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Note

Isolation of the insect metabolite trehalose by high-performance liquid chromatography

KATSUMI IIDA and MASAHIRO KAJIWARA*
Department of Medicinal Chemistry, Meiji College of Pharmacy, 1–22–1 Yato-cho, Tanasi-shi, Tokyo 188 (Japan)

Several approaches are used in our laboratory for the preparation of labelled compounds[1,2]. We are interested in a biological process for preparing ^{13}C-labelled compounds. Trehalose, which is a non-reducible disaccharide, was discovered in the blood of *Antheraea polyphemus* by Wyatt and Kalf[3]. Today, it is well known that trehalose is generally the blood sugar of insects. Candy and Kilby[4,5] discovered that trehalose is formed from glucose *in vivo* by insects. [1-^{13}C]-D-Glucose was converted into [1,1′-$^{13}C_2$]-D-trehalose in live *Gryllodes sigillatus*, the Japanese Kamado cricket. The time course of the metabolism of [1-^{13}C]-D-glucose to [1,1′-$^{13}C_2$]-D-trehalose was studied *in vivo* by ^{13}C NMR spectrometry. The isolation[6] of [1,1′-$^{13}C_2$]-D-trehalose was performed by high-performance liquid chromatography (HPLC).

EXPERIMENTAL

Materials

Gryllodes sigillatus were adult males. [1-^{13}C]-D-Glucose (99 atom-% ^{13}C) was supplied by Cambridge Isotope Laboratories. Ethanol and acetonitrile were distilled before use. Water was ion exchanged and distilled before use.

HPLC system

A SSC Flow System 3100J pump, equipped with an Erma ERC-7520 refractive index detector, and a Senshu Pak NH_2-1251-N (5 μm) column (25 cm × 0.46 cm I.D.) was used. The mobile phase was acetonitrile–water (70:30). The flow-rate of the mobile phase was maintained at 1 ml/min. The column pressure was 75 kg/cm^2 and the column temperature was kept at 22°C.

Assay of trehalose

^{13}C NMR spectra were recorded on a JEOL GSX-400 (100 MHz) spectrometer and referenced from $CDCl_3$ (77.0 ppm) as an external standard. Fast atom bombardment mass spectrometry (FAB-MS) was performed on a JMS-DX302 spectrometer.

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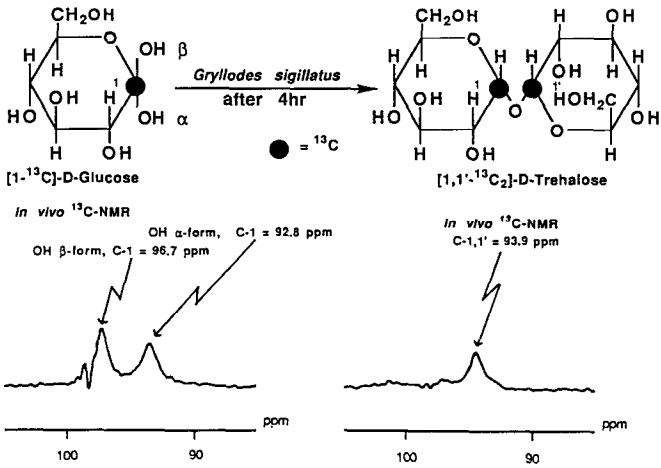

Fig. 1. ^{13}C NMR spectra of the *in vivo* transformation of [1-^{13}C]-D-glucose to [1,1'-$^{13}C_2$]-D-trehalose in *Gryllodes sigillatus*.

RESULTS AND DISCUSSION

[1-^{13}C]-D-Glucose (150 mg) was dissolved in distilled water (500 μl). The solution (10 μl) was injected intra-abdominally into each cricket, *Gryllodes sigillatus* (50 specimens). The metabolism to [1,1'-$^{13}C_2$]-D-trehalose (93.9 ppm)[7] from [1-^{13}C]-D-glucose (OH α-form = 92.8 ppm, OH β-form = 96.7 ppm)[8] was followed *in vivo* by using ^{13}C NMR spectrometry (Fig. 1).

After 4 h, the crickets were immersed in ethanol-saturated dry-ice for instanta-

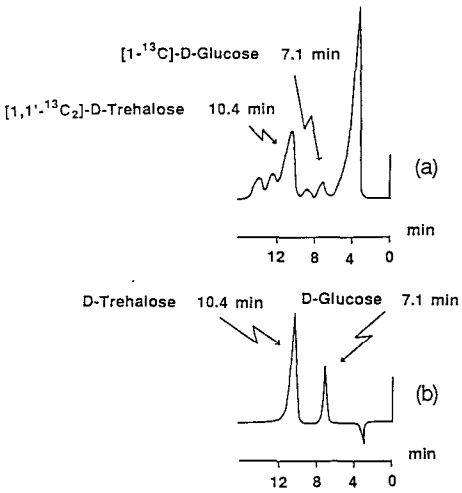

Fig. 2. Purification of extracted [1,1'-$^{13}C_2$]-D-trehalose by HPLC. (a) Separation of extracted [1-^{13}C]-D-glucose and [1,1'-$^{13}C_2$]-D-trehalose using HPLC. (b) Separation of authentic samples of D-glucose and D-trehalose using HPLC.

neous freezing, then homogenized in 70% ethanol. The suspension was centrifuged at 10 000 *g* for 20 min. The supernatant solution was passed through a cellulose acetate filter (20 μm) before freeze-drying. The residue was dissolved in mobile phase (800 μl) and the solution (20 μl) was injected onto the column. After purification by HPLC (Fig. 2), 20 mg of [1,1′-$^{13}C_2$]-D-trehalose were obtained in 27% yield. $^{12}C_{10}{}^{13}C_2H_{10}O_{11}$: mol.wt. = 344.31. FAB-MS: m/z = 343 ($M^+ - 1$, 3%). ^{13}C NMR: 93.9 ppm.

CONCLUSION

Based on the metabolic function of the insects, [1-^{13}C]-D-glucose was converted into [1,1′-$^{13}C_2$]-D-trehalose, which was isolated by HPLC. [1,1′-$^{13}C_2$]-D-Trehalose will be useful in the study of insect sugars.

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