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PINOCEMBRIN 7-NEOHESPERIDOSIDE FROM *NIEREMBERGIA HIPPOMANICA*

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Key Word Index—*Nierembergia hippomanica*; Solanaceae; flavanone glycoside; pinocembrin 7-neohesperidoside; synthesis.

Abstract—A flavanone glycoside isolated from *Nierembergia hippomanica* was characterized by spectroscopic methods as pinocembrin 7-neohesperidoside. The structure was confirmed by total synthesis of the glycoside.

There have been no previous reports on the chemical constituents of the genus *Nierembergia*, although it belongs to the Solanaceae family, which has been extensively studied chemically. *N. hippomanica* (Miers.), which grows in several regions of Argentina, is known to have a toxic effect on cattle. In the present study, pinocembrin 7-neohesperidoside was identified. The structure of the glycoside was elucidated by spectroscopic methods and confirmed by synthesis via direct condensation of the aglycone and the disaccharide.

Although pinocembrin (5,7-dihydroxyflavanone) is widely distributed in heartwoods, principally in *Pinus* [1] and *Prunus* [2, 3] species, its glycosides have rarely been recorded. Pinocembrin 5-glucoside (verecundin) has been isolated only from *Prunus verecunda* [3] and *P. aequinoctialis* [4]. Pinocembrin 7-neohesperidoside has been reported [5] from *Sparattosperma vernicosum* (Bignoniaceae) although the data were not sufficient to assign the neohesperidose structure.

EXPERIMENTAL

Mps are uncorr. UV spectra were measured in MeOH. Optical rotations were determined with an automatic polarimeter. ^{1}H NMR spectra were obtained at 100 MHz with TMS as internal reference. ^{13}C NMR were measured at 25.2 MHz. IR spectra were recorded from KBr discs. MS were determined at 70 eV by direct insertion. Microanalyses were performed by Dr. B. B. de Deferrari.

Plant material. Plants of *Nierembergia hippomanica* Miers. were collected in the province of La Pampa, Argentina. Voucher specimens have been deposited in INTA under No. 1324.

Isolation of the pinocembrin 7-neohesperidoside. Dried and ground plant material was extracted with petrol and then EtOH in a Soxhlet. The EtOH extract was concd and chromatographed on a Si gel column with $CHCl_3$–MeOH (4:1) as solvent. Fractions 3 and 4 were rechromatographed to yield the glycoside which crystallized from EtOH, mp 274–276° (d); $[\alpha]_D$ −119.6° (*c* 2, Py); UV λ_{max} nm: 285, 328; +$AlCl_3$: 308 and 379 ($\Delta\lambda_I$ 23; $\Delta\lambda_{II}$ 51); +$AlCl_3$–HCl: 308 and 376; +NaOAc: 285 and 328; +NaOAc–H_3BO_3: 287 and 330; +NaOMe: 285 and 358 ($\Delta\lambda_{II}$ 30). ^{1}H NMR (DMSO-d_6): δ 1.15 (3H, *d*, Me-rhamnose); 2.6–3.0 (2H, *m*, H-3); 3.2–4.0 (5H, complex signal, sugar protons); 4.4–4.8 (4H, *m*, sugar protons); 5.1–5.4 (2H, *m*, H-1^R and H-1^G); 5.69 (1H, *dd*, J = 4, J = 12 Hz, H-2); 6.13 (1H, *d*, J = 2 Hz, H-6); 6.19 (1H, *d*, J = 2 Hz, H-8); 7.5 (5H, *m*, ring B phenyl protons); 11.8 (1H, *bs*, 5-OH). MS (*m/e*, %): 564 (M^+, 0.25); 418 (0.62); 256 (100); 255 (49.7); 179 (77.5); 152 (62.5); 147 (20.0); 129 (27.5); 124 (32.5); 85 (62.5); 73 (32.0); 71 (33.7); 60 (25.0); 57 (33.7); 55 (12.5); 45 (17.5); 43 (30.0). (Found: C, 56.57; H, 6.04. $C_{27}H_{32}O_{13}$ requires: C, 57.45; H, 5.67%).

