Complexes of Starchy Materials with Organic Compounds

Part I. Affinity Observed by Gas Chromatography*

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Estimations of affinities between starchy materials and various kinds of organic compounds are made by means of gas-solid chromatography. The starchy materials used are V-amyloses having helices of different diameters, an amorphous amylose (freeze-drying), amylopectin, and α - and β -cyclodextrins. From the results obtained it may be concluded that an attractive interaction between solid amylose and the vapor of organic compounds would occur in the interior of V-amylose helix and dipolar interaction would mainly be responsible for the complex formation of amylose with organic compound.

Numerous organic compounds were found to form complexes with starch (amylose).¹⁾ It may be considered that all of the compounds are included in the interior of amylose helices. However, the nature of the interaction between amylose and the complexing agents is still uncertain, and any quantitative description concerning the affinity of the agents with amylose can not be seen in literature. Many organic compounds of widely different types (polar and nonpolar compounds²⁾) show a similar selective precipitation ability for amylose, forming complexes, from an aqueous starch solution, while a slight difference in molecular structures of homologues results in their remarkably different abilities of the precipitation. The complexing reaction in the solution is much complicated because of many factors influencing the precipitation, *e.g.*, hydration of the complexing

agents, and solubilities of the agents and the complexes, *etc.* In order to simplify the reaction and test the affinities of the agents with starchy materials, a gas-solid chromatography using amylose as an adsorbent was carried out. The results obtained have previously been reported in brief.³⁾ This method will be expanded in this paper to investigate the affinities of a number of volatile organic compounds with several starchy materials, *i.e.*, amyloses with different helices, amylopectin, freeze-drying amylose and cyclodextrins (cyclamyloses, Schardinger dextrins).

THEORY

The theory of a gas-solid chromatography is based on the different affinities of vapors with solid. Assuming ideal adsorption equilibrium, we may derive the following equations.

An adsorption state in the chromatographic column may be expressed as

vapor phase	$C_g V_g$	$\Delta V \rightarrow$
solid phase	$C_a V_a \phi$	
$\leftarrow$ unit length $\rightarrow \leftarrow \Delta x \rightarrow$		

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1) T. J. Schoch, *Advances in Carbohydrate Chemistry*, **1**, 247 (1945); E. J. Wilson, Jr., T. J. Schoch and C. S. Hudson, *J. Am. Chem. Soc.*, **65**, 1380 (1943).

2) D. French, A. O. Pulley and W. J. Whelan, *Die Stärke*, **15**, 349 (1963).

3) T. Kuge and K. Takeo, *Agr. Biol. Chem.*, **31**, 257 (1967).

where C_g and C_a are the concentrations of sample in the vapor phase and an active adsorbent, respectively. V_g and V_a are the true volumes of vapor phase and solid phase in the column of unit length, respectively. ϕ is the volume fraction of the active adsorbent in the solid phase. Hence, the total amount of the sample, q , in the column of unit length, may be written as

$$q = C_g V_g + C_a V_a \phi + C_a' V_a (1 - \phi) \quad (1)$$

where C_a' is the sample concentration in inert solid and may be regarded as practically zero. When the sample zone moves by Δx with respect to unit length owing to the passage of carrier gas of the volume, ΔV , we can write

$$q \Delta x = C_g \Delta V \quad (2)$$

From equations (1) and (2)

$$\Delta x / \Delta V = 1 / (V_g + K V_a \phi) \quad (3)$$

where K is a distribution coefficient, *i.e.* C_a / C_g . The volume of the carrier gas required to move itself by Δx is $V_g \Delta x$. Hence, the ratio of the rate of the movement of the sample to that of the carrier gas is

$$(\Delta x / \Delta V) / (\Delta x / V_g \Delta x) = 1 / \{1 + K(V_a / V_g) \phi\} \quad (4)$$

The reciprocal of the ratio gives rise to the ratio of the retention volume of the sample, V_R , to that of the air (inert gas), V_0 , hence

$$V_R / V_0 = 1 + K(V_a / V_g) \phi \quad (5)$$

Correcting the dead space in the column,

$$(V_R - V_0) / V_0 = V_R' / V_0 = K(V_a / V_g) \phi \quad (6)$$

Therefore, there should be a linear relationship between the relative retention value, V_R' / V_0 , and ϕ with a slope of K , if an adsorption equilibrium is ideal.

