THE FLAVONOIDS OF *STEVIA REBAUDIANA*

AMRITESWORI RAJBHANDARI and MARGARET F. ROBERTS

Department of Pharmacognosy, The School of Pharmacy, London University, 29/39 Brunswick Sq., London WC1N 1AX

ABSTRACT.—Phytochemical investigation of *Stevia rebaudiana* yielded the following glycosides: apigenin-4'-O-glucoside, luteolin-7-O-glucoside, kaempferol-3-O-rhamnoside, quercitrin, quercetin-3-O-glucoside and quercetin-3-O-arabinoside. A methoxylated flavone 5, 7, 3' trihydroxy 3, 6, 4' trimethoxy flavone (centaureidin) was also isolated.

Stevia rebaudiana Bertoni is a member of a genus with some 110 reported species, the principle taxonomic work being that of Grashoff (1). This species is a woody herb native to Paraguay but now grown on a commercial scale in a number of countries, particularly Japan, Taiwan and Korea. The principle interest, both academic and commercial, has been in the sweet diterpenes which occur in *S. rebaudiana* and which can be from 5–10% of the dried leaf (2). A recent survey of the whole genus has been made for these sweet diterpenes (3). Brief reports of chromenes from *S. serrata* (4) and a pseudoquaianolide from *S. rhombifolia* (5) have also appeared in the literature, but no extensive flavonoid studies of the genus have been made. Although brief attention was paid to the flavonoid patterns in some species by Grashoff (1) no structural determinations were made, and to date the only other reported work on flavonoids is the isolation of 5, 6 dihydroxy 7, 8, 4' trimethoxy flavone from *S. berlandieri* by Dominguez (6). The present work forms part of an extended survey of the genus *Stevia* for flavonoids.

RESULTS

The plant material for the present study came from a plantation in the Philippines and has a leaf diterpene glycoside content (stevioside 5%, rebaudioside A 2% dry weight) which compares favorably with the values given in the literature (1).

The leaves of the collected plates of *S. rebaudiana* yielded seven flavonoids. The flavonoid glycosides isolated from the ethyl acetate fraction were readily identified from their color in uv, uv/NH_3 paper chromatography, uv spectroscopy (7), hydrolysis and pmr spectroscopy. Further substantiation was made by comparison of the aglycones with commercially available samples. These glycosides were as follows: apigenin-4'-O-glucoside (8), luteolin-7-O-glucoside (9), kaempferol-3-O-rhamnoside (10), quercitrin (7), quercetin-3-O-glucoside (10), and quercetin-3-O-arabinoside (10).

A methoxylated flavonoid was isolated from the chloroform extract. From the data given in the Experimental and reference to the data given in (11), this compound was identified as 5, 7, 3' trihydroxy 3, 6, 4' trimethoxy flavone (centaureidin).

EXPERIMENTAL

PLANT MATERIAL.—The material used was collected in the Phillipines on a plantation and, therefore, must be considered as cultivated. A voucher specimen is lodged in the Department of Pharmacognosy, The School of Pharmacy, London University. The plant material was air dried before extraction. Dried leaves (250 g) were available for analysis after the removal of the flowers and stems.

EXTRACTION AND SEPARATION OF FLAVONOIDS.—The leaf material was cold extracted with 85% methanol followed by 50% methanol until all pigment and other colored material was removed. The methanol extracts were concentrated under reduced pressure until only the water remained. The aqueous layer was then extracted with *n*-hexane (5 liters) followed by chloroform (5 liters) and finally ethyl acetate (10 liters). The remaining aqueous layer was

reduced to low volume and stored at −20°. Pc indicated that the hexane and aqueous layers did not contain flavonoids, and therefore these were not further investigated.

(A) *Chloroform extract:* The material from the chloroform extract (6.53 g) was chromatographed with pc and 15% acetic acid. This extract yielded one compound a 5, 7, 3' trihydroxy 3 6, 4' trimethoxy flavone (28 mg).

