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TWO ACYLATED FLAVANONE GLYCOSIDES FROM *NIEREMBERGIA HIPPOMANICA*

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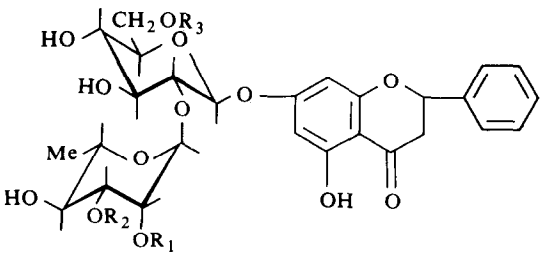
Key Word Index—*Nierembergia hippomanica*; Solanaceae; acylated flavanone glycosides; pinocembrin 7-*O*-β-(3‴-*O*-acetyl)neohesperidoside; pinocembrin 7-*O*-β-(6″-*O*-acetyl)neohesperidoside.

Abstract—Two new acylated flavanone glycosides have been isolated from *Nierembergia hippomanica* and identified by spectral data as pinocembrin 7-*O*-β-(3‴-*O*-acetyl)neohesperidoside and pinocembrin 7-*O*-β-(6″-*O*-acetyl)neohesperidoside.

INTRODUCTION

In continuation of our work on *Nierembergia hippomanica* Miers. [1,2], we now report the isolation and structural elucidation of two new acylated flavonoids, pinocembrin 7-*O*-β-(3″-*O*-acetyl)neohesperidoside (**1**) and pinocembrin 7-*O*-β-(6″-*O*-acetyl)neohesperidoside (**2**). We recently reported [2] the identification of the related compound, pinocembrin 7-*O*-β-(2‴-*O*-acetyl)neohesperidoside (**3**) from the same plant.



1 $R_1 = R_3 = H$; $R_2 = Ac$

2 $R_1 = R_2 = H$; $R_3 = Ac$

3 $R_1 = Ac$; $R_2 = R_3 = H$

RESULTS AND DISCUSSION

Repeated CC of the ethyl acetate soluble fraction of the defatted ethanol extract of the whole plant of *N. hippomanica* yielded seven main fractions. Fractions 3–7 were found to be rich in flavonoids. Pinocembrin 7-*O*-β-neohesperidoside [1] was obtained pure from fraction 7 and was also present with other flavonoids in fraction 6. **3** was obtained from fraction 3. Fractions 4 and 5 provided a mixture of acylated flavonoids which was further separated into three components **1–3**.

IR spectra of **1** and **2** showed hydroxyl, an ester carbonyl and carbonyl (C–4 of a flavonoid) absorptions at 3350, 1720 and 1630 cm^{-1}, respectively and a broad C–O stretching band in the region 1100–1000 cm^{-1} suggesting their glycosidic nature. UV spectra of **1** and **2** and the shifts obtained with the usual UV reagents were coincident with those of pinocembrin 7-*O*-β-neohesperidoside [1].

Acid hydrolysis of **1** and **2** gave 5,7-dihydroxyflavanone (pinocembrin) (mmp, co-TLC), glucose and rhamnose (GLC of the alditols). Total acetylation of **1** and **2** provided hepta-*O*-acetylpinocembrin 7-*O*-β-neohesperidoside, while treatment of **1** and **2** with sodium methoxide–methanol (deacetylation) yielded pinocembrin 7-*O*-β-neohesperidoside. These data are identical with those of **3** and suggested a common skeleton differing only in the position of acylation.

Mass spectrometry of the TMS derivatives [3] of **1** and **2** were performed to determine which of the sugar moieties was acylated. The presence of *m/z* 711 (monoacetylated TMS-neohesperidose) in both spectra indicated that only one acetyl group was attached to the disaccharide. Fragments corresponding to the loss of acetic acid, TMS-OH and TMS-OH plus acetic acid were also present.

Comparison of both spectra revealed the following differences. The mass spectrum of **1** showed fragments at *m/z* 333 corresponding to TMS-monoacetylrhamnose and *m/z* 690 (TMS-glucose attached to TMS-aglycone) indicating that no acetyl group is located on the glucose. That of **2** showed the presence of *m/z* 363 (TMS-rhamnose without acetyl group) and *m/z* 659 (TMS-monoacetylglucose attached to TMS-aglycone). From these results **1** must have one acetyl group attached either to C–3‴ or C–4‴ of the rhamnose since this compound has a different R_f from that of **3** which has an acetyl group at C–2‴ (rhamnose). **2** has one acetyl group attached either to C–3″, C–4″ or C–6″ of the glucose.

