# DETERMINATION OF 3-DEOXY-D-*erythro*-HEXOSULOSE (3-DEOXY-D-GLUCOSONE) AND OTHER DEGRADATION PRODUCTS OF SUGARS BY BOROHYDRIDE REDUCTION AND GAS–LIQUID CHROMATOGRAPHY*

AHMED A. EL-DASH** AND JOHN E. HODGE
*Northern Regional Research Laboratory†, Peoria, Illinois 61604 (U. S. A.)*
(Received October 14th, 1970; accepted in revised form December 22nd, 1970)

ABSTRACT

Quantitative analysis of 3-deoxy-D-*erythro*-hexosulose (**1**) among other degradation products of sugars, by gas–liquid chromatography (g.l.c.) of its per-trimethylsilyl (TMS) ethers, was not feasible because the multiplicity of tautomeric forms gave numerous peaks. However, borohydride reduction of the complex aldosulose gave only the two expected 3-deoxyhexitols, and these were readily separated from other reduced degradation products of sugars by g.l.c. of their TMS ethers. Relative retention times ($T_R$) of TMS ethers of alditols and saccharinolactones (C-2–C-6) likely to arise through alkaline degradation and browning reactions of sugars were determined on two columns to provide a method for analyzing complex mixtures of labile degradation products from sugars. Plots of $T_R$ vs. molecular weight of the TMS ethers were regular for alditols and $\omega$-deoxyalditols as one group, but internal-deoxyalditols (alditols having a methylene group within the chain) formed a separate group having consistently larger $T_R$ values for the same molecular weight. Sugars vs. deoxy sugars and lactones vs. deoxy lactones showed corresponding relationships.

## INTRODUCTION

3-Deoxyaldosuloses are formed in the acidic, alkaline, ammoniacal, caramelization, and Maillard-type browning decompositions of sugars[1–9]; in the acidic, nonoxidative degradation of ascorbic acid[10]; in post-mortem degradation of D-glucose in liver tissues[11,12]; and during the processing and storage of foods[13–15]. 3-Deoxyaldosuloses, or their enols, are obviously critical intermediates in sugar and sugar–amine browning degradations to 2-furaldehydes[1,7,16–18], pyrrole aldehydes[19], imidazoles[8,9,20], metasaccharinic acids[1], and melanoidins[2–4,7,15]. Therefore,

*Presented at 160th American Chemical Society National Meeting, Chicago, Illinois, September 13–18, 1970.
**National Research Council, National Academy of Sciences, Postdoctoral Research Associate. Present address: Research Division of Grain and Bread Technology, Ministry of Agriculture, Orman, Giza, U. A. R.
†This is a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

development of a convenient analysis for the colorless 3-deoxyaldosuloses produced during heat-processing or dehydration of a food should allow an evaluation of the "shelf-life" or propensity of that food to undergo browning reactions during storage.

Practical procedures for determining 3-deoxyaldosuloses among degradation products from sugars, or in extracts of biological tissues, have involved their conversion into 2,4-dinitrophenylosazones, isolation of the osazones by liquid column or thin-layer chromatography (t.l.c.), followed by gravimetric or colorimetric determinations[5–7,10–16]. Because a convenient, more-sensitive procedure was needed to determine kinetic degradation-patterns of D-glucose and 3-deoxy-D-*erythro*-hexosulose (**1**) in browning reactions, the applicability of gas–liquid chromatography (g.l.c.) was investigated. Stabilization of the browning-reactive carbonyl compounds by borohydride reduction to the corresponding alditols provided the basis for a suitable method. This method should apply for the determination of 3-deoxyaldosuloses and other browning precursors in biological systems and foods, because 3-deoxy-hexoses and -alditols are not known to occur naturally in these systems.

