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Note

High-performance liquid chromatographic determination of *Stevia* components on a hydrophilic packed column

YOHEI HASHIMOTO and MASATAKA MORIYASU
Kobe Women's College of Pharmacy, Motoyamakita-machi, Higashinada-ku, Kobe, 658 (Japan)
and
SHIGERU NAKAMURA, SUSUMU ISHIGURO and MASAHIRO KOMURO
Showa Denko K.K., Shibadaimon-1-chome, Minato-ku, Tokyo, 105 (Japan)
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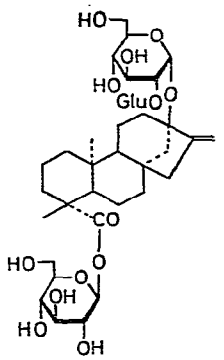
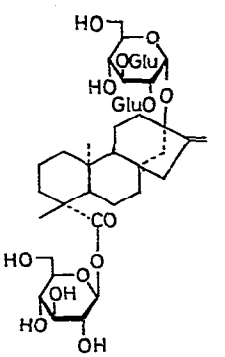
The sweet components contained in the leaves of *Stevia rebaudiana* Bertoni have recently been attracting public attention. The structures of these components have been clarified[1–4] and it has been found that stevioside (I) and rebaudioside A (II) are the main components, the latter being the sweeter of the two.



(I)



(II)

Several methods for the determination of these diterpene glycosides have been reported, but none is satisfactory[5–7]. The lack of suitable colour reagents is the main problem in the analysis of these substances.

This paper describes the determination of I and II by high-performance liquid chromatography (HPLC) utilizing a refractive index detector.

EXPERIMENTAL

Authentic samples of I and II were kindly provided by Professor O. Tanaka, Hiroshima University.

The HPLC apparatus was of our own construction. The column was a Shodex OHpak M-414 (Showa Denko, Japan; 50 cm × 4 mm I.D.), a recently developed general-purpose hydrophilic column packed with spherical beads of rigid macroporous hydroxyl polyester gel. A Shodex RI SE 11 (Showa Denko) high-sensitivity refractive index detector was used.

The sweet components in *Stevia* leaves were extracted in the following way. Dried *Stevia* leaves were crushed in a mortar and 1.0-g of the powder was weighed out. About 0.3 g of calcium carbonate and 6 ml of water were added, and the mixture was stirred to a well blended state. After maceration for 15 h, the sweet components were extracted by heating at 50° for 4 h, and then left to cool. This was followed by addition of 18 ml of acetonitrile and filtration using a Millipore filter (0.5 μm) to obtain samples for analysis.

RESULTS AND DISCUSSION

The separation of I and II was attempted with various solvent systems, and a mobile phase consisting of acetonitrile–water (4:1) at a flow-rate of 0.5 ml/min yielded satisfactory results. The chromatogram of a standard sample containing I and II, with sucrose and glucose present for comparison, is shown in Fig. 1a. The detection of about 2 μg of I and II was possible. It was found that a linear relationship exists between amount of sample and peak height (and also peak area) for the amounts of sample tested (up to 100 μg). Hence linear calibration graphs for I and II were obtained and the reproducibility was good.

The sweet components in the extract of *Stevia* leaves were determined under the conditions described above. A typical chromatogram is shown in Fig. 1 (b).

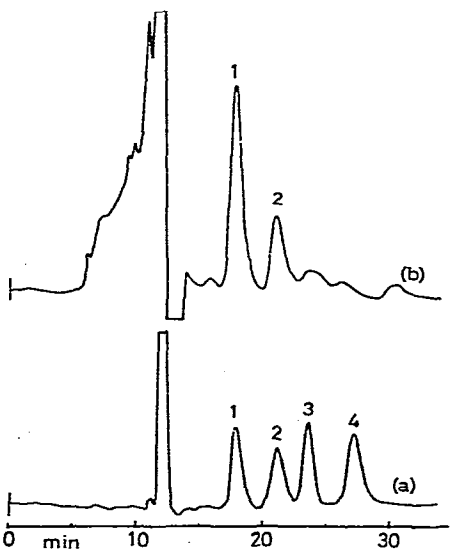


Fig. 1. Separation of stevioside and rabaudioside A. (a) Standard sample (containing 20 μg of each substance); (b) extract of *Stevia* leaves. Peaks: 1 = stevioside; 2 = rebaudioside A; 3 = glucose; 4 = sucrose.

TABLE I

CONTENTS OF STEVIOSIDE AND REBAUDIOSIDE A FOUND IN *STEVIA* LEAVES

Sample	*Occurrence*	*Stevioside (%)*	*Rebaudioside A (%)*
a	Japan	5.4	1.5
b	Japan	2.0	0.8
c	Japan	7.7	1.9
d	Japan	7.4	2.3
e	Korea	2.6	1.9
f	Brazil	6.0	1.8
g	Brazil	5.5	1.6
h	Paraguay	6.1	2.9

The small peaks of compounds other than I and II may be attributable to other minor sweet components. The content of I and II in various *Stevia* samples are given in Table I.

This determination of the sweet components in *Stevia* leaves by HPLC is advantageous because the tedious pre-treatment of the sample can be omitted. Also, the sensitivity of the determination is good when the high-sensitivity refractive index detector is used.

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