Heats of Adsorption of Small Molecules on Lactose

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This study has demonstrated that a wide variety of organic compounds, including esters, aldehydes, ketones, alcohols, and hydrocarbons, adsorb on stable, anhydrous α-lactose. The heats of adsorption of these compounds on stable, anhydrous α-lactose have been determined by the gas chromatographic method, and are taken as indications of the strengths of adsorption. On this basis it has been established that for a given number of carbon atoms, alcohols adsorb most strongly, hydrocarbons least strongly, and all other types of adsorbate examined adsorb with intermediate strengths. The formation of hydrogen bonds between adsorbent and adsorbate has been suggested as a major factor contributing to the strength of adsorption.

Lactose has the ability to adsorb aromas. This characteristic led to speculation that lactose might be useful as a flavor carrier. Flavor volatiles escaping from a food during dehydration could be trapped by lactose; the flavor-carrying lactose would be added back to the dried product, and natural flavors would be retained longer. Recently Nickerson and Dolby (1971) measured the amounts of diacetyl adsorbed on various forms of lactose and other sugars. They found that anhydrous forms of α-lactose were superior to the other sugars examined in adsorbing diacetyl. Similar studies with coffee and chocolate volatiles in industrial plants also indicated that anhydrous lactose was better than other sugars examined for adsorbing odors (Nickerson and Dolby, 1971).

Yabumoto et al. (1975) used stable anhydrous lactose to adsorb banana volatiles.

Lee et al. (1975) have shown that stable anhydrous α-lactose has a high capacity for adsorbing volatile compounds. The amount adsorbed varied greatly, but increased linearly with an increase in carbon number within homologous series. They showed the amount adsorbed decreased in going from a series of alcohols, to methyl esters, and to methyl ketones.

The first reported determination of heats of adsorption by gas chromatography was made by Greene and Pust (1958). The method offers simplicity of apparatus and of experiment. Heats of adsorption of low-boiling gases on charcoal and light hydrocarbons on alumina and silica gel were found to agree well with calorimetric results.

Since that time, several authors, Hoare and Purnell (1956), Kiselev and Yashin (1969), Gale and Beebe (1964), have derived equations which relate thermodynamic quantities to gas chromatographic–retention time data. From the enthalpy and entropy of adsorption, it is possible to calculate the Gibbs energy and hence the retention time on a given adsorbent for any temperature. Okamura and Sawyer (1972) propose this technique as a means of optimizing gas chromatographic peformance.

It was the purpose of this study to learn more about the types of flavors which will adsorb on lactose and to measure the strength of the adsorptions from heats of adsorption using gas chromatography.

EXPERIMENTAL SECTION

Preparation of Stable, Anhydrous α-Lactose. Stable, anhydrous α-lactose was prepared by an adaptation of the technique described by Lim and Nickerson (1973). USP grade lactose was suspended in absolute methanol in a ratio of one part lactose to ten parts methanol and heated under reflux for 2 hr. The resulting product was vacuum filtered and dried for 15 hr in a vacuum oven at 70° (58 Torr). Microscopic examination of the product revealed minute, needle-shaped crystals. Identity of product was confirmed by measurement of optical rotation as was done by Lim and Nickerson (1973). Though this form of lactose is not as hygroscopic as other anhydrous forms, it was stored in a desiccator. Just prior to use it was sieved through a U.S. Standard 100 mesh screen to remove large clumps of crystals.

Preparation of Chromatographic Columns. 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 packed with the stable, anhydrous α-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 column coiled and installed in either a Varian 1520 or a Hewlett-Packard 5711A gas chromatograph. Columns were flushed with dry nitrogen and aged as indicated in Table I.

Apparatus and Procedure. Operating conditions are shown in Table I. All gases were passed through filters containing molecular sieve 5A just prior to use to remove trace impurities. Gas flow rates were measured with a soap-bubble flow meter at room temperature and atmospheric pressure.

Nominal oven temperatures for the columns were selected by the instrument controls which were adjustable to each degree centigrade, with a temperature stability given as ±0.1°. 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 =8662 portable potentiometer. The thermocouple was calibrated by setting emf to 0.000 mV at 0° (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.

Compounds studied were commercially obtained, reagent grade chemicals. 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-μl syringe was flushed several times with the saturated headspace vapors and then 2 μl of the headspace was 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

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Table I. Chromatographic Conditions for the Two Gas Chromatographs and the Three Lactose Columns

	Varian 1520	Hewlett-Packard 5711A	
Column temp	Varied	Varied	
Injector temp, °C	Equals column temp	100	
Detector temp, °C	155	150	
Detector	FID	FID	
Gas flow rates, ml/min			
Nitrogen	25	25	
Hydrogen	25	25	
Air	250	250	
Stainless steel columns	#1	#2	#3
Length, cm	240	153	137
Inside diameter, mm	2.5	2.0	2.0
g of lactose	3.9	1.1	0.95
Aged[a]			
Time, hr	15	15	15
Temp, °C	150	120	100

[a] With nitrogen flow of 25 ml/min.

recorded retention times was calculated. Retention times were found to be reproducible to within 0.2%.