The position of the acyl substituents could also be determined by comparison of the ^{13}C NMR spectra of the acylated glycosides with that of the deacylated flavonoid, taking into account that the signal bearing

an acyloxy group is shifted by *ca* +2.0 ppm and the adjacent carbons by *ca* −2.0 ppm [4]. The ^{13}C NMR spectrum of **3** revealed a downfield shift of C-2‴ (1.66 ppm) and upfield shifts of C-3‴ and C-1‴ (1.99 and 2.90 ppm, respectively). These results confirm the structure of **3** as pinocembrin 7-*O*-β-(2‴-*O*-acetyl)neohesperidoside.

The ^{13}C NMR spectrum of **1** showed a large downfield shift of C-3‴ (3.87 ppm) and large upfield shifts of C-2‴ and C-4‴ (2.79 and 3.18 ppm, respectively) proving the attachment of the acetyl group at C-3‴. The ^1H NMR data confirmed this point. Thus, ^1H NMR spectrum (in C_5D_5N at 300 MHz) of **1** gave rise to a 1H double doublet at δ 5.83 which was shifted downfield from its position in the ^1H NMR spectrum of pinocembrin 7-*O*-β-neohesperidoside (δ 4.52). This double doublet assigned to H-3‴ shows the normal *ca* 1 ppm downfield shift caused by acetylation and shows the coupling constants (J_{ae} = 3 Hz and J_{aa} = 10 Hz) attributable to partitions with an equatorial (H-2‴) and an axial (H-4‴) proton. This shift was also observed in the ^1H NMR spectrum in $CDCl_3$ of the TMS derivative of **1** but the H-3‴ signal was partially superimposed with those of H-1‴ and H-1″. The signals from H-2‴ and H-4‴ also showed minor downfield shifts (0.15 and 0.13 ppm, respectively), because they are adjacent to the carbon bearing the acetoxyl group. Hence, **1** is pinocembrin 7-*O*-β-(3‴-*O*-acetyl)neohesperidoside.

The ^{13}C NMR spectrum of **2** resembled that of pinocembrin 7-*O*-β-neohesperidoside, except that signals of C-6″ and C-5″ were shifted (+2 ppm and −3.21 ppm, respectively). Moreover, the ^1H NMR spectrum of the TMS derivative of **2** showed a broad signal at δ 4.40 (H-6″) that underwent the *ca* 0.50 ppm downfield shift due to acetylation at C-6″ (glucose); H-5″ also evidenced a minor downfield shift (0.20 ppm), while the remaining signals were practically identical with those of pinocembrin 7-*O*-β-neohesperidoside. Compound **2** is, therefore, pinocembrin 7-*O*-β(6″-*O*-acetyl)neohesperidoside.

EXPERIMENTAL

General details have been previously described [2].

Isolation of **1–3**. The EtOAc soluble portion of the defatted EtOH extract of dried ground *N. hippomanica* was concd and chromatographed on a Si gel column using gradients of C_6H_6–Me_2CO as eluents. Seven main fractions were obtained. Fraction 3 was repeatedly chromatographed on a Si gel column using C_6H_6–Me_2CO (3:2) as solvent, yielding **3**. Upon repeated CC of fractions 4 and 5 with the same eluent **1** and **2**, respectively, were obtained. **1** was the major component followed by **3**, whilst **2** was present in small amounts. [Si gel TLC, C_6H_6–Me_2CO (1:2), R_f: **1**, 0.40; **2**, 0.34; **3**, 0.47; pinocembrin 7-*O*-neohesperidoside 0.12].

