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## Heats of Adsorption of Small Molecules on Various Forms of Lactose, Sucrose, and Glucose

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The heats of adsorption of a variety of organic compounds were determined by the gas chromatographic method on regular, anhydrous  $\alpha$ -lactose (*r*-lactose),  $\beta$ -lactose, anhydrous  $\alpha$ -D-glucose, and sucrose and compared with heats of adsorption previously determined for stable, anhydrous  $\alpha$ -lactose (*s*-lactose). *r*-Lactose showed greater heats of adsorption than *s*-lactose for all compounds studied except for formate esters. All compounds studied except the hydrocarbons showed larger heats of adsorption on  $\beta$ -lactose than on *s*-lactose. Heats of adsorption on  $\beta$ -lactose were generally greater than those on *r*-lactose. Sucrose showed much lower heats of adsorption than the three lactoses. Anhydrous  $\alpha$ -D-glucose generally showed greater heats of adsorption than *s*-lactose and were of the same order as those for *r*- and  $\beta$ -lactose. The bonding mechanisms appear to be similar to those proposed for *s*-lactose. Gibbs energies and entropies were calculated for all lactose reactions studied.

Lactose has been cited in the literature as possessing unique adsorptive properties which might enable it to be used as a flavor carrier in foods (Arbuckle, 1970; Nickerson and Dolby, 1971; Nickerson, 1965). The adsorptive properties of stable, anhydrous  $\alpha$ -lactose (*s*-lactose) have been extensively studied by McMullin et al. (1975). One may ask, do other sugars and other crystalline forms of lactose adsorb odors and flavors as does *s*-lactose? In order to examine this further, comparisons were made of the adsorptive behavior of  $\alpha$ -lactose hydrate (which, under the conditions employed, dehydrated to regular, anhydrous  $\alpha$ -lactose),  $\beta$ -lactose, sucrose, and anhydrous  $\alpha$ -D-glucose with that of *s*-lactose.

### EXPERIMENTAL SECTION

The sucrose was obtained commercially (California and Hawaiian Sugar Company, San Francisco, CA). The anhydrous  $\alpha$ -D-glucose was reagent grade (Mallinckrodt), and the  $\alpha$ -lactose hydrate was U.S.P. grade (Foremost-McKesson Company). All were size graded to 100/120 mesh before use. The  $\beta$ -lactose (98%, Matheson, Coleman, and Bell) was recrystallized by dissolving in cold (1 °C) distilled water and quickly adding absolute methanol until

Table I. Experimental Chromatographic Conditions for the Hewlett-Packard 5711A Gas Chromatograph and the Four Columns Used

column temperature, °C	varied, 100-120
injector temperature, °C	100
detector temperature, °C	150
detector	FID
gas flow rates, mL/min	
nitrogen	25
hydrogen	25
air	250
stainless steel columns	
length, cm	152
inner diameter, mm	2.36
g of sugar/column	
sucrose	6.8
anhydrous $\alpha$ -D-glucose	6.3
$\alpha$ -lactose hydrate	6.4
$\beta$ -lactose	3.4
aged	
time, h	12
temperature, °C	70
1 mV potentiometric recorder	
chart speed, cm/h	25

a 50:50 water/methanol mixture was reached. The  $\beta$ -lactose then began to crystallize out. The crystalline  $\beta$ -lactose was filtered off using a vacuum filter system and dried in a vacuum oven at 70 °C (127 torr). The  $\beta$ -lactose was then sieved 100/120 mesh before use.

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Stainless steel tubing, with the specifications given in Table I, was washed successively with detergent, distilled water, methanol, acetone, benzene, and pentane to remove dirt and grease, and was air-dried at room temperature. The straight lengths of tubing were each packed with one of the four sugars to be studied: sucrose, anhydrous  $\alpha$ -D-glucose,  $\alpha$ -lactose hydrate, and  $\beta$ -lactose at atmospheric pressure and room temperature. The columns were vibrated continuously during the filling procedure to achieve uniform packing. Column ends were plugged with glass wool and the columns coiled for installation in the chromatograph. Columns were flushed with dry nitrogen and aged as indicated in Table I.

Sucrose and anhydrous  $\alpha$ -D-glucose have melting points of 185 and 146 °C, respectively, which are much closer to the experimental temperatures used than are the melting points of  $\beta$ -lactose and  $\alpha$ -lactose hydrate, 252 and 202 °C, respectively. The melting points of the sucrose and anhydrous  $\alpha$ -D-glucose samples used were determined experimentally in our laboratory to learn if any decomposition or discoloration would occur at the column temperatures used. No discoloration or decomposition of the sugar samples occurred at the temperatures used experimentally. Additionally, the sucrose and anhydrous  $\alpha$ -D-glucose columns were first heated at 120 °C for two 30-min intervals prior to aging to observe whether any melting would occur during the experiment. No melting was observed during the two 30-min intervals.

Chromatographic conditions are shown in Table I.

Adsorptions on each sugar were examined at five column temperatures: 100, 105, 110, 115, and 120 °C. The column temperatures were selected by the instrument control, which could be adjusted to each degree centigrade. To obtain a more exact measurement of the column temperature, a copper-constantan thermocouple was inserted into the oven and the emf was read from a Leeds and Northrup Model No. 8662 portable potentiometer. The thermocouple was calibrated by setting emf to 0.000 mV at 0 °C (ice and water in equilibrium). Readings of the emf were taken at the center of the oven on two occasions separated by 2 months time. These corrected oven temperatures were used for all subsequent calculations.

