Phytochemistry, Vol. 24, No. 2, pp. 347–349, 1985.
Printed in Great Britain.

0031–9422/85 $3.00 + 0.00

FLAVONOIDS FROM *ERYTHROXYLON ARGENTINUM*

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(*Received* 8 *May* 1984)

Key Word Index—*Erythroxylon argentinum*; Erythroxylaceae; flavonoids; 7,4′-dimethylquercetin 3-rutinoside-5-glucoside; 7,4′-dimethylquercetin 3-rutinoside; quercetin 3-α-L-rhamnoside; quercetin 3-rutinoside.

Abstract—Quercetin 3-rutinoside, quercetin 3-α-L-rhamnoside, 7,4′-dimethylquercetin 3-rutinoside and the novel glycoside 7,4′-dimethylquercetin 3-rutinoside-5-glucoside have been identified from aerial parts of *Erythroxylon argentinum*.

INTRODUCTION

The genus *Erythroxylon* (family Erythroxylaceae; section *Archerythroxylum*) is widespread in tropical regions including areas of South America and Madagascar. The occurrence of tropane alkaloids, terpenoids, tannins and flavonoids in this genus has recently been reviewed [1, 2]. Flavonoid aglycones have been characterized from hydrolysed extracts of *Erythroxylon* species [3] but very few flavonoid glycosides have been identified [4–6].

Erythroxylon argentinum Schulz is a perennial shrub native to northwestern Argentina which belongs to the same section as *E. coca* and *E. novogranatense*. No chemical studies on this species were known until we recently reported [7] lineal hydrocarbons and alcohols, squalene, cholesterol, campesterol, sitosterol, β-amyrin palmitate, α- and β-amyrins and *trans*-4-hydroxy-*N*-methyl-L-proline from its aerial parts.

In continuation of this research on *E. argentinum*, we now report the characterisation of the following flavonoids: quercetin 3-rutinoside (rutin) (**1**), 7,4′-dimethylquercetin (ombuin) 3-rutinoside-5-glucoside (**2**), quercetin 3-α-L-rhamnoside (quercitrin) (**3**) and ombuin 3-rutinoside (**4**). Ombuin 3-rutinoside-5-glucoside is reported for the first time in nature. Ombuin 3-rutinoside has previously been characterized in *E. rufum* [4], *E. novogranatense* var. *novogranatense* and var. *truxillense* [4] and also in *Flyriella* (Compositae) [8]. However, no spectral data of this flavonoid were available.

RESULTS AND DISCUSSION

The defatted methanolic extract of *E. argentinum* was concentrated and successively percolated over polyamide with chloroform, water and methanol. Column chromatography of the methanolic percolate afforded two main Shinoda positive fractions. Fraction 1 was chromatographed further to give **1** and **2** while fraction 2 provided **3** and **4**. **1** was identified as rutin by acid hydrolysis, spectral data and comparison with a standard.

The UV spectrum of **2** showed that this glycoside was a 3,5,7,3′,4′-pentasubstituted flavonol without an *ortho*-dihydroxyl grouping [9]. Acid hydrolysis of **2** gave glucose, rhamnose and an aglycone. The UV spectrum of the aglycone was similar to that of the glycoside except for the presence of free 3- and 5-hydroxyls (formation of acid-stable complexes with $AlCl_3$), which must therefore be glycosylated in **2**. Furthermore, the 7- and 4′-hydroxyls either absent or substituted (no shift with NaOAc; stable NaOMe UV spectrum). The mass spectrum of the aglycone of **2** exhibited a $[M]^+$ (also base peak) at *m/z* 330 and diagnostic peaks at *m/z* 315 $[M-Me]^+$, 287 $[M-Me-CO]^+$, 167 $[A_1+H]^+$ and 151 $[B_2]^+$ in agreement with a flavonol with one methoxyl and one hydroxyl in both ring A and ring B. The ^1H NMR spectrum confirmed the 3,5,7,3′,4′-pentasubstitution of the aglycone, which was therefore identified as 7,4′-di-*O*-methylquercetin (ombuin).