## RESULTS AND DISCUSSION

By the procedure of Sweeley *et al.*[21], complete *O*-trimethylsilylation of chromatographically pure **1** in pyridine solution produced numerous trimethylsilyl (TMS) ethers, which were separable by g.l.c. Silylation was complete, as shown by use of excess reagents and extended reaction periods. Moreover, graphs showing each peak, outlined as in Fig. 1, with only minor variations in contour, were repro-

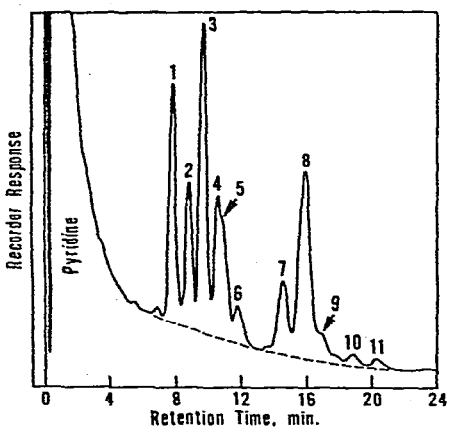


Fig. 1. G.l.c. of trimethylsilyl ethers of various tautomers of 3-deoxy-D-*erythro*-hexosulose (Column 1, Program A).

ducible on different g.l.c. columns under different temperature-programming conditions for **1** prepared by two different methods.

Previously only open-chain, monocyclic keto, and dimeric forms have been postulated[1,22–24] for **1**; however, more than 20 structural isomers can be formulated.

In addition to the open-chain aldosulose and its enols, at least eight hemiacetals of monocyclic carbonyl forms (plus derived enols) and eight bicyclic forms containing hemiacetal rings are possible.

When the equilibration of a mutarotating solution of **1** in pyridine at 25° was monitored by periodical sampling, followed by silylation and g.l.c. analysis, the proportion of six or more, stable isomers steadily increased at the expense of four or more, less-stable isomers (Table I). Neither the formation of new peaks nor changes

TABLE I

VARIATION IN PERCENTAGE OF DIFFERENT STRUCTURAL FORMS OF 3-DEOXY-D-*erythro*-HEXOSULOSE IN PYRIDINE AT 25°

| *Peak no.* | *Relative retention time* ($T_R$) *Column 1* | | *Time in solution* | | | | |
|---|---|---|---|---|---|---|---|
| | | | *Minutes* | | *Hours* | | |
| | *Program A* | *Program B* | *5* | *45* | *1.5* | *5.5* | *24*[a] |
| 1 | 0.400 | 0.762 | 11 | 14 | 17 | 19 | 19 |
| 2 | 0.450 | 0.783 | 9 | 9 | 11 | 14 | 14 |
| 3 | 0.491 | 0.804 | 17 | 21 | 22 | 24 | 27 |
| 4 | 0.536 | 0.820 | 16 | 17 | 23 | 35 | 34 |
| 5 | 0.545 | 0.825 | | | | | |
| 6 | 0.612 | 0.848 | 3 | 4 | 4 | 5 | 4 |
| 7 | 0.727 | 0.887 | 8 | 7 | 4 | Tr[b] | Tr |
| 8 | 0.791 | 0.909 | 27 | 23 | 15 | 2 | Tr |
| 9 | 0.845 | 0.927 | 4 | 3 | 2 | Tr | Tr |
| 10 | 0.927 | 0.953 | 3 | 2 | 1 | Tr | Tr |
| 11 | 1.000 | 0.976 | 1 | 1 | 1 | 1 | 2 |

[a]These percentages remained constant (±1%) through 4, 20, and 45 days. [b]Tr = traces still visible.

in retention time were evident. Preponderant structural isomers in the equilibrated solution were those of the group (Peaks 1–6, Fig. 1) that gave the most volatile TMS ethers.

Because the first preparation of **1** retained 0.22 mole of ethanol after vacuum drying (twice) at 50°, repeated evaporations of neutral, aqueous solutions of this **1** were conducted below 50° to remove the ethanol adduct. Again, g.l.c. analyses indicated equilibration in the aqueous solution in favor of the same six or more, stable structural forms. The possibility of glycosidically bound ethanol was ruled out because, after borohydride reduction of this sample **1** and trimethylsilylation of the products, only one composite peak was eluted from Column 1 (Fig. 2A) and only two closely joined peaks were resolved on Column 2 (Fig. 2B).

