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CHRYSOERIOL 7-(2″-*O*-β-D-ALLOPYRANOSYL)-β-D-GLUCOPYRANOSIDE FROM *SIDERITIS GRANDIFLORA*

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Key Word Index—*Sideritis grandiflora*; Labiatae; ^{13}C NMR; chrysoeriol 7-(2″-*O*-β-D-allopyranosyl-β-D-glucopyranoside).

Abstract—The aerial parts of *Sideritis grandiflora* yielded a new flavone glycoside, identified as chrysoeriol 7-(2″-allosylglucoside).

As part of our programme on the study of the chemical components of *Sideritis* species we had occasion to investigate the flavonoid fraction of *S. grandiflora*. This species is endemic in the south-west of the Iberian peninsula. Methanol extraction of the dried aerial parts of this plant yielded a mixture of flavonoid glycosides (0.67%), the main component of which was isolated as the acetate by prep. TLC of the total acetylated mixture as well as that of the glycosidic fraction itself. Acid hydrolyses of this glycoside (SG-1) yielded chrysoeriol as the aglycone and glucose and a second hexose were detected as the sugars. Elemental analysis ($C_{46}H_{50}O_{25}$) and mass spectrometry of the peracetate (M^+ m/z 1002) also confirmed the presence of two hexose residues in the molecule.

Methylation of the natural product SG-1 with dimethyl sulphate–potassium carbonate in acetone and subsequent hydrolysis yielded a compound whose properties were the same as those reported for 7-hydroxy-5, 3′, 4′-trimethoxyflavone [1]. This thus established that the chrysoeriol is linked at position 7 with a disaccharide moiety. The ^1H NMR spectrum of the chrysoeriol bioside in deuteropyridine at 300 MHz showed two doublets for the anomeric protons at δ 5.75 ($J = 7.1$ Hz) and at 5.88 ($J = 8.2$ Hz), respectively. The values of the coupling constant indicate that the two hexose moieties are linked in a 1, 2-*trans* diequatorial orientation and are in the pyranose forms. The lack of resonances in the region δ 2.0 ± 0.5 indicated the absence of acetyl groups. The nature of the second hexose residue as well as the position of the interglycosidic linkage in the natural product was determined by ^{13}C NMR spectroscopy. The signal at δ 67.3 in the ^{13}C NMR spectrum of the compound for an oxymethine carbon atom in a β-hexopyranosyl residue, was indicative of an unsubstituted C-4 in a β-allopyranosyl moiety [2]. Acidic hydrolysis of the glycoside and subsequent GC analysis of the sugars confirmed that the second hexose unit was indeed allopyranose. The R_f values of allose and glucose, on TLC and PC, in most solvent systems are very similar and the former can very easily be mistaken for the latter which is of more widespread occurrence. The signal at δ 82.3 must be clearly that of the sugar carbon atom bearing the second glycosyl moiety. This value is more downfield than would be expected if glucose were the terminal sugar and linked to allose in the positions C-2, C-3 or C-6. The chemical shift values for these carbon atoms are δ 70.6, 71.3 and 61.4, respectively [3]. Alternatively, if allose is the terminal sugar in the

natural product, then the position of linkage must be C-2″ of the glucosyl moiety. This is analogous with the chemical shift of C-2″ in kaempferol-3-*O*-β-glucosyl-(1→2)-β-glucoside which is at δ 82.3 [4]. Comparison of the chemical shift of C-1″ in apigenin-7-*O*-β-D-glucopyranoside (δ 100.2) [4] with that of the corresponding carbon resonance in the natural product (δ 99.2) shows that the glucose anomeric carbon (C-1″) signal has moved upfield on 2″-*O*-β-D-allopyranosylation. There are reports that in flavonoid *O*-glucosides, further glycosidation at C-2″ results in an upfield shift of the C-1″ resonance depending upon the nature of the second sugar and the anomeric configuration [4]. The signals for the anomeric carbon atoms in SG-1 at δ 92.2 and 102.2, for C-1″ and C-1‴ respectively, were clearly identified by a SFORD experiment in which the decoupler frequency was set in the range of the anomeric proton resonances. This reduces the residual coupling for these carbons well below the instrument resolution and the signals therefore appear as apparently sharp signals of relatively greater intensity. The chemical shift of C-1″ in the spectra of the natural product as well as its peracetate further confirmed that the intersugar linkage must be (1→2). On the basis of these conclusions we postulate the structure chrysoeriol 7-*O*-(2″-*O*-β-D-allopyranosyl-β-D-glucopyranoside) for SG-1. This is the second report of the occurrence in nature of a flavonoid glycoside with this disaccharide moiety [3].

