[CONTRIBUTION FROM PULP MILLS RESEARCH, DEPARTMENT OF CHEMISTRY AND CHEMICAL ENGINEERING, UNIVERSITY OF WASHINGTON]

A Chromatographic Study of Sulfite Waste Liquor Sugars[1]

BY P. K. MULVANY,[2a] H. D. AGAR,[2b] Q. P. PENISTON AND JOSEPH L. MCCARTHY

The sugars in a sulfite waste liquor have been recovered nearly quantitatively and free of ionic impurities by dialysis and ion exchange resin treatments of the liquor. Separation of these recovered sugars and of known sugar mixtures by partition chromatography on starch columns using a 1-butanol–water system has been studied. Behavior of the sugars investigated has been found to be in accord with a theory of chromatographic processes and relative elution rates are satisfactorily correlated with independently determined partition coefficients. The sugars present in a paper pulp sulfite waste liquor derived from 85% Western Hemlock and 15% White Fir are found to be distributed as follows: mannose, 48%; glucose, 15%; xylose, 15%; galactose, 10%; arabinose, 6%; fructose, less than 2%; unaccounted for, 4%.

Introduction

Although the nature of the several sugars present in sulfite waste liquors has been established in prior investigations,[3-6] quantitative uncertainties have remained due to difficulties of separation of all of the sugars from other sulfite waste liquor components, and to difficulties of analysis of complex sugar mixtures by classical methods. Recently we have developed new procedures for isolation of mixed sugars in high yield and purity from sulfite waste liquors using dialysis and anion exchange resins, for separation of individual sugars by partition chromatography, and for quantitative determination of individual sugars. Since these procedures apparently permit avoidance of uncertainties of earlier methods, and since they may prove useful in other wood chemistry investigations, we now report studies on known sugar mixtures and also results for the sugars present in a certain sulfite waste liquor.

Experimental

Isolation of Sugars from Sulfite Waste Liquor.—A commercial paper pulp calcium sulfite waste liquor[7] was first exhaustively dialyzed in a continuous apparatus under conditions previously shown[8] to retain about 50% of the lignin sulfonic acids as non-dialyzable material and to yield quantitative recovery of sugars in admixture with other low molecular weight substances in the dialyzate. This dialyzate was concentrated at reduced pressure to about 200 g. of total solids per liter, completely deashed using Amberlite IR-100 H and then exhaustively extracted with ether.

Removal of anionic substances was accomplished by passage of the dialysate over columns (12 mm. i.d. × 30 cm.) of Duolite A-2[9] anion exchange resin ground and screened to 65–100 mesh and previously treated with 2% sodium hydroxide solution followed by distilled water until a neutral effluent was obtained. After addition of 5 ml. of concentrated dialyzate to the column, distilled water was allowed to flow through at about 300 ml. per hour to wash the unabsorbed sugars from the resin. With 100 and 250 ml. of effluent collected in two experiments, the reducing value recovered was 87.5 and 88.5% of that present in the deashed dialysate. It is believed that this represents the fraction of the reducing value of the sulfite waste liquor associated with true sugars and that the remaining 12 or 13% is attributable to other components. Effluents from the resin columns were completely clear and colorless and showed a specific conductance at 25° of about $5.0 \times 10^{-6}\ \Omega^{-1}$ cm.$^{-1}$.

The reducing value calculated as glucose was found to be 84% of the total solids, thus leaving about 16% as non-ionic, non-reducing material.

Chromatographic Separation of Sugars.—Columns were prepared by mixing potato starch (75 g.) and Dicalite filter aid (75 g.) with 250 ml. of 1-butanol saturated with water. The solvent was removed and the cake twice again suspended in the same volume of fresh solvent. The final slurry was poured into a glass column (3.45 sq. cm. cross section) to a settled height of one meter. Butanol saturated with water was then passed through the column (0.04 to 0.05 ml. per sq. cm. per min.) for 4 days to obtain equilibrium between phases.

Two such columns were charged with a wet butanol solution (19.8 ml. each) containing the above-described sulfite waste liquor sugars at a concentration of 2.62 g. total sugars calculated as glucose per liter. After absorption of the sample and rinsing, the columns were attached to constant head reservoirs supplying wet butanol and samples, each consisting of the eluant over a 2-hour period, were collected automatically.

Using substantially identical procedures, chromatographic separations were also conducted in duplicate on known mixtures of xylose, mannose, glucose and galactose.

Eluant samples were analyzed for total reducing sugars by the Shaffer–Somogyi method[10] using a 0.65-ml. aliquot measured with a calibrated micro pipet.

