TAMARIND SEED JELLOSE[1] : FERMENTATIVE DEGRADATION

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It has already been reported by Rao, Ghose and Krishna[2] that tamarind seed jellose undergoes complete hydrolysis when boiled with 5 per cent. sulphuric acid for 4 hours, yielding xylose, glucose and galactose. Experiments on the fermentative degradation of the jellose under the influence of fungus have now been carried out, with a view to obtaining the probable structural unit of which the jellose molecule is built.

The fungus is initiated by exposing a solution of the jellose to air for a few moments. After incubation it develops on the surface of the solution into a greenish-grey velvety mat consisting mostly of *Cladosporium herbarum* (Pers.) Link. The colony, after establishment in malt agar, is used as inoculum for subsequent experiments.

Under the action of this culture, an aqueous solution of tamarind seed jellose undergoes fermentative degradation. Amongst the degradation products is found a hexasaccharide. This carbohydrate, which may be called *Tamarindose*, differs in its properties from the original jellose as shown below:—

	The original jellose	The degradation product
Appearance ..	Amorphous	Microcrystalline
Solubility :		
(*a*) Water ..	Colloidal solution	Clear solution
(*b*) 70% alcohol ..	Insoluble	Soluble
Specific rotation ..	+71·4°	+73·8°
Fehling's solution ..	Blue gelatinous precipitate	Reduced to cuprous oxide
Iodine ..	Blue gel in strong solutions ; pale yellow on dilution	No apparent action
Borax ..	A thick jelly	No action
Sugar ..	A firm jelly	No action
Sugar and citric acid ..	A firm jelly	No action
Molecular weight ..	Very high	About 975

The carbohydrate nature of the compound is indicated by its response to the characteristic Dreywood reaction by developing a green colour on treatment with anthrone and sulphuric acid.[3] It is a reducing carbohydrate

and its reducing power of the Fehling's solution corresponds to that of a hexasaccharide. It undergoes complete hydrolysis when boiled with 4 per cent. sulphuric acid for 3 hours and yields 107·2 per cent. of reducing sugars expressed as glucose. The hydrolysate contains only xylose, galactose and glucose, and quantitative estimations show that the first two sugars are 30·0 and 16·7 per cent. respectively, so that glucose comes to 53·3 per cent. by difference. These percentages approximately correspond to the molecular proportion of 2 of xylose, 1 of galactose and 3 of glucose. The molecular weight is determined by employing Kiliani reaction, modifying slightly the procedure recommended by Militzer for quantitative estimation of sugars,[4] and, though slightly higher, it seems to offer support to the hexasaccharide formula. It appears, therefore, that tamarindose is a hexasaccharide composed of xylose, galactose and glucose in the molecular proportion of 2 : 1 : 3.

The hexasaccharide undergoes acetylation easily when boiled with acetic anhydride and anhydrous sodium acetate, and the product which is freely soluble in acetone, alcohol, chloroform, acetic acid, etc., has a specific rotation of 43·6° in methyl alcoholic solution at 30° C.

The question of the relative proportion of the constituting sugars in the original jellose has also been reexamined now. From the specific rotation of the hydrolysate it was originally suggested by Rao, *et. al.*[2] that the sugars might be present in equimolecular proportion, while Savur and Sreenivasan[5] concluded from quantitative estimations that xylose, galactose and glucose corresponded approximately to the molecular proportion of 2 : 1 : 3. The percentage of xylosan had already been reported to be 30·5 from these laboratories.[2] Galactan has now been estimated and is found to be 17·2 per cent. Making calculations on the basis of pure anhydrous jellose, the glucosan in it comes by difference to be 52·3 per cent. These results are in agreement with those reported by Savur and Sreenivasan.[5]

EXPERIMENTAL

Preparation of tamarindose.—A 2 per cent. solution of tamarind seed jellose was sterilised for 15 minutes at 15 lbs. pressure, and when cooled the sterile solution was exposed to air for a few moments during the month of June, when the local maximum and minimum temperatures were respectively 100° F. and 75° F. and the relative humidity was 50 per cent. After incubation for about a week at 30° C., a greenish-grey velvety mat appeared on the surface of the solution. The colony which was found to consist mostly of the hyphæ and spores of *Cladosporium herbarum* was established in a culture medium consisting of 2·5 g. of malt and 2·0 g. of agar in 100 c.c.

of distilled water. This culture was used as inoculum for subsequent experiments.