The compounds examined on each sugar were reagent grade chemicals obtained commercially. Samples for injection were obtained from the headspace of laboratory chemical bottles. To obtain saturated vapor, a bottle containing a sample was shaken for several seconds. A 10- $\mu$ L syringe was flushed several times with the saturated headspace vapors and then 2  $\mu$ L of the headspace were taken for injection into the gas chromatograph. On-column injections were made to minimize feed volume and reduce peak broadening. Timing was started with a stopwatch at the moment of injection, and the timing was completed when the peak maximum emerged. At a given temperature three injections were made for each compound, and the mean of the recorded retention times was calculated. Retention times were found to be reproducible to within 0.2%.

The limits of error for heat of adsorption determinations on *s*-lactose, *r*-lactose, and  $\beta$ -lactose were found to be  $\pm 1.5$  kcal/mol and thus in keeping with those reported previously by McMullin et al. (1975).

## RESULTS AND DISCUSSION

### $\alpha$ -Lactose Hydrate/Regular, Anhydrous $\alpha$ -Lactose.

It should be noted that  $\alpha$ -lactose hydrate is easily dehydrated to one of the anhydrous forms of  $\alpha$ -lactose, but there is disagreement in the literature as to the temperature at which it is safe to work with  $\alpha$ -lactose hydrate

without losing its molecule of water (Nickerson, 1965). Vapor pressure measurements have indicated that the water may be lost at 85 °C (14.0 torr) and even at 80 °C (10.4 torr) (Nickerson, 1965). After several days at 100 °C, the water of crystallization is almost entirely lost from the powder, and at 130 °C the water of hydration is quickly lost. The experimental temperatures of 100 to 120 °C were clearly within the appropriate temperature range where conversion to the anhydrous form would be suspected. At the conclusion of these studies, examination of the sugar within the column revealed that it had solidified and yellowed slightly. The following two experiments were performed to determine whether the effective adsorbent was the  $\alpha$ -lactose hydrate or one of the anhydrous forms of  $\alpha$ -lactose.

A new stainless steel column was packed as previously described (Experimental Section) with a fresh sample of  $\alpha$ -lactose hydrate and weighed to determine the amount of sugar in the column. The column was installed in the gas chromatograph and heated for 16 h at 120 °C, with a constant stream of dry nitrogen passing through the column. After this period of heating, the column showed a weight loss of 5.2%, indicating that the water of crystallization was lost from the  $\alpha$ -lactose hydrate during the gas chromatographic procedure.

In the second experiment, duplicate samples of the  $\alpha$ -lactose hydrate unpacked from the original  $\alpha$ -lactose hydrate column were weighed; and the samples placed in a vacuum oven at 120 °C and 150 torr and held for 16 h. The samples were removed, cooled in a desiccator for 30 min and reweighed. The samples had lost 5% of their weight.

The first experiment indicated that one of the anhydrous forms of  $\alpha$ -lactose was the effective adsorbent during the gas chromatographic procedure but did not establish which one. In the second experiment, the anhydrous form must have rapidly rehydrated to  $\alpha$ -lactose hydrate during the time required for unpacking. These two experiments indicated that the effective adsorbent was regular, anhydrous  $\alpha$ -lactose (*r*-lactose). This form is produced by heat in vacuo from  $\alpha$ -lactose hydrate and is hygroscopic. *s*-Lactose can also be produced from  $\alpha$ -lactose hydrate by heat, but this form is not appreciably hygroscopic and must be dissolved in water before reforming the  $\alpha$ -lactose hydrate. Using chromatographic procedures to determine heats of adsorption, the temperatures used experimentally would have to be lowered substantially, perhaps to below 80 °C, for  $\alpha$ -lactose hydrate to be examined. However, in order to obtain comparative adsorption data, the same temperatures must be used for all sugars studied. This is due to changes in column pressure with temperature change and to lowered heat capacity resulting in lowered heat of adsorption with decreased temperature.

The heats of adsorption for *r*-lactose are compared to those for *s*-lactose in Table II. The heats of adsorption were significantly greater on *r*-lactose—that is, the difference exceeded the  $\pm 1.5$  kcal/mol maximum experimental error determined for both sugars—for the alcohols, methyl esters, ketones, aldehydes, and hydrocarbons. The heats of adsorption for the formates on *r*-lactose did not differ significantly from those determined on *s*-lactose.