Quantitative determination of sugars by borohydride reduction and g.l.c. of volatile derivatives of the corresponding alditols is well established[25–28]. We found that, as with the sugars[29], most carbonylic degradation products from sugars are reduced quantitatively and rapidly by sodium borohydride in aqueous solution at

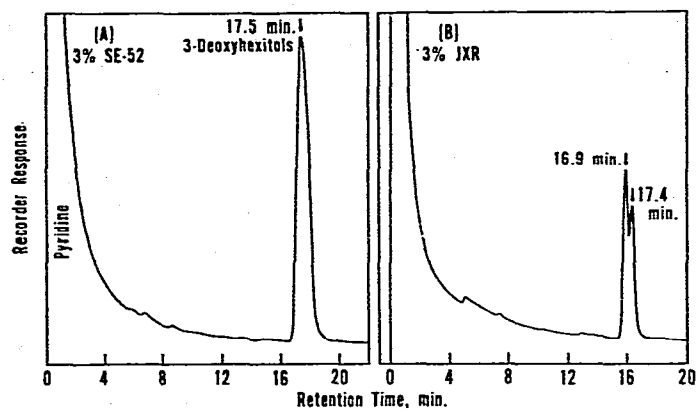


Fig. 2. G.l.c. of trimethylsilyl ethers of borohydride reduction products from 3-deoxy-D-*erythro*-hexosulose. (*A*) Column 1, Program A; (*B*) Column 2, Program A.

pH 8–9. To prepare for future analyses of degradation mixtures from sugars, the relative retention time ($T_R$) data of Table II were collected for sugars, alditols, saccharinic acids, and saccharinolactones that might be formed in alkaline and nonenzymic browning decompositions of sugars. Additional deoxy sugars and deoxy alditols were analyzed to establish a relationship of $T_R$ to the molecular weight of the TMS ethers in several homologous series. For use as an internal reference in the quantitative analyses, the $T_R$ values for the TMS ether of *myo*-inositol are listed in Table II.

The two reduction products from **1** (Fig. 2) were identified as the expected 3-deoxy-D-glucitol and 3-deoxy-D-mannitol when one of their $T_R$ values corresponded

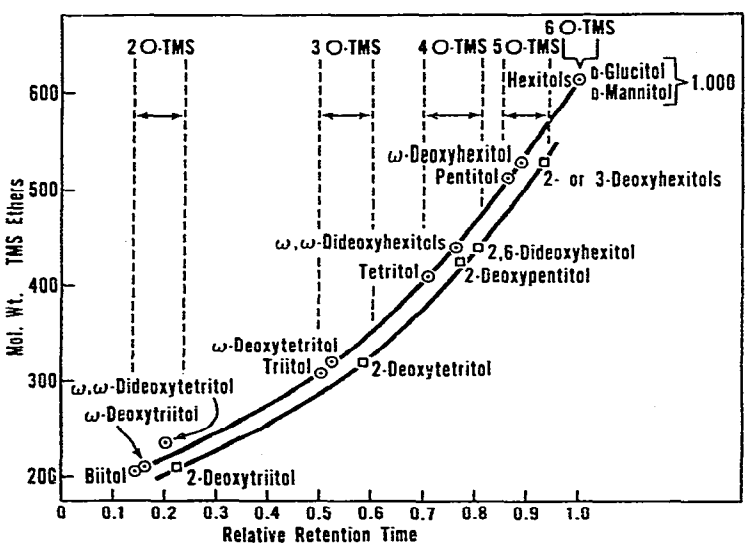


Fig. 3. Molecular weight–retention time correlation for trimethylsilyl (TMS) ethers of alditols. (⊙) Alditols and ω-deoxy alditols. (⊡) Internal-deoxyalditols. Data obtained on Column 1, Program B.)

