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Volume 1

Edited by
H. WAGNER

HIROSHI HIKINO

NORMAN R. FARNSWORTH

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H. WAGNER
Institut für Pharmazeutische Biologie
der Universität München
München, West Germany

HIROSHI HIKINO
Pharmaceutical Institute
Tohoku University
Sendai, Japan

NORMAN R. FARNSWORTH
Program for Collaborative Research in
the Pharmaceutical Sciences
College of Pharmacy, Health Sciences Center
University of Illinois at Chicago
Chicago, Illinois, U.S.A.

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Contributors

Numbers in parentheses indicate the pages on which the authors' contributions begin.

NORMAN R. FARNSWORTH (87, 155), Program for Collaborative Research in the Pharmaceutical Sciences, College of Pharmacy, Health Sciences Center, University of Illinois at Chicago, Chicago, Illinois 60680, U.S.A.

HIROSHI HIKINO (53), Pharmaceutical Institute, Tohoku University, Sendai 980, Japan

A. DOUGLAS KINGHORN (1, 155), Program for Collaborative Research in the Pharmaceutical Sciences, College of Pharmacy, Health Sciences Center, University of Illinois at Chicago, Chicago, Illinois 60680, U.S.A.

A. PROKSCH (113), Institut für Pharmazeutische Biologie der Universität München, D-8000 München 2, West Germany

HIROSHI SAITO (217), Faculty of Pharmaceutical Sciences, University of Tokyo, Tokyo 113, Japan

SHOJI SHIBATA (217), Meiji College of Pharmacy, Tokyo 154, Japan

JUNZO SHOJI (217), School of Pharmaceutical Sciences, Showa University, Tokyo 142, Japan

DJAJA D. SOEJARTO[1] (1, 155), Program for Collaborative Research in the Pharmaceutical Sciences, College of Pharmacy, Health Sciences Center, University of Illinois at Chicago, Chicago, Illinois 60612, U.S.A.

OSAMU TANAKA (217), Institute of Pharmaceutical Sciences, School of Medicine, Hiroshima University, Hiroshima 734, Japan

H. WAGNER (113), Institut für Pharmazeutische Biologie der Universität München, D-8000 München 2, West Germany

[1]Research Associate, Field Museum of Natural History—Botany, Chicago, Illinois 60605, U.S.A.

DONALD P. WALLER (87, 155), Program for Collaborative Research in the Pharmaceutical Sciences, College of Pharmacy, Health Sciences Center, University of Illinois at Chicago, Chicago, Illinois 60680, U.S.A.

LOURENS J. D. ZANEVELD[2] (87), Program for Collaborative Research in the Pharmaceutical Sciences, College of Pharmacy, Health Sciences Center, University of Illinois at Chicago, Chicago, Illinois 60680, U.S.A.

[2]Present address: Department of Obstetrics and Gynecology, Rush Presbyterian Hospital, Chicago, Illinois 60680, U.S.A.

Preface

The varied character of natural plant products, and indeed their very existence, pose fundamental questions to scientists. Many books have been published concerning the chemical aspects of these products; however, it is exceptional to find discussed within a single volume most aspects of particular genera or of particular pharmacological classes of natural substances, all having economic potential.

Thus, the intent of this volume is to identify areas of research in natural plant products that are of immediate or projected importance from a practical point of view and to review these areas in a concise and critical manner.

We feel that these topics will be of great interest to graduate students, research workers, and others interested in the discovery of natural products and in their further utilization as drugs, pharmacological tools, models for synthetic efforts, or other economic purposes. We hope decision makers in industry, government agencies, philanthropic foundations, and elsewhere will benefit from these timely reviews and consider these and related projects as worthwhile endeavors for further research.