A plot of the heats of adsorption calculated for *r*-lactose vs. the number of carbon atoms in the adsorbates shows that there is a linear relationship between heats of adsorption and chain length within each homologous series studied. The alcohols adsorbed with the highest heats of adsorption while the hydrocarbons adsorbed with the lowest heats of adsorption for a given number of carbon

Table II. Heats of Adsorption (kcal/mol) on Stable, Anhydrous  $\alpha$ -Lactose (McMullin et al., 1975) (*s*), Regular, Anhydrous  $\alpha$ -Lactose (*r*),  $\beta$ -Lactose ( $\beta$ ), Sucrose (Suc), and Anhydrous  $\alpha$ -D-Glucose (Gluc) Determined by the Gas Chromatographic Method

compd	<i>s</i>	<i>r</i>	$\beta$	Suc	Gluc
methanol	10.6	9.8	16.9		
ethanol	11.6	14.0	18.1		
1-propanol	13.4	15.6	18.4		
1-butanol	14.2	16.7	18.0		17.6
1-pentanol	15.7	18.1	19.2		
1-hexanol	17.1	19.3	20.3		
1-heptanol				14.0	23.4
methyl acetate	8.2	12.4	13.5		
methyl propionate	9.4	12.3	12.2		
methyl butyrate	10.9	14.1	14.7		
methyl pentanoate	12.0	14.9	15.7		
methyl hexanoate	13.6	15.7	16.7	5.8	17.7
propanal	7.2	10.7	11.9		
butanal	8.8	12.2	13.1		
pentanal	10.5	13.1	13.4		
hexanal	11.6	13.8	14.8		
heptanal	12.5	15.1	16.1	7.3	16.5
2-propanone	8.0	12.0	14.2		
2-butanone	9.4	13.0	14.9		
2-pentanone	10.8	14.1	15.7		14.1
2-hexanone	12.0	15.0	16.8		
2-heptanone	13.1	16.1	17.7	5.5	17.4
methyl formate	6.3	7.5	8.2		
ethyl formate	8.3	9.4	11.5		
propyl formate	9.2	9.9	13.9		
butyl formate	10.5	10.7	14.5		
pentyl formate	11.6	11.4	15.6		
hexyl formate	13.1	13.3	16.7	8.4	16.9
heptane	6.4	10.7	6.9		
octane	8.1	11.9	7.6		
nonane	9.2	12.9	9.2		
decane	10.6	13.9	10.2	9.7	14.7
undecane	11.8	15.0	11.6		
dodecane	13.0	16.2	12.9	11.0	17.1
tridecane	14.5	17.3	13.8		

atoms. The methyl esters, ketones, aldehydes, and formates adsorbed with intermediate heats of adsorption for an equal number of carbon atoms. This same pattern of adsorption behavior was found for *s*-lactose by McMullin et al. (1975), who indicated that there was no significant difference between the heats of adsorption of the methyl esters, ketones, aldehydes, and formates for an equal number of carbon atoms on *s*-lactose. The formate heats of adsorption differed significantly on *r*-lactose from the other compounds giving intermediate heats of adsorption.

Gibbs energies were found to increase with increasing temperature for the compounds studied on *r*-lactose. This was found to be true for *s*-lactose and indicates that higher temperatures are less favorable to adsorption. This behavior is characteristic of physical adsorption while the opposite behavior may be observed in chemical adsorption (Moore, 1962). Gibbs energy determinations at 110 °C for *r*-lactose are shown in Table III.

Entropy calculations depend largely on the heats of adsorption and to a lesser extent on the Gibbs energies. The calculated entropies are probably not accurate enough to explain the small differences in entropy between compounds, but an overall pattern was discerned. The entropy was found to be negative for all the compounds examined, indicating that randomness and freedom of motion were decreased on adsorption. The alcohols as a group had more negative entropies for a given number of carbon atoms (-28 to -46 cal/K mol) than the other groups of compounds studied. The entropy became more negative with increasing chain length within each homologous series. That the entropy is not a function of temperature can be seen from Table IV. (A single value for the heat of ad-

Table III. Retention Data and Thermodynamic Quantities Determined for Regular, Anhydrous  $\alpha$ -Lactose at 110 °C<sup>a</sup>

compound	$t_{\text{corr}}$ , s	$V_s$ , mL/m <sup>2</sup>	$-\Delta G$ , cal/mol	$-\Delta H$ , kcal/mol	$-\Delta S$ , cal/K mol
methanol	9.0	0.28	-969	9.8	28.1
ethanol	10.0	0.31	-891	14.0	38.9
1-propanol	21.5	0.66	-317	15.6	41.6
1-butanol	50.5	1.54	329	16.7	42.8
1-pentanol	112.8	3.44	941	18.1	44.8
1-hexanol	243.9	7.44	1530	19.3	46.4
methyl acetate	5.0	0.15	-1440	12.4	36.2
methyl propionate	9.8	0.30	-916	12.3	34.5
methyl butyrate	20.6	0.63	-352	14.1	37.7
methyl pentanoate	44.4	1.36	234	14.9	38.3
methyl hexanoate	94.3	2.88	805	15.7	38.9
propanal	3.3	0.10	-1750	10.7	32.5
butanal	7.4	0.23	-1120	12.2	34.8
pentanal	16.9	0.52	-498	13.1	35.5
hexanal	37.7	1.15	107	13.8	35.8
heptanal	77.7	2.37	657	15.1	37.7
2-propanone	5.6	0.17	-1350	12.0	34.9
2-butanone	10.6	0.32	-867	13.0	36.2
2-pentanone	22.8	0.70	-272	14.1	37.5
2-hexanone	48.6	1.48	298	15.0	38.4
2-heptanone	105.5	3.22	890	16.1	39.7
methyl formate	2.0	0.06	-2140	7.5	25.2
ethyl formate	4.4	0.14	-1500	9.4	28.5
propyl formate	9.7	0.30	-916	9.9	28.2
butyl formate	21.1	0.64	-339	10.7	28.8
pentyl formate	45.1	1.38	245	11.4	29.1
hexyl formate	93.7	2.86	800	13.3	32.6
heptane	6.1	0.19	-1260	10.7	31.2
octane	13.0	0.40	-697	11.9	32.9
nonane	27.3	0.83	-142	12.9	34.1
decane	56.9	1.74	422	13.9	35.2
undecane	118.5	3.62	979	15.0	36.6
dodecane	247.3	7.55	1540	16.2	38.3