For the isolation of tamarindose, a litre of a 2 per cent. solution of crude tamarind seed jellose was sterilised as before, and was inoculated with the fungus colony. It was kept in an incubator at 30° C. During the first 2 or 3 days, a sediment settled down at the bottom of the flask and the fungus which grew very rapidly spread itself into a mat on the surface of the solution. The latter became thinner and thinner as the fermentation progressed and there was also a fall in the pH at the rate of 0·1 per day. When the pH value fell to 4·9, which happened in about 2 weeks, the solution became quite watery and transparent. It was then filtered through fluted filters and concentrated on a water-bath to about 200 c.c. It was then boiled with a little animal charcoal in order to remove the colloidal impurities still present and also the small amount of colour that developed during concentration. The clear solution was then treated with 3 times its volume of 95 per cent. alcohol, filtered, and distilled under vacuum. When the solution assumed a syrupy consistency, it was transferred into a porcelain basin and the evaporation continued in a vacuum desiccator. For purification, a concentrated aqueous solution of the solid was treated with twice its volume of absolute alcohol, filtered, and concentrated as before in vacuum. For crystallization a concentrated aqueous solution of the compound was treated with absolute alcohol till opalescence appeared, filtered and then kept in a stoppered flask, when solid separated out gradually as microcrystalline rectangular plates. From 20 g. of the crude jellose, 8 g. of tamarindose were obtained.

The carbohydrate was freely soluble in water and 70 per cent. alcohol, moderately soluble in glacial acetic acid and insoluble in absolute alcohol. On heating it shrank and sintered at 100–10° C. (dehydration) and the anhydrous material decomposed at 228–30° C. It was more insipid than sweet in taste. (Found in the sample dried at 105° C. under vacuum: C, 43·90; H, 6·80; $C_{34}H_{58}O_{29}$ requires C, 43·87; H, 6·24 per cent.) The crystals seemed to possess 6 molecules of water of crystallization. (Loss on heating at 105° under vacuum: 10·8; $C_{34}H_{58}O_{29}$, $6\,H_2O$ requires a loss of 10·4 per cent. on dehydration.) Its aqueous solution was dextrorotatory, the specific rotation, $[\alpha]_D^{30°}$, being 73·8°. It reduced Fehling's solution, 100 mg. liberating 38 to 40 mg. of cuprous oxide.

Hydrolysis.—1 g. of a purified sample of tamarindose was heated for 3 hours with 50 c.c. of 4 per cent. sulphuric acid at the temperature of boiling water bath. After cooling the clear solution was neutralized with sodium

carbonate, the volume made upto 100 c.c. and the reducing sugars were estimated. Calculated as glucose, the sugars totalled 107·2 per cent. on the basis of anhydrous material.

The only sugars that could be identified in the hydrolysate were xylose, galactose and glucose. When a neutral solution was treated with phenyl hydrazine in the usual way, a mixture of the osazones separated out, the first fraction being xylosazone and the last fraction galactosazone. The presence of xylose was confirmed by the preparation of the characteristic dibenzylidene dimethyl acetal derivative according to the method of Breddy and Jones.[6] A small quantity (0·5 g.) of the perfectly dry product of hydrolysis was treated in a dry flask with 10 c.c. of a reagent prepared by dissolving benzaldehyde (40 c.c.) in a mixture of 2·6 N methanolic hydrogen chloride (20 c.c.) and anhydrous methanol (120 c.c.). The mixture was left at the laboratory temperature with frequent shaking. The substance went into solution during the course of 2 days and then the derivative began to separate out gradually. The deposition was complete after the 7th day. It was filtered, washed successively with water (200 c.c.) and methanol (40 c.c.). It was obtained as narrow rectangular plates, some of them tapering at the ends, and melted at 210–11° C. Mixed melting point with an authentic sample prepared from pure xylose was undepressed.

Xylose content.—The xylose content was estimated according to the method of Krober as modified by Angell, Norris and Resch[7]. 0·5 g. of tamarindose was taken and distilled with 100 c.c. of 12 per cent. hydrochloric acid over a glycerol bath at 175–80° C. More of 12 per cent. hydrochloric acid was added gradually so that the original volume was maintained as the distillation continued. 360 c.c. of the distillate were collected in about 2 hours, filtered and then treated with 0·5 g. of phloroglucinol dissolved in hydrochloric acid. The precipitate was allowed to stand overnight, filtered in a glass-sintered crucible and weighed after drying. From the amount of the phloroglucide obtained, the xylose content was calculated and found to be 30·0 per cent.