EXPERIMENTAL

Plant material was collected in June 1979 near Barbate (Cadiz), Spain, and voucher specimens were deposited in the Herbarium of the Faculty of Pharmacy, Madrid 'Complutense' University. Mps were determined on a Kofler block and are uncorr. The mass spectra (EIMS) were run on a Hitachi–Perkin–Elmer RMU 6 MG instrument. GC was performed on a Perkin–Elmer 3920 gas chromatograph (column: capillary, SE-30, 30 m; temp. 180°; N_2 carrier gas).

Isolation of SG-1. Dried and defatted aerial parts of *S. grandiflora* (300 g) were extracted (Soxhlet) with MeOH for 60 hr. On cooling the soln yielded a ppt (2 g, 0.67%) which was a mixture of several flavonoid glycosides. The major fraction was separated by prep. TLC on Si gel ($CHCl_3$–MeOH, 3:2). The glycoside SG-1 was crystallized from MeOH–Me_2CO–*n*-hexane, mp 161–167°, $[\alpha]_D^{18}$ −50.1° (MeOH; *c* 0.255). IR ν_{max}^{KBr} cm^{-1}: 3700–2300 (*br*, OH), 2930, 1725, 1660, 1600, 1510, 1500, 1070, 1030, 840. UV λ_{max}^{MeOH} nm (log ε): 272 (4.21), 320 sh (4.19), 333 (4.23); λ_{max}^{MeONa} nm (log ε): 258 sh (4.2), 397 (4.28); $\lambda_{max}^{MeOH+AlCl_3}$ (log ε): 278 (4.23), 335 sh (4.16), 357 (4.26); $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm (log ε): 278 (4.22), 335 sh (4.2), 350 (4.25); $\lambda_{max}^{MeOH+NaOAc}$ nm (log ε): 268 (4.23), 310 (4.12), 331 (4.11), 397 (4.07) ^1H NMR (300 MHz, C_5D_5N, TMS int. standard) δ 13.6 (1H, *s*, OH-5), 7.75 (1H, *d*, *J* = 2.4 Hz, H-2′), 7.67 (1H, *dd*, J_{ortho} = 8.2 Hz, J_{meta} = 2.4 Hz, H-6′), 7.29 (1H, *d*, *J* = 8.4, H-5′), 7.27 (1H, $W_{1/2}$ = 2.5 Hz, H-8), 7.07 (1H, *d*, *J* = 2.4 Hz, H-6), 6.98 (1H, *s*, H-3), 5.88 (1H, *d*, *J* = 8.2 Hz, H-1‴), 5.75 (1H, *d*, *J* = 7.1 Hz), 4.8–4.1 (12H, sugar protons), 3.9 (3H, *s* OMe). ^{13}C NMR (80 MHz, DMSO-d_6) δ 181.9 (C-4), 164.2 (C-2), 162.9 (C-7), 161.1 (C-5), 156.9 (C-9), 150.9 (C-3′), 148.1 (C-4′), 127.6 (C-1′), 120.6 (C-6′), 116.0 (C-5′), 110.8 (C-2′), 105.5 (C-10), 103.5 (C-3), 102.2 (C-1‴), 99.8 (C-6), 99.2 (C-1″), 95.6 (C-8), 82.3 (C-2″), 77.1 (C-5″), 75.8 (C-3″), 74.6 (C-5‴), 71.9 (C-3‴), 70.9 (C-4″), 69.6 (C-2‴), 67.3 (C-4‴), 61.4 (C-6‴), 60.8 (C-6″).