<sup>a</sup>  $t_{\text{corr}}$  = corrected net retention time,  $V_s$  = specific retention volume,  $-\Delta G$  = Gibbs energy of adsorption,  $-\Delta H$  = heat of adsorption,  $-\Delta S$  = entropy of adsorption.

Table IV. Entropy Values for Regular, Anhydrous  $\alpha$ -Lactose Indicating Temperature Independence<sup>a</sup>

compd	temp, °C	$-\Delta S$ , cal/K mol
1-pentanol	100	44.8
	105	44.8
	110	44.8
	115	44.8
	120	44.8
methyl butyrate	100	37.8
	105	37.8
	110	37.7
	115	37.7
	120	37.8
pentanal	100	35.5
	105	35.5
	110	35.5
	115	35.5
	120	35.5
2-pentanone	100	37.6
	105	37.6
	110	37.5
	115	37.6
	120	37.6
butyl formate	100	29.0
	105	28.7
	110	28.8
	115	28.8
	120	29.1

<sup>a</sup>  $-\Delta S$  = entropy of adsorption.

sorption is utilized in the entropy calculations so it would be more valid to explain that the entropy calculations are

Table V. Heats of Adsorption for Five-Carbon Compounds on Regular, Anhydrous  $\alpha$ -Lactose and  $\beta$ -Lactose

compd	heat of adsorption, kcal/mol
regular, anhydrous $\alpha$ -lactose	
1-pentanol	18.1
methyl <i>n</i> -butyrate	14.1
pentanal	13.1
2-pentanone	14.1
butyl formate	10.7
pentane (calcd <sup>a</sup> )	8.6
$\beta$ -lactose	
1-pentanol	19.2
methyl butyrate	14.7
pentanal	13.4
2-pentanone	15.7
butyl formate	14.5
pentane (calcd <sup>a</sup> )	4.6

<sup>a</sup> Calculated from equations in Table VII.

independent of temperature, assuming that the heats of adsorption are independent of temperature.) This same entropy pattern was found for *s*-lactose (McMullin et al., 1975). It was concluded for *s*-lactose that alcohols lose more rotational and/or vibrational freedom of motion than the other compounds studied. Loss of translational motion will result in a decrease in entropy which is proportional to the logarithm of the weight of the molecule. These entropy findings were consistent with the proposal that alcohols were held on *s*-lactose by the formation of two hydrogen bonds. This type of bonding is more rigid than that with one hydrogen bond or van der Waals attractive forces—bonding postulated for the other compounds examined on *s*-lactose. Entropy calculations at 110 °C for *r*-lactose are shown in Table III.

The compounds adsorbed on *s*-lactose were described as members of three classes (McMullin et al., 1975). The first class, the hydrocarbons, were thought to be attracted to the surface by nonspecific van der Waals forces, but to retain considerable vibrational and rotational freedom and may even be mobile on the lactose surface. A second class comprised the esters, aldehydes, and ketones which would interact with the lactose surface by van der Waals forces plus alignment of dipoles and possible small specific interactions plus a hydrogen bond between the oxygen on the adsorbate and the lactose hydroxyl group proton. Lactose has eight hydroxyl groups for possible hydrogen bonding with negatively charged adsorbate centers, such as the oxygen in the functional groups of the esters, aldehydes, ketones, and alcohols. The alcohols formed a third group and were proposed to be attracted to the lactose surface by the above interactions plus a second hydrogen bond between the alcoholic proton and the lone electron pairs of the numerous oxygen atoms which would further reduce rotation and vibration. It is possible that these two hydrogen bonds form with one lactose molecule or between two lactose molecules. These postulated bonding mechanisms were derived from the differences in the heats of adsorption between the compounds examined (Table V), since the sum of the nonspecific and specific interactions determine the strength of adsorption and, therefore, the heat of adsorption. The esters, aldehydes, and ketones had heats of adsorption of about 6 kcal/mol higher than those for hydrocarbons of an equal number of carbon atoms. Hydrogen bonds have energies of formation of about 3–10 kcal/mol—generally 5 kcal/mol (Brescia et al., 1966). The alcohols had heats of adsorption of about 5 kcal/mol higher than those determined for the

