Phytochemistry, 1978, Vol. 17, pp. 299-300 Pergamon Press. Printed in England.

NEW *C*-GLYCOSYLFLAVONES FROM *MOLLUGO DISTICA*

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(*Received* 11 *July* 1977)

Key Word Index—*Mollugo distica*; Aizoaceae; *C*-glycosylflavones; isoswertisin; molludistin; 8-*C*-α-L-arabinopyranosylgenkwanin; ^{13}C-NMR spectra; isowertisin 2″-rhamnoside; molludistin 2″-rhamnoside.

Abstract—Four *C*-glycosylflavones isolated from *Mollugo distica* were identified as 8-*C*-β-D-glucopyranosylgenkwanin, 8-*C*-α-L-arabinopyranosylgenkwanin and their 2″-rhamnosides.

INTRODUCTION

The Order Centrospermae contains eleven families, of which nine produce only betalain pigments and two synthesize only anthocyanins [1]. Since few chemical studies have attempted to further distinguish the two anthocyanin families, the Caryophyllaceae and Molluginaceae, from the betalain families, we have initiated a comparative flavonoid study of selected members from all eleven families. Here we report the isolation and characterization of four glycoflavones (including two new ones) from *Mollugo distica*. The anthocyanin-producing genus, *Mollugo* [2], is of particular interest in that it was until recently treated as a member of the betalain family, the Aizoaceae [3]. A member of the latter family, *Sesuvium portulacastrum*, is known to contain eupalitin [4], an unusual 6-oxygenated flavonol [5], and a flavonoid type not detected in any of the six species of *Mollugo* we have examined.

RESULTS AND DISCUSSION

Four flavonoids were isolated from the aerial parts of *Mollugo distica* (for details see Experimental). Two of the compounds (A and B) remained unchanged after acid hydrolysis and both showed very similar chromatographic and spectral properties. The UV spectra and diagnostic shifts [6] were characteristic of 7-*O*-substituted apigenins and their mobility in H_2O on PC indicated a glycosidic structure which was confirmed as *C*-glycosidic by the NMR spectra [7] in d_6-DMSO which showed the presence of six aromatic protons as two doublets (H-2′,6′ and -3′,5′) and two singlets (H-3, H-6 or 8), one aromatic OMe and sugar protons. $FeCl_3$ oxidation [8] of a mixture of the two compounds gave glucose and arabinose which were identified by GLC of their TMS derivatives. The MS of the permethyl (PM) derivatives of the two compounds indicated a sugar in the 8-position. PM compound A gave the MS of a PM 8-*C*-hexosylapigenin: *m/e* 530 (M^+, 74%), 355 (M-175, 100%) and PM compound B the MS of a PM 8-*C*-pentosylapigenin: *m/e* 486 (M^+, 92%), 355 (M-131, 100%) [9]. Direct chromatographic comparison of the PM derivatives with PM 8-*C*-β-D-glucopyranosylapigenin [10], PM 8-*C*-β-D-xylopyranosylapigenin [11] and PM 8-*C*-α-L-arabinopyranosylapigenin [12] showed PM compound A to be identical with the first standard and PM compound B with the third. The pyranosyl structure and the equatorial bonding of the arabinosyl residue in compound B were confirmed by the 250 MHz NMR spectrum of the perdeuteriomethylated derivative, in which H-1″ (δ 4.88) appeared as a doublet (J = 9.5 Hz) showing the trans-diaxial relationship of H-1″ and H-2″.

Thus it may be concluded that compounds A and B are respectively 8-*C*-β-D-glucopyranosyl and 8-*C*-α-L-arabinopyranosyl 5,4′-dihydroxy-7-methoxyflavones, i.e. 8-*C*-glucosyl- and 8-*C*-arabinosylgenkwanin. The former (isoswertisin) is already known as the acid isomerization product of swertisin from *Swertia japonica* [13] and as an acid hydrolysis product of the blue pigment from *Centaurea cyanus* [14]. The latter, 8-*C*-arabinosylgenkwanin, is a new compound and represents the first report of a natural mono-*C*-arabinosylflavone. We wish to name it molludistin after the plant source.

The ^{13}C-NMR spectra of isoswertisin and molludistin showed that the signals of the *C*-glycosyl moiety could be clearly distinguished from those of the flavone. The latter could be tentatively assigned from a previous spectrum of genkwanin [15] in which C-5 and C-9 were reversed [16] and from the 9 ppm down field shift of luteolin C-6 by *C*-glycosylation in iso-orientin (6-*C*-β-D-glucopyranosyl luteolin) [17]. Moreover, comparison of the ^{13}C-NMR spectra of isoswertisin and iso-orientin showed the chemical shifts of the *C*-glucosyl C atoms were neither affected by the 6- or 8-positions of the sugar nor by the methylation of the 7-OH group. Therefore it appears that ^{13}C-NMR spectra may be successfully used in the identification of the sugars in *C*-glycosylflavones.

