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On the Asymmetric Interaction of the Optical Antipodes with Amylose

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The asymmetric adsorption of D, L-mandelic acid and its homologues has been demonstrated on amylose by means of the column chromatographic and static techniques. The D-(−)-isomer of mandelic acid has been found to be adsorbed more weakly than the L-(+)-isomer; the separation factor of the two antipodes has been estimated to be 1.09 at 0°C. This value is greater than that on starch, suggesting that amylose may have a somewhat higher selectivity for the asymmetric adsorption of the optical antipodes than amylopectin. The anomalous interaction of D, L-atrolactic acid has been observed on the amylose much as in the case with starch reported previously.

It has been demonstrated,[1,2] that the optical antipodes of mandelic acid and its homologues are selectively adsorbed on starch. Since starch is composed of amylose and amylopectin, one question of interest would be which constituent is responsible for the asymmetric interaction. Another point of interest is what modes of selectivity are found in the interaction of the optical antipodes with these starch constituents.

In the present report, the authors will describe the asymmetric adsorption of D, L-mandelic acid and its homologues on amylose. The distinct difference in the adsorption affinity will be revealed between the two optical antipodes by means of the column chromatographic and static techniques. Some information concerning the asymmetric adsorption on starch will then be deduced from the results obtained.

Experimental

The Preparation of the Adsorbent.—The following route of pretreatment was taken in order to obtain the water-insoluble amylose. Commercially-available amylose was immersed in distilled water at room temperature for 24 hr. The precipitates were filtered and repeatedly washed with distilled water until the optical rotation due to water-soluble fractions of the adsorbent completely disappeared from the mother liquor. Water-insoluble precipitates were obtained and then dried in air at room temperature. The amylose sample thus obtained was 20.1% moisture. This sample was characterized in an anhydrous state by measuring the limiting viscosity number, $[\eta]$, in a dimethyl sulfoxide solution at 25°C using an Ubbelohde-type viscometer.[3] The $[\eta]=0.257\ \overline{P}_w^{0.82}$,[4] relation, was employed to calculate the mean degree of polymerization, $\overline{P}_w$, from which the viscosity-average molecular weight was estimated to be about 210000.

Chromatographic Procedure.—The compounds subjected to the chromatographic separation were as follows; DL-mandelic acid, DL-methyl mandelate, DL-ethyl mandelate and DL-atrolactic acid.

Each racemate (1—2 g.) in 50% aqueous methanol was put on an amylose column (1.6×42 cm.) at room temperature and then eluted with the same solvent. The flow rate was 4.0 ml./min. The optical rotation of each fraction (1.6 ml.) was determined by a Zeiss-Kreis polarimeter, and the concentration of mandelic acid was determined by the optical absorption at 257 mμ. In the case of DL-atrolactic acid, the optical rotation in each fraction was too small to affirm the asymmetric interaction. Therefore, some enriched L-(+)- or D-(−)-isomer samples were subjected separately to chromatographic separation.

The Determination of Adsorption Equilibrium.—3.5 g. of the pretreated amylose was immersed in 30 ml. of a DL-mandelic acid solution. The system was allowed to stand at 0°C for 48 hr., by which time equilibrium had been attained. The supernatant liquid drawn off was then analyzed. The optical rotation of the solution was measured by a Yanagimoto Model OR-20-type photomagnetic polarimeter, and the concentration of the acid was determined by the optical absorption, as has been mentioned already.

Results

The asymmetric adsorption on amylose has been distinctly revealed by the column chromatography. Figures 1 and 2 show the specific rotation, $[\alpha]_D$, of mandelic acid and its homologues with respect to each fraction of the effluent. The observed angle of rotation, α_D, and the concentration of the adsorbates, c, in g. per 100 ml. of an aqueous methanol solution are given for some of the fractions.

It is apparent that (−)-isomers of mandelic acid and its esters were eluted first, followed by (+)-isomers. On the other hand, the order of elution was reversed with DL-atrolactic acid. The same situation was observed when starch was used as

1) M. Ohara, K. Ohta and T. Kwan, This Bulletin, **37**, 76 (1964).
2) M. Ohara, C.-Y. Chen and T. Kwan, ibid., **39**, 137 (1966).
3) W. W. Everett and J. F. Foster, *J. Am. Chem. Soc.*, **81**, 3464 (1959).
4) W. Burchard, *Makromol. Chem.*, **64**, 110 (1963).