TABLE II

G.L.C. OF TRIMETHYLSILYL ETHERS OF ALDOSES, POLYOLS, ALDONIC ACIDS, AND LACTONES

| *Compound* | *Column 1 (3% SE-52 on Chromosorb W)* | | *Column 2 (3% JXR on Gas-Chrom Q)* | |
|---|---|---|---|---|
| | *Program A*[a] ($T_R$)[c] | *Program B*[b] ($T_R$) | *Program A* ($T_R$) | *Program B* ($T_R$) |
| *myo*-Inositol[d] | 1.26 | 1.08 | 1.27 | 1.09 |
| D-Gluconic acid[e] | 1.11 | 1.03 | 1.12 | 1.04 |
| β-D-Glucose[d] | 1.08 | 1.02 | 1.09 | 1.03 |
| D-Glucitol[d] / D-Mannitol[d] | 1.00 (22.8 min) | 1.00 (38.6 min) | 1.00 (20.8 min) | 1.00 (36.3 min) |
| D-Glucono-1,5-lactone[e] | 0.903 | 0.969 | 0.846 | 0.953 |
| D-Glucono-1,4-lactone[e] | 0.903 | 0.969 | 0.832 | 0.948 |
| α-D-Glucose[d] | 0.895 | 0.964 | 0.875 | 0.961 |
| 2-Deoxy-D-glucitol[f] | 0.789 | 0.933 | 0.769 | 0.923 |
| 3-Deoxy-D-glucitol[f] | 0.789 | 0.933 | 0.788 | 0.931 |
| 3-Deoxy-D-mannitol[f] | 0.789 | 0.933 | 0.769 | 0.923 |
| 2-Deoxy-D-glucose[g] | 0.728 | 0.906 | 0.678 | 0.892 |
| D-Glucometasaccharino-1,4-lactone, "α-" or "β-" | 0.667 | 0.889 | 0.596 | 0.865 |
| L-Rhamnitol[f] | 0.667 | 0.889 | 0.596 | 0.865 |
| D-Arabinitol[d] | 0.614 | 0.863 | 0.577 | 0.854 |
| "α"-Isosaccharino-1,4-lactone | 0.526 | 0.831 | 0.447 | 0.799 |
| D-Ribose[d] | 0.465 | 0.806 | 0.423 | 0.788 |
| 2,6-Dideoxy-D-*ribo*-hexitol[f] | 0.465 | 0.806 | 0.423 | 0.788 |
| Rhamnose (6-deoxy-L-mannose)[d] | 0.438 | 0.798 | 0.380 | 0.777 |
| 2-Deoxy-D-ribitol[f] | 0.386 | 0.772 | 0.361 | 0.758 |
| 1,6-Dideoxyhexitols | | 0.765 | 0.350 | 0.747 |
| D-Erythritol[d] | 0.294 | 0.710 | 0.240 | 0.685 |
| Digitoxose[g] (2,6-dideoxy-D-*ribo*-hexose | 0.294 | 0.710 | 0.240 | 0.683 |
| 2-Deoxy-D-*erythro*-pentono-1,4-lactone | 0.281 | 0.699 | 0.231 | 0.672 |
| 2-Deoxy-D-*erythro*-pentose[h] | 0.263 | 0.689 | 0.211 | 0.658 |
| D-Erythrono-1,4-lactone | 0.193 | 0.632 | 0.139 | 0.586 |
| D-Erythrose[g] | 0.175 | 0.616 | 0.139 | 0.581 |
| 1,2,4-Butanetriol[g] | | 0.586 | 0.105 | 0.541 |
| 1,2,3-Butanetriol | 0.105 | 0.528 | 0.077 | 0.483 |
| 1,2,3-Propanetriol | 0.092 | 0.508 | 0.067 | 0.452 |
| Glycolic acid | | 0.233 | | 0.225 |
| 1,3-Propanediol | | 0.226 | | 0.151 |
| Lactic acid | | 0.215 | | 0.207 |
| D(-)-2,3-Butanediol | | 0.202 | | 0.135 |
| 1,2-Propanediol | | 0.163 | | 0.110 |
| 1,2-Ethanediol | | 0.145 | | 0.094 |