Dead time is the time that a molecule resides in the column in the nonadsorbed state, and can be measured by passing a nonadsorbing gas through the column and measuring the time taken from injection to peak maximum. Methane was used for this purpose because it showed no variation in retention time with changes in temperature.

Approximate calculations of amount of substance in an injected sample can be made from vapor pressure data at room temperature. Such calculations were carried out for several compounds and indicate that about 10^{-6} to 10^{-8} g of sample is contained in 2 μl of saturated vapor. Such low concentrations are prerequisite for use of this experimental technique.

A change in the column temperature results in changes in flow rate of the carrier gas and pressure at the head of the column. Thus, whenever temperature was changed, the flow rate was readjusted to the desired value. The pressure at the head of the column was then measured using a calibrated Bourdon pressure gauge.

The specific surface area of stable, anhydrous α-lactose has been determined by others in three studies. Berlin and coworkers (1972) obtained values of 2.19 and 2.04 m^2/g. A subsequent determination of Berlin (1973) yielded a value of 1.26 m^2/g. The Micromeritics Corporation (Hinsch, 1973) performed for us a determination of specific surface area for stable, anhydrous α-lactose and obtained a value of 2.88 m^2/g. For the present study the mean of these four values, 2.09 m^2/g, was taken as the specific surface area of the lactose adsorbent.

RESULTS AND DISCUSSION

The adsorbates studied, which included esters, aldehydes, ketones, alcohols, and hydrocarbons, were chosen for several reasons. Together they represent a cross-section of organic compounds, including many that are important as flavor components. The temperature range of 100 to 120° facilitated rapid elution of most adsorbates, yet was low enough that adjacent homologs in a given series had distinctly different retention times.

Fifty-six adsorbate compounds were studied by injecting samples individually onto a lactose column at five different temperatures (5° intervals from 100 to 120°). For each compound a plot was made of the natural logarithm of corrected net retention time or natural logarithm of net

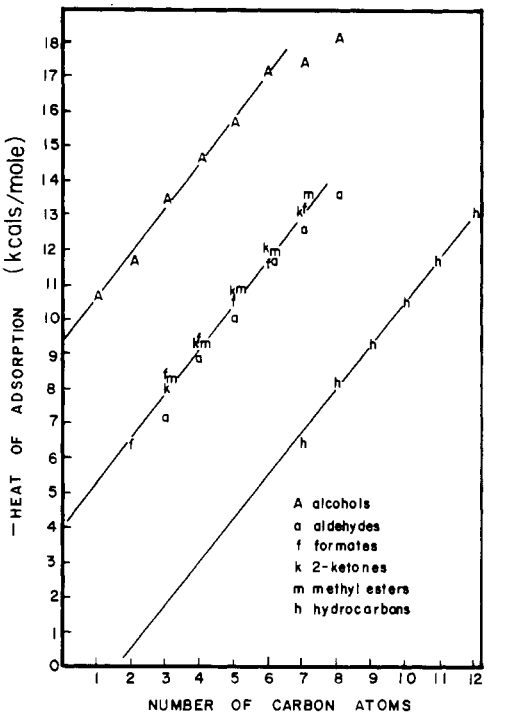

Figure 1. Heats of adsorption on lactose (from Table V) vs. the number of carbon atoms in the molecule.

retention time vs. the reciprocal absolute temperature of the column. In every case the plot was linear; correlation coefficients describing the fit were usually 0.997 or better. In a few cases for compounds with low boiling points, especially methyl formate and propionaldehyde, the correlation dropped to about 0.98. Slopes of the lines were determined and were multiplied by the gas constant to obtain heats of adsorption (from $\ln t_{corr} = -\Delta H/RT + C$) (Gale and Beebe, 1964). All heats determined are shown in Table II. Heats of adsorption on lactose for the compounds studied are seen to range from about 6 to 18 kcal/mol.

On the basis of experience with the experimental technique for determining heats of adsorption by gas chromatography, limits of errors for several experimental parameters were estimated. From these limits it was possible to obtain, by a propagation-of-errors treatment, the limits of errors for the thermodynamic quantities calculated in this study. It was concluded that the limit of error of a heat of adsorption determination was ±1.5 kcal/mol. It should be emphasized that reproducibility was found to be far better than this, usually within ±0.5 kcal/mol.