Table VI. Particle Sizes and Surface Areas of the Sugars Examined by the Gas Chromatographic Method (The Surface Area of  $\alpha$ -Lactose Hydrate Is Included for Comparison with Stable, Anhydrous  $\alpha$ -Lactose and Regular, Anhydrous  $\alpha$ -Lactose)

sugar	particle size, mm	surface area, m <sup>2</sup> /g
$\alpha$ -lactose hydrate	0.125–0.149	0.33 (Berlin et al., 1964)
		0.18 (Berlin et al., 1972)
sucrose	0.074–0.125	0.36 (Berlin et al., 1972)
anhydrous $\alpha$ -D-glucose	0.074–0.125	0.78 (Berlin et al., 1972)
$\beta$ -lactose	0.125–0.149	0.83 (Berlin et al., 1964)
		0.64 (Berlin et al., 1972)
stable, anhydrous $\alpha$ -lactose	≤0.149	1.26 (Berlin 1973)
		2.04 (Berlin et al., 1972)
		2.19 (Berlin et al., 1972)
		2.88 (McMullin et al., 1975)
	mean	2.09
regular, anhydrous $\alpha$ -lactose	0.125–0.149	2.04 (Berlin et al., 1972)
		2.19 (Berlin et al., 1972)
	mean	2.12

esters, aldehydes, and ketones—suggesting formation of a second hydrogen bond. The difference between the 6 kcal/mol for the first hydrogen bond and the 5 kcal/mol for the second hydrogen bond was explained by other interactions, such as alignment of permanent or induced dipoles which contribute about 1 kcal/mol. Simultaneous formation of two hydrogen bonds between an alcohol and lactose might produce ring strain, reducing the energy of bond formation.

Study of heats of adsorption on *r*-lactose in Table II shows that the heats of adsorption of the methyl esters, aldehydes, and ketones were about 5 kcal/mol above the heats of adsorption of the hydrocarbons. The heats of adsorption of the alcohols were 4–5 kcal/mol higher than the heats of adsorption of the methyl esters, aldehydes, and ketones of an equal number of carbon atoms. It would appear from the heat of adsorption and entropy determinations that the bonding mechanisms on *r*-lactose are the same as those proposed by McMullin et al. (1975) for *s*-lactose.

This similarity in bonding mechanisms might lead one to conclude that there should be no difference in adsorption on *s*-lactose and *r*-lactose. However, *r*-lactose shows greater adsorption based on heat of adsorption values when compared with *s*-lactose. The two sugars were sieved to comparable particle sizes before being examined experimentally (Table VI). Although the mean surface area for *r*-lactose appears greater than mean surface area value for *s*-lactose, as shown in Table VI, their surface areas are essentially identical. Both the anhydrous forms of  $\alpha$ -lactose have a greater surface area than does  $\alpha$ -lactose hydrate (Table VI). The difference in adsorption ability between the two anhydrous forms is probably due to the manner in which they are prepared from  $\alpha$ -lactose hydrate. The increased adsorptive properties and expanded surface area of *r*-lactose as compared to  $\alpha$ -lactose hydrate have been proposed by Berlin et al. (1972) to be due to increased porosity. Berlin et al. (1972) have concluded from density data that desiccation of  $\alpha$ -lactose hydrate forming *r*-lactose leads to a porous form obtained by loss of water from the crystal lattice without complete rearrangement to form a stable crystal. Berlin's explanation for the increased porosity is the development of large cracks in the crystalline particles due to escaping water vapor during

Table VII. Equations for Predicting Heats of Adsorption for Straight Chain Molecules on Stable, Anhydrous  $\alpha$ -Lactose (McMullin et al., 1975), Regular, Anhydrous  $\alpha$ -Lactose, and  $\beta$ -Lactose<sup>a</sup>

stable, anhydrous $\alpha$ -lactose	
alcohols	$-\Delta H = 1.32n + 9.21$ kcal/mol
esters, aldehydes, ketones	$-\Delta H = 1.31n + 3.84$ kcal/mol
hydrocarbons	$-\Delta H = 1.30n - 2.54$ kcal/mol
regular, anhydrous $\alpha$ -lactose	
alcohols	$-\Delta H = 1.33n + 11.4$ kcal/mol
methyl esters, aldehydes, ketones	$-\Delta H = 0.97n + 8.8$ kcal/mol
formates	$-\Delta H = 1.20n + 5.2$ kcal/mol
hydrocarbons	$-\Delta H = 1.07n + 3.2$ kcal/mol
$\beta$ -lactose	
alcohols	$-\Delta H = 0.65n + 16.3$ kcal/mol
esters, aldehydes, ketones	$-\Delta H = 0.95n + 10.2$ kcal/mol
hydrocarbons	$-\Delta H = 1.25n - 1.0$ kcal/mol

<sup>a</sup>  $-\Delta H$  = heat of adsorption,  $n$  = number of carbon atoms.

dehydration. Rehydration results in only partial loss in surface area. It is believed that these wide pores, which might form in the  $r$ -lactose particles, could be of appropriate dimensions to be significant in the adsorption of volatiles (Berlin et al., 1972).  $s$ -Lactose is commonly formed from  $\alpha$ -lactose hydrate from alcohol solvents, and dehydration results in a stable rearrangement of the crystal structure. The result is an expanded surface area, but with no increase in porosity due to fissuring. Thus, the increased adsorption ability of  $r$ -lactose compared with  $s$ -lactose may be due to the trapping and adsorption of the adsorbate within these fissures. Since all of the compounds studied on  $r$ -lactose showed increased adsorption, except for the case of the formates where there was no significant increase, the effect of the  $r$ -lactose, increased adsorptive ability, may be an increase in nonspecific adsorption.

Nickerson and Dolby (1971) found that  $r$ -lactose adsorbed larger amounts of diacetyl than any of the other sugars they examined. It is important to note that although one may intuitively correlate greater heats of adsorption with increased amounts of adsorbate adsorbed, this concept remains to be experimentally verified.