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1

Current Status of Stevioside as a Sweetening Agent for Human Use

A. DOUGLAS KINGHORN
DJAJA D. SOEJARTO
Program for Collaborative Research in the Pharmaceutical Sciences
College of Pharmacy, Health Sciences Center
University of Illinois at Chicago
Chicago, Illinois USA

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I. INTRODUCTION

Stevioside and extracts prepared from the leaves of the plant *Stevia rebaudiana* have been used widely in Japan since the mid-1970s as sweetening agents, taste modifiers, and sugar substitutes. They are currently accepted for general use as food additives in Japan, and to date there have been no adverse effects reported from the use of *Stevia* products by humans.

The sweet properties of *Stevia rebaudiana* have been known for centuries, being first realized in Paraguay, the natural habitat for this plant. Stevioside, the major sweet substance of this plant, was discovered in 1905 but was never seriously considered as a sugar substitute until the early 1970s, when a group of Japanese industrial firms specializing in the manufacture of food products decided to form a consortium in order to commercialize stevioside and *Stevia* extracts for the food industry in that country.

This chapter is concerned with *Stevia rebaudiana* and its sweet principles relative to their application in foods. The history, botany, chemistry, and pharmacology of extracts prepared from this species are discussed, as well as analytical methods for quantifying the stevioside content of such extracts. Additionally, all applicable pharmacologic data on stevioside and related sweet principles are discussed and evaluated in the context of human safety. Stability studies of stevioside under a variety of conditions are summarized, and the legitimate uses and applications of these materials in the food industry are presented.

Stevia rebaudiana and stevioside have periodically been the subject of reviews over the past 40 years. Topics such as the cultivation, constituents, and commercialization prospects for *S. rebaudiana,* as well as the chemistry, biological effects, safety, and utilization of stevioside as a sweetener have been addressed by Thomas (1937), Samaniego (1946), Klages (1951), Bell (1954), Fletcher (1955), Jacobs (1955), Nieman (1958), Sumida (1973), Matsumi (1974), Brucher (1974), Kato (1975), Yoshino (1975), Seidemann (1976a), Abe and Sonobe (1977), Chueh (1977), Felippe (1977), Akashi (1977), Okazaki *et al.* (1977), Morita (1977a), Kazuyama (1979), T. Fujita (1979), Ochi (1979), Hsin *et al.*

(1979), Tanaka (1979, 1980), Fujita and Edahiro (1979a,b), Tsuchiya (1979), H. Fujita (1980), Toffler and Orio (1981), Huang (1981), and Sakaguchi and Kan (1982). Most of the more recent papers are in Japanese, however. These papers are referred to when relevant, throughout this chapter. In addition, a number of general review articles on natural-product sweeteners including stevioside and the other *S. rebaudiana* sweet diterpene glycosides (Farnsworth, 1973; Seidemann, 1976b; Crosby, 1976; Morris, 1976; Inglett, 1976, 1978; Seidemann, 1977; Crammer and Ikan, 1977; Unterhalt, 1978; Bragg *et al.*, 1978; Lee, 1979; Crosby and Wingard, 1979; Kojima, 1980; Miyoshi, 1980a,b; Crosby and Furia, 1980; Tanaka, 1981; Heraud, 1981; DuBois, 1982) are mentioned when appropriate.

II. THE SWEET PRINCIPLES OF *STEVIA REBAUDIANA*

A. BOTANICAL ASPECTS

Stevioside is a sweet *ent*-kaurene glycoside constituent of *Stevia rebaudiana* (Bertoni) Bertoni, a plant native to elevated terrain at latitude ~25° S in the Amambay and Iguaçu districts on the borders of Brazil and Paraguay (Sumida, 1973; Soejarto *et al.*, 1983a). M. S. Bertoni originally described this plant, a herb of the Compositae (daisy family), which reaches a height of 80 cm when fully grown, as *Eupatorium rebaudianum* Bertoni, but later reassigned it to the genus *Stevia* (Bertoni, 1905). The correct name for this taxon therefore is *S. rebaudiana* (Bertoni) Bertoni, rather than *S. rebaudiana* (Bertoni) Hemsley (Hemsley, 1906), for reasons of priority (Soejarto *et al.*, 1983a). *Stevia rebaudiana* is known in the Guarani language in Paraguay as *Caá-êhé* (Gosling, 1901), *Kaá Hê-é* (Bertoni, 1905, 1918; Gosling, 1901; Hemsley, 1906), *Caá-êhé* and *Azucá-caá* (Cabrera, 1939), *Caá-hê-hê* or *Caá-enhem* (Mors and Rizzini, 1966), and *Ka-á Hê-e* (Soejarto *et al.*, 1983a), all of which mean "sweet herb."

