ISOLATION AND CHARACTERIZATION OF THE SWEET PRINCIPLE FROM *DIOSCOREOPHYLLUM CUMMINSII* (Stapf) DIELS

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1. Introduction

The fruit of *Dioscoreophyllum cumminsii* (Stapf) Diels has an intensely sweet taste. The plant, first described in 1895 [1], is found in several regions of tropical Africa. In the Congo the fruit is eaten by the natives.

Inglett and May [2], who called the fruit 'serendipity berry', used pectinase digestion to facilitate extraction of the sweet principle by homogenization. Fractionation on Sephadex G-50 and G-200 indicated that the sweetener was bound to fruit proteins. Digestion with bromelain yielded a sweet-tasting, lower-molecular-weight material, which was assumed to be a carbohydrate substance.

The isolation and characterization of the sweet principle concerned is described below. The results indicate that this substance, which has a sweetness intensity about 1500 × that of sucrose on a weight basis, is not a carbohydrate but a protein.

2. Methods

Protein in purified berry extracts was determined by the biuret method [3] with BSA as standard. For ultrafiltration, a UM 2 membrane (Amicon) was used. Electrophoresis was performed on 15% polyacrylamide gel in 0.03 M potassium acetate buffer of pH 5.0 [4], for 4 hr at 75 V, using Amido Black 10B as staining agent; or (when the gel was to be tasted) on 12% starch slabs in 0.02 M potassium acetate buffer of pH 4.3, for 3 hr at 120 V (6 V/cm). The isoelectric point was determined by isoelectric focusing in 7% polyacrylamide gel containing 1% Ampholine carrier ampholytes being isoelectric between pH 8 and 10 [5]. The sugar content was estimated qualitatively by the Molisch reaction and quantitatively by the carbazole method [6] as well as by the anthrone method [7] using glucose as a standard. Absorption spectra were measured with a Zeiss PMQ II spectrophotometer.

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3. Procedure and results

The fruit originating from Nigeria was stored at −20°. 100 g of berries were cut open and the seeds with attached mucilaginous pulp were removed from the berries and immediately brought into 100 ml of distilled water. This mixture was allowed to stand for 2 hr at room temp with periodic shaking. The solid material was allowed to settle and the supernatant decanted. The sweet-tasting substance was precipitated from the supernatant by adding 4 vol of ethanol, collected by centrifugation (3000 *g*, 10°), dissolved in water and then subjected to ultrafiltration.

Fractionation of the crude concentrate thus obtained in a column of Sephadex G-100, equilibrated with 0.1 M NaCl, gave 3 peaks (fig. 1a). Fractions 35–52, having a sweet taste, were collected, concentrated and desalted by ultrafiltration (concentrate I). On polyacrylamide gel electrophoresis, concentrate I appeared to consist of 1 major and 3 minor components

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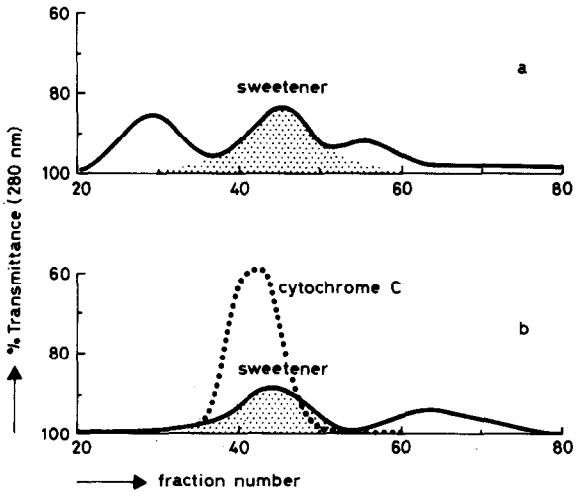

Fig. 1. Gel filtration on Sephadex (equilibrated with 0.1 M NaCl): (a) 25 mg crude berry extract in 3.5 ml 0.1 M NaCl on Sephadex G-100; bed 1.5 × 38 cm, flow rate 13.5 ml/hr, fraction vol 1.35 ml; (b) 7 mg concentrate I in 1.5 ml 0.1 M NaCl on Sephadex G-50; bed 1 × 90 cm, flow rate 3.75 ml/hr, fraction vol 0.75 ml.

