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FLAVONOL 3-*O*-NEOHESPERIDOSIDES OF *NERISYRENIA LINEARIFOLIA* AND *N. GRACILIS*

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Key Word Index—*Nerisyrenia linearifolia*; *N. gracilis*; Cruciferae; flavonol 3-*O*-neohesperidosides.

Flavonol 3-*O*-neohesperidosides are rare in nature. Indeed, only one, quercetin 3-*O*-neohesperidoside, has been unequivocally identified to date [1] while the kaempferol derivative has been provisionally identified [2]. During the course of a chemosystematic study of the genus *Nerisyrenia*, the 3-*O*-neohesperidosides of quercetin (**1**), kaempferol (**2**) and isorhamnetin (**3**) have been isolated and identified. This is the first report of the latter flavonoid, **3**, as a natural product, and all compounds are reported for the first time from the Cruciferae.

The standard set of six UV spectra [3] for each compound indicated that all had hydroxyl groups at positions 5, 7 and 4′. The presence of *B*-ring *ortho*-oxygen functions in **1** and **3** was indicated by the presence of a shoulder on the long wave length side of band II in the MeOH spectrum of each compound. An *o*-dihydroxyl group was established for **1** by the 82 nm bathochromic shift of band I in $AlCl_3$ (relative to band I in MeOH) followed by a hypsochromic shift of 35 nm on addition of HCl; also, the bathochromic shift of 20 nm for band I in $NaOAc/H_3BO_3$ (relative to band I in MeOH) confirmed the presence of a 3′, 4′-*o*-hydroxyl system.

Acid hydrolysis of each compound afforded the respective aglycone (co-chromatography with an authentic sample by PC and UV spectra) and glucose and rhamnose in a 1:1 ratio (GLC of the trimethylsilylated sugars) [3]. Moreover, comparison of the UV spectra for the natural products with those for the aglycone indicated that in all three compounds, the disaccharide must be *O*-linked at C_3. Hydrolysis with β-glucosidase failed for each natural product, indicating that in each the rhamnose was terminal in the disaccharide. The NMR spectra of the trimethylsilyl ethers [3] of **1** and **2** readily confirmed rhamnose to be one of the sugars in each by the presence of a 3 proton doublet (J 6·0) at δ 0·83–0·85 ppm; in addition a 1→2 interglycosidic linkage was indicated for each compound by the presence of a singlet at 4·81–4·83 attributable to the rhamnose H-1 proton [3] and a glucose H-1 proton signal at 5·75 ppm. These data confirmed that 1→6 linkages were not present in any of the three rhamnoglucosides. The identification of the disaccharide was subsequently established by treating each compound with H_2O_2; in each case, the oxidative cleavage gave a disaccharide identical with authentic neohesperidose (co-chromatography and co-electrophoresis).

EXPERIMENTAL

Voucher specimens for *N. linearifolia* (Wats.) Greene (*Bacon and Hartman 1355* collected from U.S.A.: TEXAS: Culberson Co.: 7·1 mi SE of jct. FM 1108 and 652, on 652), from which **1** and **2** were isolated, and *N. gracilis* I. M. Johnston (*Bacon and Hartman 1335* collected from Mexico: San Luis Potosi: gypsum plain W of Hwy. 57, 3·5 mi N of Matehuala), from which **3** was isolated, are on deposit in the Univ of Texas Herbarium (TEX).

Air-dried, ground leaf material (600 g for *N. linearifolia*, 250 g for *N. gracilis*) was extracted at room temp, 1 litre, 24 hr × 2,

with $CHCl_3$ and 1 l., 24 hr × 2 with 85% aqueous MeOH. The $CHCl_3$ extracts contained no flavonoids and were discarded. The aqueous MeOH extracts were concentrated to 200 ml; this solution was extracted in each case with EtOAc, 500 ml × 5. The EtOAc extracts were evaporated to dryness. For each extract the residue was dissolved in MeOH, and the soln was applied as narrow bands on paper (Whatman 3 MM). The chromatograms were developed one-dimensionally in 15% HOAc for 5 hr. The lowermost band was cut from the paper and eluted 2 × 24 hr with MeOH. The eluate was concentrated and applied to a small column (i.d. 2·5 cm) packed with 10 g of polyamide (Polyclar AT). Elution was accomplished with $CHCl_3$–MeOH (2:1). The concentrate from *N. linearifolia* gave two well separated bands, detected by UV light (366 nm) during the column chromatography; the first band gave **2** (30 mg) while the second gave **1** (20 mg). The concentrate from *N. gracilis* yielded only **3** (8 mg).