Galactose content.—The galactose content was estimated according to the method of Tollens.[8] 5 g. of tamarindose were treated with 50 c.c. of nitric acid of sp. gr. 1·15 in a beaker, 6 cm. in diameter, and the solution was concentrated to one-third of its volume on a water bath. After cooling, the solution was diluted with 100 c.c. of water and then seeded with 0·5 g. of pure mucic acid to facilitate the crystallization of the mucic acid formed during the oxidation. After 2 days' standing with occasional stirring the solid that separated out was collected on a weighed filter, washed twice

with 5 c.c. of cold water, dried at 100°C. and weighed. 0·57 g. of mucic acid was obtained which was equivalent to 0·74 g. of galactose. Hence the galactose present in tamarindose on the basis of anhydrous material was 16·7 per cent.

Molecular weight estimation.—The carbohydrate (2 g.) was dissolved in 18 c.c. of 0·1 N acetic acid in a glass-stoppered 250 c.c. Erlenmeyer flask and treated with 20 c.c. of 0·2 N potassium cyanide solution. The reaction flask was immediately stoppered, sealed with glycerol and kept in a cool place for 24 hours. After this period the stopper was carefully removed, and about 20 c.c. of 6 N ammonium hydroxide were added. The stopper was replaced for a few minutes and the flask shaken well for a good mixing. After adding 0·2 g. of potassium iodide the solution was titrated to the first perceptible turbidity with 0·1 N silver nitrate. A blank was also run simultaneously. The difference in the titre values between the blank and the sample experiment multiplied by 13 represented the number of milligrams of potassium cyanide consumed by the carbohydrate, and it came to be 118·4 milligrams. Hence the molecular weight of the carbohydrate would be 975 nearly. A hexasaccharide composed of two molecules of xylose, one of galactose and three of glucose, ($C_{34}H_{58}O_{29}$), requires 930 for its molecular weight.

Acetyl tamarindose.—Tamarindose underwent easy acetylation when treated with acetic anhydride and anhydrous sodium acetate for 4 hours at the temperature of water-bath. The acetyl derivative was soluble in methyl and ethyl alcohols, acetic acid, acetone, etc., but heating with hydroxy solvents like alcohol and acetic acid brought about its decomposition. For purification the substance was dissolved in chloroform and the solution was treated with petroleum ether in drops. All the impurities separated out first as a brownish oily liquid. After the removal of the oily liquid, the chloroform solution was further diluted with petroleum ether, when acetyl tamarindose separated as a colourless fluffy precipitate. For crystallization the dry acetate was dissolved in hot acetic anhydride and the solution treated in the cold with an equal amount of absolute alcohol. After two days the acetate separated as a microcrystalline powder. On heating it sintered at 154° C., became glassy as the temperature was raised and decomposed at 172–74° C. In methyl alcoholic solution its specific rotation was 43·6° at 30° C. (Found: C, 50·37; H, 5·98; $C_{34}H_{40}O_{11}$ $(OCOCH_3)_{18}$ requires C, 49·82; H, 5·58 per cent.) The acetyl group was estimated according to the method of Eberstadt.[9] (Found: $-COCH_3$, 46·45; $C_{34}H_{40}O_{11}$ $(OCOCH_3)_{18}$ requires $-$ $COCH_3$, 45·91 per cent.)

Galactan content of the jellose.—The estimation was carried out according to the method of Tollens as described under tamarindose. The weight of mucic acid formed multiplied by 1·197 gave the amount of galactan. From 5 g. of the pure dry material 0·72 g. of mucic acid was obtained so that the jellose contained 17·2 per cent. of galactan.

SUMMARY

Tamarind seed jellose undergoes fermentative degradation in aqueous solution yielding a hexasaccharide which is composed of xylose, galactose and glucose in the molecular proportion of 2:1:3. The carbohydrate, which may be named Tamarindose, decomposes at 228–30° C. and has a specific rotation of + 73·8° at 30°. Its acetate melts with decomposition at 172–74° and has a specific rotation of + 43·6° in methyl alcoholic solution at 30°.

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