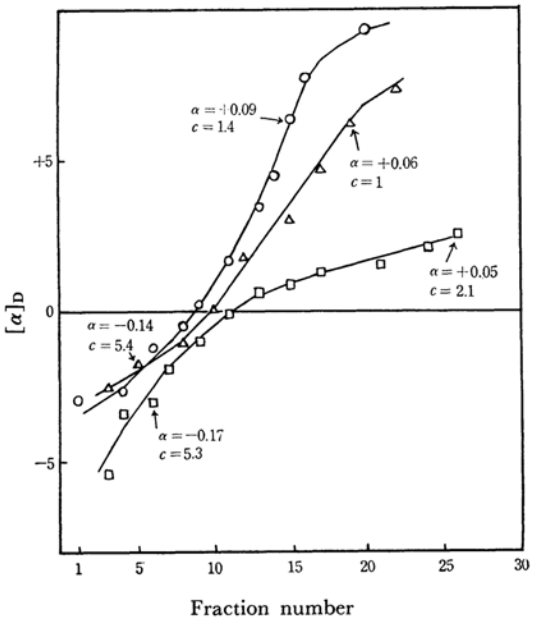

Fig. 1. The specific rotation $[\alpha]_D$ of mandelic acid and its esters against fraction numbers.
□ Mandelic acid 2.0 g.
○ Methyl mandelate 1.0 g.
△ Ethyl mandelate 1.0 g.

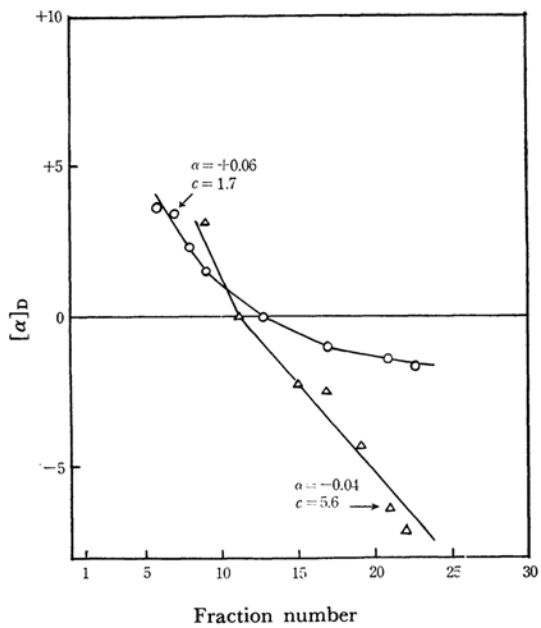

Fig. 2. The specific rotation $[\alpha]_D$ of atrolactic acid against fraction numbers.
○ (+)-enriched sample
△ (−)-enriched sample

the adsorbent.[1] This behavior of the enantiomers on amylose indicates that there is a parallel relationship between adsorption affinity and configuration, since optical isomers with the same sign can be assigned the same configuration for mandelic acid and its esters.[1] The adsorption affinity of D, L-atrolactic acid may be considered to be reversed on the basis of the generally-established theory that optical isomers of mandelic acid and atrolactic acid with the same sign can be assigned to the same configuration.[5]

Table I lists typical adsorption equilibrium data obtained by a static method. The adsorption data on starch[2] are also shown for the sake of comparison.

Table I. Adsorption equilibrium data of D, L-mandelic acid on amylose and starch (T=0°C)

Absorbent	Amylose	Starch
Amount of adsorbent, g.	3.5	9.0
Initial concentration of D, L-mandelic acid, C_i, mg./ml.	100.0	100.3
Equilibrium concentration of D, L-mandelic acid, C_e, mg./ml.	95.9	91.8
Amount of D, L-mandelic acid adsorbed, W, mg./g.	64	57
Optical rotation of the solution α_D	−0.036	−0.060
Specific rotation of the solution $[\alpha]_D$	−0.375	−0.66
$\Delta W/W$ %	3.8	2.6
Separation factor f	1.09	1.06

