Journal of Natural Products
Vol. 47, No. 2, pp. 316-319, Mar-Apr 1984

FLAVONOIDS WITH ANTI-CATARACT ACTIVITY FROM *BRICKELLIA ARGUTA*[1]

KARL-HEINZ A. ROSLER,* ROBERT S. GOODWIN,

Department of Medicinal Chemistry/Pharmacognosy, School of Pharmacy, University of Maryland at Baltimore, Baltimore, MD 21201

TOM J. MABRY,

Department of Botany, University of Texas at Austin, Austin, TX 78712

SHAMBHU D. VARMA,

Department of Ophthalmology, School of Medicine, University of Maryland at Baltimore, Baltimore, MD 21201

and JOHN NORRIS

Department of Botany, University of Texas at Austin, Austin, TX 78712

ABSTRACT.—Six flavonoids were isolated from *Brickella arguta* and identified using chemical and spectral methods. The isolation and spectral data of a new flavonoid, 6-methoxykaempferol 3-O-β-D-robinobioside (**3**), are reported for the first time. Three of these flavonoids were tested and showed inhibition of rat lens aldose reductase.

Brickellia is a member of the Eupatorieae tribe of the Compositae and is distributed from the southwestern Canadian border, through Mexico, and into Central and South America (1). Recently, the generic alignments in the Eupatorieae have been reassessed on the basis of the flavonoid chemistry pattern of the respective species (2).

Many flavonoids have been established as effective inhibitors of lens aldose reductase, the enzyme that converts D-glucose and D-galactose to sorbitol. This inhibitory activity is of importance, because this property imparts potential therapeutic value to flavonoids in the treatment of diabetic and galactosemic cataract (3).

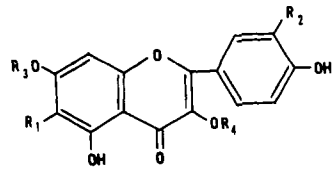
Previous chemotaxonomical work on *Brickellia* indicated that this genus produced flavonoids which, based on established structure-activity relationships, would be potent inhibitors of aldose reductase (4,5). However, a practical difficulty with the use of flavonoids as therapeutic agents is their insolubility in water. Being phenolic compounds, flavonoids are water soluble at a basic pH, but they are frequently unstable under such conditions. A chemical investigation of *Brickellia arguta* was, therefore, undertaken in order to determine if any physiologically active flavonoids, with emphasis on water-soluble compounds, could be isolated in good yield.

RESULTS AND DISCUSSION

Four flavonoids (**1-4**) were isolated from the aqueous phase including a new glycoside, 6-methoxykaemferol 3-O-β-D-robinobioside. Compounds **1-4** were the major components of the EtOAc extract; three glycosides of patuletin and 6-methoxykaempferol were also detected in this extract. Aglycones **5** and **6** were isolated from the $CHCl_3$ extract. In addition, trace amounts of two unidentified flavonoids were detected in the $CHCl_3$ extract.

Two of the flavonoids, including the new 6-methoxykaempferol 3-O-β-D-robinobioside (**4**), contained a disaccharide, consisting of β-D-galactose and α-L-rhamnose with the rhamnose being the terminal sugar. Glc and spectroscopic data es-

[1]This work was performed in partial fulfillment of the requirements for the degree of Doctor of Philosophy for Robert S. Goodwin.



1 R_3=H, R_2=OH, R_1=OCH_3, R_4=β-D-galactose
2 R_1=R_3=H, R_2=OCH_3, R_4=β-D-glucose
3 R_2=R_3=H, R_1=OCH_3, R_4=β-D-robinobiose
4 R_3=H, R_2=OH, R_1=OCH_3, R_1=β-D-robinobiose
5 R_3=R_4=CH_3, R_1=R_2=OCH_3
6 R_1=R_3=H, R_4=CH_3, R_2=OCH_3

tablished the structure of the monosaccharides and the 1-6 linkage, respectively. The confirmation that this rhamnogalactose is identical with the known robinobiose was obtained by direct glc comparison of the trimethysilylethers of the disaccharides and authentic robinobiose. Robinobiose was obtained from robinin and the disaccharides from **3** and **4** by partial hydrolysis with 15% HOAc (6).

