL of ethanol were heated under reflux for 2 h. After cooling and evaporation of the organic solvent, the product was separated on a thin-layer silica gel plate [developing solvent ethyl acetate/methanol (9:1, v/v)]. The UV-absorbing band (254 nm) with R_f 0.7 was separated from the plate, and piperidino hexose reductone **8a** was eluted with methanol. Compound **8a** was identified by comparison of its MS and IR spectral data with data of an authentic sample (Weygand et al., 1958). Compound **8a** was obtained in 30% yield as determined by UV absorption measurement at 320 nm.

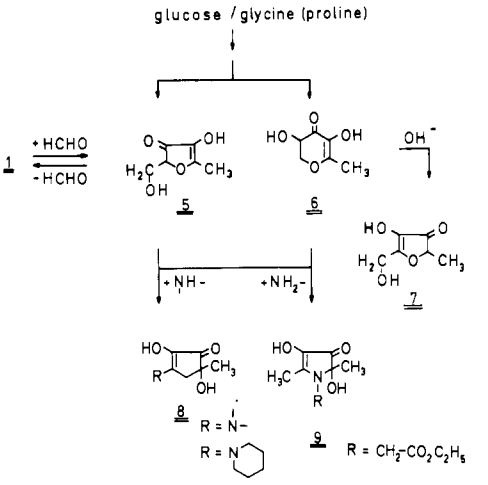

Figure 3.

3. Reaction of 5 and 6 with Glycine Ethyl Ester. Portions (50 mg) of **5** and **6** and 60 mg of glycine ethyl ester hydrochloride salt were dissolved in 5 mL of water buffered with sodium acetate/acetic acid (pH 5.6). The solution was heated under reflux for 2 h. After cooling, water was removed under vacuum at 40 °C. Separation of the residue was performed with HPLC [stainless steel column, 0.8 (i.d.) × 25 cm, packed with LiChrosorb RP 18, 7 m, Merck 9394; elution solvent water/methanol (7:3, v/v)] and UV detection at 320 nm (flow rate 2 mL/min). Compound **9a** was eluted in the fraction with R_t 12.5. Spectral data (MS, IR) were identical with those obtained from a substance prepared from acetylformoin (**16**) and glycine ethyl ester (Ledl and Fritsch, 1984). Compound **9a** was formed in 20% yield as determined by HPLC peak area.

4. Formation of Furanone 1 from 5. An aqueous solution (5 mL) of 50 mg of **5** and 20 mg of glycine (phosphate buffer, pH 7.0) was kept under reflux for 3 h. After cooling, the reaction mixture was extracted three times with 30 mL of methylene chloride. The combined organic solvent was dried over anhydrous sodium sulfate, filtered, and concentrated to a syrup. A part was dissolved in methanol and separated by capillary GC (R_t(1) = 3.74). The other part was silylated and also analyzed with capillary GC (R_t(silylated **1**) = 8.35). MS of silylated **1**: 258 (72, M$^+$), 243 (45), 184 (10), 169 (17), 149 (18), 147 (35), 133 (22), 73 (100). The identity of compound **1** was confirmed by comparing the R_f values with those of an underivated and silylated authentic sample (Peer et al., 1968). **1** was obtained in 10% yield as determined by GC peak area.

RESULTS

In order to facilitate the separation of more hydrophilic sugar decomposition products we extracted concentrated aqueous reaction mixtures of glucose and glycine (or proline) with acetonitrile and separated the obtained fraction by chromatography. Thus, it was possible to isolate 4-hydroxy-2-(hydroxymethyl)-5-methyl-3(2*H*)-furanone (**5**) as a pure crystalline substance. The structure is derived from spectral data (MS, ^{1}H NMR, IR, UV). Earlier, Ledl (1979) prepared compound **5** from dihydrofuranone **1** and formaldehyde. The substance obtained from a glucose reaction mixture and that prepared from **1** proved to be identical (see the Experimental Section). According to Mills (1978), isomeric furanone **7** is formed when di-