The values of the optical rotation described here were ascribed to the net rotation values of mandelic acid in the solution, which were determined by using a glycolic acid solution (concentration: 143 mg./ml.), as has been reported previously.[2] From these negative values, it may be inferred that the D-(−)-isomer is less adsorbable than the L-(+)-isomer. This finding is consistent with those revealed by the column chromatography. The amount of D, L-mandelic acid adsorbed per gram of amylose, W, was calculated much as that absorbed on starch[2] by assuming that the amounts of water bound to the two adsorbents were of the same magnitude. ΔW is the excess amount of the (+)- over the (−)-isomer adsorbed, so that $\Delta W/W$ may indicate the degree of the asymmetric adsorption of the optical antipodes. The separation factor, f, is defined by the ratio of the adsorption equilibrium constants of the two enantiomers and is expressed as:

$$f = \frac{W_d/C_{ed}}{W_l/C_{el}}$$

where W_d and W_l are, respectively, the amounts of the L- or D-isomer adsorbed, while C_{ed} and C_{el} are the concentrations of the L- and D-isomers,

5) See Footnotes 3—6 in a previous article by Ohara et al.[1]

respectively, in the liquid phase at equilibrium. It was found to be greater on amylose than on starch.

Discussion

It is interesting to note that amylose possesses the same mode of selectivity as starch for the asymmetric adsorption of the optical antipodes.

Separate static experiments[6] similar to that on amylose have been made using potato amylopectin and starch as the adsorbents in the methanol phases. In these cases, the asymmetric adsorption of D, L-mandelic acid has also been revealed on both adsorbents, and the orders of the adsorption affinity for the enantiomers have been shown to be as follows:

$$\text{L-}(+)\text{-isomer} > \text{D-}(-)\text{-isomer}$$

Accordingly, for the enantiomers of mandelic acid, it can be concluded that the modes of selectivity on amylose and amylopectin are identical with that on starch.

Now, let us consider which constituent of the starch is more responsible for the asymmetric adsorption of D, L-mandelic acid. It is generally accepted that amylose and amylopectin constitute 20—25% and 75—80% portions of the starch granule respectively.[7] By considering that the degree of the asymmetric adsorption, $\Delta W/W$, on amylose and on starch are 3.8% and 2.6% respectively, and by assuming that the amylose portion in the granular starch possesses the same $\Delta W/W$ value as the amylose employed in this experiment, the $\Delta W/W$ value ascribed to the amylopectin portion may be estimated as 2.2—2.3%. It is suggested, therefore, that amylose may have a somewhat higher selectivity for the asymmetric adsorption of D, L-mandelic acid than amylopectin. However, no large difference in selectivity can be seen between that on amylose and that on amylopectin. These facts may be explained from the fact that there is not essentially a large difference in structure between amylose and amylopectin, although amylose (with a linear-chain structure) has a somewhat more regular structure than amylopectin (with a branched-chain structure).

Finally, we want to refer to the anomalous interaction of amylose with atrolactic acid. This phenomena cannot be found in β-cyclodextrin, which is known to be a cycloheptaose connected with α-1, 4 glucosidic linkage. It was found[8] that β-cyclodextrin builds inclusion compounds stereospecifically with a series of mandelic acid homologues. Both in the case of ethyl mandelate and in the cases of atrolactic acid and ethyl atrolactate, (−)-isomers were proved to be more strongly interacting than (+)-isomers. Cramer and Dietsche elucidated the parallelism between the affinities of the two enantiomers to β-cyclodextrin and their configurations in terms of the generally-established theory,[5] as has been mentioned already.

However, this is not the case with amylose. This may be explained from the fact that the structure of β-cyclodextrin is fixed, while that of amylose is flexible.[9]

Consequently, it may be inferred that, in the case of β-cyclodextrin, the normal interaction of D, L-mandelic acid homologues can be observed. On the other hand, in the case of amylose, the adsorption structures of the optical isomers of mandelic acid are probably different from those of atrolactic acid.

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7) J. Nikuni, "Starch Handbook" (Denpun Handbook), Ed. by J. Nikuni, Asakura Pub. Co., Tokyo (1960), Chapter 1, p. 54.

8) F. Cramer and W. Dietsche, *Chem. Ber.*, **92**, 378 (1959).

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