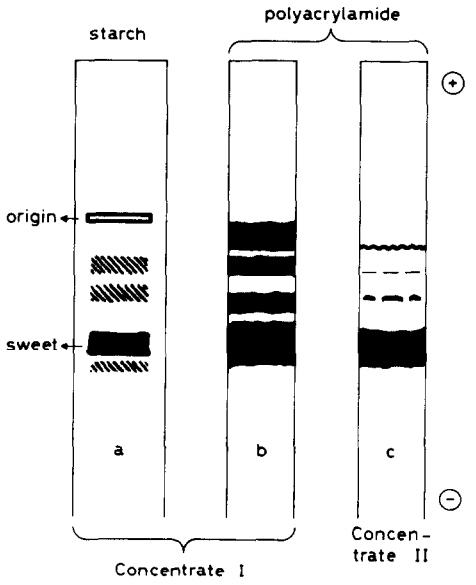

Fig. 2. Polyacrylamide gel and starch gel electrophoresis of concentrates I and II.

(fig. 2b). Starch gel electrophoresis showed a similar pattern (fig. 2a); the major component tasted very sweet but the other 3 did not taste sweet at all.

Fractionation of concentrate I on a column of Sephadex G-50, equilibrated with 0.1 M NaCl, yielded 2 peaks (fig. 1b), the first of which tasted very sweet. After concentrating and desalting of the combined sweet-tasting fractions by ultrafiltration, concentrate II was obtained, which was shown by polyacrylamide-gel electrophoresis to be an almost pure preparation of the sweet principle (fig. 2c). According to this procedure about 150 mg of the pure material was obtained from 1 kg berries.

Its molecular weight was estimated at about 10,000 by comparing the elution volume of concentrate II with that of cytochrome *c* (M.W. 13,000) (fig. 1b). Its isoelectric point was about 8.7. Its absorption spectrum showed a maximum at 278 nm at pH 5.6, shifting to 288 nm at pH 13 ($E^{1\%}_{1\,cm}$ at 278 nm = 16.2; at 288 nm = 17.6). As assessed by the biuret method, in concentrate II nearly 100% polypeptide material was found. In order to obtain an impression of the effect of proteolytic enzymes, 10 ml of a 0.05% solution of concentrate II in a 0.02 M phosphate buffer (pH 7.0) was mixed with 2.5 ml of a 0.05% trypsin solution in the same buffer. During 24 hr incubation the sweet taste practically fully disappeared. No carbohydrate could be detected in concentrate II, neither qualitatively nor quantitatively (anthrone method, less than 0.5% of carbohydrate and carbazole method, less than 1% of carbohydrate).

4. Discussion

The proteinaceous character of the macromolecular sweet principle from the fruit of *D. cumminsii* is proved by the strong absorption maxima at 278 and 288 nm, by the biuret reaction, the staining with Amido Black, and the disappearance of the sweet taste after incubation with trypsin. This result is in contrast with the findings of Inglett and May [2] who found a carbohydrate substance with a lower molecular weight.

The fact that the sweet material is a protein is noteworthy because a sweet-tasting protein ('thaumatin') has also been isolated from the fruits of

Thaumatococcus daniellii Benth [8]. Moreover, when compared on a molar basis, both proteins are the sweetest compounds known.

During the preparation of this paper, Drs. James A. Morris and Robert H. Cagan of the Monell Chemical Senses Center of the University of Pennsylvania kindly sent me a report on this matter (prior to publication). They independently isolated and characterized the sweet principle from *D. cumminsii* and their results are in good agreement with those reported above.

Acknowledgement

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