Results and Discussion

For nearly quantitative recovery of sulfite waste liquor sugars substantially uncontaminated by lignin sulfonates and other cations or anions, removal of high molecular weight lignin sulfonates by exhaustive dialysis is desirable since these substances are not readily absorbed by the subsequent anion exchange resin treatment. The alternative removal of these substances by precipitation as heavy metal or amine salts seems more likely to cause sugar loss or destruction. Ether extraction of the concentrated deashed dialyzate serves to remove resins, fatty acids, and other substances which might not be retained by the anion resin. This extraction may not be necessary to the success of the chromatographic separation of sugars. No characterization has yet been made of the non-reducing material present to the extent of about 16% in the purified sugar fractions.

The chromatographic elution curves obtained for known sugar mixtures and for sulfite waste liquor sugars are shown in Fig. 1. Identification of the sugars giving rise to peaks in the elution curves was confirmed by consideration of their characteristic elution rates as controlled by their respective partition coefficients between butanol

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(2) (a) California Research Corp., Richmond, California. (b) Microbiology Department, University of Washington, Seattle, Washington.

(3) H. Krause, *Chem. Ind. (Berlin) Nachr.-Ausgabe*, **29**, 217 (1906).

(4) E. Hägglund, *Biochem. Z.*, **70**, 416 (1915).

(5) P. Klason, "Kempe—Festschrift von Skogsvårdsföreningens Tidskrift," Stockhlom, 1917, p. 224.

(6) E. Hägglund, F. W. Klingstedt, T. Rosenquist and H. Urban, *Z. Physiol. Chem.*, **177**, 248 (1928).

(7) Kindly provided by the Puget Sound Pulp and Timber Co., Bellingham, Washington.

(8) Q. P. Peniston and J. L. McCarthy, THIS JOURNAL, **70**, 1324 (1948).

(9) Chemical Process Co., San Francisco, California.

(10) Shaffer and Somogyi, *J. Biol. Chem.*, **100**, 695 (1933).

and water and by the phase volume ratio in the chromatographic columns.[11]

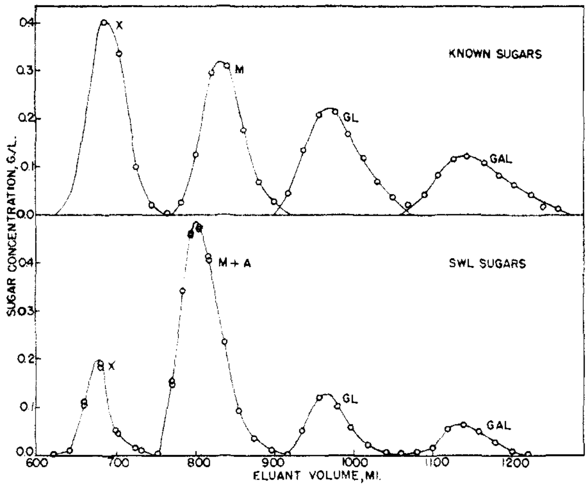

Fig. 1.—Chromatographic elution curves for sugars: X, M, A, Gl and Gal designate xylose, mannose, arabinose, glucose and galactose, respectively.

Partition coefficients for the five principal sugars of sulfite waste liquor were determined at 25° as shown in Table I for the system butanol–water when about 6.7 g. of sugar was present per liter of aqueous phase. These experimental partition coefficients are found to be in satisfactory relation with R_F values (ratios of solute to solvent migration distances) determined by Partridge and Westfall[12] for partition chromatography on filter paper strips using the same system in the presence of a little ammonia. Thus using the expression of Consden and co-workers[13]

$$K = \frac{AL}{A_s}\left(\frac{1}{R_F} - 1\right)$$

where AL/A_s is the ratio of areas of mobile and immobile phases, the experimental values of K and of R_F for glucose were substituted to compute the area ratio and then values for K were calculated for other sugars from Partridge's R_F data with results shown in Table I.

TABLE I

PARTITION COEFFICIENTS OF SUGARS

Sugar	$K_{\text{expt.}}$[a]	R_F[b]	$K_{\text{calcd.}}$[a,c]
Xylose	17.1	0.125	16.0
Arabinose	21.5	.100	20.6
Mannose	21.6	.100	20.6
Glucose	30.5	.070	Ref.
Galactose	36.2	.060	36.0

[a] K = grams sugar per liter of water phase/grams sugar per liter of butanol phase. [b] R_F values reported by Partridge and Westfall.[12] [c] Computed from R_F values shown referred to glucose.