Heats of adsorption increase fairly uniformly as molecular weight increases. This type of behavior has been previously reported (Kiselev and Yashin, 1969). More revealing information is shown in Table III. Here are tabulated the heats of adsorption for straight-chain compounds all containing four carbon atoms, but with various functional groups. *n*-Propyl formate, methyl *n*-propionate, *n*-butanal, and 2-butanone all have nearly equal heats of adsorption, about 9 kcal/mol. *n*-Butyl alcohol has a considerably higher heat of adsorption, 14.69 kcal/mol, while the value for the hydrocarbon *n*-butane (obtained by extrapolation of the data for other normal hydrocarbons) is much lower, ~2.7 kcal/mol. These data lead to the conclusion that the functional group of a molecule is important in determining its heat of adsorption on lactose.

The patterns described in the preceding paragraph are graphically demonstrated in Figure 1, where heats of adsorption of members of six homologous series of compounds are plotted vs. the number of carbon atoms. It is seen that these compounds fall into three classes. Alcohols

Table II. Retention Data and Thermodynamic Quantities Determined by the Gas Chromatographic Method[a]

Compound	t_{corr}, sec	V_s, ml/m^2	$-\Delta G$, cal/mol	$-\Delta H$, kcal/mol	$-\Delta S$, cal/(K mol)
	A. Column #1 (See Table I), 109°				
Methyl formate	2.67	0.14	−1488	7.30	23.0
Ethyl formate	6.03	0.31	−889	8.34	24.2
n-Propyl formate	11.38	0.58	−413	9.17	25.0
n-Butyl formate	23.10	1.18	126	10.46	27.1
n-Pentyl formate	47.80	2.44	677	11.72	28.9
n-Hexyl formate	98.42	5.03	1226	13.13	31.2
Methanol	7.78	0.40	−696	10.2	28.5
Ethanol	13.81	0.71	−260	11.55	30.9
1-Propanol	26.89	1.37	239	13.01	33.4
2-Propanol	22.60	1.15	106	12.69	32.9
1-Butanol	60.75	3.10	859	14.69	36.2
tert-Butyl alcohol	35.64	1.82	355	14.15	35.9
1-Pentanol	129.6	6.62	1435	16.03	38.2
1-Hexanol	271.4	13.87	1996	17.57	40.8
Propanal	5.59	0.29	−940	5.82	17.7
n-Butanal	9.96	0.51	−511	8.29	23.0
n-Pentanal	19.68	1.01	8	9.99	26.1
n-Hexanal	39.69	2.03	537	11.63	29.0
2-Propanone	8.30	0.42	−658	7.99	22.6
2-Butanone	13.85	0.71	−260	9.37	25.2
2-Pentanone	26.49	1.35	228	10.81	27.7
2-Hexanone	55.08	2.81	784	12.03	29.4
2-Heptanone	114.6	5.86	1342	13.09	30.8
Methyl acetate	7.33	0.37	−755	7.65	22.0
Methyl *n*-propionate	12.31	0.63	−351	9.12	24.8
Methyl *n*-butyrate	23.53	1.20	138	10.65	27.5
Methyl *n*-pentanoate	48.20	2.46	683	12.14	30.0
Methyl hexanoate	97.20	4.97	1217	13.57	32.3
	B. Column #2, 108°				
Propanal	1.14	0.21	−1181	7.51	22.8
n-Butanal	2.46	0.45	−605	9.26	25.9
Methyl formate	0.59	0.10	−1700	5.3	19
n-Propyl formate	2.75	0.50	−525	9.19	25.5
n-Pentyl formate	12.25	2.22	604	11.30	28.1
2,3-Pentanedione	4.83	0.87	−105	9.89	26.2
2,3-Butanedione	2.26	0.41	−675	9.67	27.2
n-Heptane	1.49	0.27	−991	6.38	19.3
n-Octane	3.02	0.55	−453	8.12	22.5
n-Nonane	6.09	1.10	72	9.24	24.1
n-Decane	12.60	2.28	624	10.55	26.1
n-Undecane	25.73	4.66	1165	11.75	27.8
n-Dodecane	52.45	9.49	1704	13.01	29.7
n-Tridecane	108.1	19.57	2253	14.45	32.0
Cyclohexane	0.70	0.13	−1500	6.1	20
Cyclohexane	0.64	0.12	−1600	5.9	20
1-Hexene	0.82	0.15	−1400	8.7	27
1-Hexene	0.82	0.15	−1400	7.1	22
1,3-Cyclohexadiene	0.88	0.16	−1400	7.1	22
Cyclopentanone	13.10	2.37	653	12.29	30.5
Cyclohexanone	28.22	5.11	1235	13.06	31.0
Cycloheptanone	59.19	10.71	1794	14.30	32.8
Cyclooctanone	99.03	17.92	2184	15.14	34.0
Cyclopentanol	30.47	5.52	1293	15.35	36.9
Benzene	1.03	0.19	−1257	6.60	20.6
Benzene	0.97	0.18	−1300	7.2	22
Toluene	2.34	0.42	−657	8.34	23.6
Ethylbenzene	4.51	0.82	−150	9.48	25.3
n-Propylbenzene	8.61	1.56	337	10.51	26.7
n-Butylbenzene	17.43	3.15	869	11.43	27.7
	C. Column #2, 115°				
n-Hexanal	8.81	1.59	358	11.7	29.1
n-Heptanal	17.66	3.20	897	12.51	29.9
n-Octanal	35.29	6.39	1430	13.53	31.2

