

FLAVONOIDS FROM THE TWIGS OF *EUCRYPHIA GLUTINOSA*

SILVIA SEPULVEDA-BOZA,* SUALAHEEN DELHVI and BRUCE K. CASSELS†

Institut für Physiologische Chemie der Universität Bonn, Bonn, F.R.G.; †Departamento de Química, Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago 1, Chile

(Received in revised form 21 August 1992)

Key Word Index—*Eucryphia glutinosa*; Eucryphiaceae; twigs; flavonoids; dihydroquercetin 3-*O*- β -D-xyloside; reynoutrin; jaceidin 5-*O*- β -D-glucoside; caryatin 7-*O*- β -D-glucoside.

Abstract—The rare dihydroquercetin 3-*O*- β -D-xyloside, caryatin 7-*O*- β -D-glucoside and the previously unknown 6-methoxylated flavonoid glycoside jaceidin 5-*O*- β -D-glucoside were isolated from twigs of *Eucryphia glutinosa* where they co-occur with the widespread reynoutrin.

INTRODUCTION

The leaf flavonoids of *Eucryphia cordifolia* Cav. and *E. glutinosa* (Poepp. et Endl.) Baillon, from south central Chile, have been shown to differ markedly from those of the three Australasian species of this small, Southern genus. Both Chilean species are characterized by the presence of quercetin-5-methyl ether (azaleatin), 3-glycosides of this unusual flavonol methyl ether, and the very rare quercetin 3,5-dimethyl ether (caryatin) in their leaves, as well as a number of quercetin glycosides and unidentified constituents [1]. More recently, the trunk bark of *E. cordifolia* was studied and shown to contain the 3-*O*- α -L-rhamnosides of dihydroquercetin (astilbin, the major component) and of dihydrokaempferol (engelitin), as well as a new chromone 3-*O*- α -L-rhamnoside named eucryphin; no *O*-methylated flavonoids were found, however [2]. *Eucryphia glutinosa* is the only other species growing on the American continent, where it is known as 'guindo santo' ('holy cherry tree') because of its appearance when covered with large white blossoms. It is only found near rivers and streams west of the Andes between latitudes 36° and 38° south, north of the range of the much larger *E. cordifolia* [3]. We now report the results of an analysis of the butanol-soluble fraction of the twigs of *E. glutinosa*.

RESULTS AND DISCUSSION

The crude butanol extract was subjected to chromatography on a silica gel column to give two main flavonoid-containing fractions. The larger of these, after column chromatography on Sephadex LH-20 and TLC on silica gel, afforded two pure compounds. The major constituent

was found to be dihydroquercetin 3-*O*- β -D-xyloside (**1**) by spectroscopic methods and by acid hydrolysis and chromatographic comparison of the products with standards. This substance had been found, but not fully characterized, as a component of an unresolved mixture of dihydroquercetin monoglycosides in leaves of the Tasmanian *E. milliganii* [1] and later reported as a constituent of *Leucothoe keiskei* (Ericaceae) [4] and of *Cordia obliqua* (Boraginaceae) [5]. Its abundance in *E. glutinosa* twigs parallels that of the analogous dihydroquercetin rhamnoside in the bark of *E. cordifolia* [2]. The second known substance now reisolated from this fraction of *E. glutinosa* is the widely distributed quercetin 3-*O*- β -D-xyloside (reynoutrin), which may be assumed to be biogenetically related to its dihydro derivative.

The minor flavonoid fraction was further fractionated by successive chromatographic runs on Sephadex and on silica gel which afforded a small amount of the 7-*O*- β -D-glucoside of caryatin (**2**) and the previously undescribed compound **3**. The complete ^1H and ^{13}C NMR spectral assignments of **2**, based on 2D experiments, are summarized in Tables 1 and 2.

Compound **3** was laevorotatory in pyridine. Its UV spectrum showed maxima at 254 (log ϵ 3.52), 275 (log ϵ 3.60), and 353 nm (log ϵ 3.76). Upon addition of the usual reagents [6], NaOMe produced a 42 nm bathochromic shift indicating the presence of a C-4' free phenolic function, and AlCl_3 gave no change indicating the absence of C-3 or C-5 hydroxyl groups or a catechol moiety. The EI mass spectrum showed the molecular ion of the aglycone at m/z 360 (100%) and large peaks at m/z 359 (84%), typical of C-3-methoxylated flavonoids [7], and at m/z 345 (68%), consistent with the presence of a methoxyl group at C-6 or C-8 [8]. In its ^1H NMR spectrum three methoxyl resonances were clearly distinguishable, and two of these appearing at relatively high fields (δ 3.74 and 3.77 m) could be assigned to groups attached to C-3 and the sterically hindered C-6 or C-8. A one-proton singlet at

*Author to whom correspondence should be addressed.
Present address: Departamento de Química, Facultad de Ciencia, USACH, Casilla 307, Santiago 2, Chile.