McMullin et al. (1975) developed a method for predicting the heats of adsorption on  $s$ -lactose. This method was based on the observed linear relationship between the heat of adsorption and chain length within an homologous series. McMullin et al. (1975) used the following equation of a straight line,  $-\Delta H = nx + y$ , for predicting the heats of adsorption for any homologous series on  $s$ -lactose where  $x$  is the slope and  $y$  is the intercept of a plot of the heat of adsorption vs. carbon number for a homologous series. The carbon number is equal to  $n$ . The heat of adsorption would consist of  $x$  kcal/mol contributed from the hydrocarbon portion and  $y$  kcal/mol contributed by the functional group of the adsorbate. The equations for predicting the heats of adsorption on  $s$ -lactose are shown in Table VII. This same method was used to derive equations for predicting the heats of adsorption on  $r$ -lactose (Table VII).

**$\beta$ -Lactose.** Adsorption on  $\beta$ -lactose was investigated using the same groups of compounds. Data were obtained and treated as for  $r$ -lactose and  $s$ -lactose. The  $\beta$ -lactose

Table VIII. Retention Data and Thermodynamic Quantities Determined for  $\beta$ -Lactose at 110 °C<sup>a</sup>

compd	$t_{\text{corr}}$ , s	$V_s$ , mL/m <sup>2</sup>	$-\Delta G$ , cal/mol	$-\Delta H$ , kcal/mol	$-\Delta S$ , cal/K mol
methanol	5.5	0.91	-72	16.9	44.3
ethanol	12.0	1.99	524	18.1	45.9
1-propanol	22.9	3.80	1020	18.4	45.4
1-butanol	47.3	7.85	1570	18.0	42.9
1-pentanol	101.1	16.78	2150	19.2	44.5
1-hexanol	219.5	36.44	2740	20.3	45.9
methyl acetate	3.8	0.63	-352	13.5	36.2
methyl propionate	6.9	1.14	100	12.2	31.6
methyl butyrate	14.3	2.38	660	14.7	36.7
methyl pentanoate	30.3	5.03	1230	15.7	37.8
methyl hexanoate	63.3	10.51	1790	16.7	38.9
propanal	2.4	0.40	-697	11.9	32.9
butanal	5.1	0.85	-124	13.1	34.5
pentanal	11.1	1.84	464	13.4	33.8
hexanal	23.7	3.94	1043	14.8	35.9
heptanal	49.2	8.17	1600	16.1	37.9
2-propanone	5.2	0.86	-115	14.2	37.4
2-butanone	8.9	1.48	298	14.9	38.1
2-pentanone	18.4	3.06	851	15.7	38.8
2-hexanone	38.2	6.34	1410	16.8	40.2
2-heptanone	82.9	13.76	2000	17.7	41.0
methyl formate	1.2	0.20	-1220	8.2	24.6
ethyl formate	3.0	0.50	-527	11.5	31.4
propyl formate	6.2	1.03	23	13.9	36.2
butyl formate	13.1	2.18	593	14.5	36.3
pentyl formate	27.2	4.52	1150	15.6	37.7
hexyl formate	55.9	9.28	1880	16.7	39.2
heptane	1.9	0.32	-867	6.9	20.3
octane	3.8	0.63	-352	7.6	20.8
nonane	7.9	1.31	206	9.2	23.5
decane	16.2	2.69	753	10.2	24.7
undecane	33.2	5.51	1300	11.6	26.9
dodecane	68.3	11.34	1850	12.9	28.9
tridecane	138.9	23.06	2390	13.8	29.8

<sup>a</sup>  $t_{\text{corr}}$  = corrected net retention time,  $V_s$  = specific retention volume,  $-\Delta G$  = Gibbs energy of adsorption,  $-\Delta H$  = heat of adsorption,  $-\Delta S$  = entropy of adsorption.

packed within the column can be considered stable since the only change possible during the gas chromatographic procedure would be one of heat-induced decomposition. Visual examination of the sugar within the  $\beta$ -lactose column at the conclusion of the experiment showed that it had remained free flowing without discoloration.

The heats of adsorption on  $\beta$ -lactose are compared to those determined on  $s$ -lactose and  $r$ -lactose in Table II.

All of the compounds studied, except the hydrocarbons, showed higher heats of adsorption on  $\beta$ -lactose than on  $s$ -lactose. The heats of adsorption calculated for the hydrocarbons on both sugars were not significantly different based on the  $\pm 1.5$  kcal/mol maximum error for the heat of adsorption calculations. The heats of adsorption for  $\beta$ -lactose were in general greater than those found on  $r$ -lactose, although the difference was not always significant.

A plot of the heats of adsorption on  $\beta$ -lactose vs. carbon number shows that  $\beta$ -lactose exhibits the same pattern of adsorption behavior as was found for  $s$ -lactose and  $r$ -lactose, with the exception that the formates renewed their position relative to the aldehydes (Table II).

The Gibbs energy calculations for  $\beta$ -lactose follow the same pattern observed for  $s$ -lactose and  $r$ -lactose. The entropy values for  $\beta$ -lactose are consistent with those calculated for  $s$ -lactose and  $r$ -lactose (Table VIII).