Table II *(Continued)*

Compound	t_{corr}, sec	V_s, ml/m^2	$-\Delta G$, cal/mol	$-\Delta H$, kcal/mol	$-\Delta S$, cal/(K mol)
1-Heptanol	130.6	23.64	2437	17.38	38.5
1-Octanol	254.9	46.14	2953	18.22	39.3
	D. Column #3, 108°				
Methyl formate	0.39	0.08	−1900	6.1	21
Methyl acetate	1.22	0.26	−1020	8.82	25.8
Methyl *n*-propionate	2.35	0.49	−540	9.61	26.6
Methyl *n*-butyrate	4.96	1.04	30	10.90	28.5
Methyl *n*-pentanoate	10.31	2.17	587	11.83	32.6
Propanal	0.83	0.17	−1300	8.2	25
n-Butanal	1.66	0.35	−795	8.92	25.5
Benzene	0.71	0.15	−1400	7.1	22
Toluene	1.66	0.35	−795	8.76	25.1
Ethylbenzene	3.09	0.65	−326	9.04	24.6
n-Propylbenzene	6.03	1.27	181	9.98	25.7
Methanol	1.43	0.30	−911	11.1	31.4
1-Propanol	6.27	1.32	210	13.7	35.5
1-Pentanol	30.92	6.49	1416	15.37	36.6
1-Heptanol	131.6	27.64	2512	16.99	38.0
1-Octanol	269.4	56.57	3055	17.22	37.2

[a] t_{corr} = corrected retention time; V_s = specific retention volume; ΔG = Gibbs energy of adsorption; ΔH = heat of adsorption; ΔS = entropy of adsorption.

Table III. Heats of Adsorption of Four-Carbon Molecules on Stable, Anhydrous α-Lactose; Determined by Gas Chromatography

Compound	−Heat of adsorption, kcal/mol[a]
1-Butanol	14.69
n-Propyl formate	9.18
Methyl *n*-propionate	9.36
n-Butanal	8.82
2-Butanone	9.37
n-Butane (extrapolated)	~2.7

[a] Mean values from Table II.

have the highest heats of adsorption for a given number of carbon atoms, hydrocarbons have the lowest, while esters, aldehydes, and ketones occupy an intermediate position. The methods used do not permit a further subdivision of the latter class of compounds. The heat of adsorption determinations are not precise enough to indicate, for example, that the heat of adsorption of 2-propanone is greater or less than the heat of adsorption of methyl acetate, ethyl formate, or *n*-propanal (Table II).

On first examination of Figure 1 it may occur to the observer that the heats determined by this method may be merely a reflection of boiling points and that the quantity being measured results from condensation as well as adsorption. This would seem to be supported by the fact that a plot of boiling point vs. number of carbon atoms follows a pattern very similar to the plot of heat of adsorption vs. number of carbon atoms (Figure 1). However, an indication that boiling point is not the controlling factor in the heat of adsorption determinations is that a plot of heat of adsorption vs. boiling point is not a single curve, but is divided into the same three classes of compounds (Figure 1). Clearly there is some other factor which determines the heat of adsorption. The same deduction can be made by examining available vapor pressure data and correlating it with the heats of adsorption. Vapor pressure is a property related to boiling point in that they both are functions of the attraction of molecules to others of their own kind. When vapor pressure is plotted vs. heat of adsorption for methyl esters, formates, ketones, and alcohols it is once again found that alcohols stand alone while the esters and ketones are grouped together. This is an additional indication that it is not adsorbate-adsorbate interaction, but some other factor which gives alcohols higher heats of adsorption.

Kiselev and Yashin (1969) have classified adsorbents and adsorbates on the basis of the types of interactions in which they can engage. Adsorbents are grouped into three types, I, II, and III, depending on type of charge and its distribution. Similarly adsorbates are categorized as group A, B, C, or D depending on electron distribution.

The above classification contributes to visualizing the types of interactions which can occur between adsorbent and adsorbate. For any situation involving a type I adsorbent or a group A adsorbate there will be nonspecific interactions only, resulting from van der Waals or dispersion forces. Other combinations will result in not only the nonspecific attraction but also specific interactions. Specific interactions will result from attraction of opposite charges and alignment of permanent or induced dipoles.

The sum of the nonspecific and specific interactions determines the strength of the adsorption, and hence the heat of adsorption. Thus, from knowledge of the heats of adsorption and simple consideration of the structures of the compounds involved, the types of interactions taking place in the adsorption process may be deduced.