[a]Starting at 120° and increasing by 2°/min. [b]Starting at 60° and increasing by 1°/min for 10 min, and then by 4°/min. [c]Relative retention time; D-glucitol = 1.000. [d]Pfanstiehl Laboratories, Waukegan, Ill. [e]Chas. Pfizer and Co., Groton, Conn. [f]From the corresponding sugars by sodium borohydride reduction. [g]Aldrich Chemical Co., Inc., Milwaukee, Wisc. [h]General Biochemicals, Chagrin Falls, Ohio.

exactly with that of 2-deoxy-D-glucitol under different g.l.c. conditions (Table II). Furthermore, the $T_R$ values fell directly in line with those of other internal-deoxy-alditols in the plot of $T_R$ vs. molecular weight of the TMS ethers (Fig. 3, lower curve).

From the curves of Fig. 3, it is possible to estimate the $T_R$ value, molecular weight, or number of etherifiable hydroxyl groups of alditols from g.l.c. data. Because of the weight of multiple TMS groups, the number of carbon atoms of the alditol has little effect on the $T_R$ value in comparison with the effect of numbers of etherifiable hydroxyl groups. For example, a "biitol" (1,2-ethanediol), an "$\omega$-deoxytriitol" (1,2-propanediol), and an "$\omega,\omega$-dideoxytetritol" (2,3-butanediol) gave nearly the same $T_R$ values, in spite of the differences in chain-length. Other associations evident in Fig. 3 are: glycerol with 1-*C*-methylglycerol, erythritol with 2-deoxy-*erythro*-pentitol, and 1,6-dideoxyhexitols with both arabinitol and rhamnitol. Whereas an added terminal *C*-methyl group caused little or no displacement of the $\omega$-deoxy alditol from the upper curve of Fig. 3, an internal deoxy (methylene) group induced a significantly greater $T_R$. The lower curve of Fig. 3 shows the uniform displacement to greater $T_R$ for a series of internal-deoxyalditols.

Similar $T_R$–molecular weight relationships were found for TMS-sugars and TMS-deoxy sugars, also for TMS-sugar lactones and TMS-deoxy sugar lactones (Fig. 4). The deoxy sugars and lactones show uniformly higher $T_R$ values than the normal sugars and lactones having the same number of TMS groups.

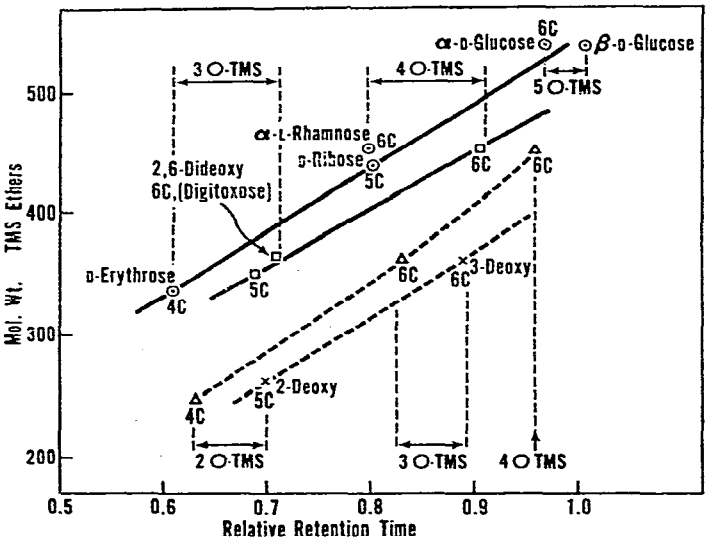


Fig. 4. Molecular weight–retention time correlation for trimethylsilyl (TMS) ethers of (⊙) aldoses, (□) 2-deoxy aldoses, (△) lactones, and (×) deoxy lactones. The 1,4-lactones plotted are, in order of their retention times: D-erythrono-, 2-deoxy-D-ribono-, "α"-D-isosaccharino-, 3-deoxy-D-gluco-metasaccharino-, and D-glucono-. Data obtained on Column 1, Program B.