Pinocembrin 7-O-β-(3‴-O-*acetyl*)*neohesperidoside* (**1**). UV λ_{max}^{MeOH} nm: 285, 328; +$AlCl_3$: 308, 379; +$AlCl_3$–HCl: 308, 376; +NaOAc: 285, 328; +NaOAc–H_3BO_3: 287, 330; +NaOMe: 285, 358. IR ν_{max} cm^{-1}: 1720 (C=O of an ester). ^1H NMR (DMSO-d_6; δ 1.20 (3H, *d*, C-6^R); 2.00 (3H, *s*, MeCO); 2.80–3.35 (2H, *m*, H-3); 3.30–4.00 (9H, *m*, sugar protons); 4.40–5.20 (3H, *m*, H-3^R, H-1^R, H-1^G); 5.70 (1H, *dd*, *J* = 4, *J′* = 12 Hz, H-2); 6.15 (1H, *d*, *J* = 2 Hz, H-6); 6.19 (1H, *d*, *J* = 2 Hz, H-8); 7.47 (5H, *m*, ring B phenyl protons). ^1H NMR (300 MHz, C_5D_5N): δ 1.80 (3H, *d*, C-6^R); 2.02 (3H, *s*, MeCO); 3.05 (2H, *dq*, J_{gem} = 16, J_{gauche} = 4, J_{anti} = 12 Hz, H-3); 4.00–5.00 (7H, *m*, sugar protons); 4.54 (1H, *t*, J_{aa} = 10 Hz, H-4^R); 5.00(1H, *dd*, J_{ee} = 1.5, J_{ea} = 3 Hz, H-2^R); 5.40 (1H, *dd*, *J* = 4, *J′* = 12 Hz, H-2); 5.73 (1H, *d*, *J* = 10 Hz, H-1^G); 5.83 (1H, *dd*, J_{ea} = 3, J_{aa} = 10 Hz, H-3^R); 6.34 (1H, *d*, J_{ee} = 1.5 Hz, H-1^R); 6.63 (1H, *d*, *J* = 2 Hz, H-6); 6.68 (1H, *d*, *J* = 2 Hz, H-8); 7.40–7.56 (5H, *m*, ring B phenyl protons). ^{13}C NMR (25.15 MHz, DMSO-d_6): δ 17.83 (*q*, C-6^R); 21.04 (*q*, MeCO); 42.13 (*t*, C-3); 60.34 (*t*, C-6^G); 67.62 (*d*, C-2^R); 68.27 (*d*, C-5^R); 68.62 (*d*, C-4^R); 69.53 (*d*, C-4^G); 74.20 (*d*, C-3^R); 76.39 (*d*, C-2^G); 76.81 (*d*, C-3^G and C-5^G); 78.49 (*d*, C-2); 95.09 (*d*, C-8); 96.39 (*d*, C-6); 97.04 (*d*, C-1^G); 100.36 (*d*, C-1^R); 103.32 (*s*, C-10); 126.62 (*d*, C-2′, C-3′, C-5′ and C-6′); 128.53 (*d*, C-4′); 138.40 (*s*, C-1′); 162.48 (*s*, C-9); 162.85 (*s*, C-5); 164.71 (*s*, C-7); 170.05 (*s*, MeCO); 196.66 (*s*, C-4). ^{13}C NMR (75.46 MHz, C_5D_5N): δ 18.84 (*q*, C-6^R); 21.06 (*q*, MeCO); 43.41 (*t*, C-3); 62.01 (*t*, C-6^G); 69.86 (*d*, C-5^R); 70.10 (*d*, C-2^R); 70.73 (*d*, C-4^G); 71.09 (*d*, C-4^R); 76.51 (*d*, C-3^R); 78.34 (*d*, C-5^G); 78.91 (*d*, C-2^G and C-3^G); 79.45 (*d*, C-2); 96.19 (*d*, C-8); 97.84 (d, C-6); 99.01 (*d*, C-1^G); 102.60 (*d*, C-1^R); 104.20 (*s*, C-10); 126.81 (*d*, C-3′ and C-5′); 128.99 (*d*, C-4′); 129.11 (*d*, C-2′ and C-6′); 139.36 (*s*, C-1′); 163.36 (*s*, C-9); 164.43 (*s*, C-5); 166.11 (*s*, C-7); 170.85 (*s*, MeCO); 196.58 (*s*, C-4).

Trimethylsilylation of **1**. HMDS and TMCS (1:1) in pyridine were used as previously described [2]. ^1H NMR ($CDCl_3$): δ 1.18 (3H, *d*, C-6^R); 2.06(3H, *s*, MeCO); 2.90 (2H, *dq*, J_{gem} = 16, J_{gauche} = 4, J_{anti} = 12 Hz, H-3); 3.45-4.10 (9H, *m*, sugar protons); 4.70 (1H, *dd*, J_{ae} = 3, J_{aa} = 10 Hz, H-3^R); 4.80–4.90 (2H, *m*, H-1^R, H-1^G); 5.25 (1H, *dd*, *J* = 4, *J′* = 12 Hz, H-2); 6.03 (1H, *d*, *J* = 2 Hz, H-6); 6.30 (1H, *d*, *J* = 2 Hz, H-8); 7.34 (5H, *m*, ring B phenyl protons). MS *m/z* (%): 711 (TMS-monoacneohesperidose, 1.3); 690 (TMS-pinocembrin 7-Glc − TMS-O + H, 1.0); 651 (711 − HOAc, 1.5); 621 (711 − TMS-OH, 14.0); 561 (621 − HOAc, 7.0); 471 (561 − TMS-OH, 2.0); 421 (TMS-AcRha − H, 2.6); 400 (TMS-pinocembrin, 2.0); 385 (400 − 15, 6.5); 333 (421 + H − TMS-O, 55.0); 328 (400 + H − TMS, 37.0); 313 (328 − 15, 19.0); 273 (333 − HOAc, 40.0); 261 (333 − TMS + H, 10.0); 256 (pinocembrin, 1.5); 243 (333 − TMS-OH, 98.0); 201 (273 − TMS, 30.0); 73 (TMS, 100.0).