Table 1. Assignment of the ¹H NMR and HH-COSY spectra of compound 2 (400 MHz, DMSO-*d*₆)

H	δ (ppm)	H–H correlation
2'	7.53 <i>d</i> (2.5)	7.53–7.42
6'	7.42 <i>dd</i> (8.5, 2.5)	7.42–6.88–7.42
5'	6.88 <i>d</i> (8.5)	7.42
8	6.80 <i>d</i> (2.4)	6.57
6	6.57 <i>d</i> (2.4)	6.80–3.86*
1''	5.05 <i>d</i> (7.4)	3.25
OMe-5	3.86 <i>s</i> —	6.57*
OMe-3	3.72 <i>s</i> —	—
6''	3.71 <i>m</i> —	3.47
4''	3.47 <i>m</i> —	3.71–3.18
5''	3.30 —	—
2''	3.25 <i>m</i> —	5.02
3''	3.18 <i>t</i> —	3.47–3.25

*Long-range.

Coupling constants (*J* in Hz) in parentheses.

δ6.86 was in good agreement with the chemical shift expected for this atom at C-8. Furthermore, the signal corresponding to the anomeric hydrogen atom appeared at δ5.16, showing that the sugar moiety is bonded to an aromatic ring and not to C-3. A 5,7,4'-trihydroxy-3,6,3'-trimethoxyflavone (jaceidin) 5-*O*-hexoside structure was therefore indicated. The ¹³C NMR spectrum lent further support to this interpretation, as two of the methoxyl carbon resonances appeared at 'normal' field values (δ56.1 and 56.8) and can be assigned to the groups attached to C-3' and C-6 (but not C-8) [Breitmaier, E., personal communication], while the third appeared at δ60.3, as expected for a methoxyl bonded to C-3. Upon acetylation, a hexaacetate was obtained for which a mass spectrum was recorded, showing the expected molecular ion peak at *m/z* 775.2. Hydrolysis with β-glucosidase gave glucose (identified chromatographically) and a substance which, after purification, melted at 126–130°. This value is in agreement with that expected for jaceidin [8–11] and differs markedly from those of its positional isomers centaureidin (5,7,3'-trihydroxy-3,8,4'-trimethoxyflavone: 196–197°) [11, 12] and 5,7,4'-trihydroxy-3,8,3'-trimethoxyflavone (215–217°) [13]. The mass spectrum of the aglycone agreed well with that described for jaceidin [9], and so did the UV spectra recorded with different shift reagents [6].

The presence of caraytin 7-*O*-β-D-glucoside in the bark of *E. glutinosa* can be related to the occurrence of its aglycone in the leaves of both Chilean species of this genus. On the other hand, the fact that this glycoside was found in rather small amounts in comparison with the strongly dominant dihydroquercetin xyloside agrees to some extent with the apparent absence of *O*-methylated flavonoids in *E. cordifolia* bark.

EXPERIMENTAL

General. The plant material was collected around Bullileo dam (36° 17' S, 71° 24' W), in the month of

Table 2. Assignment of the ¹³C NMR and CH-HETCOR spectra of compound 2 (100.2 MHz DMSO-*d*₆)

C	δ (ppm)	C–H correlation
4	172.3	—
7	161.5	—
8a	160.5	—
5	157.9	—
4''	152.6	—
3'	148.2	—
2	145.3	—
3	140.1	—
1'	121.3	—
6'	120.2	7.42
2'	115.8	6.88
5'	115.5	7.53
4a	109.3	—
1''	100.0	5.00
6	97.0	6.57
8	95.8	6.80
4''	77.4	3.47
5''	76.7	3.30
2''	73.3	3.25
3''	69.9	3.18
6''	60.9	3.71
OMe-3	59.3	3.72
OMe-5	56.3	3.83

February (summer). A voucher specimen is preserved in the herbarium of the National Museum of Natural History, Santiago, Chile. Chromatographic columns were packed with silica gel 60 or Sephadex LH-20. ¹H NMR spectra were run at 90 MHz; chemical shifts are given in ppm (δ) from TMS as int. standard.

