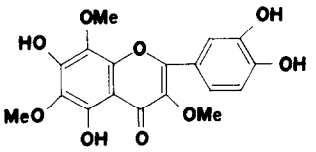
entina). *Uses.* It has been reported to have medicinal perties [2,3]. *Previous work.* Phytochemical screening]. Essential oil [7–9]. Pharmacological activity [10]. *resent work.* From the $CHCl_3$ extract we have iso- d a new flavone, whose structure has been determined , [11], IR, NMR, MS and methylation product) as ',4'-tetrahydroxy-3,6,8-trimethoxyflavone (**1**). This is first report of this compound as a natural product.



(1)

EXPERIMENTAL

ir dried, ground material (600 g) was extracted 24 hr at n temp. with 3 × 5.5 l 25% aq MeOH. The aqueous OH extracts were evaporated to dryness, taken into hot) and partitioned with petrol and $CHCl_3$. The petrol act contained no flavonoids and was discarded. The Cl_3 extract was evaporated to dryness and applied to a mn packed with Sephadex LH_{20} and eluted with C_6H_6, Cl_3 and MeOH. The MeOH eluates were concentrated applied as bands on cellulose TLC and developed with HOAc. The lowermost band was scrapped from the plate, ed with MeOH and taken to dryness. This band afforded ',4'-tetrahydroxy 3,6,8-trimethoxyflavone which crystal- l from ETOH as yellow crystals mp 167–169°. ',4'-Tetrahydroxy-3,6,8-trimethoxyflavone; (a) purple (UV) ellow–brown (UV/NH_3); R_fs: TBA 0.96, HOAc 15% 0.41; λ_{max} (nm): MeOH, 260, 275 *sh*, 345; NaOMe, 270, 282 85; $AlCl_3$, 277, 305 *sh*, 365 *sh*, 435; $AlCl_3$–HCl, 265, 282, *sh*, 365; NaOAc, 270, 380; NaOAc–H_3BO_3, 265, 367. R (60 MHz), (DMSO-d_6) using TMS as internal standard, als at δ 7.60 (2H, *d*, *J* 16 Hz), δ 6.85 (1H, *d*, *J* 8 Hz), 97, 3.85, 3.80 (9H, 3 OMe). MS, principal peaks in *m/e* (100%) (M^+), 361 (100%) ($M^+ - 15$), 344 (50%), 331 (10%), (10%), 301 (7%), 180 (10%), 153 (10%), 137 (25%), 121 %).

The MS spectrum of the compound showed a parent peak at *m/e* 376 ($C_{18}H_{16}O_9$ required 376) with a base peak at *m/e* 361 (M − 15) diagnostic for 3,6,8 methoxylated flavones [12,13]. Methylation with CH_2N_2 afforded 5,6,7,8,3,3',4'-heptamethoxyflavone; pale yellow prisms from Et_2O–petrol mp 131–132° (lit. 130–131°) UV λ_{max} (nm) 340, 255 [14]. No shifts with NaOMe, $AlCl_3$, $AlCl_3$–HCl, NaOAc and NaOAc–H_3BO_3; NMR corresponding to this heptamethoxyflavone.

Acknowledgements—This work was supported in part by Consejo Nacional de Investigaciones Cientificas y Técnicas 6855/74. We wish to thank Dra. Stella Beatriz Sorarú, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, for botanical identification and Prof. Dr. Frank Stermitz, Colorado State University, for recording the MS spectrum.

REFERENCES

1. Cabrera, A. L. (1963) *Flora de la Provincia de Buenos Aires*, Colección Científica del Inta, 135, Buenos Aires.
2. Hieronymus J. (1882) *Boletín de la Academia Nacional de Ciencias*, Tomo **IV**, 343.
3. Parodi, D. (1881) *Ensayo de Botánica Médica Argentina Comparada*, Tesis, **48**, P. Coni, Buenos Aires.
4. Domínguez, J. A. (1928) *Contribuciones a la Materia Médica Argentina*, **128**, Peuser, Buenos Aires.
5. Rey, F. P. (1930) *Rev. Farm.* **73**, 544.
6. Bandoni, A. L., Rondina, R. V. D. and Coussio, J. D. (1972) *Rev. Invest. Agropec. Ser.* 2, **9**, 49.
7. Talenti, E. C. J., Manzi, R., Tedone, F. A., Aringoli, E. and Yunes, R. A. (1969) *Rev. Fac. Ing. Quim.* **38**, 251.
8. Talenti, E. C. J. and Voltero, Z. R. de (1974) *An. Soc. Cient. Arg.* Tomo CXCVIII, 11.
9. Talenti, E. C. J., Orellana, J. A. de and Priano, L. (1975) *An. Soc. Cient. Arg.*, Tomo CXCIX, 31.
10. Udaondo, B. C., Goñalons, G. P., Basile, A. R., Zunino, H. and Lacour, J. J. (1937) *Boletín de la Academia Nacional de Medicina de Buenos Aires* **20**, 441.
11. Mears, J. A. and Mabry, T. J. (1972) *Phytochemistry* **11**, 411.
12. Bowie, J. H. and Cameron, D. W. (1966) *Australian J. Chem.* **19**, 1627.
13. Kingston, D. G. I. (1971) *Tetrahedron* **27**, 2691.
14. Böhme, H. and Völcker, P. E. (1959) *Arch. Pharm.* **292**, 529.