The two other flavonoids (C and D) which were isolated from the direct aerial part extract, were more hydrophilic than compounds A and B. On acid hydrolysis compound C gave isoswertisin and compound D gave molludistin. Rhamnose was identified as the sugar in both cases. The O″-rhamnosylation in both compounds was inferred from the UV diagnostic shifts showing OH-4′ and 5 to be free and from the MS of the PM derivatives [18]. Moreover the 2″ position of rhamnose in compound C was in agreement with R_f and MS identity of its PM derivative with the PM derivative of synthetic 8-*C*-neohesperidosylacetin [19] and the MS similarity of their PM derivatives strongly suggests a 2″

position for the rhamnose in compound D. Compounds C and D are thus identified as isoswertisin and molludistin 2″-rhamnosides respectively. Isoswertisin 2″-rhamnoside was recently identified [20] in nature as a compound (F1) previously isolated from *Avena sativa* [21].

EXPERIMENTAL

PMR spectra were recorded on CAMECA (250 MHz) and Varian A-60 D (60 MHz) instruments, ^{13}C-NMR spectra on a Varian XL-100 (25.2 MHz) and MS on an AEI MS 902 (70 eV). For permethylation and purification of PM derivatives see [9]. *Mollugo distica* Lam. (Voucher specimen No. 3/76 deposited at Jawaharlal Institute) was collected from Pondicherry.

Isolation. Shade dried aerial parts of *M. distica* (2 kg) were extracted ×3 with hot 90% EtOH and the concentrate (1.5 l.) shaken with petrol (40–60°), Et_2O and EtOAc. The EtOAc concentrate was run on TLC Si gel in $CHCl_3$–MeOH (5:1). The 4 bands corresponding to compounds A, B, C and D were eluted with hot MeOH and further purified by preparative-PC in BAW. The aq. mother liquor also contained all four components, but after hydrolysis (2 N HCl, 2hr, 100°) deposited a light yellow solid which on TLC showed the presence of only A and B besides a sterol. A and B were best separated, after recrystallization of the mixture from EtOAc–petrol, by preparative TLC (SiO_2, $CHCl_3$–MeOH).

Compound A (isoswertisin). Mp 282–83°; UV λ_{max}^{MeOH} nm 267, 333; +NaOAc 267, 350, 394 *sh*; +NaOMe 250, 267, 300 *sh*, 389; +$AlCl_3$ 276, 304, 343, 388; +$AlCl_3$+HCl 274, 302, 338, 385; IR ν_{max}^{KBr} cm^{-1} 3350 *br*, 2875, 1650, 1600, 1575, 1500, 1280, 1245, 1210, 1180, 1110, 1075, 1015, 890, 830, 805, 765, 660, 570; PMR (d_6-DMSO) $\delta_{TMS}^{10^{-6}}$ 7.94 (2H, *d*, *J* = 9 Hz) H-2′, 6′, 6.77 (2H, *d*, *J* = 9 Hz) H-3′, 5′; 6.70 (1H, *s*) H-3; 6.39 (1H, *s*) H-6; 5.0–3.0 (*m*) sugar protons; 3.77 (3H, *s*) 7-OMe; ^{13}C-NMR (d_6-DMSO) $\delta_{TMS}^{10^{-6}}$ 181.9 (C-4), 164 (C-7?), 163 (C-2?), 161 (C-4′, C-5), 155 (C-9), 128.8 (C-2′, C-6′), 121.2 (C-1′), 115.6 (C-3′, C-5′), 105.5 (C-10), 104.6 (C-3?), 102.2 (C-8?), 94.8 (C-6), 81.7, 78.4, 72.9, 70.6, 70.3, 61.2 (Glc), 56.3 (7-OMe); PC (Whatman No. 1, asc., 28°) R_f 0.09 (H_2O), 0.28 (15% HOAc), 0.60 (BAW), 0.65 (TBA); TLC (Si gel) R_f 0.75 (EtOAc–C_5H_5N–H_2O–MeOH, 80 20:10.5), 0.28 ($CHCl_3$–MeOH, 5:1). Acetate: mp 190–91°. Permethyl ether: MS (*m/e*) 530 (M^+, 74%), 369 (M-161, 9%), 355 (M-175, 100%), 341 (M-189, 19%), 325 (M-205, 10%), 311 (M-219, 10%); TLC (Si gel) R_f 0.46 ($CHCl_3$–EtOAc–Me_2CO, 5:1:4).