Table 1 compares the inhibitory activity of three flavonoids isolated from *B. arguta* with that of 1,3-dioxo-[1H]-benz de isoquinoline (alrestatin), which is regarded as one of the most promising water-soluble aldose reductase inhibitors (7). The three flavonoids show a level of activity similar to alrestatin. Also of significance is the fact that patuletin 3-O-β-D-robinobioside is water soluble at neutral pH at pharmacologically active concentrations. It, thus, represents an easily isolable, natural product with potential therapeutic utility.

TABLE 1. Activity of Aldose Reductase Inhibitors

Compound	Inhibition Percentage[a]		
	10^{-5}M	10^{-6}M	10^{-7}M
Patuletin 3-O-β-D-robinobioside	86	33	11
Patuletin 3-O-β-D-galactoside	84	38	7
6-Methoxykaempferol 3-O-β-D-robinobioside	63	32	9
1,3-Dioxo-1H-benz de isoquinoline[b]	90	40	0

[a]All compounds tested inhibited the enzyme activity almost completely at 10^{-4} M. The numbers indicate percentage of inhibition of the aldose reductase activity as compared to controls when the reaction was carried out in the absence of inhibitors.

[b]Reproduced from Varma *et al.* (13).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Chemical structures of compounds isolated in sufficient yield were elucidated using spectral and chromatographic techniques in conjunction with comparisons of appropriate reference compounds when available. Uv and pmr spectra were taken according to the methods of Mabry *et al.* (8). Sugars were identified from their gas-liquid chromatograms after hydrolysis and trimethylsilylation (9) and by enzymatic hydrolysis. The linkage of the disaccharides was determined from ms and the pmr spectra of the acetyl derivatives (10). Tlc on polyamide (11) and silica gel and two-dimensional pc (8) were also utilized.

Uv spectra were recorded on a Gilford 2600 Ultraviolet-Visible spectrophotometer. Pmr spectra of the trimethylsilylethers were recorded on a Varian T-60A spectrometer in CCl_4 with TMS as internal standard unless otherwise noted. Mass spectra were obtained with an AEI/Kratos MS-50 mass spectrometer using fast atom bombardment. Gc was performed on a Varian Aerograph series 2100 instrument equipped with a flame ionization detector. As liquid phase, 3% SE-30 on Chromosorb W was used in a 2-mm glass column of 1.8 m length. The column temperature was 180° for the monosaccharides and 230° for

robinobiose, and the flow rates were 17.6 ml/min and 23.1 ml/min, respectively. Silica gel 60 tlc plates used were from EM Laboratories Inc., Darmstadt, Germany. Melting points were obtained with a Kofler micro melting point apparatus and are uncorrected. β-D-Glucosidase and β-D-galactosidase were supplied by Sigma Chemical Co., St. Louis, MO.

PLANT MATERIAL.—Specimens of *Brickellia arguta* variety *odontolepis* B.L. Robins. were collected in May 1981, in Baja California del Norte about six miles east of La Rumerosa. A voucher specimen (Norris #278) was deposited at the Lundell Herbarium, University of Texas at Austin.