Acetate of SG-1. The total flavonoid glycoside mixture was acetylated with pyridine and Ac_2O and worked-up in the usual manner and the product subjected to prep. TLC on Si gel using EtOAc as eluent. The major constituent was separated and crystallized from EtOH mp 129–131°, $[\alpha]_D^{22}$ −36.0° ($CHCl_3$; *c* 1.03) calcd for. $C_{46}H_{50}O_{25}$: C, 55.08; H, 5.02. (Found: C, 55.09; H, 5.08%.) UV ν_{max}^{MeOH} nm (log ε): 235.5 (4.29), 257 (4.14), 307 (4.25). MS (70 eV. direct inlet) *m/z* (%): 1003 (MH^+, 40), 961 (100), 919 (80), 673 (8), 631 (22), 613 (28), 539 (10), 385 (84), 343 (85), 331 (99), 301 (52), 289 (58), 243 (78), 213 (65), 169 (85), 139 (98), 127 (80), 111 (71), 109 (70). ^1H NMR (100 MHz, $CDCl_3$) δ 7.43 (1H, *dd*, J_{ortho} = 8 Hz, J_{meta} = 2 Hz, H-6′), 7.39 (1H, *d*, *J* = 2 Hz, H-2′), 7.13 (1H, *d*, *J* = 8 Hz, H-5′), 6.90 (1H, *J* = 2.5 Hz, H-8), 6.67 (1H, *d*, *J* = 2.5 Hz, H-6), 6.55 (1H, *s*, H-3), 5.6 (1H, *m*, H-3‴), 5.4–4.70 and 4.30–3.90 (11H, sugar protons), 3.90 (3H, *s*, OMe), 2.40 (3H, *s*, OAc-5), 2.30 (3H, *s*, OAc-4′), the other sugar acetoxyls appeared at 2.11 (3H), 2.08 (3H), 2.01 (9H), 1.99 (3H), 1.97 (3H). ^{13}C NMR (25.2 MHz, $CDCl_3$) δ aglycone part: 176.0, 161.4, 160.2, 158.1, 151.5, 150.4, 142.5, 129.8, 123.2, 119.0, 112.7, 110.2, 109.3, 108.5, 102.5, 56.1; sugar carbons: 99.1 (C-1″), 98.1 (C-1‴), 77.9, 74.2, 71.9, 70.3, 68.6 (double signal), 68.3, 66.1, 61.9 (double signal); acetyl carbons: 170.3, 170.1, 169.4, 169.3, 169.1, 168.8, 168.2 (CO), 21.0, 20.5, 20.3 (Me).

Acid hydrolysis of SG-1. The glycoside was subjected to acidic hydrolysis with 2 N HCl–EtOH (1:1) for 1 hr at 100°, diluted with H_2O and then extracted with Et_2O. The Et_2O phase was dried and evapd to yield an aglycone and the aq. soln neutralized with Amberlite MB-3 and evapd to yield the sugar fraction. The latter was dried in vacuum, converted to the trimethylsilyl ethers and subjected to GC. The monosaccharides were identified as glucose and allose by comparison with authentic samples. The aglycone was identified as chrysoeriol on the basis of its mp, UV spectrum and comparison with an authentic sample. It crystallized from Me_2CO–*n*-hexane, mp 324–326°.

Chrysoeriol acetate. The aglycone was acetylated with pyridine and Ac_2O and worked-up in the usual manner to yield the tri-*O*-acetate mp 220–222°; Calcd for $C_{22}H_{18}O_9$: C, 61.97; H, 4.26%. (Found: C, 61.78; H, 4.20). UV λ_{max}^{MeOH} nm (log ε): 239 (4.15), 255 sh (4.06), 309 (4.14). ^1H NMR (60 MHz, $CDCl_3$) δ 7.50 (1H, *dd*, J_{ortho} = 8.5 Hz, J_{meta} = 2.5 Hz, H-6′), 7.44 (1H, *d*, *J* = 2.5 Hz, H-2′), 7.40 (1H, *d*, *J* = 2.5 Hz, H-8), 7.22 (1H, *d*, *J* = 8.5 Hz, H-5′), 6.91 (1H, *d*, *J* = 2.5 Hz, H-6), 6.67 (1H, *s*, H-3), 3.94 (3H, *s*, OMe), 2.46 (3H, *s*, OAc-5), 2.35 (6H, *s*, OAc-7 and OAc-4′). MS (75 eV, direct inlet) *m/z* (%): 426 (M^+, 4), 384 (25), 342 (61), 300 (100), 285 (3), 271 (8), 257 (7), 229 (6), 153 (10), 148 (8), 133 (6), 123 (5), 105 (3).