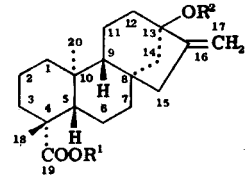
Stevia is an entirely New World genus belonging to the tribe Eupatoriae within the Compositae (King and Robinson, 1967; Grashoff, 1972; Robinson and King, 1977). The distribution range of *Stevia* extends from the southwestern United States to northern Argentina, through Mexico, Central America, the South American Andes, and the Brazilian highlands. Records indicate that *Stevia* is not represented in the West Indies and Amazonia (King and Robinson, 1967; Grashoff, 1972). While more than 80 species of *Stevia* are known to occur in North America (Grashoff, 1974), the South American species do not appear to have

been studied taxonomically in recent years. Estimates on the total number of species in the genus range from 150 to 300 (King and Robinson, 1967; Grashoff, 1972, 1974; Robinson and King, 1977; Soejarto *et al.*, 1983a).

B. CONSTITUENTS OF *STEVIA REBAUDIANA*

The dried leaves of *Stevia rebaudiana* contain ~42% (w/w) of water-soluble constituents (Bell, 1954). Chemical work to isolate the water-soluble sweet constituents of the plant, following preliminary observations by Bertoni (1905), began with experiments by Rasenack (1908) and Dieterich (1909). Dieterich (1909) isolated two sweet components, one of which, eupatorin, was renamed stevin by Bertoni (1918), in view of the nomenclatural change of the plant from *Eupatorium rebaudianum* to *S. rebaudiana* (Bertoni, 1918).

In a series of papers, Bridel and Lavieille reported several observations on the chemical nature of the sweet principles of *Stevia rebaudiana*, and stevin (= eupatorin) was renamed stevioside (Fig. 1, **1**) with preliminary

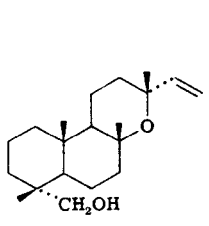


	R^1	R^2
1	β-Glu	β-Glu-β-Glu (2→1)
2	—H	β-Glu-β-Glu (2→1)
3	β-Glu	β-Glu-β-Glu (2→1) β-Glu (3→1)
4	—H	β-Glu-β-Glu (2→1) β-Glu (3→1)
5	β-Glu	β-Glu-α-Rha (2→1) β-Glu (3→1)
6	β-Glu-β-Glu (2→1)	β-Glu-β-Glu (2→1) β-Glu (3→1)
7	β-Glu-β-Glu (2→1)	β-Glu-β-Glu (2→1)
8	β-Glu	β-Glu-α-Rha (2→1)

(Glu = glucose; Rha = rhamnose)

FIG. 1 Structures of some *Stevia rebaudiana* constituents.

structural information on this glycoside and its aglycone being established (Bridel and Lavieille, 1931a–h). In 1952, a research group at the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland, renewed the investigation into the chemical structure of stevioside. This group was able to establish that one sugar unit of stevioside occurs as a glucopyranose function attached β to a carboxyl group, and a second sugar unit occurred as a sophorose [2-*O*-(β-D-glucopyranosyl)-D-glucose] function attached β to an alcoholic hydroxyl group of the aglycone (Wood *et al.*, 1955; Wood and Fletcher, 1956; Vis and Fletcher, 1956). The structures and stereochemistry of the true aglycone moiety steviol (13-hydroxy-*ent*-kaurenoic acid) (Fig. 2, **17**) and of isosteviol (Fig. 2, **18**), a product obtained on treatment of stevioside with boiling dilute sulfuric acid, were also established by this