EXTRACTION AND ISOLATION.—Air-dried stems, flowers, and leaves (717 g) were extracted four times with 600-ml portions of petroleum ether. The marc was air-dried and extracted five times with 700-ml portions of absolute MeOH until the extract became colorless. The two extracts were then taken to dryness. The residue from the MeOH extract was suspended in 300 ml of H_2O and partitioned five times with 150-ml portions of $CHCl_3$. The aqueous phase was then extracted five times with 150-ml of EtOAc. Finally, the $CHCl_3$ and EtOAc extracts were taken to dryness. The aqueous phase was acidified to pH 2 and passed over an Amberlite XAD-2 column.[2] Various impurities were eluted with 1200 ml of H_2O at pH 2. After washing with 1 liter of H_2O to neutrality, the flavonoid-containing fraction was eluted with 1200 ml each of 50% and 100% MeOH. The methanolic eluate was then chromatographed sequentially over two Polyclar AT columns[3] using 90% MeOH, followed by chromatography over a Sephadex LH-20 column[4] in absolute MeOH. Four compounds (**1-4**) were isolated from the aqueous phase.

Patuletin 3-O-β-D-galactoside (**1**) *(66 mg).*—Uv λ max (MeOH) 260, 271sh, 359; (NaOMe) 273, 339, 413; ($AlCl_3$) 277, 306sh, 441; ($AlCl_3$/HCl) 270, 309sh, 381, 403; (NaOAc) 273, 340sh, 406; (NaOAc/H_3BO_3) 265, 379 nm; pmr 7.76 (dd, J=9,2 Hz, H-6′), 7.36 (d, J=2 Hz, H-2′), 6.80 (d, J=9 Hz, H-5′), 6.51 (s, H-8). 5.61 (d, J=8 Hz, galactose H-1), 3.94 (s, 3H, OCH_3), 3.90-3.13 (m, 5H, galactose).

Isorhamnetin 3-O-β-D-glucoside (**2**) *(8 mg).*—uv λ max (MeOH) 256, 269, 358; (NaOMe) 273, 331, 413; ($AlCl_3$) 270, 304sh, 362sh, 405; ($AlCl_3$/HCl) 270, 301sh, 362sh, 405; (NaOAc) 274, 328, 409; (NaOAc/H_3BO_3) 256, 269sh, 359 nm; pmr 7.85 (brd, H-2′), 7.41 (dd, J=8, 2 Hz, H-6′), 6.82 (d, J=8 Hz, H-5′), 6.43 (d, J=2 Hz, H-8), 6.18 (d, J=2 Hz, H-6), 5.82 (m, glucose H-1), 3.88 (s, 3H, OCH_3), 3.87-3.40 (m, 5H, glucose).

6-Methoxykaempferol 3-O-β-D-(1,6)-robinobioside (**3**) *(238 mg).*—mp 244-247°; uv λ max (MeOH) 271, 302sh, 346; (NaOMe) 276, 331, 399; ($AlCl_3$) 276, 308sh, 370, 408sh; ($AlCl_3$/HCl) 278, 307sh, 364, 409sh; (NaOAc) 275, 320sh, 332, 399; (NaOAc/H_3BO_3) 273, 350 nm; pmr 7.93 (d, J=9 Hz, 2H, H-2′, 6′) 6.82 (d, J=9 Hz, 2H, H-3′, 5′), 6.50 (s, H-8), 5.74 (d, J=7 Hz, galactose H-1), 4.34 (characteristic of rhamnose H-1) 3.67 (s, 3H, OCH_3), 3.78-3.36 (m, 10H, galactose and rhamnose), 1.09 (bd, rhamnose CH_3); pmr (acetyl derivative, $CDCl_3$) 8.15 (d, J=8 Hz, 2H, H-6′, 2′), 7.28 (s, H-8), 7.22 (d, J=8 Hz, 2H, H-3′,5′), 5.43-4.97 (m, 7H, galactose and rhamnose), 4.51 (bd, rhamnose H-1), 4.03-3.40 (m, 4H, galactose and rhamnose), 3.87 (s, 3H, OCH_3), 2.57-1.87 (m, 27H, acetyl), 1.12 (d, J=7 Hz, 3H, rhamnose CH_3); ms, *m/z* (rel. int.) 625 (MH^+, 3), 479 (MH^+ −rhamnose, 1), 317 (MH^+ −rhamnogalactoside, 8); glc for robinobiose. Retention-times (min): peak 1, 5.7 (18.4%), peak 2, 6.9 (46.08%) peak 3, 8.3 (35.52%).

