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AN ACYLATED FLAVONOID FROM *NIEREMBERGIA HIPPOMANICA*

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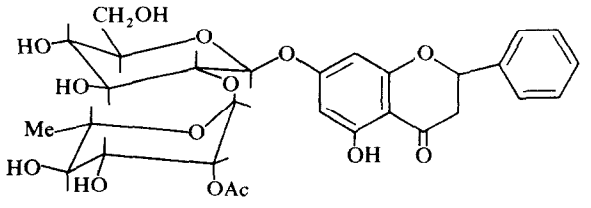
Key Word Index—*Nierembergia hippomanica*; Solanaceae; acylflavonoid; pinocembrin 7-*O*-β-(2‴-*O*-acetyl)neohesperidoside.

Abstract—A new acylflavonoid has been isolated from *Nierembergia hippomanica* and identified by chemical and spectral data as pinocembrin 7-*O*-β-(2‴-*O*-acetyl)neohesperidoside.

We have previously reported [1] the isolation of pinocembrin 7-*O*-β-neohesperidoside from *Nierembergia hippomanica* Miers. (Solanaceae). Further studies on this species led to the isolation of another flavonoid (**1**) which was shown to be acylated. The present communication describes its characterisation as pinocembrin 7-*O*-β-(2‴-*O*-acetyl)neohesperidoside and is the first report of this compound. Several acylated anthocyanins have been isolated from the Solanaceae [2], most of them with a cinnamic acid derivative as the acylating moiety. Acylated flavonoids have also been isolated from *Petunia hybrida* [3], which belongs to the same tribe and subtribe as the genus *Nierembergia*. As far as we know flavonoids acylated with acetyl groups have not been described for the Solanaceae family, but have been reported from the Vitaceae [4–6], Compositae [7–9], Crassulaceae [10, 11], Pinaceae [10, 11] and Gramineae [12].

The IR spectrum of the new acyl flavonoid (**1**) showed, among other signals typical for a flavonoid glycoside, a peak at 1720 cm^{-1} in agreement with a carbonyl of an ester. Its UV spectrum is characteristic of a flavanone; on addition of $AlCl_3$ a bathochromic shift was observed, which was maintained in the presence of $AlCl_3$/HCl indicating the presence of a free 5-hydroxyl. No shift was observed with NaOAc indicating that the 7-hydroxyl be substituted. The UV spectral data were identical with those of pinocembrin 7-*O*-β-neohesperidoside [1]. The ^{1}H NMR spectrum in DMSO-d_6 showed a singlet at δ 2.03 (3 H) assigned to an acetate methyl group; a multiplet at δ 2.6–3.0 (2 H) was ascribed to H-3, whilst a double doublet at δ 5.70 (1 H) accounted for H-2. Aromatic proton signals appeared at δ 6.13 (1 H, *d*, J = 2 Hz) and 6.19 (1 H, *d*, J = 2 Hz) corresponding to H-6 and H-8, respectively; a multiplet at δ 7.47 (5 H) indicated an unsubstituted B ring. Proton sugars appeared at δ 1.18 (Me-rhamnose), 3.0–4.0 (9 H, *m*), 4.4–5.2 (3 H, *m*, two H are H-1^R and H-1^G).



1

Acid hydrolysis of **1** gave pinocembrin (5,7-dihydroxyflavanone), rhamnose and glucose. Total acetylation of **1** afforded hepta-*O*-acetyl pinocembrin 7-*O*-neohesperidoside [1], indicating that it is a derivative of pinocembrin 7-*O*-neohesperidoside. ^{1}H NMR spectrum of the total acetylated glycoside revealed the presence of two kinds of acetyl groups, a phenolic one (δ 2.45) and those attached to the sugar portion of the glycoside (from δ 2.02 to 2.21).

The IR signal at 1720 cm^{-1} and the ^{1}H NMR peak at δ 2.03 show the presence of an aliphatic acetate group, which must be located on the sugar moiety. According to these results, **1** is pinocembrin 7-*O*-acetylneohesperidoside. This structure was confirmed by deacetylation with NaOMe–MeOH to give pinocembrin 7-*O*-neohesperidoside.

In an attempt to locate the acetyl group on one of the sugars, the MS of the trimethylsilylated derivative [13] of **1** was determined. A fragment at *m/z* 711, assigned to monoacetylated TMSi-neohesperidose, indicated that only one acetyl group is attached to the disaccharide. Fragments at *m/z* 651, 621, 561 and 471 correspond to the loss of AcOH, TMSiOH, TMSiOH and AcOH respectively, as well as AcOH and two TMSiOH. The presence of *m/z* 689 (TMSi-glucose attached to TMSi-aglycone) indicates that no acetyl group is attached to the glucose. A fragment at *m/z* 333 accounts for a TMSi-rhamnose with one acetyl group; the other fragments are typical for subsequent losses of TMSiOH and AcOH, and for fission of the aglycone. Accordingly, there is only one acetyl group which is attached either to C-2, C-3 or C-4 of rhamnose.