Pinocembrin 7-O-β-(6″-O-*acetyl*)*neohesperidoside* (**2**). UV λ_{max}^{MeOH} nm: 285, 328; when adding the UV reagents the same shifts were observed as those described for **1**. IR ν_{max} cm^{-1}: 1720 (C=O of ester). ^1H NMR (300 MHz, C_5D_5N): δ 1.78 (3H, *d*, C-6^R); 1.98 (3H, *s*, MeCO); 3.04 (2H, *dq*, J_{gem} = 16, J_{gauche} = 4, J_{ante} = 12 Hz, H-3); 4.10–5.05 (8H, *m*, sugar protons); 5.45 (1H, *dd*, *J* = 4, *J′* = 12 Hz, H-2); 5.60 (2H, *m*, H-6^G); 5.72 (1H, *d*, *J* = 10 Hz, H-1^G); 6.35 (1H, *d*, *J* = 1.5 Hz, H-1^R); 6.68 (1H, *d*, *J* = 2 Hz, H-6); 6.72 (1H, *d*, *J* = 2 Hz, H-8); 7.45–7.50 (5H, *m*, ring B phenyl protons). ^{13}C NMR (25.15 MHz, DMSO-d_6): δ 17.93 (*q*, C-6^R); 20.80 (*q*, MeCO); 42.18 (*t*, C-3); 62.30 (*t*, C-6^G); 68.30 (*d*, C-5^R); 70.05 (*d*, C-4^G); 70.33 (*d*, C-3^R); 70.44 (*d*, C-2^R); 71.84 (*d*, C-4^R); 73.66 (*d*, C-5^G); 76.14 (*d*, C-2^G); 76.96 (*d*, C-3^G); 78.70 (*d*, C-2); 95.26 (*d*, C-8); 96.55 (*d*, C-6); 97.38 (*d*, C-1^G); 100.42 (*d*, C-1^R); 103.35 (*s*, C-10); 126.60, 126.88 (each *d*, C-2′, C-3′, C-5′ and C-6′); 128.50 (*d*, C-4′); 138.37 (*s*, C-1′); 162.55 (*s*, C-9); 162.83 (*s*, C-5); 164.63 (*s*, C-7); 169.65 (*s*, MeCO); 196.71 (*s*, C-4). ^{13}C NMR (75.46 MHz, C_5D_5N): δ 18.81 (*q*, C-6^R); 20.46 (*q*, MeCO); 43.50 (*t*, C-3); 64.35 (*t*, C-6^G); 69.92 (*d*, C-5^R); 71.27 (*d*, C-4^G); 72.35 (*d*, C-3^R); 72.71 (*d*, C-2^R); 74.02 (*d*, C-4^R); 75.61 (*d*, C-5^G); 77.56 (*d*, C-2^G); 79.09 (*d*, C-3^G); 79.63 (*d*, C-2); 96.43 (*d*, C-8); 97.78 (*d*, C-6); 99.46 (*d*, C-1^G); 102.45 (*d*, C-1^R); 104.46 (*s*, C-10); 126.84 (*d*, C-3′ and C-5′); 129.08 (*d*, C-2′ and C-6′); 139.00 (*s*, C-1′); 163.34 (*s*, C-9); 164.09 (*s*, C-5); 166.08 (*s*, C-7); 196.64 (*s*, C-4).

