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A simple chemical synthesis of β-methylfructofuranoside, a β-fructofuranosidase substrate

A number of authors have reported the synthesis of β-methylfructofuranoside, but most of these preparations were proven to be syntheses of the "γ" variety, namely, a mixture of the α and β stereoisomers (AUGESTAD *et al.*[1]). The first preparation of the pure β isomer was reported by SCHLUBACH AND BARTELS[2]. Enzymic synthesis of this compound employing β-fructofuranosidase (invertase, EC 3.2.1.26) has been reported by BACON[3] and ISHIZAWA AND MIWA[4]. The chemical synthesis described below results in a relatively high yield and is rapid and simple.

Three solvent systems were used in the identification of the products by paper chromatography: Solvent 1: *n*-butanol–glacial acetic acid–water (4:1:5, v/v); upper phase; Solvent 2: *n*-propanol–1 N NH_4OH (7:2, v/v); Solvent 3: *n*-butanol–pyridine–water (5:5:2, v/v).

After development, the air-dried chromatograms were sprayed with a solution of benzidine (0.4 %) in glacial acetic acid and heated at 80° for 15 min, which rendered the reducing sugars visible. Following this, the chromatograms were sprayed with 0.1 N H_2SO_4 in 90 % ethanol and were again heated for 15 min, which cleaved the non-reducing fructosides, and resulted in their detection. The chromatographic behavior of the relevant compounds in three solvent systems is presented in Table I.

In the quantitative determination of β-methylfructofuranoside, oxalic acid hydrolysis was used as described by BACON AND BELL[5]. The reducing sugar resulting from the hydrolysis was measured by the method of SOMOGYI[6]. The highly purified β-fructofuranosidase used in the hydrolysis was prepared by the method of METZENBERG[7].

TABLE I

R_F VALUES OF ENZYMICALLY AND CHEMICALLY SYNTHESIZED β-METHYLFRUCTOFURANOSIDE IN DIFFERENT SOLVENT SYSTEMS

	Solvents		
	No. 1	*No. 2*	*No. 3*
Enzymically synthesized	0.42	0.57	0.74
Chemically synthesized	0.42	0.56	0.74
Glucose	0.25	0.34	0.50
Fructose	0.28	0.40	0.54
Sucrose	0.20	0.31	0.46

Dowex-50 H^+ (AG-50W-X8, 200–400 mesh) and Dowex-1 Cl^- (AG1-X8, 200–400 mesh) were purchased from Bio-Rad Laboratories. Dowex-1 was converted to the borate form essentially as described by KHYM AND ZILL[8].

The measurements for the determination of specific rotation were made on a PH 7 F Pellin polarimeter using a 40-cm glass cell.

Synthesis of β-methylfructofuranoside. Sucrose (0.2 mole), anhyd. methanol (1.5 l) and dry Dowex-50 H^+ (10 g) were refluxed together with constant stirring. After 1 h, the reaction mixture was cooled and the Dowex-50 was removed by filtration. The solution was evaporated to a thick syrup, which was redissolved in 1 l of water.

The pH was adjusted to 7 with 0.1 N NH_4OH both before and after the vacuum evaporation. Paper chromatography in Solvent 1 revealed the presence of glucose, β-methylfructofuranoside, and an unidentified fast-moving component ($R_F = 0.81$) in the crude reaction mixture. No sucrose or free fructose could be detected. The unknown component was extractible by organic solvent, although this was not a necessary step in the purification of β-methylfructofuranoside.

The aqueous solution was introduced at the top of a column of Dowex-1 borate (4 × 20 cm) and fractions of the self-eluate were collected. The elution of β-methylfructofuranoside began after the hold-up volume of the column, and the progress of the elution was assessed qualitatively by paper chromatography of the eluates in Solvent 2. At the end of the self-elution, both glucose and unidentified fructosides began to appear in the eluate. These fractions were discarded.

Fractions which contained β-methylfructofuranoside alone were pooled and the pH was adjusted to approx. 7. The pooled fractions were evaporated *in vacuo* to a syrup. Boric acid was removed as the volatile methyl borate by dissolving the preparation in methanol (25 ml) followed by evaporation *in vacuo*. This process was repeated 4 times.

Comparison of the enzymically and the chemically synthesized β-methylfructofuranoside was made in Solvents 1, 2, and 3. The results shown in Table I suggest the identity of the enzymically and chemically synthesized compounds. Both preparations were totally hydrolyzed by Neurospora β-fructofuranosidase at comparable rates, and yielded fructose as the sole sugar, as revealed by chromatography in Solvent 2. A stock solution of the chemically synthesized material was found to yield 14.6 μmoles of fructose per ml by oxalic acid hydrolysis, and 14.8 μmoles of fructose per ml by total enzymic hydrolysis, suggesting that all the fructose was of the β-fructofuranoside configuration.

The specific rotation $[\alpha]_D^{20}$ was found to be $-60° \pm 3°$.

[^{14}C]β-methylfructofuranoside was prepared from uniformly labelled sucrose (500 μmoles, 20 μC) by a small-scale modification of the above-described procedure. Chromatograms were prepared from aliquots of the fractions eluted from Dowex-1 borate, and the developed chromatograms were examined by autoradiography with Kodak No-Screen X-ray film. Those fractions which contained β-methylfructofuranoside only were pooled and freed of borate as before. The radiochemical yield of the pure compound was 50% based on the fructose moiety of sucrose.

A sample of the radioactive fructoside was mixed with 200 parts of enzymically synthesized β-methylfructofuranoside and the mixture was subjected to chromatography in Solvents 1, 2, and 3. Autoradiograms were prepared, following which the chromatograms were sprayed as usual. In each case, a single spot was seen on the paper, which corresponded both in position and in shape with that on the autoradiogram, providing further evidence of the identity and purity of the chemically synthesized material.

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Department of Physiological Chemistry,
University of Wisconsin Medical School,
Madison, Wisc. (U.S.A.)

ANDREW E. HORVATH
ROBERT L. METZENBERG

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