The position of the acetyl group was determined by comparing the ^{1}H NMR spectra of the TMSi derivative of pinocembrin 7-*O*-neohesperidoside with the TMSi derivative of **1**. In the latter spectrum a double doublet centred at δ 5.12 (J = 2 Hz and J' = 4 Hz), not present in the spectrum of TMSi-pinocembrin 7-*O*-neohesperidoside, was assigned to the proton directly attached to the carbon carrying the acetyl group. The values of the coupling constants agree with those observed for a sugar

proton coupled to vicinal ones in equatorial–equatorial ($J = 1.8$ Hz) and equatorial–axial ($J = 3.5$ Hz) spacial arrangement. Only H-2 of the rhamnose show these coupling constants [14] with H-1 and H-3, respectively. Thus the acetyl group must be attached to C-2 of the rhamnose and therefore, **1** is identified as pinocembrin 7-*O*-*β*-(2‴-*O*-acetyl)neohesperidoside.

EXPERIMENTAL

General details have been previously described [1].

Isolation of **1**. Dried and ground plant material was successively extrd with petrol and EtOH in a Soxhlet. Upon concn of the EtOH extract the residue obtained was suspended in H_2O and successively extracted with $CHCl_3$ and EtOAc. The EtOAc extract was concd and chromatographed on a Si gel column using gradients of C_6H_6–Me_2CO as eluants when 7 main fractions were obtained. Fraction 3 was repeatedly chromatographed on Si gel columns using C_6H_6–Me_2CO (3:2) as solvent, yielding **1**.

Pinocembrin 7-O-β-(2‴-O-acetyl)neohesperidoside (**1**). $C_{29}H_{33}O_{14}$. mp 130–135° $[\alpha]_D^{25}$ − 62° (pyridine; *C* 0.2). 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 1.18 (3 H, *d*, Me-rhamnose); 2.03 (3 H, *s*, MeCO); 2.60–3.00 (2 H, *m*, H-3); 3.00–4.00 (9 H, *m*, sugar protons); 4.40–5.20 (3 H, *m*, H-2^R, H-1^R and H-1^G); 5.70 (1 H, *dd*, $J = 4$, $J = 12$ Hz, H-2); 6.13 (1 H, *d*, $J = 2$ Hz, H-6); 6.19 (1 H, *d*, $J = 2$ Hz, H-8); 7.47 (5 H, *m*, ring B phenyl protons).

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

Trimethylsilylation of **1**. HMDS and TMCS (1:1) in pyridine were used. After 5 min at room temp, the mixture of **1** and silylating agents was concd to dryness and extracted with dry $CHCl_3$. Salts were filtered off, the filtrate was concd and the residue obtained was used for ^1H NMR and MS. ^1H NMR ($CDCl_3$): δ 1.21 (3H, *d*, $J = 6$ Hz, Me-rhamnose): 2.06 (3H, *s*, MeCO); 2.84 (2H, *dq*, $J_{gem} = 16$, $J_{gauche} = 4$, $J_{anti} = 12$ Hz, H-3); 3.30–4.10 (12 H, complex signals, sugar protons except H-1^R and H-1^G); 4.96 (1 H, *d*, $J = 2$ Hz, H-1^R); 5.07 (1 H, *d*, $J = 7$ Hz, H-1^G); 5.12 (1 H, *dd*, $J_{ee} = 2$, $J_{ea} = 4$ Hz, H-2^R); 5.30 (1 H, *dd*, $J_{gauche} = 4$, $J_{anti} = 12$ Hz, H-2); 6.03 (1 H, *d*, $J = 4$ Hz, H-6); 6.35 (1 H, *d*, $J = 4$ Hz, H-8); 7.36 (5 H, *m*, ring B phenyl protons). MS *m/z* (%): 711 (TMSi-mono-Ac-neohesperidose, 2.0); 689 (TMSi-pinocembrin 7-glucoside − TMSiO, 1.3); 651 (711 − AcOH, 0.7); 621 (711 − TMSiOH, 13.0); 561 (621 − AcOH, 5.2); 471 (561 − TMSiOH, 5.8); 421 (TMSi-Ac-rhamnose − H, 4.3); 400 (TMSi-pinocembrin, 5.0); 385 (400 − 15, 7.3); 333 (421 + H − TMSiO, 52.1); 328 (400 + H − TMSi, 28.0); 313 (328 − 15, 10.0); 273 (333 − AcOH, 32.0); 261 (333 − TMSi + H, 8.0); 256 (pinocembrin, 1.0); 243 (333 − TMSiOH, 98.0); 103 ($C_6H_5CH{=}CH_2$, 35.0); 73 (TMSi, 100.0).

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

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