

TAO dissolves readily in *N*/10 hydrochloric acid (a concentration corresponding to that found in normal gastric juice), but neither gastric juice nor *N*/10 hydrochloric acid could be used as solvents of TAO for assays of sensitivity, since these reagents alone produce narrow zones of inhibition of bacterial growth and may cause considerable alteration and discoloration of complex media.

A series of tests with a wide range of hydrochloric acid concentrations showed that *N*/100 hydrochloric acid had no effect on media or micro-organisms, and dissolved TAO satisfactorily. To provide a margin of safety, however, *N*/200 hydrochloric acid is recommended as the solvent for TAO in sensitivity assays.

Triacetyl-oleandomycin requires an acid medium for its solubilization, and because of this one may speculate whether the speed of absorption and effectiveness of this antibiotic could be increased by dissolving it beforehand or supplementing it with dilute hydrochloric acid during its oral administration, especially in patients with achlorhydria or hypochlorhydria.

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Crystalline α -Kojibiose

KOJIBIOSE (2-O- α -D-glucopyranosyl-D-glycopyranose) was first detected in saké and its moromi¹, and it was isolated from hydrol², koji extract³ and honey⁴ as its crystalline α - and β -octaacetates. Chemical synthesis^{5,6} of this sugar was also achieved. But the crystallization of free sugar was hitherto unsuccessful, so its properties such as melting point, specific rotation or its form of crystal were still unknown. We have now succeeded in crystallizing kojibiose and examining its properties.

The same method as reported previously⁷ was used for preparation of the kojibiose; but in this work the culture medium of *Leuconostoc mesenteroides* contained sucrose (9 per cent) and lactose (15 per cent) instead of sucrose (2 per cent) and lactose (10 per cent), and then a branched trisaccharide (O- β -D-galactopyranosyl-(1 \rightarrow 4)-O-[α -D-glucopyranosyl-(1 \rightarrow 2)]-D-glucopyranose) and lactosucrose^{8,9} (O- β -D-galactopyranosyl-(1 \rightarrow 4)-O- α -D-glucopyranosyl-(1 \rightarrow 2)-D- β -fructofuranoside) were obtained together. After removal of most of the lactose and dextran by addition of an equal volume of ethanol, the supernatant was concentrated and fractionated by carbon column chromatography. 95 gm. of lactosucrose and 140 gm. of the branched trisaccharide contaminated with a small amount of lactosucrose were obtained from 15 litres of the culture medium. The contaminated lactosucrose in the branched trisaccharide fraction was hydrolysed to lactose and fructose with 0.5 *N* sulphuric acid at 60° C. for 30 min. and was removed by carbon column chromatography. 97 gm. of the branched trisaccharide was obtained. 10 gm. of the lactase preparation of *Saccharomyces fragilis* (kindly supplied by Dr. Henry Roberts) and 90 gm. of the above branched trisaccharide were incubated in 1 litre of phosphate buffer (*pH* 6.2), and 48 gm. of amorphous kojibiose was obtained by carbon column chromatography.

This crude kojibiose was dissolved in 200 ml. of hot methanol, and the solution was refluxed gently, followed by filtration of the insolubles on 'Celite', and was left to cool at room temperature and then

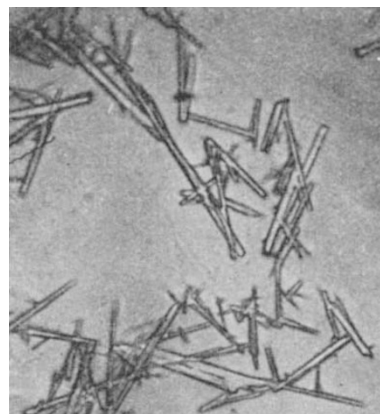


Fig. 1

the kojibiose crystallized. 30.5 gm. of crystalline free sugar was obtained. Recrystallization was carried out by dissolving 10 gm. of the above crystals in 15 ml. of hot water and adding 35 ml. of methanol after being decolorized with charcoal. The crystals obtained took the form of fine prisms (Fig. 1). On drying in vacuum at 100° C. for 2 hr. over phosphorus pentoxide, crystalline kojibiose, which was somewhat hygroscopic, showed a melting point of 187–88° C. (uncorrected) and $[\alpha]_D^{18} = +162^\circ \rightarrow 137^\circ$ (17 hr.) (c., 2.1 in water) (calculated for C₁₂H₂₂O₁₁: C, 42.11; H, 6.48 per cent; found: C, 42.22; H, 6.48 per cent). The downward mutarotation showed that this crystalline kojibiose was α -isomer. This fact was further confirmed by formation of its acetyl derivative. Namely, only α -kojibiose octaacetate (m.p. 166° C.) was obtained when the acetylation was carried out at a low temperature by using pyridine as catalyst, while both α -kojibiose octaacetate (m.p. 166° C.) and β -kojibiose octaacetate (m.p. 117° C.) were obtained by the usual method using sodium acetate. Neither acetate showed depression of melting point on admixture with the authentic samples synthesized chemically by Matsuda⁵.

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PHYSIOLOGY

Plastic Changes of Spinal Monosynaptic Responses from Tenotomized Muscles in Cats

In an attempt to reduce muscle afferent activity, the tendons of some hind-limb muscles of cats were cut. It was postulated that this would prevent the development of effective tension, reduce passive stretching, and so induce relative disuse of the central synaptic connexions of the muscle afferent nerves.