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chemistry, 1976, Vol. 15, pp. 1087–1088. Pergamon Press. Printed in England.

FLAVONOL GLYCOSIDES OF *NERISYRENIA* (CRUCIFERAE)

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(*Received* 28 *November* 1975)

Key Word Index—*Nerisyrenia camporum*; *N. linearifolia*; Cruciferae; flavonol glycosides.

our chemosystematic survey of the genus *Nerisyrenia* ded several flavonol glycosides including the pre- usly reported quercetin, kaempferol and isorhamnetin -neohesperidosides [1]. We report here the isolation l identification of four additional flavonol glycosides, 3-*O*-glucoside 7-*O*-gentiobiosides of kaempferol (**1**), isohamnetin (**2**) and quercetin (**3**) along with quercetin 3-*O*-neohesperidoside 7-*O*-glucoside (**4**).

Acid hydrolysis of each compound afforded the respective aglycone (i.e. kaempferol from **1**, isorhamnetin from **2** and quercetin from **3** and **4**) as determined by PC co-chromatography with authentic aglycone samples and

by UV spectroscopy and, for **1**, **2** and **3**, glucose and for **4** glucose and rhamnose in a 2:1 ratio (GLC of the trimethylsilylated sugars) [2]. Furthermore, comparison of the standard set of six UV spectra [2] for the natural products with those for their aglycones indicated that all natural products must have free 5 and 4′ hydroxyl groups and sugar moieties *O*-linked to C_3 and C_7 and not elsewhere. Hydrolysis of **1**, **2** and **3** with β-glucosidase also afforded the respective aglycone and glucose; however, enzyme hydrolysis of **4** produced glucose and quercetin 3-*O*-neohesperidoside (UV spectra and co-chromatography with an authentic sample by PC).

The NMR spectra of the trimethylsilyl ethers of **1** and **2** confirmed the oxygenation-substitution pattern of each of the aglycones and established that each compound contained three hexose moieties. On the basis of chemical shifts for the signals for the glucose H_1 protons, **1** and **2** each could be assigned a glucosyl moiety *O*-linked to C_3 and to C_7 [2]. Thus, the question remaining concerned the assignment of the third glucosyl group, that is, the location of the di- and monosaccharides. Controlled acid hydrolysis of **1**, **2** and **3** and subsequent PC of the hydrolysate (removal of hydrolysate at 2, 6 and 15 min intervals) gave, in all cases, two 7-*O*-glycosides which were fluorescent yellow when viewed in UV light, 366 nm, in addition to the original compound and the expected aglycone. These results indicated the disaccharide must be *O*-linked to C_7 since each compound produces a 7-*O*-monoglucoside and a 7-*O*-diglucoside. On the basis of the rapid rate by which the compounds were hydrolyzed with β-glucosidase (complete hydrolysis in 6 hr) [3], the disaccharide in **1**, **2** and **3** is assigned a gentiobiose structure.

EXPERIMENTAL

Voucher specimens for *Nerisyrenia 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 **3** and **4** were isolated, and *N. camporum* (Gray) Greene (*Richardson 1686* collected from Mexico: Chihuahua: 10.3 miles S of K 240, from Ojinaga) from which **1** and **2** were isolated, are on deposit in The Lundell Herbarium, The University of Texas at Austin (TX).

Air-dried, ground leaf material (600 g for *N. linearifolia*, 165 g for *N. camporum*) was extracted at room temp., 1 l. 24 hr × 2, with $CHCl_3$ and 1 l. 24 hr × 2, with 85% aq MeOH. The aq MeOH extracts were concentrated to 150 ml; this solution was extracted, in each case, with EtOAc, 500 ml × 5; the fractions thus obtained were set aside. In each case, the remaining water fraction was concentrated to a volume of 30 ml and applied to a column (i.d. 4.5 cm) packed with 40 g of polyamide (Polyclar AT); elution was accomplished with methanol. The flavonoids were collected as one fraction; the fraction was concentrated and applied as narrow bands on sheets (46 × 57 cm) of paper (Whatman 3 MM). The papers were developed one-dimensionally 2 × 48 hr in TBA, air drying between each run. The papers containing the *N. camporum* extract exhibited three well defined bands (detected by UV light, 366 nm). The middle band was cut from the papers 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. Elution with $CHCl_3$–MeOH(2:1) gave two well separated bands (detected by UV light, 366 nm); the first gave **1** (12 mg) while the second yielded **2** (10 mg). The papers spotted with the *N. linearifolia* extract also gave three well defined bands. The fastest moving and intermediate migrating bands were cut from the papers, eluted with MeOH and each was subjected to column chromatography as previously described. The eluate from the fastest moving band, in addition to three trace components, afforded **4** (2 mg); the intermediate band gave **3** (1 mg) plus trace components.