The ^1H NMR spectrum of the TMSi derivative of **2** showed an AMX system due to a 3′,4′-disubstitution: a doublet at δ7.50 ($J_m = 2$ Hz), a doublet at 6.89 ($J_o = 8$ Hz) and a double-doublet at 7.58 ($J_m = 2$ Hz; $J_o = 8$ Hz) assigned to H-2′, H-5′ and H-6′, respectively. The 5,7-disubstitution was demonstrated by the presence of two doublets at δ6.49 and 6.52 each with *meta*-coupling ($J = 2$ Hz), ascribed to H-6 and H-8, respectively. Singlets at δ3.85 and 3.87 confirmed the presence of two aromatic methoxyls. A complex multiplet centred at δ0.90 and a doublet ($J = 2$ Hz) at δ4.18 were assigned to H-6‴ and H-1‴ of the rhamnose, respectively. The chemical shift of H-1‴ (δ4.18) compared with that reported for a rhamnose directly attached to an aglycone (δ5.00) indicated that rhamnose should be a second moiety of a rhamnosyl glucosyl disaccharide. Since the position of H-1‴ of the rhamnose in 7- and 3-*O*-rutinosides appears in the region δ4.20–4.40 ($J = 2$ Hz) and the H-6‴ as a complex signal in the region δ0.70–1.00 [9], this rhamnosyl glucoside must be rutinose. The anomeric proton (H-1″) of the glucose of

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this disaccharide appeared as a doublet at δ5.68 (J = 7 Hz). This chemical shift confirmed the direct attachment to the aglycone, and the diaxial coupling (J = 7 Hz) between H-1″ and H-2″ indicated β-configuration. An additional doublet at δ5.13 agreed with an anomeric proton (H-1^{iv}) of another glucose moiety attached to the 5-hydroxyl and β-linked (diaxial coupling, J = 7 Hz) to the aglycone.

The ^{13}C NMR spectrum of **2** indicated 3,5-di-*O*-glycosylation and that both glucoses were directly attached to the aglycone while rhamnose was linked to one of the glucose moieties. This fact was based on the upfield shift of C-3, which was similar to that expected for 3-*O*-glycosylation and not for 3-*O*-rhamnosylation [10]. The downfield shift (+22 ppm) of C-6″ and the slight upfield shift of C-5″ (−0.8 ppm) with respect to the ^{13}C NMR spectrum of D-glucose indicated that the C-1 of rhamnose (C-1‴) was linked to C-6 of glucose (C-6″) [10]. Furthermore, glucose was β-linked, rhamnose α-linked and both were in the pyranose form. Signals at 55.6 and 55.8 ppm confirmed the presence of a methoxyl at both the 7- and 4′-positions. Therefore, **2** was identified as 7,4′-di-*O*-methylquercetin 3-*O*-β-D-glucopyranosyl(1 → 6)-*O*-α-L-rhamnopyranoside-5-*O*-β-D-glucopyranoside (ombuin 3-rutinoside-5-glucoside).

Since this is a novel flavonol glycoside, its structure was confirmed by chemical methods. Thus, **2** was permethylated by the Hakomori method [11], further hydrolysed and the partially methylated monosaccharides were compared with standards. Some standards were prepared by permethylation of pinocembrin 7-*O*-β-neohesperidoside and rutin followed by acid hydrolysis. The chromatographic analysis of the partially methylated sugars of **2** afforded 2,3,4-tri-*O*-methyl-L-rhamnose, 2,3,4-tri-*O*-methyl-D-glucose and 2,3,4,6-tetra-*O*-methyl-D-glucose. These results confirmed that the C-1 of the rhamnose was linked to the 6-hydroxyl of the glucose to give rutinose and that the 1-hydroxyl of a second glucose moiety was involved in another linkage (5-*O*-glucosylation).

Upon acid hydrolysis of **3**, quercetin and rhamnose were obtained and the UV spectrum indicated 3-glycosylation. The ^{1}H NMR and ^{13}C NMR spectra of **3** were identical to those reported for quercetin 3-*O*-α-L-rhamnopyranoside (quercitrin) [9, 10].

Acid hydrolysis of **4** gave ombuin, glucose and rhamnose. The UV spectrum showed the presence of a free 5-hydroxyl and the ^{1}H NMR spectrum was similar to that of **2** but the doublet at δ5.13 assigned to the anomeric proton of the glucose attached to the 5-hydroxyl was absent. Shielding of H-6 was also observed, probably due to the absence of 5-glycosylation. Therefore, **4** was identified as 7,4′-di-*O*-methylquercetin 3-*O*-β-glucopyranosyl (1 → 6)-α-L-rhamnopyranoside (ombuin 3-rutinoside).

The few fragmentary studies regarding the flavonoids of the Erythroxylaceae restricted to the genus *Erythroxylon* [2] as well as this paper show that the flavonols quercetin and kaempferol as their 3-glycosides and the apparent absence of flavones are chemotaxonomic features of this genus. Only once [4] has a flavanone 7-*O*-glycoside been detected. Further studies on *E. argentinum* constituents are being carried out in our laboratories.