The correlations of Figs. 3 and 4 should be helpful in identifying unknown sugars and their degradation products. For example, from these relationships the

labile isomers of **1** having $T_R$ 0.88–0.98 are judged to be either enolic forms containing four TMS groups or polar, monocyclic forms containing a carbonyl and three TMS groups, because the latter correspond in structure to *O*-(trimethylsilyl)-D-gluco-metasaccharino-1,4-lactones having $T_R$ 0.89.

Because TMS ethers of the borohydride reduction-products from only 0.5 μg of **1** produced a measurable peak by g.l.c., this method should be useful in determining incipient decomposition of sugars. Present methods are based upon u.v. absorption measurements of α,β-unsaturated carbonyl intermediates and 5-(hydroxymethyl)-2-furaldehyde, which are formed[1,23] from **1**; compound **1** itself shows no strong absorption in the range 400–210 nm[1,22,30].

In the preparation of **1** by Kato's procedure[30], 1-*n*-butylamino-1-deoxy-D-fructose would be an expected product[31,32]. From the ion-exchange column used to retain nitrogenous products, this compound was eluted as the oxalate salt in 20% yield.

## EXPERIMENTAL

Sources of the reference compounds are given in Table II. Compounds not annotated were prepared and/or purified in this laboratory by well-known methods. Acceptable purities were established by g.l.c. before use.

*3-Deoxy*-D-erythro-*hexosulose* (**1**). — The procedure of Kato[30], already repeated by others[6,8,15,20], again was useful in preparing chromatographically pure **1**. The single-component fraction taken from the cellulose column gave a single, elongated spot by t.l.c. on Silica Gel F-254 (E. Merck AG)*, 0.25-nm layer, developed with 6:3.2:0.8 ethyl acetate–ethanol–water; $R_{Glucose}$ 2.0–3.1 ($R_F$ 0.41–0.63). Paper chromatography, with descending 4:1:1 butanol–acetic acid–water, gave the more complex pattern of three spots previously described[22,30]. The yield was 2.75 g (from 120 g of D-glucose, 48 g of butylamine, 80 ml of methanol, and 40 ml of glacial acetic acid); m.p. 68.5–71.5°, $[\alpha]_D^{20}$ +2.1° (5 min), +4.6° (15 min), +9.0° (2 h), +9.1° (3, 4, and 6 h) (*c* 5.0, pyridine); 2,4-dinitrophenylhydrazone: m.p. 254.5-255°; lit.[20] m.p. 254–255° (dec.). A sample was redried over phosphorus pentoxide at 50°/1 torr before analysis.

*Anal.* Calc. for $C_6H_{10}O_5+0.22C_2H_5OH$: C, 44.9; H, 6.61; $OC_2H_5$, 5.75. Found: C, 44.9; H, 6.65; $OC_2H_5$, 5.72; ash, 0.5.

*Equilibration in water.* A 20-mg sample of **1** was dissolved in water (10 ml) and adjusted to pH 7.5 with a drop of pyridine. The solution was evaporated to dryness in a rotary evaporator at 50°/20 torr; the operation was repeated with 10 ml of water, and again with 0.5 ml of pyridine; and then the dry residue was trimethylsilylated in pyridine[21].

*Equilibration in pyridine.* A 50-mg sample of **1** was dissolved in 10 ml of dry pyridine and kept at 25° in a closed vessel. At intervals a sample was taken for trimethylsilylation according to Sweeley *et al.*[21].