Sugar identifications utilized a stainless steel column 3 m by 3 mm (i.d.) packed with 80–100 mesh 3% SE 30 on chromosorb G in a Varian 600 D gas chromatograph with a flow rate of 25 ml of He/min. (measured at the detector end of the column) and an isothermal oven temperature of 180°. All other procedures were those as outlined by Mabry *et al.* [2].

Kaempferol 3-O-*glucoside* 7-O-*gentiobioside*. **1**. Color: purple (UV) to yellow–green (UV/NH_3); R_f's; TBA 0.12, HOAc 0.78. UV λ_{max} (nm): MeOH, 348, 320*sh*, 268; NaOMe, 395, 360*sh*, 300*sh*, 275, 248; $AlCl_3$, 398, 355, 304, 277; $AlCl_3$–HCl, 395, 345, 298, 277; NaOAc, 397, 295*sh*, 267; NaOAc–H_3BO_3, 351, 266. NMR* (CCl_4): 3.55 (*c*, 18H, sugar protons), 4.12 (1H, glucosyl H_1), 4.95 (1H, glucosyl H_1), 5.88 (1H, glucosyl H_1), 6.29 (*d*, *J* 2, 1H, H_6), 6.87 (*d*, *J* 8.5, 3H, $H_{3'}$, $H_{5'}$, and H_8), 7.93 (*d*, *J* 8.5, $H_{2'}$ and $H_{6'}$).

Isorhamnetin 3-O-*glucoside* 7-O-*gentiobioside*. **2**. Color: purple (UV) to yellow–orange (UV/NH_3); R_f's: TBA 0.11, HOAc 0.80. UV λ_{max} (nm): MeOH, 356, 266*sh*, 254, NaOMe, 416, 291 *sh*, 263; $AlCl_3$, 404, 375*sh*, 300*sh*, 268; $AlCl_3$–HCl, 400, 360, 300*sh*, 266, NaOAc, 416, 300*sh*, 262; NaOAc–H_3BO_3, 360, 266*sh*, 254. NMR* (CCl_4): 3.50 (*c*, 18H, sugar protons), 3.87 (3H, OMe 3′), 4.47 (1H, glucosyl H_1), 4.94 (1H, glucosyl H_1), 5.75 (1H, glucosyl H_1), 6.31 (*d*, *J* 2.5, 1H, H_6), 6.75 (*d*, *J* 2.5, 1H, H_8), 6.88 (*d*, *J* 8.5, 1H, $H_{5'}$), 7.44 (*dd*, *J* 8.5, *J* 2.5, 1H, $H_{6'}$), 7.96 (*d*, *J* 2.5, 1H, $H_{2'}$).

Quercetin 3-O-*glucoside* 7-O-*gentiobioside*. **3**. Color: purple (UV) to yellow–orange (UV/NH_3); R_f's: TBA 0.10, HOAc, 0.69. UV λ_{max} (nm): MeOH, 359, 269*sh*, 257; NaOMe, 398, 269; $AlCl_3$, 427, 350*sh*, 300*sh*, 276; $AlCl_3$–HCl, 401, 360, 300*sh*, 271; NaOAc, 403, 266; NaOAc–H_3BO_3, 379, 265.

Quercetin 3-O-*neohesperidoside* 7-O-*glucoside*. **4**. Color: purple (UV) to yellow–brown (UV/NH_3); R_f's: TBA 0.16, HOAc 0.88; UV λ_{max} (nm): MeOH, 354, 268*sh*, 255; NaOMe, 399, 267; $AlCl_3$, 440, 300*sh*, 274; $AlCl_3$–HCl, 404, 370, 300*sh*, 270; NaOAc, 376, 258; NaOAc–H_3BO_3, 375, 261.

Acknowledgements—This work was supported by the National Science Foundation (Grant BMS-71-01088) and the Robert A. Welch Foundation (Grant F-130).

*Values are given in ppm (δ scale) relative to TMS as internal standard; spectra were recorded for trimethylsilyl ethers on a Varian A-60 spectrometer.

REFERENCES

1. Bacon, John D., Mabry, Tom J. and Harborne, J. B. (1975) *Phytochemistry* **14**, 295.
2. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids*, Springer, Heidelberg.
3. Harborne, J. B. (1965) *Phytochemistry* **4**, 107.