EXPERIMENTAL

^{1}H NMR (100 MHz) and ^{13}C NMR (25.2 MHz) were recorded in the solvents stated on a Varian XL-100 with a Fourier-transform accessory; MS were recorded at 70 eV.

Plant material. Aerial parts of *E. argentinum* were collected at Departamento Las Talitas, Barranca Colorada, Province of Tucumán (Argentina). Voucher specimens have been deposited at the 'Miguel Lillo' Foundation (Tucumán, Argentina) under No. 8654.

Extraction and isolation of the flavonoids. Dried ground aerial parts of *E. argentinum* were successively extracted in a Soxhlet with petrol and MeOH. The petrol extract (2.4% rel. to dry plant) was worked up as described previously [7]. The methanolic extract (24.2% rel. to dry plant) was successively percolated on polyamide with $CHCl_3$ (17.3% rel. to MeOH extract), H_2O (73.0% rel. to MeOH extract) and MeOH (9.7% rel. to MeOH extract). The methanolic percolate was chromatographed on a Sephadex LH-20 column with MeOH as eluant. Two main fractions (Shinoda positive test) were obtained. Fraction 1 gave two spots on TLC [silica gel; $CHCl_3$–MeOH–HOAc (5:2:2), R_f 0.50 (**1**) and 0.31 (**2**)] which were separated on a silica gel H column using gradients of $CHCl_3$–MeOH from 5:1 to 1:1. Fraction 2 also gave two spots on TLC [silica gel, $CHCl_3$–MeOH–HOAc (5:2:2), R_f 0.61 (**3**) and 0.52 (**4**)] which were isolated on a Sephadex LH-20 column with MeOH as eluant.

O-Trimethylsilylation of the flavonoids was carried out with HMDS and TMCS (1:1) in pyridine in the usual manner [9].

Acid hydrolysis of the glycosides. Each glycoside, dissolved in 7% aq. H_2SO_4, was heated in a sealed tube at 100° for 1 hr. The aglycone was extracted with $CHCl_3$ and its spectrum recorded. The aq. neutralized layer free of salts was examined by TLC on cellulose with sugar standards. Acetate alditols were also prepared.

7,4′-Di-O-methylquercetin 3-O-β-rutinoside-5-O-β-glucoside (**2**). UV λ_{max} MeOH nm: 250, 260 (sh), 340; +NaOMe: 257, 273 (sh), 390. No shifts were observed on addition of $AlCl_3$, $AlCl_3$/HCl, NaOAc or NaOAc/H_3BO_3. ^{1}H NMR (TMSi deriv., $CDCl_3$): δ0.90 (3H, *m*, H-6″), 3.10–3.70 (complex signal, sugar protons), 3.85 (3H, *s*, OMe), 3.87 (3H, *s*, OMe), 4.18 (1H, J_{ee} = 2 Hz, H-1‴), 5.13 (1H, *d*, J_{aa} = 7 Hz, H-1^{iv}), 5.68 (1H, *d*, J_{aa} = 7 Hz, H-1″), 6.49 (1H, *d*, J_m = 2 Hz, H-6), 6.52 (1H, *d*, J_m = 2 Hz, H-8), 6.89 (1H, *d*, J_o = 8 Hz, H-5′), 7.50 (1H, *d*, J_m = 2 Hz, H-2′), 7.58 (1H, *dd*, J_m = 2 Hz and J_o = 8 Hz, H-6′). ^{1}H NMR (free glycoside, CD_3OD): δ3.93 (3H, *s*, MeO), 3.95 (3H, *s*, MeO), 6.04 (1H, *d*, J = 8 Hz, H-5′), 6.71 (1H, *d*, J = 2 Hz, H-2′), 6.76 (1H, *dd*, J = 2 Hz and J = 8 Hz, H-6′), 6.85 (1H, *d*, J = 2 Hz, H-6), 6.91 (1H, *d*, J = 2 Hz, H-8). The chemical shifts of the sugar protons were coincident with those shown above. ^{13}C NMR (TMSi deriv., $CDCl_3$): δ18.0 (C-6‴), 55.4 (OMe), 55.5 (OMe), 61.1 (C-6″), 64.1 (C-6^{iv}), 69.4 (C-5‴ and C-4″), 71.6 (C-2‴, C-3‴ and C-4^{iv}), 73.2 (C-4‴), 73.8 (C-2^{iv}), 74.8 (C-2″), 76.1* (C-5″), 76.5* (C-3″), 76.9 (C-3^{iv}), 77.8 (C-5^{iv}), 93.9 (C-8), 100.1† (C-1‴ and C-1^{iv}), 100.3† (C-1″), 109.9 (C-6), 110.9 (C-10), 115.2‡ (C-2′), 115.6‡ (C-5′), 121.6§ (C-6′), 123.0§ (C-1′), 133.2 (C-3), 145.2 (C-3′), 148.5 (C-4′), 157.3 (C-2 and C-9), 162.9 (C-5 and C-7), 177.7 (C-4). (Assignments bearing the same superscript may be interchanged.)