The structure of lactose suggests that it is not a type I adsorbent, capable of only nonspecific interactions. This is shown to be true by the fact that molecules of equal number of carbon atoms but of various functional groups have very different heats of adsorption on lactose (see Table III and Figure 1). The saturated hydrocarbons adsorb on lactose by van der Waals attraction only, so that an adsorbate of this type would have the minimum possible heat of adsorption for a given number of carbon atoms. We observe in Figure 1 that this is true. In the same figure we see that aldehydes, ketones, and esters have heats of adsorption which are about 6 kcal/mol higher than the heats for hydrocarbons of an equal number of carbon atoms. These three functional groups all contain at least one oxygen atom, and thus may be classified as group B adsorbates because the oxygen atoms have lone pairs of electrons. These lone pairs of electrons are proba-

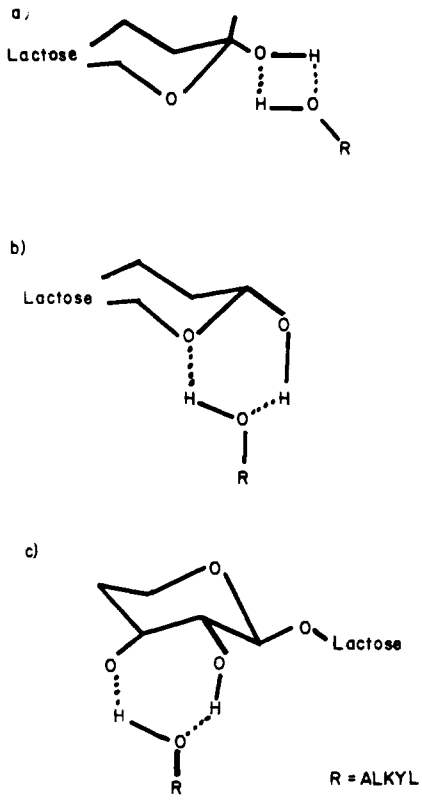

Figure 2. Three possible arrangements for the simultaneous existence of two hydrogen bonds between lactose and an alcohol: (a) four-membered ring; (b) six-membered ring; and (c) seven-membered ring. (Bond lengths are not drawn to scale.)

bly the source of a specific interaction with lactose which gives these group B adsorbates higher heats of adsorption on lactose. A lone electron pair of the adsorbate molecule would be attracted to a localized positive charge on the adsorbent. Lactose has eight hydroxyl groups, and the protons of these hydroxyl groups are the most likely locations to which a negatively charged adsorbate center would be attracted. It is thus likely that a hydrogen bond forms. Hydrogen bonds have energies of formation in the range of 3–10 kcal/mol, but generally about 5 kcal/mol (Breschia et al., 1966). Thus, the observed energy difference of 6 kcal/mol between the heats of adsorption of a saturated hydrocarbon and a group B molecule of similar size is in agreement with the suggestion that a hydrogen bond is formed between the lactose and the group B molecule.

Alcohols also have oxygen atoms with lone pairs of electrons which could engage in the same hydrogen bonding mentioned above. Yet Figure 1 indicates that, for a given number of carbon atoms, alcohols have heats of adsorption on lactose which are an additional 5 kcal/mol above the heats for the group B adsorbates. Alcohols apparently undergo yet another specific interaction with lactose. It is logical to suppose that alcohols form a second hydrogen bond with lactose. Alcohols are group D adsorbates. The alcoholic proton is an effective localization of positive charge which would be attracted to a localization of negative charge on the lactose molecule. Lactose does have such negative charge centers in the lone electron pairs of the numerous lactose oxygen atoms. Thus, two hydrogen bonds could exist simultaneously between lactose and an alcohol. Three possible arrangements for the simultaneous existence of two hydrogen bonds between lactose and an alcohol are shown in Figure 2. The four-membered ring shown seems less likely than the six- or seven-membered rings on the basis of ring strain. For hydrocarbons the six-membered ring is more stable than the seven-membered

Table IV. Interactions between Lactose and Adsorbates

Adsorbate	Type of interaction
Hydrocarbons	Nonspecific interaction, van der Waals type
Esters, aldehydes, and ketones	Van der Waals attraction, as with hydrocarbons; *plus* alignment of dipoles, possible small specific interaction; *plus* hydrogen bond between oxygen on adsorbate and lactose hydroxyl group proton
Alcohols	Van der Waals attraction; *plus* alignment of dipoles, as above; *plus* hydrogen bond, as above; *plus* second hydrogen bond, involving alcoholic proton and oxygen on lactose; *minus* possible decrease in stability due to strained ring from simultaneous formation of two hydrogen bonds

ring by about 1 kcal/mol (Noller, 1957), but with the longer hydrogen bonds it may be that the seven-membered ring is more stable. It is also possible, of course, that an alcohol forms one hydrogen bond with each of two neighboring lactose molecules.