(B) *Ethyl acetate extract:* The material from the ethyl acetate extract (5.3 g) was chromatographed over a polyamide column (5 x 60 cm). The elution was with methanol-water (3:13) with increasing amounts of methanol to 100%. The flavonoids isolated sequentially from this column were as follows: 1. Two compounds separated by pc, apigenin-4'-O-glucoside (26 mg) and luteolin-7-O-glucoside (23 mg). 2. Kaempferol-3-O-rhamnoside (20 mg). 3. A mixture of quercitrin and quercitin-3-O-glucose (238 mg). 4. Quercetrin and quercetin-3-O-arabinose (425 mg). All compounds were finally purified before spectral analysis on columns of Sephadex LH20 with either methanol or methanol-water (8:2).

STRUCTURAL DETERMINATIONS.—Flavonoid structures were determined by standard methods of uv, pmr and ms spectroscopy[1] (11); partial and total hydrolysis were carried out with 0.1 N trifluoroacetic acid or 2 N HCl. Pc and tlc were performed with the solvent systems given in reference (12). Samples were screened for sulfated compounds by means of electrophoresis, as in reference (13).

IDENTIFICATION OF CENTAUREIDIN.—When paper chromatographed with testing butyl alcohol 0.85 and acetic acid 0.25 centaureidin gave the following color: uv purple, uv/NH_3 purple brown. Centaureidin gave the following spectral data: uv λmax MeOH: nm 253, 346; NaOMe: 265, 400; $AlCl_3$: 270, 394; $AlCl_3$/HCl: 265, 366; NaOAc: 264, 400; H_3BO_3: 256, 354. Pmr $CDCl_3$ 250 MHz) δ 3.8, 3.91, 3.95, (*s* 3 OMe); δ 6.52 (*s* 1H–C8): δ 7.02 (*d* J=8.6 H–C5'); δ 7.52 (*d* J=7.7 H–C6'); δ 7.96 (*s* H–C2'). Ms: M^+ 360 (100), M^+–CH_3 345 (60), A_1^+· −H 182 (15) B_2^+· −151. (15) B_1^+· 148 (10).

ACKNOWLEDGMENT

A.R. would like to thank the British Council for a Student bursary. We would also like to thank Professor O. Tanaka for the analysis of diterpenes, Dr. H. Geiger for samples of luteolin and luteolin-7-O-glucoside and Dr. T. J. Mabry for quercetin-3-O-glucoside. We would also like to thank Mrs. E. Summers of King's College, London University for pmr spectra.

Received 25 May 1982

LITERATURE CITED

1. J. L. Grashoff, Ph.D. thesis, University of Texas (1972).
2. N. Kaneda, R. Kasai, K. Yamasaki and O. Tanaka, *Chem. Pharm. Bull.*, **25**, 2466 (1977).
3. D. D. Soejarto, A. D. Kinghorn and N. P. Farnsworth, *J. Nat. Prod.*, **45**, 590 (1982).
4. H. Kohda, K. Tamazaki, O. Tanaka and K. Nishi, *Phytochemistry*, **15**, 846 (1976).
5. T. Rios, A. Romode Vivar and J. Romo, *Tetrahedron*, **23**, 4256 (1967).
6. X. A. Dominguez, A. Gonzalez, A. Zamudio and A. Garza, *Phytochemistry*, **13**, 2001 (1974).
7. T. J. Mabry, K. R. Markham and M. B. Thomas. The Systematic Identification of Flavonoids. Springer-Verlag, New York, 1970.
8. C. G. Nordstrom and T. Swain, *Journal of the Chemical Society*, 2764 (1953).
9. S. Haltori and H. Matsuda, *Acta Phytochim*, Japan, **15**, 233 (1949).
10. J. B. Harborne, *Arch. Biochem. Biophys.*, **96**, 171 (1962).
11. R. Mues, B. N. Timmermann and T. J. Mabry, *Phytochemistry*, **18**, 1379 (1979).
12. M. F. Roberts, B. N. Timmermann and T. J. Mabry, *Phytochemistry*, **19**, 127 (1980).
13. C. A. Williams, J. B. Harborne and H. T. Clifford, *Phytochemistry*, **12**, 2417 (1973).

[1]Spectra were recorded with the following instruments: uv, Pye SP8–100; pmr, Brucker 250 MHz; ms, 3VG micromass ZablF.