Acetylation of the glycoside. The acetylation was carried out with Ac_2O–Py in the usual manner; needles from EtOH; mp 119–121°; $[\alpha]_D$ −64.4° (*c* 3, Py). UV λ_{max} nm: 267, 309; +NaOMe: 255 (sh), 275 (sh), 280, 283 (sh), 320 (sh); no shifts were observed with $AlCl_3$, $AlCl_3$–HCl, NaOAc and NaOAc–H_3BO_3. ^{1}H NMR ($CDCl_3$): δ 1.27 (3H, *d*, Me-rhamnose); 2.02, 2.10, 2.17 2.21, 2.45 (21H, *s* each, 7 acetyl groups); 2.85–3.10 (2H, *dq*, J_{gem} = 16, J_{gauche} = 4, J_{anti} = 12 Hz, H-3); 3.9–4.2 (4H, *m*, H-5^R, H-2^G, H-5^G, H-6^G); 4.7–5.2 (7H, *m*, H-1^R, H-2^R, H-3^R, H-4^R, H-1^G, H-3^G, H-4^G); 5.2–5.5 (1H, *q*, J_{gauche} = 4, J_{anti} = 12 Hz, H-2); 6.32 (1H, *d*, J = 4 Hz, H-6); 6.62 (1H, *d*, J = 4 Hz, H-8); 7.30 (2H, *s*, phenyl protons); 7.46 (3H, *s*, phenyl protons). ^{13}C NMR ($CDCl_3$) ppm: 17.38 (C-6^R)$_i$; 20.44, 20.85, 21.04 (<u>C</u>H$_3$CO); 44.71 (C-3); 61.71 (C-6^G); 66.73 (C-5^R)^a; 68.21 (C-4^G)^a; 69.82 (C-2^R)^a; 70.70 (C-3^R)^a; 72.06 (C-4^R); 73.92 (C-2^G)^b; 75.64 (C-5^G)^b; 76.41 (C-3^G)^b; 79.60 (C-2); 98.03 (C-8); 98.10 (C-6); 102.18 and 105.67 (C-1^R ^{G}); 106.20 (C-4a); 125.94 (C-2′ and C-6′); 128.68 (C-4′); 128.84 (C-3′ and C-5′); 137.78 (C-1′); 160.50 (C-5); 162.50 (C-8a); 169.75 (C-7); 178.60 (CH$_3$<u>C</u>O); 187.80 (C-4) (a and b: the assignments can be interchanged). MS (*m/e*, %): 563 (1.2); 562 (5.3); 561 (21.2); 331 (4.4); 273 (50.2); 213 (18.4); 171 (15.2); 169 (29.7); 153 (70.0); 127 (10.5); 126 (14.2); 111 (34.8); 109 (15.8); 79 (21.8); 52 (14.3); 43 (100); 42 (10.9). (Found: C, 56.96; H, 5.47. $C_{41}H_{46}O_{20}$ requires: C, 57.34; H, 5.36%).

Acid hydrolysis of the glycoside. Acid hydrolysis with 2 N HCl under reflux for 4 hr gave 5,7-dihydroxyflavanone, identified by direct comparison with an authentic sample, together with D-glucose and L-rhamnose.

Synthesis of hexa-O-acetyl-α-neohesperidosyl bromide. Hepta-O-acetyl-β-neohesperidose was prepared by the Helferich method [6, 7]. 1,3,4,6-Tetra-*O*-acetyl-α-D-glucose [8] and tri-*O*-acetyl-α-L-rhamnopyranosyl bromide [9] were added to a soln of $Hg(CN)_2$ and $HgBr_2$ in dry MeCN. The mixture was allowed to stand at room temp. for 3 hr and then evapd. $CHCl_3$ added and insoluble salts removed. The $CHCl_3$ soln was washed with N KBr, H_2O, dried and evapd. The residue was immediately dissolved in $CHCl_3$ and treated with 30% HBr–HOAc at room temp. for 30 min, $CHCl_3$ was added and the soln worked-up in the usual manner. To the residue obtained by evapn of $CHCl_3$, a soln of mercuric acetate in HOAc was added. The mixture was left at room temp. for 2 hr. $CHCl_3$ added before purification by PLC (Si gel GF_{254}), solvent C_6H_6–MeOH (92:8). The upper band provided needles of hepta-*O*-acetyl-β-D-neohesperidose, which after recrystallization from EtOH had mp 153–154°; $[\alpha]_D$ +3.65° (*c* 3.9, Me_2CO).

Hexa-O-acetyl-α-neohesperidoxyl bromide. The previous compound was treated with 30% HBr–HOAc in the usual manner to yield a syrup which was immediately dissolved in dry $CHCl_3$ and used in the condensation reaction.

Synthesis of 5,7-dihydroxyflavanone. Pinocembrin was prepared by Fries reaction [10]. Dry $AlCl_3$ was added slowly to a cooled suspension of 3,5-dihydroxyphenyl cinnamate in nitrobenzene with stirring. The mixture was left at room temp. for 24 hr, poured into dil HCl and extracted with EtOAc. The residue obtained by evapn of the solvent was purified by chromatography on a Si gel column with C_6H_6–Me_2CO. Pinocembrin was recrystallized from MeOH to mp 189–191°; $[\alpha]_D$ −2.68° (*c* 5.7, Py); The spectral data (UV, IR, ^1H NMR, MS) of synthetic and natural pinocembrin were coincident.

Synthesis of pinocembrin 7-neohesperidoside. Direct condensation of synthetic pinocembrin with hexa-O-acetyl-α-neohesperidosyl bromide. A mixture of synthetic pinocembrin, freshly prepared Ag_2CO_3 and Drierite in dry quinoline was stirred at room temp. for 30 min before addition of a soln of hexa-*O*-acetyl-α-neohesperidosyl bromide. The reaction mixture was stirred in the dark at room temp. for 4 hr when the Ag salts were removed by centrifugation, the supernatant poured into 10% HOAc to remove quinoline, and extracted with $CHCl_3$. The $CHCl_3$ extract was washed, dried and the residue dissolved in MeOH and deacylated with NaOMe; the mixture was diluted with H_2O, neutralized with HOAc, and extracted successively with hexane, Et_2O and EtOAc. The EtOAc extract was dried and evapd. The residue was purified by Si gel column chromatography using C_6H_6–Me_2CO (3:2) as solvent. Fractions having a positive Shinoda test were combined and evapd. The residue crystallized from MeOH after 2 days giving needles of mp 277–281°; $[\alpha]_D$ −3.57° (*c* 4, Py).

Direct condensation of natural pinocembrin with hexa-O-acetyl-α-neohesperidosyl bromide. The synthesis was performed as previously described for the racemic pinocembrin. In this case natural pinocembrin from *P. radiata* and *P. pinaster* was used. The product had mp 278–281°; $[\alpha]_D$ −115.2° (*c* 3.2, Py).

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