From Mayer and Tompkins' treatment of the chromatographic process,[11] it may be shown that the ratios of partition coefficients for solutes should be equal to the ratios of respective effluent volumes to the maximum concentration of the effluent. Such ratios referred to galactose are shown in Table II for xylose, mannose and glucose. These were calculated from the experimental partition coefficients and also from the experimental volumes of column effluents to elution peaks for both known sugar mixtures and for sulfite waste liquor sugars. The close agreement among these ratios together with chemical characterization of xylose as the cadmium brom xylonate,[14] confirms the identity of the individual sugars eluted from the known mixture. The recoveries of the sugars from known mixtures in duplicate experiments are shown in Table III.

TABLE II

RATIOS BETWEEN PARTITION COEFFICIENTS AND BETWEEN ELUANT VOLUMES AT MAXIMUM SOLUTE CONCENTRATIONS

Sugar	($K_{\text{sugar}}/K_{\text{galactose}}$)	(Eluant volume $_{\text{sugar}}$/ eluant volume $_{\text{galactose}}$) Known mixture	SWL sugars
Xylose	0.61	0.61	0.59
Mannose	.73	.73	.71[a]
Glucose	.84	.84	.85
Galactose	1.00	1.00	1.00

[a] Mannose in eluant is here accompanied by arabinose and possibly fructose.

TABLE III

RECOVERY OF SUGARS FROM KNOWN MIXTURES

Sugar	Amount added per column, mg.	Amount recovered, mg. Column A	Column B	Recovered, % Column A	Column B
Xylose	18.59	20.12	21.68	108	116[a]
Mannose	19.27	20.80	20.48	108	104
Glucose	18.51	19.04	19.32	103	104
Galactose	15.70	14.00	14.56	89	93
Other[b]	...	0.47	0.18	0.6	0.2
Total	72.07	74.43	76.22	103	106

[a] High recovery is probably due to summation of small errors in sugar analyses. [b] Recovered in wash water used finally to purge the column.

For the sulfite waste liquor sugars, xylose, glucose and galactose were identified as indicated above. However, the partition coefficients of mannose, arabinose and fructose are nearly identical so that these substances were eluted together. Thus the amounts of arabinose accompanying mannose were determined in appropriate eluant fractions

TABLE IV

COMPOSITION OF SULFITE WASTE LIQUOR SUGARS

Sugar	85% Western Hemlock + 15% White Fir Wood,[a] %	Spruce,[b] %
Xylose	15	17.0
Arabinose	6	
Mannose	48	42.7
Fructose	<2	4.0
Glucose	15	28.9
Galactose	10	4.2
Galacturonic acid	[c]	3.2
Unidentified	4	...
Total	100	100

[a] Average of two determinations of present study. [b] Undesignated species.[6] [c] Not investigated in present work.

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(11) Mayer and Tompkins, THIS JOURNAL, 69, 2866 (1947).

(12) S. M. Partridge and R. G. Westfall, *Biochem. J.*, 42, 238 (1948).

(13) R. Consden, A. H. Gordon and Martin, *ibid.*, 38, 224 (1944).

(14) A. W. Van der Haar, "Anleitung zum Nachweis zur trennung und Bestimmung der Monosaccharide und Aldehydsäuren," Borntraeger, Berlin, 1920, p. 59.

by a new empirically-standardized micro procedure[15] involving determination of ultraviolet absorption of dilute acidified sugar solution heated for fixed periods of time. Since the rate of furfural formation from arabinose is much greater than that of hydroxymethylfurfural from mannose, a high degree of sensitivity is achieved. The presence of fructose accompanying mannose was indicated by polarographic examination,[16] but the sensitivity of the procedure permitted only the definition of an upper limit for the amount of fructose possibly present.

In Table IV are shown the proportions of the several sugars found present in the sulfite waste liquor sample derived from about 85% Western Hemlock (*Tsuga heterophylla*) and 15% White Fir (*Abies grandis*) woods.[7] This sulfite waste liquor contains relatively less glucose and more galactose than a sulfite waste liquor from a spruce which was examined by Hägglund[6] with results also included in Table IV. Total reducing value recoveries in present chromatographic experiments were 95 and 101%.

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[15] Q. P. Peniston and H. D. Agar, unpublished work.