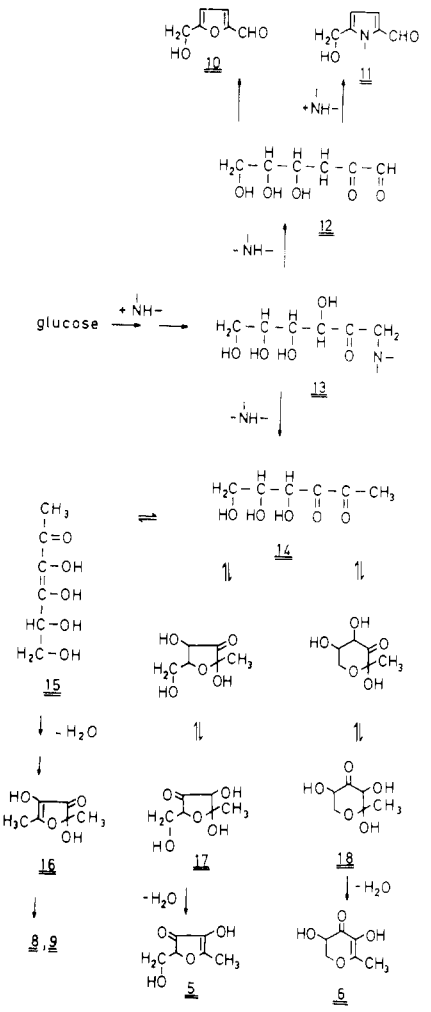

Figure 4.

hydropyranone **6** is treated with sodium hydroxide. It is remarkable that, pursuant to the observations we have made so far, **5** and **7** cannot be readily transformed into each other (Figure 3).

As far as we have observed, furanone **5** is an unstable intermediate. When **5** is heated with glycine in an aqueous solution at pH 7 (phosphate buffer), a retro aldol reaction occurs and furanone **1** is partly formed, to some extent. When piperidine is allowed to react with **5**, the so-called piperidino hexose reductone **8a** is obtained. A different reaction proceeds with primary amines. Thus, pyrrolinone **9a** is obtained from **5** and glycine ethyl ester. A separate paper will be published regarding the formation of **5** in different types of food.

DISCUSSION

Furanone **5** can be classified as an important sugar degradation product. The first stages of the Maillard reaction are well-known and have been thoroughly investigated (Paulsen and Pflughaupt, 1980). With primary and secondary amines, glucose forms glycosylamines, which are transformed into the corresponding N-substituted 1-deoxy-1-aminofructose (**13**) by an Amadori rearrangement (Figure 4). When amines from substances of the general structure **13** are split two types of dicarbonyl compounds are obtained that are important also in terms of quantity. Anet (1959) and Kato (1962) succeeded in isolating pure 3-deoxyosone **12**. 3-deoxyosones of hexoses are easily decomposed forming (hydroxymethyl)furfural (**10**) (Anet, 1964) or pyrrole aldehyde **11** (Jurch and Tatum, 1970) in the presence of primary amines. So far, however attempts to isolate 1-deoxy-2,3-hexodiulose (**14**) from sugar reaction mixtures have failed. But the formation of some degradation products can be explained best via compound **14**. The formulation of dicarbonyls **12** and **14** in open-chain structure is a simplification; in fact, they rather exist as cyclic semi-acetals. Formation of dihydropyranone **6** can be explained via the 6-ring enediol **18**, whereas furanone **5** originates from dihydrofuran **17**. At present, we are trying to isolate and characterize 1-deoxy-2,3-hexodiulose (**14**). The results will be published in a separate paper.

By tautomerism of **14**, the open-chain reductone **15** is formed. So far, this compound has only been prepared synthetically by hydrolysis of the phenylosazone of 1-deoxy-D-fructose (Ishizu et al., 1967) or by hydrolysis of 1-deoxy-4,5-*O*-isopropylidene-2,3-hexodiulose (Fisher et al., 1983). **15** is an unstable substance only available in solution and characterized by identification of decomposition products. Acetylformoin (**16**), which has been known for some time, is possibly formed via **15** (Tatum et al., 1967; Hodge et al., 1972).