The bonding mechanisms for volatiles on  $\beta$ -lactose are the same or similar to those proposed for both  $s$ -lactose and  $r$ -lactose based on the entropy calculations and differences between the heats of adsorption. The alcohol

heats of adsorption were 4–5 kcal/mol higher than those determined for the esters, aldehydes, and ketones on  $\beta$ -lactose. The hydrocarbons had heats of adsorption on  $\beta$ -lactose which did not vary significantly from those found on *s*-lactose. Since the esters, aldehydes, and ketones had heats of adsorption on  $\beta$ -lactose which were significantly greater than those for *s*-lactose, the difference between the heats of adsorption of these intermediate strength compounds and the hydrocarbons on  $\beta$ -lactose was approximately 9–10 kcal/mol—still within range of the energy of formation of a hydrogen bond (Table V).

The larger heats of adsorption found on  $\beta$ -lactose cannot be explained on the basis of particle size or surface area. Referring once again to Table VI, the particle sizes for all the sugars studied were similar; and  $\beta$ -lactose does not have a larger surface area as compared to *s*-lactose and *r*-lactose. The greater heats of adsorption found on  $\beta$ -lactose may be accounted for, at least in part, by the  $\beta$  configuration of the anomeric carbon atom. It is proposed that the  $\beta$  configuration with its equatorial hydroxyl group (Fries et al., 1971) allows an additional possibility for hydrogen bonding. All of the compounds which showed higher heats of adsorption on  $\beta$ -lactose are postulated to bond to the lactose surface via hydrogen bonding. The hydrocarbons, which are proposed to be attracted to the lactose surface by van der Waals forces, did not show heats of adsorption significantly greater than those found for *s*-lactose.

Equations were derived for predicting the heats of adsorption on  $\beta$ -lactose, as described previously for *s*-lactose, and are shown in Table VII.

**Sucrose.** Adsorption data were obtained using the same compounds treated in the same manner as discussed for *s*-lactose, *r*-lactose, and  $\beta$ -lactose. Most of these compounds exhibited extremely short observed retention times on sucrose. Sufficiently long observed retention times could be recorded to allow for reasonable accuracy and large enough corrected net retention times to be used in thermodynamic calculations for seven of the volatile compounds studied on the lactose columns: 1-heptanol, hexyl formate, heptanal, methyl hexanoate, 2-heptanone, decane, and dodecane. The heats of adsorption for these compounds on sucrose are compared to those found for *s*-lactose, *r*-lactose, and  $\beta$ -lactose in Table II. Sucrose had much lower heats of adsorption than those found for the lactose columns. The differences between the heats of adsorption on sucrose and on the lactose columns were greater in most cases than the  $\pm 1.5$  kcal/mol maximum error for heats of adsorption determinations on the lactose columns. There was insufficient data to calculate the limits of error for heat of adsorption determinations on sucrose.

Gibbs energy and entropy calculations for sucrose followed the same patterns observed for the lactose columns.

The heat of adsorption values for sucrose indicate that sucrose is not comparable to *s*-lactose, *r*-lactose, and  $\beta$ -lactose with regard to strength of adsorption.

Visual examination on conclusion of these studies of the sugar within the sucrose column revealed that the adsorbent had slowly decomposed, for it was no longer free flowing, having yellowed and sintered. Although the melting point of sucrose (185 °C) is well above the temperatures used in these studies, the sucrose column was not as heat stable as the lactose columns at the same temperatures. The decomposition was apparently heat induced and not oxidative in nature since the column was constantly flushed with dry nitrogen during the thermodynamic determinations. Naturally, it would have been desirable to conduct the study at a still lower temperature, but the sugars must be examined at the same temperatures

Table IX. Results of Repeated Heat of Adsorption Determinations on Anhydrous  $\alpha$ -D-Glucose

compd	heats of adsorption (kcal/mol)	
	initial	after 4 months
1-butanol	17.6	7.3
1-heptanol	23.4	14.5
2-heptanone	17.4	11.0
methyl hexanoate	17.7	11.3
heptanal	16.5	10.3
hexyl formate	16.9	10.1
decane	14.6	10.6
dodecane	17.1	13.5

for comparable adsorption data to be obtained.

Nickerson and Dolby (1971) found that only  $\alpha$ -lactose hydrate, spray-dried amorphous lactose glass, and heated sucrose adsorbed smaller amounts of diacetyl than untreated sucrose. *r*-Lactose, *s*-lactose, and  $\beta$ -lactose adsorbed larger amounts of diacetyl than did sucrose. If amounts of adsorbate adsorbed correlate with heats of adsorption, then the Nickerson and Dolby (1971) findings are consistent with the lower heats of adsorption found for sucrose. The heated sucrose used to adsorb diacetyl is not totally comparable to the sucrose adsorbent used here. Although the sucrose used to adsorb diacetyl was heated at 125 °C, near the experimental temperature range for the gas chromatographic procedure, it would have been subject to oxidative as well as heat-induced decomposition.

Since the sucrose was sieved to a particle size of the same magnitude as the other sugars examined (Table VI), the lower heats of adsorption on sucrose could probably be explained on the basis of its low surface area as compared with the surface areas of the other sugars studied.