Extraction and isolation. Powdered air-dried twigs (357 g) were soaked with EtOH (0.5 l) and then extracted (Soxhlet) with EtOH. The extract was concd and the residue (45.8 g) suspended in water (0.8 l) and partitioned into BuOH (5 × 0.35 l). The organic layers were concd to yield 32.2 g yellowish powder. Part of this mixture (15 g) was chromatographed on a silica gel column (600 g) with CHCl₃–MeOH–H₂O (74:23:3) to afford 3 frs weighing (A) 2.1, (B) 8.2 and (C) 1.5 g, respectively. Fraction A was resolved into 1 (158 mg) and caryatin 7-*O*-β-D-glucoside (2, 30 mg) by successive chromatography on Sephadex LH-20 and silica gel. Fraction B was similarly separated into dihydroquercetin 3-*O*-β-D-xyloside (1, major constituent) and reynoutrin (minor). Fraction C was not analysed.

*Dihydroquercetin 3-*O*-β-D-xyloside (1)* Amorphous powder, mp 170–175°. UV λ^{MeOH}_{max} (log ε) nm: 232 (4.14), 292 (4.25), 336 sh (3.68); λ^{NaOMe}_{max} 248, 330; λ^{NaOAc}_{max} 253, 290, 330; λ^{AlCl₃}_{max} 225, 316, 383; λ^{AlCl₃+HCl}_{max} 223, 315, 385; EIMS *m/z* (rel. int.) 304 (dihydroquercetin [D]⁺) (40%), 286 [D–H₂O]⁺ (12), 275 [D–CHO]⁺ (56), 165 (28), 153 (100), 152 (24), 150 (33), 123 (55). ¹H NMR (Me₂CO-*d*₆): δ3.3–4.5 (4H, *m*), 4.88 (1H, *d*, *J* = 10 Hz), 5.29 (1H, *d*, *J* = 10 Hz), 6.0 (2H, *s*), 6.87 (2H, *m*), 7.1 (1H, *m*), 11.72 (1H, *s*,

exchangeable with D₂O). Acid hydrolysis gave xylose, identified chromatographically.

Caryatin 7-O-β-D-glucoside (2). Amorphous powder, mp 287–291°, [α]_D –16.7° (MeOH; c 0.12). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm: 253, 266 sh, 348; $\lambda_{\text{max}}^{\text{NaOMe}}$ 231, 258, 396; $\lambda_{\text{max}}^{\text{NaOAc}}$ 253, 266, 348; $\lambda_{\text{max}}^{\text{AlCl}_3}$ 228, 257, 375; $\lambda_{\text{max}}^{\text{AlCl}_3 + \text{HCl}}$ 253, 266 sh, 348, 430, (weak). ¹H NMR (400 MHz, MeOH-*d*₄): δ 3.18 (1H, t), 3.25 (1H, m), 3.47 (1H, m), 3.71 (1H, m), 3.72 (1H, s), 3.86 (1H, s), 5.05 (1H, d, *J* = 7.4 Hz), 6.57 (1H, d, *J* = 2.4 Hz), 6.80 (1H, d, *J* = 2.4 Hz), 6.88 (1H, d, *J* = 8.5 Hz), 7.42 (1H, dd, *J* = 2.4, 8.5 Hz), 7.53 (1H, d, *J* = 2.5 Hz). Acid hydrolysis gave glucose, identified chromatographically, and caryatin; UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm: 253, 266 sh, 298 sh, 347; $\lambda_{\text{max}}^{\text{NaOMe}}$ 266, 314, 392; $\lambda_{\text{max}}^{\text{NaOAc}}$ 253, 266 sh, 300, 348; $\lambda_{\text{max}}^{\text{AlCl}_3}$ 257, 297, 370, 475; $\lambda_{\text{max}}^{\text{AlCl}_3 + \text{HCl}}$ 253, 266 sh, 304, 350, 430.

5,7,4'-Trihydroxy-3,6,3'-trimethoxyflavone (jaceidin) 5-O-β-D-glucoside (3). Yellow amorphous powder, mp 285–290°, [α]_D²⁰ –89° (pyridine; c 1). EIMS *m/z* (rel. int.) 360.0855 (jaceidin [J]⁺, calc. C₁₈H₁₆O₈ 360.0860) (100%), 359 [J–H]⁺ (84), 345 [J–Me]⁺ (68), 343 [J–OH]⁺ (14), 342 [J–H₂O]⁺ (30). ¹H NMR (DMSO-*d*₆): δ 3.35 (4H, m), 3.74 (3H, s), 3.77 (3H, s), 3.84 (3H, s), 4.5–5.0 (2H, m), 5.16 (1H, d, *J* = 2 Hz), 6.86 (1H, s), 6.96 (1H, d, *J* = 8.0 Hz), 7.63 (1H, dd, *J* = 2.0, 8.0 Hz), 7.71 (1H, d, *J* = 2.0 Hz).