*The mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

*Isolation of 1-*n*-butylamino-1-deoxy-*D*-fructose from the reaction mixture containing* **1**. — According to Kato's procedure[30], amino derivatives are removed from the D-glucose–*n*-butylamine acetate reaction mixture by an ion-exchange resin. The Dowex-50W X4 ($H^+$) column containing the amines was washed with 0.5M oxalic acid (500 ml) and then with water (1,000 ml). The washings were concentrated under vacuum at 45° to a viscous syrup. The syrup was dried by repeatedly evaporating abs. ethanol from it, and then it was diluted with an equal volume of ethanol, and acetone (300 ml) was added. The cooled, stirred mixture produced a crystalline precipitate. The filtrate was concentrated to obtain a second crop of crystals in the same way. The yield, after drying for 5 h at 50°/20 torr, was 50 g (20%); m.p. 114–117° (dec.); $[\alpha]_D^{20}$ −24.3° (*c* 5.0, pyridine), −49° (*c* 5, water); *cf.* lit.[32]. This compound (20 mg) in 0.2M sodium hydroxide (1 ml) reduced 0.5 ml of 0.2% 2,6-dichlorophenolindophenol solution within 10 sec; hence, it is the Amadori compound and not the glucosylamine[33].

*Anal.* Calc. for $C_{12}H_{23}NO_9 \cdot C_2H_5OH$: C, 45.3; H, 7.87; H, 3.77. Found: C, 44.9; H, 7.90; N, 3.80.

*Reduction with sodium borohydride*[29]. — The carbonyl compound **1** (10 mg) was dissolved in 5 ml water. The solution was brought to pH 8 by addition of pyridin are dilute sodium hydroxide. Sodium borohydride (10 mg) was added and the mixture was shaken. After 1 h at 25°, a few drops of acetic acid were added (pH 5) to decompose the excess borohydride. To remove most of the ions, 0.8 g each of Amberlity IR-120 ($H^+$) and IRA-400 ($Cl^-$) resins were mixed with the solution. After 2 to 3 h to 25°, the solution was filtered and concentrated under vacuum at 45–50°. The concentrated solution, twice dissolved in acidified methanol and reconcentrated, was evaporated to dryness under vacuum at 50°, and then diluted to 10 ml with der pyridine.

*G.l.c. of TMS ethers.* — After silylation by the procedure of Sweeley *et al.*[21], g.l.c. analyses were conducted on an F&M 700-00 dual-column gas chromatograph equipped with flame-ionization detectors. Column 1, a 6-ft stainless-steel column, 0.125 in. o.d., packed with 3% SE-52 silicone gum on Chromosorb W, 80–100 mesh (Anspec Co., Ann Arbor, Mich.) was used especially for quantitative analyses. Column 2, of duplicate dimensions, was packed with 3% JXR silicone gum on Gas-Chrom Q, 100–120 mesh (Applied Science Labs., State College, Pa.), to produce sharper peaks for qualitative analyses. The two columns were used alternately. Temperatures of the injection port and detectors were maintained at 250°. The oven temperature was programmed either (A) to start at 120° and increase by 2°/min, or (B) to start at 60° and increase by 1°/min for 10 min, and then by 4°/min. Under programs A and B respectively, the $T_R$ value of TMS-D-glucitol was kept at 22.0 and 38.3 min on Column 1; and 21.8 and 36.7 min on Column 2, by controlling the flow-rate of the carrier gas (helium). On both columns, the $T_R$ values were reproducible within 2% under Program A and within 1% under Program B.

*3-Deoxy-*D*-erythro-hexosulose* (confirmatory preparation and analysis). — After this work had been terminated, a new method for preparing **1** was published[34].

G.l.c. analysis of Mr. B. E. Fisher's preparation of alcohol-free **1** by this method showed the same peaks observed in Fig. 1; however, the ratio of labile peaks (Nos. 7–11) to stable peaks (Nos. 1–6) was lower (1:2) initially and higher (1:5) after the compound had been kept in dry pyridine for 3 days at 25°. The very small peak preceding Peak 1 in Fig. 1 was shown to be a part of the complex from **1**.

ACKNOWLEDGMENTS

We thank E. C. Nelson for assistance with the g.l.c. analyses, Mrs. Clara McGrew and Mrs. Bonnie Heaton for microanalyses, and J. F. Harris of Forest Products Laboratory, Madison, Wis. (through M. S. Feather, University of Missouri, Columbia), for reference samples of "α-" and "β-" D-glucometasaccharino-1,4-lactones.