7,4′-Di-O-methylquercetin (ombuin). UV λ_{max}^{MeOH} nm: 253, 267 (sh), 355; +NaOMe: 274 (sh), 415; +$AlCl_3$: 264, 300 (sh), 405; +$AlCl_3$/HCl: 262, 297 (sh), 405. No shifts were observed on addition of NaOAc or NaOAc/H_3BO_3. MS *m/z* (rel. int.): 330 $[M]^+$ (100), 315 $[M-Me]^+$ (20), 287 $[M-Me-CO]^+$ (12), 167 $[A_1+H]^+$ (2); 151 $[B_2]^+$ (7); 123 $[B_2-CO]^+$ (3).

Total methylation of **2**. The methansulfinyl carbanion of DMSO [12] (0.5 ml) was added to a soln of **2** (3 ml) in dry DMSO (0.5 ml) and stirred at 60° for 1.5 hr. After cooling, MeI (0.5 ml) was added and the mixture extracted twice with $CHCl_3$ (4 ml). The $CHCl_3$ layer was dried and the I_2 eliminated with toluene

under vacuum. Permethylated **2** was hydrolysed with the Kiliani mixture (HOAc–HCl–H_2O, 7:3:10) overnight in a sealed tube at 85°, MeOH was added and the acids were eliminated by percolation through a weak basic resin (AG3-X4A, HO^- form). The eluate was evapd to dryness and the residue chromatographed on silica gel HP TLC plates. The same procedure was applied to pinocembrin 7-*O*-neohesperidoside and rutin to get standards. Silica gel HP TLC, toluene–MeOH (10:2.5), R_f: 2,4-di-*O*-methylglucose, 0.20; 3,4-di-*O*-methylglucose, 0.20; 2,3,4-tri-*O*-methyl-D-glucose, 0.53; 3,4,6-tri-*O*-methyl-D-glucose, 0.59; 2,3,4,6-tetra-*O*-methyl-D-glucose, 0.65; 2,3,4-tri-*O*-methylrhamnose, 0.76; partial methylated sugars from **2**: three spots: 0.53; 0.65 and 0.76.

7,4′-Di-O-methylquercetin (ombuin) 3-O-β-rutinoside (**4**). UV λ_{max} MeOH nm: 255, 264 and 349; + NaOMe: 266, 295 (sh), 326 and 370; + $AlCl_3$: 268, 298 and 390 nm; + $AlCl_3$/HCl: 266, 297, 355 and 385. No shifts were observed on addition of NaOAc or NaOAc/H_3BO_3. ^{1}H NMR (TMSi deriv., $CDCl_3$): δ0.90 (3H, *m*, H-6‴), 3.10–3.70 (complex signal, sugar protons), 3.86 (3H, *s*, OMe), 3.89 (3H, *s*, OMe), 4.30 (1H, *d*, J = 2 Hz, H-1‴), 5.70 (1H, *d*, J_{aa} = 7 Hz, H-1″), 6.33 (1H, *d*, J = 2 Hz, H-6), 6.40 (1H, *d*, J = 2 Hz, H-8), 6.52 (1H, *d*, J_m = 2 Hz, H-2′), 6.92 (1H, *d*, J_o = 8 Hz, H-5′), 7.62 (1H, *dd*, J_m = 2 Hz, J_o = 8 Hz, H-6′).

Acknowledgements—We wish to thank Professor D.I.A. de Iglesias (Universidad Nacional de Tucumán, Tucumán, Argentina) for plant material, Professor P. Legname (Miguel Lillo Foundation, Tucumán, Argentina) for identification of the plant material, UMYMFOR (CONICET, Buenos Aires, Argentina) for GLC, MS, ^{1}H NMR and ^{13}C NMR measurements and CONICET (Argentina) for financial support. One of us (R.P.A.I.) is also indebted to CONICET for a research fellowship.

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