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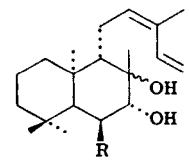
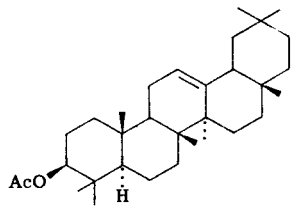


(or its enantiomer)
10 (R = OH)
11 (R = OAc)



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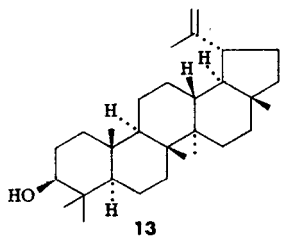

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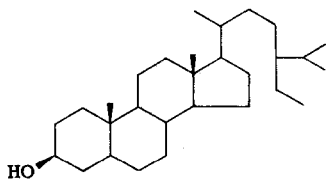

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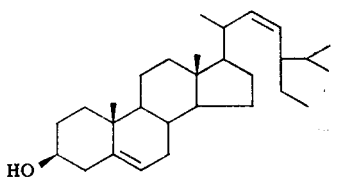

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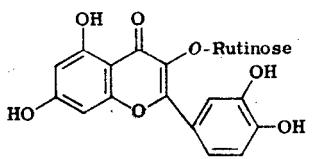

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FIG. 1 *Continued*

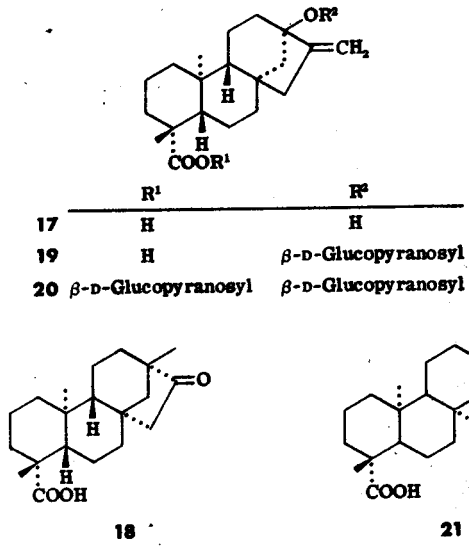

FIG. 2 Structures of steviol, isosteviol, and other hydrolytic products of stevioside.

group (Mosettig and Nes, 1955; Dolder *et al.*, 1960; Mosettig *et al.*, 1961, 1963; Djerassi *et al.*, 1961). Steviol can be converted to isosteviol by a reaction involving a proton-catalyzed Wagner–Meerwein rearrangement (Mosettig *et al.*, 1963).

During the 1970s, further insight was gained into both the sweet and nonsweet constituents of *Stevia rebaudiana.* An additional seven sweet diterpene glycosides, based on the *ent*-kaurene skeleton (Fig. 1, **2–8**), were isolated from this plant by Japanese groups at Hiroshima University and Hokkaido University, including rebaudioside A (Fig. 1, **3**), which reportedly is sweeter than stevioside and has a more pleasant taste (Kohda *et al.*, 1976b; Yamasaki *et al.*, 1976; Kobayashi *et al.*, 1977; Sakamoto *et al.*, 1977a,b). Structural assignments of these new sweet principles relied heavily on carbon-13 nuclear magnetic resonance spectroscopy (Yamasaki *et al.*, 1976; Sakamoto *et al.*, 1977a,b). Nonsweet labdane diterpenes (Fig. 1, **9–11**), triterpenes (Fig. 1, **12**, **13**), sterols (Fig. 1, **14**, **15**), and a flavonoid (Fig. 1, **16**), have also been detected in the leaves or callus tissue of the plant (Nabeta *et al.*, 1976; Yamasaki *et al.*, 1976; Tanaka, 1979, 1980; Sholichin *et al.*, 1980). A total of 31 constituents of the volatile oil of *S. rebaudiana* herb have been identified by a combination of gas chromatography, infrared spectroscopy, and mass spectrometry (Fujita *et al.*, 1977). The chemical constituents of *S. rebaudiana* characterized to date are summarized in Table I. Table II provides