EXPERIMENTAL

Materials

Amyloses. Amylose was isolated by *n*-butyl alcohol from potato starch by a similar method as described by Schoch⁴⁾ and was recrystallized five times. The amylose was dehydrated by washing with *n*-butyl alcohol repeatedly and dried over phosphorus pentoxide at 80°C in vacuum for several days. X-ray powder diagram of the amylose showed a V-pattern which indicated a close packing of helical amylose, and the spacings observed closely agreed to those given by

Rundle.⁴⁾ The diameter of the helix was evaluated to be 13.0 Å. This helix, accordingly, seems to consist of six glucose residues per turn and is denoted as 6₁-helix. Another type of amylose was also prepared from potato starch by *l*-menthone method,⁵⁾ and dehydrated by *tert*-butyl alcohol. Amylose crystal thus obtained, after drying at 80°C in vacuum for 20 hr, showed a X-ray diffraction pattern similar to that of *tert*-butyl alcohol-amylose complex reported by Zaslow.⁶⁾ Accordingly, the crystal may be packed with helical amylose whose diameter is 14.7 Å. One turn of this helix may consist of seven glucose residues, hence, the amylose crystal is denoted as 7₁-helix amylose.

Amylopectin. Amylopectin was precipitated by adding an equal volume of acetone to a remaining supernatant after a separation of amylose complex by the *l*-menthone method. The precipitate was successively washed with ethyl alcohol and ethyl ether, followed by drying in vacuum at 80°C. The amylopectin obtained showed only a halo in X-ray powder pattern indicative of its amorphous structure.

Freeze-drying amylose. Wet amylose obtained by *l*-menthone method was dissolved in water, and *l*-menthone was steam-distilled off. The solution was further boiled for two hours to achieve random configuration of the amylose in the solution.⁷⁾ The solution was then suddenly chilled, freeze-dried and lyophilized. The product had the similar X-ray diffraction pattern to that of amylopectin, indicating an amorphous structure of the amylose.

Cyclodextrins. Crude cyclodextrin was the gift of Dr. J. A. Thoma and fractionated into α - and β -dextrins. The dextrins were recrystallized from their respective aqueous solutions three times each, and used in the experiment after drying in vacuum at 80°C for 20 hr. The α - and β -dextrins has specific rotatory power of $[\alpha]_D^{25} = +152.0^\circ$ and $[\alpha]_D^{25} = +162.0^\circ$, respectively.

Cyclodextrin complexes. The complexes were prepared using the purified dextrins. The preparation of these complexes and their X-ray analyses will

4) R. E. Rundle and F. C. Edwards, *J. Am. Chem. Soc.*, **65**, 2200 (1943).

5) Presented at the Meeting of the Kansai Division of the Agricultural Chemical Society of Japan, Kyoto, June 24, 1967, and will be published.

6) B. Zaslow, *Biopolymers*, **1**, 165 (1963).

7) T. Kuge and S. Ono, *Bull. Chem. Soc. Japan*, **34**, 1264 (1961); T. Kuge, *Bull. Univ. Osaka Prefecture Series B*, **11**, 121 (1961).

be reported in detail elsewhere.⁸⁾

Reagents. The reagents were of the purest grade of commercially available and used without further purification.

Methods

Gas chromatography. Gas chromatograms were recorded using a Shimadzu type GC-2C gas chromatographic apparatus. Gas chromatographic column used was of 750 mm in length and 3 mm in diameter U type stainless-steel tube. Each of the adsorbents (amyloses, amylopectin, freeze-drying amylose, cyclodextrins and complexes of cyclodextrins) and teflon powder (Shimalite F, 30–80 mesh), which can serve as an inert solid, were thoroughly mixed in various proportions and packed in the column. Helium was used as a carrier gas at a constant flow rate of 20 ml per minute. A detector was a thermal conductivity cell. The column temperature was 80°C in this experiment unless otherwise mentioned. Retention volumes were measured changing injection volume (0.2, 0.4 and 0.8 μ l of each sample). Plotting the retention volume against the injection volume, an extrapolation to zero injection volume was made, which enables us to determine a retention volume indifferent to the injection volume. This might also minimize error in a retention volume due to an uncompletion of adsorption equilibrium. The retention volume thus obtained can be regarded as V_R appeared in the above theory.

X-ray diffraction measurements.⁸⁾ X-ray diffraction diagrams of powder were obtained by a Shimadzu GX-8 recording X-ray diffractometer with nickel-filtered Cu-K α radiation.