Hydrolysis of compound 3 with β-glucosidase. Compound 3 was suspended in H₂O and incubated with β-glucosidase for 48 hr at 37°. Glucose was identified chromatographically. After freeze-drying and prep. chromatography on silica gel (CHCl₃–MeOH, 10:1) the dark yellow, gummy product was further purified by chromatography on Sephadex LH-20 (MeOH–CHCl₃, 20:1) to afford jaceidin as an amorphous powder melting at 126–130°. EIMS *m/z* (rel. int.) 360 [M]⁺ (100%), 359 [M–H]⁺ (54), 345 [M–Me]⁺ (68); UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm: 257, 273, 352; $\lambda_{\text{max}}^{\text{NaOMe}}$ 273, 335, 416; $\lambda_{\text{max}}^{\text{AlCl}_3}$ 272, 281, 298 sh, 383; $\lambda_{\text{max}}^{\text{AlCl}_3 + \text{HCl}}$ 260, 280, 299 sh, 368, 412.

5,7,4'-Trihydroxy-3,6,3'-trimethoxyflavone 5-O-β-D-glucoside (3) hexaacetate. Compound 3 dissolved in pyridine and treated with Ac₂O at room temp. for 48 hr, afforded a single product which crystallized as needles, mp 187–189° (CHCl₃–MeOH), [α]_D²⁰ –54° (CHCl₃; c 1). IR ν_{max} cm^{–1}: 1770, 1655. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm: 246, 265, 298 sh, 337. FAB-MS *m/z* (rel. int.) 775.2 [M]⁺ (4), 460 (3), 445 (7), 331 (2), 307 (24), 290 (13), 271 (1.5), 242 (2), 229 (1.5), 169

(6), 165 (5), 155 (25), 154 (100), 152 (11), 139 (12), 138 (29), 137 (53), 136 (69), 135 (7), 125 (8), 120 (11), 107 (22), 95 (3). ¹H NMR (CDCl₃): δ 2.02 (3H, s), 2.04 (6H, s), 2.06 (3H, s), 2.33 (3H, s), 2.35 (3H, s), 3.85 (3H, s), 3.86 (3H, s), 3.97 (3H, s), 4.15 (2H, m), 5.1–5.4 (4H, m), 6.55 (1H, s), 7.13 (1H, d, *J* = 8.4 Hz), 7.57 (1H, dd, *J* = 2.0, 8.4, Hz), 7.70 (1H, d, *J* = 2.0 Hz). ¹³C NMR (CDCl₃): δ 56.1 (OMe-3'), 56.8 (OMe-6), 60.3 (OMe-3).

Acknowledgements—Thanks are due to Mr Rodrigo Urzúa for collecting and Mr Godofredo Sepúlveda for preparing the plant material, and Prof. Eberhard Breitmaier and Dr Gert Eckhardt for providing the NMR and mass spectra. S.D. thanks the Friedrich Naumann Foundation for a fellowship.

REFERENCES

1. Bate-Smith, E. C., Davenport, S. M. and Harborne, J. B. (1967) *Phytochemistry* **6**, 1407.
2. Tschesche, R., Delhvi, S., Sepúlveda, S. and Breitmaier, E. (1979) *Phytochemistry* **18**, 867.
3. Muñoz Pizarro, C. (1966) *Flores Silvestres de Chile*. Universidad de Chile, Santiago.
4. Ogiso, A. and Kashida, I. (1972) *Phytochemistry* **11**, 3545.
5. Srivastava, S. K. (1980) *Indian J. Pharm. Sci.* **42**, 95.
6. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids*. Springer, Berlin.
7. Kingston, D. G. I. (1971) *Tetrahedron* **27**, 2691.
8. Roitman, J. N. and James, L. F. (1985) *Phytochemistry* **24**, 835.
9. Harborne, J. B., Mabry, T. J. and Mabry, H. (1975) *The Flavonoids*. Chapman & Hall, London.
10. Fukui, K., Matsumoto, T., Nakamura, S. and Nakayama, H. (1968) *Bull. Chem. Soc. J.* **41**, 1413.
11. Fárkás, L., Hörhammer, L., Wagner, H., Rösler, H. and Gurniak, R. (1964) *Chem. Ber.* **97**, 610.
12. Fárkás, L., Hörhammer, L., Wagner, H., Rösler, H. and Gurniak, R. (1964) *Chem. Ber.* **97**, 1666.
13. Horie, T., Tsukuyama, M., Kawamura, Y. and Yamamoto, S. (1988) *Phytochemistry* **27**, 1491.