Sugar identification utilized a stainless steel column 3 m × 3 mm (i.d.) packed with 80–100 mesh 3% SE 30 on chromosorb G installed in a Varian 600 D gas chromatograph having a flow rate of 25 ml of He/min (measured at the detector end of the column) and an isothermal oven temperature of 180°. The disaccharide released after H_2O_2 oxidation was co-chromatographed with authentic neohesperidose (prepared from natural naringenin 7-*O*-neohesperidoside) in four solvents; co-electrophoresis of the sugars was accomplished on paper in borate buffer pH 10 at 15 V/cm for 6 hr. All sugars were identical with neohesperidose. All other procedures were those as outlined in Mabry *et al.* [3].

Quercetin 3-O-*neohesperidoside* **1**. Color test: purple (UV) to yellow-brown (UV/NH_3); R_fs: TBA 0·54, HOAc 0·78, UV, λ_{max} (nm): MeOH, 354, 296sh, 266sh, 255; NaOMe, 401, 325 272; $AlCl_3$, 436, 302sh, 275; $AlCl_3$ HCl, 401, 360, 296sh, 270; NaOAc, 386, 322, 272; NaOAc–H_3BO_3, 374, 308sh, 259. NMR* (CCl_4) 0·83 (*d*, *J* 6·0, 3 H, rhamnosyl Me), 3·65 (*c*, 10 H, sugar protons), 4·81 (1 H, rhamnosyl H-1), 5·75 (1 H, glucosyl H-1), 6·25 (*d*, *J* 2·5, 1 H, H_6), 6·42 (*d*, *J* 2·5, 1 H, H_8), 6·83 (*d*, *J* 8·5, 1 H, H_5), 7·72 (*d*, *J* 8·5, 2 H, H_3 and H_6).

Kaempferol 3-O-*neohesperidoside* **2**. Color test: purple (UV) to green-brown (UV/NH_3); R_fs: TBA 0·70, HOAc, 0·79; UV λ_{max} (nm): MeOH, 348, 298sh, 265; NaOME, 394, 324, 274; $AlCl_3$, 398, 351, 304, 274; $AlCl_3$–HCl, 397, 344, 301, 275; NaOAc, 380, 306, 273; NaOAc–H_3BO_3, 350, 315sh, 266. NMR* (CCl_4): 0·85 (*d*, *J* 6·0, 3 H, rhamnosyl CH_3), 3·65 (*c*, 10 H, sugar protons), 4·83 (1 H, rhamnosyl H-1), 5·75 (1 H, glucosyl H-1), 6·12 (*d*, *J* 2·5, 1 H, H_6), 6·45 (*d*, *J* 2·5, 1 H, H_8), 6·85 (*d*, *J* 9, 2 H, H_3 and H_5), 8·10 (*d*, *J* 9, 2 H, H_2 and H_6).

Isorhamnetin 3-O-*neohesperidoside* **3**. Color test: purple (UV) to yellow-brown (UV/NH_3); R_fs: TBA 0·57, HOAc 0·81, UV λ_{max} (nm): MeOH, 350, 300sh, 268sh, 252; NaOMe, 406, 326, 273; $AlCl_3$, 403, 365sh, 303, 270; $AlCl_3$ HCl, 400, 356, 302, 270; NaOAc, 376, 318, 274; NaOAc–H_3BO_3, 353, 302sh, 263sh, 252.

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* Values are given in ppm (δ scale) relative to TMS as internal standard; spectra were recorded for trimethylsilyl ethers.

REFERENCES

1. Williams, C. A., Harborne, J. B. and Clifford, H. T. (1971) *Phytochemistry* **10**, 1059.
2. Vancraenenbroeck, R., Callewaert, W., Gorissen, H. and Lontie, R. (1969) Europ. Brew. Conv., *Proc. 12th Congr. Interlaken*, p. 29, Elsevier, Amsterdam.
3. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids*, Springer, Heidelberg.

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PENTACYCLIC TRITERPENES AND TYPICAL STEROL PRECURSORS IN *CUCUMIS SATIVUS* SEEDLINGS

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Previous work on triterpenoids. 4-desmethylsterols (mainly stigmasta-7,22,25-trien-3β-ol and stigmasta-7,25-dien-3β-ol) in seeds [1] and seedlings [2]; cucurbitacins B and C in seedlings [3]. We decided to examine the fraction of sterol precursors of *C. sativus*, since it had been suggested that a different sequence of intermediates may be involved in the biosynthesis of Δ^{25}-sterols than for typical phytosterols such as sitosterol or stigmasterol [4]. A possible role of parkeol (an isomer of cycloartenol) as a biogenetic precursor of cucurbitacins had been considered [5].

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