It has been proposed above that one hydrogen bond contributes 6 kcal/mol to the heat of adsorption, while a second such bond adds only 5 kcal/mol. To explain this difference it should be recognized that there may be other interactions which contribute about 1 kcal/mol, such as alignment of permanent or induced dipoles. It might also be the case that the simultaneous formation of two hydrogen bonds between an alcohol and lactose results in ring strain, thereby reducing slightly the energies of formation of the bonds. The interactions discussed above are summarized in Table IV.

The suggestion that up to 1 kcal/mol may be contributed to the heat of adsorption by alignment of dipoles can be evaluated by comparing known dipole moments with heats of adsorption. The dipole moments for a number of compounds were examined. While alcohols and esters have dipole moments of about 1.7 D, aldehydes and ketones have values of about 2.8 D. Esters, aldehydes, and ketones have roughly equal heats of adsorption for a given number of carbon atoms. It can therefore be concluded either: (1) that if dipole moment contributes significantly to the heats of adsorption of ketones and aldehydes, then some other factor not yet mentioned contributes to the heats of adsorption of esters to bring them up to the level of aldehydes and ketones, or (2) that dipole moment does not contribute significantly to the heat of adsorption on lactose.

As noted above, it is the expected pattern to find a linear increase in heat of adsorption with molecular weight for a given homologous series of compounds. Considering the case for the alcohols, as an example, the heat of adsorption of methanol will consist of a contribution of x kcal/mol from the hydrocarbon portion, $-CH_3$, and y kcal/mol from the functional group, -OH. For the ethanol molecule there will be the same contributions, plus an additional contribution from the added hydrocarbon segment, $-CH_2-$. The contribution from a methylene group should be very nearly x kcal/mol because there is little difference in size or character between a methylene and a methyl group. Similar increases of x cal/mol will result from further additions of methylene groups, so that the heat of adsorption of a normal alcohol of n carbon atoms can be calculated from $-\Delta H = nx + y$. This is the equation of a straight line, and such an equation can be found

Table V. Scheme for Predicting Heats of Adsorption of Straight-Chain Molecules on Stable, Anhydrous α-Lactose[a]

Alcohols	$-\Delta H = 1.32N + 9.21$ kcal/mol
Ketones, esters, aldehydes	$-\Delta H = 1.31N + 3.84$ kcal/mol
Hydrocarbons	$-\Delta H = 1.30N - 2.54$ kcal/mol

[a] ΔH = heat of adsorption; N = number of carbons atoms.

for any homologous series. Such equations may be useful for predicting heats of adsorption in a series for which some, but not all, determinations have already been made. Table V gives the equations which fit the data obtained for the adsorption on lactose of five homologous series of compounds. The slopes are very nearly equal in each case, verifying that the contribution to the heat of adsorption by a methylene group is constant, regardless of the functional group of the molecule.

In addition to the series of normal alcohols, two branched alcohols were studied. The heats of adsorption of 2-propanol and *tert*-butyl alcohol were found to be 12.7 and 14.2 kcal/mol, respectively, while the respective heats for 1-propanol and 1-butanol were 13.4 and 14.7 kcal/mol. These results indicate that, all else being equal, the heats of adsorption of branched molecules are less than those of straight-chain molecules. This result is expected, because a branched molecule is less likely to have its entire structure in close proximity to the absorbent.

Cyclic analogs of ketones, an alcohol, and some hydrocarbons were examined in order to compare their heats of adsorption with those of the noncyclic compounds. These values are shown in Table II. The differences between the heats of adsorption of cyclic and noncyclic analogs are variable. In no case do these differences greatly exceed the level of uncertainty in the heat of adsorption determinations. For the compounds examined, whether a molecule is cyclic or noncyclic does not appear to affect greatly the strength of its adsorption on lactose.

According to the classification of adsorbates given by Kiselev and Yashin (1969), molecules with electrons in π bonds have localized electron density and should be classified as group B molecules. Such molecules should be able to interact specifically with localized positive charges in type II adsorbents. The magnitude of such a specific interaction could be determined by comparing the heat of adsorption of an unsaturated hydrocarbon with that of the corresponding saturated hydrocarbon. Reference is once again made to Table II to compare heats of adsorption. Although the difference of about 2.6 kcal/mol between the heats of adsorption of *n*-hexane (obtained by extrapolation, see Table IV) and 1-hexene is significant, similar significant differences are not observed between cyclohexane and unsaturated cyclic hydrocarbons. It is clear that stepwise increases to two, four, and six π electrons do not result in constant significant increases in the heat of adsorption. If these so-called group B molecules do have specific interactions with lactose, then the interaction is evidently much weaker than that of the group B molecules containing oxygen atoms.

Gregg (1951) has observed that heats of physical adsorption tend to be about one to two times the latent heat of condensation of the adsorbate. With minor exceptions the heats of adsorption determined in this study fall in the range of one to two times the heats of condensation of the adsorbates. This is another indication that the heats of adsorption as determined by the present method are at least approximately of the correct magnitude.