Patuletin 3-O-β-D-robinobioside (**4**) *(524 mg).*—uv λmax (MeOH) 258, 270sh, 300sh, 360; (NaOMe) 273, 336, 413; ($AlCl_3$) 276, 309sh, 336sh, 436; ($AlCl_3$/HCl) 271, 306sh, 393; (NaOAc) 273, 337sh, 406; (NaOAc/H_3BO_3) 266, 379 nm; pmr 7.77 (dd, J=9, 2 Hz, H-6′), 7.35 (d, J=2 Hz, H-2′), 6.82 (d, J=9 Hz, H-5′), 6.52 (s, H-8), 5.62 (d, J=7 Hz, galactose H-1), 4.38 (characteristic of rhamnose H-1), 3.72 (s, 3H, OCH_3), 3.92-3.38 (m, 10H, galactose and rhamnose), 1.15 (d, J=3 Hz, rhamnose CH_3), pmr (acetyl derivative, $CDCl_3$) 8.13-7.90 (m, 2H, H-6′, 2′), 7.43-7.20 (m, 2H, H-5′, 8), 5.43-4.93 (m, 7H, galactose and rhamnose), 4.53 (bd, rhamnose H-1), 4.03-3.40 (m, 4H, galactose and rhamnose), 3.88 (s, 3H, OCH_3), 2.57-1.87 (m, 30H, acetyl), 1.12 (d, J=7 Hz, 3H, rhamnose CH_3); glc for robinobiose, see **3**.

The EtOAc extract (5 g) was chromatographed sequentially over two Polyclar columns using 90% MeOH and yielded additional quantities of compounds **1-4** and trace amounts of unidentified glycosides of patuletin and 6-methoxykaempferol.

The $CHCl_3$ extract (18 g) was suspended in a mixture of 155 ml of petroleum ether and 120 ml of MeOH and partitioned with 100 ml of H_2O. This mixture was then centrifuged to separate the two phases. The H_2O-MeOH phase was filtered over Celite[5] and taken to dryness. This residue was then chromato-

[2]Amberlite XAD-2 was supplied by Mallinckrodt, St. Louis, MO.

[3]Polyclar AT was supplied by GAF Corporation, New York.

[4]Sephadex LH-20 was obtained from Pharmacia Fine Chemicals AB, Uppsala, Sweden.

[5]Celite was supplied by Fisher Scientific Company, Fair Lawn, NJ.

graphed sequentially over two Polyclar columns with 90% MeOH followed by 50% MeOH. Four compounds were isolated from the $CHCl_3$ extract, two of which were obtained in sufficient yield to permit total structure elucidation.

Quercetagetin 3.6.7.3′-tetramehyl ether (**5**) *(4 mg).*—uv λ max (MeOH) 257, 275, 294sh, 348; (NaOMe) 275, 422; ($AlCl_3$) 270, 281sh, 305sh, 387; ($AlCl_3$/HCl) 268, 281, 373; (NaOAc) 270, 406; (NaOAc/H_3BO_3) 258, 271, 352 nm; pmr 7.55 (dd, J=10, 2 Hz, 2H, H-2′, 6′), 6.85 (d, J=10 Hz, H-5′), 6.50 (s, H-8), 3.91 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.73 (s, 3H, OCH_3).

Quercetin 3.3′-dimethyl ether (**6**) *(7 mg).*—uv λ max (MeOH) 255, 268, 295sh, 356; (NaOMe) 271, 330, 406; ($AlCl_3$) 268, 302, 368sh, 406; ($AlCl_3$/HCl) 267, 276sh, 301sh, 359, 402; (NaOAc) 274, 325, 406; (NaOAc/H_3BO_3) 255, 268, 357 nm; pmr 7.57 (dd, J=10, 2 Hz, 2H, H-2′, 6′), 6.89 (d, J=10 Hz, H-5′), 6.45 (d, J=2 Hz, H-8), 6.14 (d, J=2 Hz, H-6), 3.83 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3).