[16] J. Heyrovsky and I. Smöler, *Collection Czechoslov. Chem. Commun.*, **4**, 521 (1932).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF SOUTHERN CALIFORNIA]

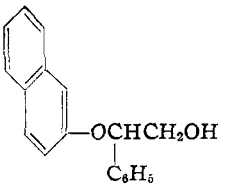
The Acid-Catalyzed Reaction of Styrene Oxide with Phenol and with 2-Naphthol

BY CYRUS O. GUSS,[1] HOMER R. WILLIAMS[2a] AND LEONARD H. JULES[2b]

The acid-catalyzed reaction of styrene oxide with phenol formed, in addition to ether-alcohols, two types of products: those expected from the isomerization of the oxide to phenylacetaldehyde, which then reacted with phenol; and those that are postulated to result from a unimolecular ring opening accompanied by nuclear attack on the phenol to give isomeric phenol-alcohols. With 2-naphthol a similar course was indicated by the products isolated; the phenol-alcohol initially formed here, however, cyclized to a dihydrofuran so that the latter was found among the reaction products.

Ether-alcohols are the expected products from the reaction of olefin oxides with hydroxylic compounds. The observation[3] that a mixture of alkali-soluble materials, not identified, resulted from the acid-catalyzed reaction of styrene oxide with phenol suggested the present investigation.

In this study of the acid-catalyzed reaction of styrene oxide with phenols, 2-naphthol was utilized initially in order to reduce the formation of isomers, which would complicate the isolation of pure products. Unexpectedly, however, the products isolated from the reaction of styrene oxide with excess 2-naphthol at 140–145°, in the presence of *p*-toluenesulfonic acid, were alkali-insoluble. This result was in apparent contradiction to the phenol–styrene oxide reaction mentioned. That the formation of ether-alcohols was not an explanation was shown when 2-(2-naphthoxy)-2-phenylethanol (I), m. p. 107–108°, and 2-(2-naphthoxy)-1-phenylethanol (II), m. p. 87–88°, were prepared[4] and found to be unlike any of the alkali-insoluble products at hand from the acid-catalyzed experiment. Neither were I nor II converted into any of the products isolated from the acid-catalyzed run under the same experimental conditions.



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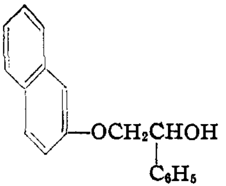
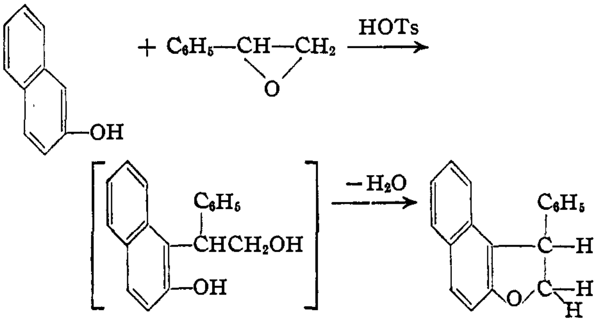


II

One of the alkali-insoluble compounds, m.p. 95–96°, isolated from the acid-catalyzed interaction of styrene oxide with 2-naphthol in 43% yield, gave analytical values for carbon and hydrogen that corresponded to either I or II less a molecule of water. It was presumed, therefore, that nuclear attack had occurred, followed by intramolecular loss of water to form a furan. The preparation[5] of III and its cyclization[5] by acid catalysis to 1-phenyl-1,2-dihydronaphtho(2,1-b)furan (IV), m.p. 95–96°, identical with that obtained from styrene oxide and 2-naphthol, was considered to validate this view. Later it was shown[4] that the base-catalyzed reaction of styrene oxide with 2-naphthol could be made to give III as the major product as well as some of I.



III IV

Another alkali-insoluble product, m.p. 81–82°, obtained in small amount once from the acid-catalyzed reaction had the correct analysis for an isomer of IV. Its structure was not elucidated.

A third component, m.p. 178–178.5°, was isolated from the alkali-insoluble fraction in 21.5% yield. This compound had the correct analysis for

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[1] Department of Chemistry, Colorado A. and M. College, Fort Collins, Colorado.

[2] (a) The experiments with phenol are from a portion of the M.S. thesis (August, 1950) of Homer R. Williams, Department of Chemistry, Stanford University, Palo Alto, California. (b) The work with 2-naphthol is based on a portion of the M.S. thesis (August, 1949) of Leonard H. Jules, Sahyun Laboratories, 316 Castillo St., Santa Barbara, California.

[3] C. O. Guss, THIS JOURNAL, **71**, 3460 (1949).

[4] C. O. Guss and L. H. Jules, *ibid.*, **72**, 3878 (1950).

[5] C. O. Guss and L. H. Jules, *ibid.*, **72**, 3462 (1950).