Table II shows the results obtained from calculations of Gibbs energies and entropies of adsorption at 110°. The Gibbs energy determinations depend largely on the corrected retention volume and on the surface area of the lactose. Combined uncertainties in these quantities give the calculated Gibbs energies a large degree of uncertainty compared to the uncertainty in the heats of adsorption. Entropy calculations depend mainly on the result obtained for the heat of adsorption, and to a lesser degree on the Gibbs energy. The accuracy of the entropies is probably not great enough to warrant attempts to explain small differences observed between compounds. Important to note, however, are some gross features of the tabulated entropy values. First, in every case the entropy change for adsorption has a negative sign, indicating the expected result that randomness and freedom of motion are decreased by adsorption. Secondly, while most of the compounds studied had entropies of adsorption in the range of −30 to −20 cal K^{-1} mol^{-1}, the alcohols as a group had more negative entropies of adsorption, from −40 to −30 cal K^{-1} mol^{-1}. This would indicate that on adsorption, alcohols lose significantly more freedom of motion than the other compounds studied. Loss of translational motion will result in a decrease in entropy, this decrease being proportional to the logarithm of the weight of the molecule. Because on adsorption an alcohol shows a greater decrease in entropy than other compounds of similar molecular weight, it must be concluded that alcohols lose more rotational and/or vibrational freedom of motion than the other compounds examined. This is consistent with the proposal that alcohols are held to lactose by two hydrogen bonds, a situation lending more rigidity than the more common case of one hydrogen bond attachment, or simply van der Waals attractions.

The overall picture of three classes of compounds which adsorb on lactose may thus be described as: (1) hydrocarbons that are attracted to the surface by van der Waals forces, but have considerable vibrational and rotational freedom, and may even be mobile on the lactose surface; (2) group B molecules containing oxygen that are more firmly attached to the surface by a hydrogen bond, thus limiting rotation and vibration to some extent; and (3) alcohols that are anchored in two places to the surface, further reducing rotation and vibration.

Gibbs energies of adsorption and entropies of adsorption were calculated at five temperatures for a few representative compounds. Gibbs energies of adsorption were found to increase with increasing temperature, indicating that higher temperatures are less favorable to adsorption. This is characteristic behavior for physical adsorption, whereas chemical adsorption may invoke opposite behavior, that is, lower Gibbs energies of adsorption at higher temperatures (Moore, 1962).

Our observations indicate that the entropy of adsorption is not a function of temperature. It should be pointed out that a single value for the heat of adsorption was utilized in the calculations of entropies for a given adsorbate. Thus, it would be more accurate to state that, assuming heat of adsorption is not a function of temperature, then entropy of adsorption is also independent of temperature.

To check the temperature dependence of the heat-of-adsorption determinations, selected samples were injected over a wider range of temperatures than in the general experiments. Seven compounds were each injected on lactose column no. 2 (described in Table I) at 65, 80, 100, 105, 110, 115, and 120°. For each of these compounds heats of adsorption were determined from the slope of ln t_{net} vs. $1/T$ in the 65–100° range, in the 100–120° range, and over the entire 65–120° range. In nearly every case the heat of adsorption is determined to be greater in the lower temperature range. Such behavior has been noted previously by Habgood (1967), who states that it is the difference in heat capacities between the adsorbed and gaseous states of the adsorbate that may cause the observed increase in heat of adsorption at lower temperatures. Whatever the cause, it appears from the very limited data that heats of adsorption as determined by gas chromatography

are dependent on temperature. In the 100–120° range there was no noticeable temperature dependence, so it may be that in the lower temperature range (65–100°) equilibrium was not reached. Such a situation might lead to curvature of the ln t_{net} or ln t_{corr} vs. $1/T$ plots. Further studies should be made to determine the exact nature of the temperature dependence.

Because in this study determinations of thermodynamic quantities depend on retention time and retention volume, and because retention data depend directly on the flow rate of the carrier gas, it is important to ascertain that the thermodynamic quantities as determined here are not functions of flow rate. In the general experiments a flow rate of 25 ml/min was used. For three compounds experiments were made in which each compound was studied at flow rates of 20, 25, and 30 ml/min. Though retention times vary widely with flow rate, as expected, the corrected retention volumes were remarkably constant for a given temperature, so that Gibbs energies of adsorption as derived from corrected retention volumes will not be dependent on flow rate. Heats of adsorption were observed to vary only slightly within experimental error, for different flow rates.