Trimethylsilylation of **2**. Method as described above. ^{1}H NMR ($CDCl_3$): δ 1.20 (3H, *d*, $J = 6$ Hz, C-6^R); 1.90 (3H, *s*, MeCO); 3.05 (2H, *dq*, H-3); 3.50–4.10 (8H, *m*, sugar protons); 4.40 (2H, *m*, H-6^G); 4.90 (1H, *d*, $J = 1.5$ Hz, H-1^R); 5.10 (1H, *d*, $J = 7$ Hz, H-1^G); 5.30 (1H, *dd*, $J = 4$, $J' = 12$ Hz, H-2); 6.07 (1H, *d*, $J = 2$ Hz, H-6); 6.35 (1H, *d*, $J = 2$, H-8); 7.35 (5H, *m*, ring B phenyl protons). MS *m/z* (%): 711 (TMS-monoacneohesperidose, 2.0); 651 (711 − HOAc, 1.2); 621 (711 − TMS-OH, 2.5); 561 (621 − HOAc, 1.0); 451 (TMS-Rha − H, 4.0); 400 (TMS-pinocembrin, 1.2); 385 (400 − 15, 1.5); 363 (451 + H − TMSO, 29.0); 328 (400 + H − TMS, 4.0); 313 (328 − 15, 5.0); 273 (363 − TMS-OH, 16.0); 73 (TMS, 100.0).

Total acetylation of **1** *and* **2**. This was performed with Ac_2O–pyridine in the usual manner, The product obtained in both cases was shown to be (IR, NMR, MS) hepta-*O*-acetylpinocembrin 7-*O*-β-neohesperidoside [1].

Deacetylation of **1** *and* **2**. Deacetylation was carried out with NaOMe–MeOH in the usual manner. Pinocembrin 7-*O*-β-neohesperidoside [1] was obtained.

Pinocembrin 7-O-β-(2‴-O-acetyl)neohesperidoside (**3**). ^{1}H NMR (300 MHz, C_5D_5N): δ 1.82 (3H, *d*, C-6^R); 1.98 (3H, *s*, MeCO); 3.05 (2H, *dq*, $J_{gem} = 16$, $J_{gauche} = 4$, $J_{anti} = 12$ Hz, H-3); 4.00–4.90 (8H, complex signal, sugar protons); 4.70 (1H, *dd*, $J_{ea} = 3$, $J_{aa} = 10$ Hz, H-3^R); 5.40 (1H, *dd*, $J = 4$, $J' = 12$ Hz, H-2); 5.69 (1H, *d*, $J_{aa} = 10$ Hz, H-1^G); 6.10 (1H, *dd*, $J_{ee} = 1.5$, $J_{ea} = 3$ Hz, H-2^R); 6.30 (1H, *d*, $J_{ee} = 1.5$ Hz, H-1^R); 6.70 (1H, *d*, $J = 2$ Hz, H-6); 6.75 (1H, *d*, $J = 2$ Hz, H-8); 7.40–7.55 (5H, *m*, ring B phenyl protons). ^{13}C NMR (25.15 MHz, DMSO-d_6) δ 17.83 (*q*, C-6^R); 20.90 (*q*, MeCO); 42.16 (*t*, C-3); 60.33 (*t*, C-6^G); 68.34 (*d*, C-5^R and C-3^R); 69.68 (*d*, C-4^G); 72.07 (*d*, C-2^R and C-4^R); 75.91 (*d*, C-2^G); 76.61 (*d*, C-5^G); 76.88 (*d*, C-3^G); 78.59 (*d*, C-2); 95.25 (*d*, C-8); 96,49 (*d*, C-6); 97.43 (*d*, C-1^G and C-1^R); 103.42 (*s*, C-10); 127.25 (*d*, C-2′ and C-6′); 128, 32 (*d*, C-3′ and C-5′); 128.61 (*d*, C-4′); 138.46 (*s*, C-1′); 162.56 (*s*, C-9); 162.95 (*s*, C-5); 164.82 (*s*, C-7); 169.66 (*s*, MeCO); 196.76 (*s*, C-4). ^{13}C NMR (75.46 MHz, C_5D_5N): δ 18.57 (*q*, C-6^R); 21.04 (*q*, MeCO); 43.00 (*t*, C-3); 61.79 (*t*, C-6^G); 69.83 (*d*, C-5^R); 70.17 (*d*, C-3^R); 70.94 (*d*, C-4^G); 73.80 (*d*, C-4^R and C-2^R); 77.80 (*d*, C-2^G); 78.35 (*d*, C-5^G); 78.55 (*d*, C-3^G); 79.39 (*d*, C-2); 96.28 (*d*, C-8); 97.73 (*d*, C-6); 99.14 (*d*, C-1^G); 99.26 (*d*, C-1^R); 104.36 (*s*, C-10); 126.84 (*d*, C-3′ and C-5′); 128.68 (*d*, C-4′); 129.41 (*d*, C-2′ and C-6′); 139.11 (*s*, C-1′); 163.39 (*s*, C-9); 164.11 (*s*, C-5); 165.99 (*s*, C-7); 171.08 (*s*, MeCO); 196.80 (*s*, C-4).

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