BIOASSAY.—Partially purified rat lens aldose reductase was prepared by homogenizing rat lenses in H_2O (one lens/ml) and centrifuging at 16,000 g for 15 min.[6] The supernatant was used for the determination of activity of aldose reductase. The reaction mixture contained the following: 0.1 M sodium phosphate buffer, pH 6.2, 0.2 ml; NADPH, 2.5×10^{-4} M, 0.05 ml; *D,L*-glyceraldehyde, 1.5×10^{-3} M, 0.1 ml; the lens homogenate supernatant, 0.1 ml; and H_2O. The total volume of the reaction mixture was 1 ml. Activity was expressed as the rate of decrease in absorbance of NADPH at 340 nm in the reaction:

$$\text{glyceraldehyde}+\text{NADPH} \xrightarrow{\text{aldose reductase}} \text{glycerol}+\text{NADP}$$

The reference blank consisted of all of the above compounds except the substrate. The effect of inhibitors on the enzyme activity was determined by including in the reaction mixture the compound being tested at the desired concentration. Appropriate blanks were run to correct for nonspecific reduction of NADPH and absorption by the compounds tested (12).

ACKNOWLEDGMENTS

This investigation was supported in part by Research to Prevent Blindness, Inc., and by the School of Pharmacy Biomedical Research Grant (NIH 2S07RR057704). In addition, it was partially supported by grants to T.J.M. (NIH HDO-4488 and NSF DEB 8102043). Mass spectral determinations were carried out at the Middle Atlantic Mass Spectrometry Laboratory, a National Science Foundation Shared Instrumentation Laboratory.

LITERATURE CITED

1. B.L. Robinson, "A Monograph of the Genus *Brickellia*," in: "Memoirs of the Gray Herbarium of Harvard University," Cambridge, MA: Harvard University Press, 1917, p. 4.
2. T.J. Mabry, B.N. Timmermann, N. Heil, and A.M. Powell, *Plant System Evol.*, **137**, 275 (1981).
3. S.D. Varma, *Curr. Topics. Eye Res.*, **3**, 91 (1980).
4. R. Mues, B.N. Timmermann, N. Ohno, and T.J. Mabry, *Phytochemistry*, **18**, 1379 (1979).
5. S.D. Varma and J.H. Kinoshita, *Biochem. Pharmacol.*, **25**, 2505 (1976).
6. G. Zemplen and A. Gerecs, *Ber. Dtsch. Chem. Ges.*, **71**, 2520 (1938).
7. D. Dvornik, N. Simard-Duquesne, M. Krami, K. Sestanj, K.H. Gabbay, J.H. Kinoshita, S.D. Varma, and L.O. Merola, *Science*, **182**, 1146 (1973).
8. T.J. Mabry, K.R. Markham, and M.B. Thomas, "The Systematic Identification of Flavonoids," New York: Springer-Verlag, 1970.
9. C.C. Sweeley, R. Bentley, M. Makita, and W.W. Wells, *J. Am. Chem. Soc.*, **85**, 2497 (1963).
10. H. Rosler, T.J. Mabry, M.F. Cranmer, and J. Kagan, *J. Org. Chem.*, **30**, 4346 (1965).
11. H. Rosler, W. Heinrich, and T.J. Mabry, *J. Chromatogr.*, **78**, 432 (1973).
12. S. Hayman and J.H. Kinoshita, *J. Biol. Chem.*, **240**, 877 (1965).
13. S.D. Varma, I. Mikuni, and J.H. Kinoshita, *Science*, **188**, 1215 (1975).

Received 18 April 1983

[6]All animals used were Sprague-Dawley rats.