Reactions of dihydrofuranone **5** with amines or proteins (preferably with the lysine side chain) are of special interest. It has been known for some time that reductone-type substances are formed during Maillard reaction (Franzke and Iwainsky, 1954). In foodstuff, reductones have a stabilizing effect. Until recently, the actual structures of these substances are unknown. Weygand et al. (1958) isolated and identified the so-called piperidino hexose reductone **8a** from reaction mixtures of different sugars with piperidine. At first the assumption that these hexose reductones with carbocyclic structure are also formed with primary amines suggested itself. But according to our subsequent analysis, this proved to be incorrect (Ledl et al., 1982). From a reaction mixture of acetylformoin (**16**) with glycine ethyl ester Ledl and Fritsch (1984) isolated 2,3-dihydro-2,4-dihydroxy-2,5-dimethyl-3-oxo-1*H*-pyrrole-1-acetic acid ethyl ester (**9a**) as a crystalline compound. **9a** was also identified in sugar reaction mixtures. When secondary amines are heated with acetylformoin (**16**), however, type **8** compounds are produced (Simon and Heubach, 1965). We observed analogous behavior with sugar decomposition products **5** and **6**. While from **5** and **6** reductones of type **8** are obtained with secondary amines, pyrrolinones of structure **9** are formed with primary amines. Since primary amines (amino acids, lysine side chains in proteins) predominate in food, reductones **9** will mainly be formed. Only with comparatively large amounts of proline (beer, wort) or hydroxyproline amino reductones of structure **8** can this also be expected.

Registry No. 1, 19322-27-1; **5**, 17678-20-5; **6**, 28564-83-2; **8a**, 34421-11-9; **9a**, 91887-16-0; H-Gly-OH, 56-40-6; H-Pro-OH, 147-85-3; H-Gly-OEt·HCl, 623-33-6; glucose, 50-99-7; piperidine, 110-89-4.

LITERATURE CITED

Anet, E. F. L. *Aust. J. Chem.* **1959**, *12*, 491.
Anet, E. F. L. *J. Adv. Carbohydr. Chem.* **1964**, *19*, 181.
Büchi, G.; Demole, E.; Thomas, A. F. *J. Org. Chem.* **1973**, *38*, 23.
Fisher, B. E.; Sinclair, H. B.; Goodwin, J. C. *Carbohydr. Res.* **1983**, *116*, 209.
Franzke, C.; Iwainsky, H. *Dtsch. Lebensm.-Rundsch.* **1954**, *50*, 251.
Hodge, J. E. *Proc. Am. Soc. Brew. Chem.* **1963**, 84.
Hodge, J. E.; Mills, F. D.; Fisher, B. E. *Cereal Sci. Today* 1972, 2, 34.
Ishizu, A.; Lindberg, B.; Theander, O. *Carbohydr. Res.* **1967**, *5*, 329.
Jurch, G. R.; Tatum, I. H. *Carbohydr. Res.* **1970**, *15*, 233.
Kato, H. *Agric. Biol. Chem.* **1962**, *26*, 187.
Ledl, F. *Z. Lebensm. Unters. Forsch.* **1979**, *169*, 176.
Ledl, F.; Severin, Th. *Z. Lebensm. Unters. Forsch.* **1978**, 167, 410.

Ledl, F.; Fritsch, G. *Lebensm. Unters. Forsch.* **1984**, *178*, 41.
Ledl, F.; Fritsch, G.; Severin, Th. *Z. Lebensm. Unters. Forsch.* **1982**, *175*, 208.
Mills, F. D. *J. Agric. Food Chem.* **1978**, *26*. 894.
Mills, F. D.; Weisleder, D.; Hodge, J. E. *Tetrahedron Lett.* **1970**, *15*, 1243.
Paulsen, H.; Pflughaupt, K. W. In *Carbohydrates IB*; Pigman, W., Horton, D., Eds.; Academic: New York, London, Toronto, Sydney, San Francisco, 1980; p 881.
Peer, H. G.; van den Ouweland, G. A. M.; De Groot, C. N. *Recl.: J. R. Neth. Chem. Soc.* **1968**, *87*, 1011.
Pisarnitskii, A. F.; Egorov, I. A.; Egofarova, R. Kh.; Erygin, G. D. *Prikl. Biokhim. Mikrobiol.* 1979, 15, 902.
Severin, Th.; Seilmeier, W. *Z. Lebensm. Unters. Forsch.* **1967**, *134*, 230.
Severin, Th.; Seilmeier, W. *Z. Lebensm. Unters. Forsch.* **1968**, *137*, 4.
Severin, Th.; Krönig, U. *Chem. Mikrobiol. Technol. Lebensm.* **1973**, *2*, 49.
Simon, H.; Heubach, G. *Chem. Ber.* **1965**, *98*, 3703.
Tatum, J. H.; Shaw, E. P.; Berry, R. E. *J. Agric. Food Chem.* **1967**, *15*, 773.
Tonsbeek, C. H. T.; Plancken, A. J.; van de Weerdhof, T. J. *J. Agric. Food Chem.* **1968**, *16*, 1016.
Van den Ouweland, G. A. M.; Peer, H. G. *Recl.: J. R. Neth. Chem. Soc.* **1970**, *89*, 750.
Weygand, F.; Simon, H.; Bitterlich, W.; Hodge, J. E.; Fisher, B. E. *Tetrahedron* **1958**, *6*, 123.