RESULTS AND DISCUSSION

According to the theory, the relative retention value, V_R'/V_0 , would increase with increasing amylose content in the solid phase if an attractive interaction would exist between amylose and testing compound. Relationships observed between the relative values of several compounds and the contents of 6₁-helix amylose in the column are representatively shown in Fig. 1. As can be seen from the figure, the values of *n*-butyl alcohol and *n*-amyl

FIG. 1. Relationship between Relative Retention Value and Amylose Content in Column.

Amylose, 6₁-helix amylose; Column temperature, 100°C.

alcohol increase exponentially with increasing amylose content (group 1). On the contrary to this, the relationships are almost linear with slopes of practically zero in the cases of *n*-hexane and cyclohexane (group 3). The remaining compounds presented in Fig. 1 show intermediate behavior. Hence, it seems that compounds might be classified into three groups according to their chromatographic behavior observed. Table I shows the classification of compounds examined. Group 1 consists of molecules having a polar group in addition to a predominant nonpolar portion, group 2 mainly aromatic compounds and halogenated hydrocarbons, and group 3 saturated hydrocarbons. When 7₁-helix amylose are used instead of 6₁-amylose, the situation is slightly changed, as seen in Fig. 2. There is no rapid exponential increase of the relative value even in the case of group 1 compounds.

Since the slope of the curve in these figures should be proportional to the distribution coefficient of a given compound between vapor and solid phases, the binding affinity of the compound to amylose can be reflected upon the slope. Even when the curve is not linear,

8) Presented at the Meeting of the Kansai Division of the Agricultural Chemical Society of Japan, Osaka, July 15, 1967, and will be published.

indicating an adsorption equilibrium is not complete, a qualitative argument may be possible from the slope. Consequently, it may be concluded that the vapor of nonpolar com-

pound can scarcely interact with solid amylose and the binding affinity (attractive interaction) would increase as polar or polarizable property of molecule increases.

Group 1 and group 2 compounds gave misshapen peaks having tails which became longer as the amylose content in the column was increased, in extreme case, it was impossible to determine the peak position because of its vigorous broadening, while group 3 compounds always gave sharp symmetric peaks (Fig. 3). These phenomena⁹⁾ may confirm the above conclusion.

In the case of a column containing amylopectin or freeze-drying amylose, both are amorphous, any of compounds tested did not show tailings in their chromatograms and their relative retention values were nearly constant almost independent of the content of such adsorbents. This fact seems to be suggestive evidence that the attractive interaction above observed would occur in the interior of amylose helix, in other words, the inclusion complexes of amylose and organic compounds would be formed in the chromatographic column. This opinion is further established by the following observations. The chromatography, in which α -dextrin, β -dextrin, α -dextrin-cyclohexane complex or β -dextrin-*n*-propyl alcohol complex was used as an active adsorbent, also indicated no appreciable interaction between these starchy materials and the reagents used. X-ray powder patterns of these complexes were found to be closely agreed with those of 6₁- and 7₁-helix amylose crystals, respectively, but such was not the case with α - and β -dextrins. Within the complexes, however, cylindrical cavities prepared presumably by stacking alignment of the dextrin molecules may be occupied with guest molecules. It seemed not unlikely that the complexes were so stable⁸⁾ that the guest molecules could not be replaced by the agent injected. On the contrary to

TABLE I. CLASSIFICATIONS OF ORGANIC COMPOUNDS BY THEIR CHROMATOGRAPHIC BEHAVIOR

Adsorbent: 6 ₁ -helix amylose		
group 1	group 2	group 3
<i>n</i> -butyl alc.	<i>tert</i> -butyl alc.	<i>n</i> -hexane
<i>n</i> -amyl alc.	○ benzene	isopentane
isoamyl alc.	× toluene	○ cyclohexane
cyclohexanol	<i>o</i> -xylene	○ cyclopentane
cyclohexanone	× <i>m</i> -xylene	○ cyclohexene
	× <i>p</i> -xylene	○ methylcyclohexane
	× mesitylene	○ carbon tetrachloride
	× chlorobenzene	○ isopropylbromide
	○ chloroform	○ isoheptane
	○ 1,1,2,2-tetrachloroethane	
	isooctane	
	α -pinene	

○: Hydrophobic compound which complex amylose.