LITERATURE CITED

Berlin, E., U.S. Department of Agriculture, Washington, D.C., private communication, 1973.
Berlin, E., Anderson, B. A., Pallansch, M. J., *J. Dairy Sci.* **55**, 1396 (1972).
Breschia, F., Arents, J., Meirlich, H., Turk, A., "Fundamentals of Chemistry; A Modern Introduction", Academic Press, New York and London, 1966, p 297.
Gale, R. L., Beebe, R. A., *J. Phys. Chem.* **68**, 555 (1964).
Greene, S. A., Pust, H., *J. Phys. Chem.* **66**, 55 (1958).
Gregg, S. J., "The Surface Chemistry of Solids", Reinhold, New York and London, 1951, pp 17–44.
Habgood, H. W., in "The Solid-Gas Interface", Flood, E. A., Ed., Marcel Dekker, New York, N.Y., 1967, p 635.
Hinsch, D. M., Micromeritics Instrument Corp., Norcross, Ga. 30071, private communication, 1973.
Hoare, M. R., Purnell, J. H., *Trans. Farad. Soc.* **52**, 22 (1956).
Kiselev, A. V., Yashin, Y. I., "Gas-Adsorption Chromatography", translated by J. E. S. Bradley, Plenum Press, New York and London, 1969, pp 11–141.
Lee, I., Nickerson, T. A., Bernhard, R. A., *J. Dairy Sci.*, in press (1975).
Lim, S. G., Nickerson, T. A., *J. Dairy Sci.* **56**, 843 (1973).
Moore, W. J., "Physical Chemistry", 3rd ed, Prentice-Hall, Englewood Cliffs, N.J., 1962, pp 744–745.
Nickerson, T. A., Dolby, R. M., *J. Dairy Sci.* **54**, 1212 (1971).
Noller, C. R., "Chemistry of Organic Compounds", W. B. Saunders, Philadelphia, Pa., 1957, p 829.
Okamura, J. P., Sawyer, D. T., *Sep. Purif. Methods* **1**(2), 409 (1972).
Yabumoto, K., Jennings, W. G., and Pangborn, R. M., *J. Food Sci.*, in press (1975).

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Characterization of Phosphatase of Intact Maize Roots

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Conditions for optimum assay of intact maize (*Zea mays* L.) root phosphatase are described. *p*-Nitrophenyl phosphate was the most effective of all substrates tried. Phosphatase activity was linear up to 1.5 hr at 0.25 to 1.0 m*M*. At *p*-nitrophenyl phosphate concentrations above or below these linearity decreased sooner. Phosphatase activity was inhibited by *p*-nitrophenol above 0.36 m*M*, phosphorus above 0.26 m*M*, molybdenum above 0.02 m*M*, and aluminum above 0.37 m*M*. Calcium, magnesium, iron, and zinc at concentrations up to tenfold that of the original solution were not inhibitory. Optimum activity was obtained between pH 3 and 7 and at 35–50°. Fibrous roots had higher phosphatase activity than prop roots and 21-day-old roots had higher activity than younger or older roots.

Studies have shown phosphatase activities of intact roots may play a significant role in making nonavailable forms of phosphorus more available for plant use (Bieleski, 1973; Ridge and Rovira, 1971; Woolhouse, 1969). Considerable phosphatase activity in intact roots is located near root surfaces (Hall and Butt, 1968; Ridge and Rovira, 1969), especially under phosphorus deficiency conditions (Bieleski and Johnson, 1972; Reid and Bieleski, 1970). Phosphorus-deficient *Spirodela* hydrolyzed more glucose 1-phosphate in the growth medium than in the plant tissue (Bieleski and Johnson, 1972) and it was suggested that, under these conditions, the function of root phosphatase was to utilize P-esters in the growth medium. Thus, P-esters appear to be hydrolyzed by making contact with plant roots without being absorbed inside the root.

Phosphatase activity has generally been determined on tissue extracts or on purified preparations (Hollander, 1971). Information on phosphatase activities of intact roots is limited and many of the conditions for optimum activity have not been given (Bieleski and Johnson, 1972; Hall and Butt, 1968; Reid and Bieleski, 1970; Ridge and Rovira, 1971; Spencer, 1954; Woolhouse, 1969). The purpose of this study was to determine optimum conditions for phosphatase assay of intact maize roots.

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EXPERIMENTAL SECTION

Growth of Plants. The maize (*Zea mays* L.) inbred Pa36 was chosen for this study because of its use in other phosphorus nutrition studies (Clark and Brown, 1974). Plants were grown in full-strength nutrient solutions containing (m*M*) 2.6 Ca, 1.8 K, 0.6 Mg, 0.9 NH_4N, 6.9 NO_3N, 0.5 S, 0.5 Cl, 0.069 P, 0.007 Mn, 0.019 B, 0.002 Zn, 0.0006 Mo, 0.0005 Cu, and 0.038 Fe as Fe-hydroxyethylenediaminetriacetate (FeHEDTA). These nutrient levels were adequate to sustain optimum growth for 15 to 17 days. The plants were grown in a glasshouse with added light (19 klx, 30 cm from the source) in 18-l. plastic containers (4 plants/container).

Phosphatase Assay Solutions. Phosphatase assay solutions were full-strength nutrient solutions with added substrate (*p*-nitrophenyl phosphate) and treatment salts where necessary. Solutions were adjusted to pH 4.0 and