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Mass Spectrometric Method To Determine the Chain Length of Oligosaccharides Attached to Phenolic Polymers by Nonglycosidic Linkages[1,2]

James L. Minor* and Roger C. Pettersen

In many plants, a portion of the polysaccharides appears to have a very low degree of cross-linking with aromatic polymers such as lignin or flavolans. The proportion of cross-linked units may be enriched for study by enzymatically hydrolyzing the nonbonded carbohydrates. A convenient method is described for the simultaneous analysis of sugar content and apparent chain length of the oligosaccharidic fragments remaining after enzymatic hydrolysis. The analysis assumes attachment to the phenolic polymers by nonglycosidic linkages. The hemiacetal ends of the oligosaccharidic fragments are reduced with sodium borohydride while the fragments are still attached to the phenolic polymer. After acid hydrolysis, monomeric sugars are reduced with sodium borodeuteride. The mixed isotopic products are analyzed as their alditol acetate derivatives by capillary GC/MS with selected ion monitoring. The isotopic ratioing procedure is detailed and the precision determined. The method is accurate to 1 monomeric unit for oligosaccharide chain lengths of less than 10.

During a study of chemical bonding between lignin and carbohydrates in woody plants, the relatively few bonding sites were enriched by enzymatic hydrolysis of the major portion of the carbohydrate units not involved in the interpolymer bonding (Minor, 1982). The resultant material is primarily lignin with oligosaccharidic units attached. To completely characterize the bonding, it was necessary to develop a procedure to analyze the chain length of the attached oligosaccharides. The procedure involves an initial reduction of the attached oligosaccharides with sodium borohydride followed by hydrolysis and reduction of the released sugars with sodium borodeuteride. The alditols are then analyzed as their acetates by capillary gas chromatography/mass spectrometry (GC/MS) using chemical ionization and selected ion monitoring. The apparent chain length is obtained from the ratio of deuteriated to nondeuteriated alditol acetates.

This paper describes a study of the isotopic ratioing, its limitations, and the precision to be expected. The procedure is useful for oligosaccharides containing up to 10 monomeric sugar units. An alternative procedure, which can be used if a mass spectrometer is not available, involves conversion of the released sugars to their aldononitrile derivatives and analysis of both the alditol acetates and aldononitriles by gas chromatography (Baird et al., 1973).

EXPERIMENTAL SECTION

Preparation of Standards and Samples. With chloroform as solvent, two stock solutions were prepared from measured amounts of the five wood sugar alditol acetates, one in the protio and the other in the monodeuterio form. Standard mixtures of deuteriated and undeuteriated alditol acetates were prepared by mixing aliquots from the stock solutions. The extent of deuteriation from a given lot of $NaBD_4$ was checked as follows by mass spectrometry. A deuteriated derivative was prepared by reducing 1.2 mmol of sugar with 3 mmol of $NaBD_4$ in 2 mL of 2 M NH_4OH at 60 °C for 1 h. The reaction was stopped, and sodium ions were removed with cation-exchange resin (H^+). Borate was removed as the methyl ester. The residue was acetylated by the procedure of Harris et al. (1984). The yield from a satisfactory lot of $NaBD_4$ contained less than 2.0% undeuteriated compound. (It is noted that one unsatisfactory lot yielded about 30% undeuteriated compound and was replaced by the vendor.)

Kraft pulp enzyme lignins were obtained from loblolly pine kraft pulps by enzymatic hydrolysis of the pulp polysaccharides (Minor, 1986) and then were suspended in

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