×: Hydrophobic compound which does not complex amylose, according to French *et al.*, 1963.²⁾

FIG. 2. Relationship between Relative Retention Value and Amylose Content in Column.

Amylose, 7₁-helix amylose; Column temperature, 80°C.

9) J. H. Knox, "Gas Chromatography", Methuen and Co. Ltd., 1962, p. 41.

FIG. 3. Change of Gas-chromatogram Caused by Changing Amylose Content in Column.

Upper chromatograms are of *n*-butyl alcohol and lower of cyclohexane. Experimental conditions: 7₁-helix amylose; column temperature, 80°C; injection volume, 0.4 μ l; carrier gas, helium; flow rate, 20 ml/m. Amylose content; (a), 0%; (b), 3.1%; (c), 6.1%; (d), 11.0%; (e), 17.0%. The marks, $\downarrow$, show injection points.

this, the β -dextrin complex, after 20 hr evacuation at 100°C, showed an appreciable binding affinity for group 1 and 2 compounds, which were demonstrated by the broadening of the sample peaks. The evacuation resulted in 5% decrease in weight of the complex presumably due to partial removal of *n*-propyl alcohol, leaving void cavity through the dextrin molecules, since the complex structure remained mostly unchanged which was ascertained by X-ray analysis.

In this study all the starchy materials are solid and may possess no freedom to change their configuration, so appropriate spatial conditions may be of critical importance in the complex formation. Then it may be safely mentioned that the complexation does not take place unless the void cavity of suitable length has been ready by an arrangement of host molecules.

Several possibilities should be taken into account for the forces that stabilize the inclusion complex, namely, hydrophobic bonding, hydrogen bonding, dipolar interactions,

dispersion forces, and charge transfer. Among these, the authors incline to think that the complexation would be mainly due to dipolar cooperative interaction such as proposed by Stein and Rundle¹⁰ for amylose-iodine complex, since a regular alignment of host molecules may be considered to be indispensable to the complexation from the present investigation, the alignment may produce dipolar field in the cavity, and the fact that the more the polar character of guest molecule is, the more the binding affinity, can be seen. The result that the increase of the diameter of amylose helix is accompanied with a decrease of the binding affinity in the case of group 1 compounds, may also be explained by the proposition of this kind of force. For *tert*-butyl alcohol, the reverse correlation between the cavity diameter and the affinity strength are seen. This may be interpreted by supposing the possible molecular-sieve effect of amylose

10) R. S. Stein and R. E. Rundle, *J. Chem. Phys.*, **16**, 195 (1948).

helix. The molecular shape of *tert*-butyl alcohol may be rather sphere whose diameter is 4.7 Å more or less, while the cavity available in 6₁-helix may be considered to be 4.5 Å or so in diameter.¹¹⁾

In Table I are also shown hydrophobic compounds which possess precipitation abilities for amylose from aqueous solutions, data are quoted from the results of French *et al.*²¹ It seems worthy to be again noticed that those belonging to group 3 have no appreciable affinity for solid amylose. It is obvious that the failure of the complexation can not be ascribed to their too large size to fit the cavity, since a large molecule such as cyclohexanol can strongly interact with amylose. In aqueous solutions, amylose molecules may be more or less helical,^{7,12)} and complexing agents may be surrounded by water molecules. The interior of the helical amylose may be hydrophobic in nature rather than the water en-

vironment. Under such conditions, it may be possible that hydrophobic compounds would enter preferentially into the amylose helices to fulfil energetic requirements. Further thermodynamic study and argument may be needed to elucidate completely the complexing process in an aqueous solution. However, direct interaction between helical amylose and vapor of the complexing agents can be inferred from this study, remembering an earlier assumption¹³⁾ that the complexing agent should have a polar group, otherwise the complex could not be formed.

It is to be regretted that a numerical treatment as to the complexing affinity has thus far been impossible because of the nonideal adsorption isotherm in the chromatographic column. In this meaning, an extensive study concerning adsorption isotherm of solid amylose are strongly desired in future.

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11) W. J. James, D. French and R. E. Rundle, *Acta Cryst.*, **12**, 385 (1959).

12) J. Holló and J. Szejtli, *Die Stärke*, **10**, 49 (1958); V. S. R. Rao and J. F. Foster, *Biopolymers*, **1**, 527 (1963).

13) R. S. Bear, *J. Am. Chem. Soc.*, **66**, 2122 (1944).