Methylation of SG-1: 7-hydroxy-5, 3′, 4′-trimethoxyflavone. Methylation and hydrolysis using standard procedures gave 7-hydroxy-5, 3′, 4′-trimethoxyflavone, mp 279–283°. UV λ_{max}^{MeOH} nm (log ε): 241 (4.25), 265.5 (4.19), 336 (4.27). λ_{max}^{NaOMe} nm (log ε): 232 (4.35), 274 (4.4), 313 (4.13), 359 (4.14). $\lambda_{max}^{MeOH+AlCl_3}$ nm (log ε): 242 (4.27), 265.4 (4.23), 336 (4.24), 403 sh (3.61). $\lambda_{max}^{MeOH+NaOAc}$ nm (log ε): 273.5 (4.40), 313 (4.13), 356 (4.14). MS (75 eV, direct inlet) *m/z* (%): 328 (M^+, 100), 327 (70), 314 (22), 299 (46), 281 (53), 255 (20), 185 (18), 162 (22), 137 (24), 105 (18).

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TWO RHAMNETIN DIGALACTOSIDES AND AN OLEANOLIC ACID DIGALACTOSIDE FROM THE FLOWERS OF *CASSIA LAEVIGATA*

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Key Word Index—*Cassia laevigata*; Leguminosae; flowers; rhamnetin 3-galactosyl(1→4)galactoside; rhamnetin 3-galactosyl(1→6)galactoside; oleanolic acid 3-galactosyl(1→4)galactoside.

Abstract—From the flowers of *Cassia laevigata*, two new rhamnetin glycosides, the 3-galactosyl(1→4)-galactopyranoside and the related 3-galactosyl(1→6)galactopyranoside, and oleanolic acid 3-galactosyl(1→4)-galactopyranoside have been isolated. These three glycosides have not been isolated earlier from any plant source. The known compounds quercetin, docosyl alcohol, carnaubyl alcohol, ceryl alcohol and octacosanol have also been obtained.

INTRODUCTION

Species of *Cassia* are rich sources of flavonoids, anthraquinones and polysaccharides. Flavonoids[1, 2] and anthraquinones[3] have previously been isolated from *Cassia laevigata*. The plant possesses important medicinal properties [4].

RESULTS AND DISCUSSION

From the flowers of *Cassia laevigata* two flavonol glycosides (**1**) $C_{24}H_{32}O_{17}$ mp 215°(d), (**2**) $C_{24}H_{32}O_{17}$ mp 271°(d) and a triterpene-carboxylic acid glycoside (**3**) $C_{42}H_{68}O_{13}$ mp > 300°, and the known compounds quercetin docosyl alcohol carnaubyl alcohol, ceryl alcohol and octacosanol have been isolated.

1 on acid hydrolysis gave an aglycone and galactose. The aglycone $C_{16}H_{12}O_7$, mp 284°gave all characteristic colour reactions of a flavonol[5] and was identified as rhamnetin on the basis of UV, IR, NMR, mass fragmentation pattern, chemical degradation and co-chromatography with an authentic sample.

Methylation of **1** followed by acid hydrolysis gave 5, 7, 3′, 4′-tetramethylquercetin confirming the attachment of a sugar moiety at position-3. The glycoside was fully methylated, hydrolysed and the resulting partially methylated sugars were identified as 2, 3, 6-tri-*O*-methylgalactose and 2, 3, 4, 6-tetra-*O*-methylgalactose[6, 7]. This established the glycoside as a (1→4) bioside of galactose linked at position-3 of the aglycone. This was also confirmed by periodate oxidation. The glycoside was completely hydrolysed by β-glucosidase showing the presence of two β-linkages. On this basis **1** was identified as rhamnetin 3-*O*-β-D-galactosyl(1 → 4)-*O*-β-D-galactopyranoside. This glycoside has not been isolated earlier from any plant source.

2 was also a flavonol glycoside and on acid hydrolysis again gave rhamnetin and galactose. The attachment of the sugar moiety at position-3 was established as above. The permethylated glycoside gave 2, 3, 4-tri-*O*-methylgalactose and 2, 3, 4, 6-tetra-*O*-methylgalactose on hydrolysis with 4 N sulphuric acid which established that **2** is a (1→6) bioside linked at position-3 of the aglycone. This was also confirmed by periodate oxidation. The glycoside was completely hydrolysed with emulsin thereby showing the presence of β-linkages. On the above facts **2** was identified as rhamnetin 3-*O*-β-D-galactosyl(1→6) galactopyranoside. This glycoside is also new to nature.

3 was a terpene glycoside and on acid hydrolysis gave an aglycone and galactose. The aglycone, $C_{30}H_{48}O_3$, mp 306°, responded to all the colour tests for a triterpenoid and its IR spectrum showed the presence of a hydroxyl group, an acid carbonyl, a gem