TABLE I

CHEMICAL CONSTITUENTS OF *STEVIA REBAUDIANA*

Compound class	Constituents	Plant part (Fig. 1)	Yield[a]	Reference
Diterpene glycoside	—	Leaves	—	Rasenack (1908)
Diterpene glycoside	Eupatorin (= stevioside, 1)	Leaves/stems	—	Dieterich (1909); Bridel and Lavieille (1931f); Kobert (1915)
Diterpene glycoside	Stevioside (1)	Leaves	4.5	Bridel and Lavieille (1931a)
	Stevioside (1)	Leaves	7.0	Wood and Fletcher (1956)
	Stevioside (1)	Leaves	2.2	Kohda *et al.* (1976b)
	Stevioside (1)	Leaves	—	Kobayashi *et al.* (1977)
	Stevioside (1)	Callus	—	Komatsu *et al.* (1976); Kotani (1980)
Diterpene glycoside	Steviolbioside (2)	Leaves	0.04	Kohda *et al.*(1976b)
Diterpene glycoside	Rebaudioside A (3)	Leaves	1.43	Kohda *et al.* (1976b)
	Rebaudioside A (3)	Leaves	—	Kobayashi *et al.* (1977)
Diterpene glycoside	Rebaudioside B (4)	Leaves	0.44	Kohda *et al.* (1976b)
Diterpene glycoside	Rebaudioside C (5) (= dulcoside B)	Leaves	0.4	Sakamoto *et al.* (1977a)
		Leaves	0.013	Kohda *et al.* (1976b)
Diterpene glycoside	Rebaudioside D (6)	Leaves	0.03	Sakamoto *et al.* (1977a,b)
Diterpene glycoside	Rebaudioside E (7)	Leaves	0.03	Sakamoto *et al.* (1977a,b)
Diterpene glycoside	Dulcoside A (8)	Leaves	0.029	Kohda *et al.* (1976b)
Labdane diterpene	Jhanol (9)	Leaves	0.0063	Sholichin *et al.* (1980)
Labdane diterpene	Austroinulin (10)	Leaves	0.06	Sholichin *et al.* (1980)
Labdane diterpene	6-*O*-Acetylaustro-inulin (11)	Leaves	0.15	Sholichin *et al.* (1980)
Triterpene	β-Amyrin acetate (12)	Leaves	—	Sholichin *et al.* (1980)
Triterpene	Lupeol (13)[b]	Leaves	—	Sholichin *et al.* (1980)
Sterol	β-Sitosterol (14)	Leaves	—	Sholichin *et al.* (1980)
Sterol	Stigmasterol (15)	Leaves	—	Sholichin *et al.* (1980)
Sterol	Stigmasterol (15)	Callus	0.00091	Nabeta *et al.* (1976)

(*continued*)

TABLE I (*Continued*)

Compound class	Constituents	Plant part (Fig. 1)	Yield[a]	Reference
Flavonoid glycoside	Rutin (**16**)	Callus	0.0073	Suzuki *et al.* (1976)
Tannins	Unidentified	Leaves	7.8	Chung and Lee (1979)
Volatile oil	See Table II[b]	Herb[c]	0.12	Fujita *et al.* (1977)
Volatile oil	See Table II	Herb[d]	0.16	Fujita *et al.* (1977)
Volatile oil	See Table II	Inflorescence[d]	0.43	Fujita *et al.* (1977)

[a]Yields are expressed as the percentage (w/w) of a compound isolated from the dried plant part stated.
[b]Isolated in the form of three esters that were not identified.
[c]Cultivated in Paraguay.
[d]Cultivated in Japan.