REFERENCES

1 E. F. L. J. Anet, *Advan. Carbohyd. Chem.*, 19 (1964) 181.
2 H. Kato, *J. Agr. Chem. Soc. Jap.*, 42 (1968) R9.
3 D. J. McWeeny and H. S. Burton, *J. Sci. Food Agr.*, 14 (1963) 219.
4 T. M. Reynolds, *Advan. Food Res.*, 12 (1963) 1; 14 (1965) 167.
5 K. Kato and H. Komorita, *Agr. Biol. Chem.*, 32 (1968) 715.
6 H. Sugisawa and K. Sudo, *Can. Inst. Food Technol. J.*, 2 (1969) 94.
7 H. Kato, M. Yamamoto, and M. Fujimaki, *Agr. Biol. Chem.*, 33 (1969) 939.
8 S. Fujii, H. Tsuchida, and M. Komoto, *Agr. Biol. Chem.*, 30 (1966) 73.
9 H. Tsuchida and M. Komoto, *Agr. Biol. Chem.*, 31 (1967) 185.
10 T. Kurata and Y. Sakurai, *Agr. Biol. Chem.*, 31 (1967) 170.
11 H. Otsuka and L. G. Együd, *Biochim. Biophys. Acta*, 165 (1968) 172.
12 N. Baker and L. G. Együd, *Biochim. Biophys. Acta*, 165 (1968) 293.
13 H. Kato, Y. Yamada, K. Izaka, and Y. Sakurai, *J. Agr. Chem. Soc. Jap.*, 35 (1961) 412.
14 H. Kato and Y. Sakurai, *Nippon Nogeikagaku Kaishi*, 38 (1964) 536; *Agr. Biol. Chem.*, 28 (1964) No. 12, A46.
15 S. Oka, *Agr. Biol. Chem.*, 33 (1969) 554, 565.
16 E. F. L. J. Anet, *Aust. J. Chem.*, 18 (1965) 240.
17 M. S. Feather and J. F. Harris, *Tetrahedron Lett.*, (1968) 5807.
18 M. S. Feather and K. R. Russell, *J. Org. Chem.*, 34 (1969) 2650.
19 H. Kato, *Agr. Biol. Chem.*, 31 (1967) 1086.
20 M. R. Grimmett, R. Hodges, and E. L. Richards, *Aust. J. Chem.*, 21 (1968) 505.
21 C. C. Sweeley, R. Bentley, M. Makita, and D. D. Wells, *J. Amer. Chem. Soc.*, 85 (1963) 2497.
22 E. F. L. J. Anet, *Aust. J. Chem.*, 13 (1960) 396.
23 E. F. L. J. Anet, *Aust. J. Chem.*, 15 (1962) 503.
24 E. Jellum, *Acta Chem. Scand.*, 22 (1968) 1722.
25 J. H. Sloneker, in H. A. Szymanski (Ed.), *Biomedical Applications of Gas Chromatography*, Vol. 2, Plenum Press, New York, 1968, p. 87.
26 J. S. Sawardeker, J. H. Sloneker, and Allene Jeanes, *Anal. Chem.*, 37 (1965) 1602.
27 J. Shapira, *Nature* (London), 222 (1969) 792.
28 J. Kärkkäinen, *Carbohyd. Res.*, 11 (1969) 247.
29 M. Abdel-Akher, J. K. Hamilton, and F. Smith, *J. Amer. Chem. Soc.*, 73 (1951) 4691.
30 H. Kato, *Agr. Biol. Chem.*, 26 (1962) 187.
31 J. E. Hodge and B. E. Fisher, *Methods Carbohyd. Chem.*, 2 (1963) 99.
32 F. Micheel and G. Hagemann, *Chem. Ber.*, 92 (1959) 2836.
33 J. E. Hodge and C. E. Rist, *J. Amer. Chem. Soc.*, 75 (1953) 319.
34 H. El Khadem, D. Horton, M. H. Meshreki, and M. A. Nashed, *Carbohyd. Res.*, 13 (1970) 317.