**Anhydrous  $\alpha$ -D-Glucose.** The adsorption behavior of anhydrous  $\alpha$ -D-glucose (glucose) was examined for comparison with sucrose, *s*-lactose, *r*-lactose, and  $\beta$ -lactose. The adsorption data were obtained as described above. The same groups of compounds were used. Once again very short observed retention times were found for most of the compounds injected onto the glucose column. Only 1-butanol, 1-heptanol, 2-pentanone, 2-heptanone, methyl hexanoate, heptanal, hexyl formate, decane, and dodecane gave observed retention times which were large enough for accurate measurements and thus for effective use in thermodynamic calculation. The heats of adsorption for glucose are compared with those for the other sugars examined in Table II. These compounds gave heats of adsorption on glucose which were larger than those for *s*-lactose. This difference exceeded the  $\pm 1.5$  kcal/mol maximum error for the heat of adsorption determinations on *s*-lactose. Insufficient data were obtained for glucose to determine the limits of error for the heats of adsorption. The heats of adsorption for glucose were of the same magnitude as were those determined for *r*-lactose and  $\beta$ -lactose, except where the latter two sugars gave heats of adsorption similar to those determined on *s*-lactose: the formates on *r*-lactose and the hydrocarbons on  $\beta$ -lactose. The heats of adsorption for glucose, *r*-lactose, and  $\beta$ -lactose were generally within  $\pm 1.5$  kcal/mol of each other—the maximum error calculated for heat of adsorption determinations on *r*-lactose and  $\beta$ -lactose. The heats of adsorption were greater for glucose than for sucrose (Table II).

Subsequent heat of adsorption determinations on the glucose column were considerably lower than they were when first determined for the same compounds (Table IX). It would seem that while glucose initially gave larger heats of adsorption as compared to *s*-lactose, glucose is not as

stable as the crystalline forms of lactose at the temperatures used experimentally. At the conclusion of this experiment, the sugar within the glucose column was found to have yellowed and sintered. This decomposition occurred even though the experimental temperatures were below the melting point of glucose (146 °C), and the column was continuously flushed with dry nitrogen. Lower temperatures would have to be used to study the adsorption on glucose; but, of course, comparable adsorption data would not be obtained.

The Gibbs energy and entropy calculations showed the same patterns for glucose as were found for sucrose, *s*-lactose, *r*-lactose, and  $\beta$ -lactose.

The glucose particle size was comparable to the particle sizes of the other sugars examined (Table VI). The initially larger heats of adsorption on glucose cannot be explained on the basis of a large surface area (Table VI). A possible explanation for the initially stronger adsorption on glucose is similar to the one proposed for *r*-lactose. Glucose is prepared commercially at higher temperatures (above 50 °C) than is used in preparing  $\alpha$ -D-glucose hydrate in a vacuum pan while evaporation is taking place (Pigman, 1948). It is possible that fissuring occurs in the preparation of the anhydrous form during evaporation, as was postulated to occur during the formation of *r*-lactose from  $\alpha$ -lactose hydrate. The occurrence of fissures in glucose might provide additional possibilities for adsorption, as in the case of *r*-lactose. The instability of the glucose to heat would still explain the smaller heats of adsorption found in the later heat of adsorption determinations. The assumption has been made that a molecule of lactose can interact with an adsorbate, such as an ester, with both van der Waals forces and hydrogen bonding. There is also the distinct possibility that since glucose is a hexose rather than a disaccharide, a molecule of glucose is too small to

interact with an adsorbate via both van der Waals forces and hydrogen bonding. It is conceivable that an adsorbate can bridge two molecules of glucose in order to interact on the glucose surface with both van der Waals forces and hydrogen bonding. This bridging may be a consideration in understanding the initially large heats of adsorption found on glucose.

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## Analysis of Sugar Cane Saccharides by Liquid Chromatography. 1. Adsorption Chromatography with Flow Programming

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A rapid method for the determination of sucrose, glucose, and fructose in sugar cane juice by adsorption chromatography is described. A Waters Associates prepacked  $\mu$ Bondapak/carbohydrate (8-12  $\mu$ m) column of 4 mm i.d.  $\times$  30 cm is utilized. Elution is carried out with an acetonitrile/water mixture in the ratio 80:20 as the eluting solvent. The application of flow programming facilitates complete analysis in less than 12 min while achieving an adequate resolution among the sugars as detected with a differential refractometer.

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Polarimetric techniques have been traditionally employed by sugar cane industries for estimating the sucrose content of sugar cane juice. Although these techniques may be adequate under ideal conditions, they do have serious limitations (Wong-Chong, 1978). Other more accurate methods of sucrose analysis include enzymatic methods, gas-liquid chromatography with prior derivatization, paper chromatography, and liquid chromatography. However, considerations of cost and/or time per

sample have deferred acceptance of these methods by sugar cane industries. Recent developments with high-pressure liquid chromatography, on the other hand, offer rapid analysis with minimum sample preparation (Conrad and Palmer, 1976). The objective of this study, therefore, was to determine the feasibility of using pressurized liquid chromatography for analysis of sugar cane saccharides.

#### MATERIALS AND METHODS

The following is a list of equipment used in the LC system for separation of saccharides: (1) Water Associates ALC/GPC 244 liquid chromatograph instrument equipped with: (a) differential refractometer, Model R401, (b)

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