Compound B (molludistin). Mp 260–61°; UV λ_{max}^{MeOH} nm 268, 298 *sh*, 336; +NaOAc 267, 297 *sh*, 348, 400 *sh*; +NaOMe 250, 265, 300 *sh*, 390; +$AlCl_3$ 274, 303, 345, 389; +$AlCl_3$+HCl 277, 304, 342, 388; IR ν_{max}^{KBr} cm^{-1}; 3350 *br*, 3150, 2800, 2275, 1650, 1600, 1550, 1500, 1270, 1250, 1200, 1180, 1105, 1090, 1040, 1005, 872, 830, 770, 630, 570; PMR (d_6-DMSO) $\delta_{TMS}^{10^{-6}}$ 8.1 (2H, *d*, *J* = 8 Hz) H-2′, 6′; 6.77 (2H, *d*, *J* = 8 Hz) H-3′, 5′; 6.70 (1H, *s*) H-3; 6.36 (1H, *s*) H-6; 5.0–3.0 (*m*) sugar protons; 3.75 (3H, *s*) 7-OMe; ^{13}C-NMR (d_6-DMSO) [illegible] 164.4 (C-7?), 163.1 (C-2?), 162 (C-4′), 161 (C-5), 155 (C-9), 129.4 (C-2′, C-6′), 119.9 (C-1′), 115.9 (C-3′, C-5′), 105.4 (C-10), 104.2 (C-3?), 101.4 (C-8?), 94.8 (C-6), 74.9, 74.1, 71.0, 68.9, 67.8 (Ara), 56.3 (7-OMe); PC (Whatman No. 1, asc., 28°) R_f 0.06 (H_2O), 0.24 (15% HOAc), 0.62 (BAW), 0.59 (TBA); TLC (Si gel) R_f 0.75 (EtOAc–C_5H_5N–H_2O–MeOH, 80·20·10:5), 0.43 ($CHCl_3$–MeOH, 5:1). Acetate mp 142–43°. Permethyl ether: MS (*m/e*) 486 (M^+, 92%), 355 (M-131, 100%), 341 (M-145, 64%), 325 (M-161, 8%), 311 (M-175, 12%), TLC (Si gel) R_f 0.37, PM 8-*C*-β-D-xylopyranosylapigenin 0.44 ($CHCl_3$–EtOAc–Me_2CO, 5:1:4). Perdeuteriomethyl ether: 250 MHz-PMR ($CDCl_3$) $\delta_{TMS}^{10^{-6}}$ 4.88 (*d*, *J* = 9.5 Hz) H-1″; 4.32 (*q*, *J* = 13 Hz, *J*′ = 1.9 Hz); 3.98 (*s*) 7-OMe; 3.75 (*br s*); 3.5 (*br d*, *J* = 13 Hz); 3.39 (*q*, *J* = 9.5 Hz, *J*′ = 3.5 Hz). $FeCl_3$ oxidation: 0.5 g of the mixture of A and B mixed with aq. $FeCl_3$ (3 g in 10 ml H_2O) was heated under reflux at 115° for 15 min and then at 125° for 6 hr, diluted with H_2O, filtered and the pale yellow filtrate passed through a column of IRC-120 (H) (25 g) followed by IRA-400 (OH) (25 g) to remove Fe^{3+} and Cl^- ions. The neutral soln was concd to dryness and the residue subjected to trimethylsilylation (BSTFA+TMCS in Py). Sugars identified by GLC (5% SE-52): arabinose and glucose.

Compound C. UV λ_{max}^{MeOH} nm 268, 330; +NaOAc 270, 282 *sh*, 345; +$AlCl_3$ 274, 303, 347, 380; +$AlCl_3$+HCl 276, 303, 341, 376. PC: R_f 0.70 (H_2O), 0.65 (15% HOAc), 0.71 (BAW), 0.73 (TBA); TLC (Si gel) 0.10 ($CHCl_3$–MeOH, 4:1). Hydrolysis (N HCl, 1 hr) gave compound A+rhamnose. Permethyl ether. MS (*m/e*) 704 (M^+, 54%), 690 (M-14, 6%), 675 (M-29, 4%), 545 (M-159, 28%), 544 (M-160, 37%), 516 (M-188, 31%), 515 (M-189, 100%), 355 (11%), 341 (89%), 325 (29%), 311 (20%); TLC (Si gel) R_f 0.37 ($CHCl_3$–EtOAc–Me_2CO, 5:1:4).

Compound D. UV λ_{max}^{MeOH} nm 268, 335; +NaOAc: 268, 345; +$AlCl_3$ 232 *sh*, 275, 304, 344, 385; $AlCl_3$+HCl 232, 275, 302, 340, 385. PC: R_f 0.49 (H_2O), 0.68 (15% HOAc), 0.71 (BAW) 0.76 (TBA); TLC (Si gel) 0.17 ($CHCl_3$–MeOH, 4:1). Hydrolysis (N HCl, 1 hr) gave compound B+rhamnose. Permethyl ether. MS (*m/e*) 660 (M^+, 24%), 646 (M-14, 2%), 631 (M-29, 3%), 501 (M-159, 14%), 500 (M-160, 26%), 472 (M-188, 29%), 471 (M-189, 86%), 355 (5%), 341 (100%), 325 (17%), 311 (22%); TLC (Si gel) R_f 0.33 ($CHCl_3$–EtOAc–Me_2CO, 5:1:4).

Acknowledgements—This work was supported at the University of Lyon by the C.N.R.S., at the Jawaharlal Institute by the U.G.C., New Delhi, and at the University of Texas by the National Science Foundation (Grant DEB 76-09320) and the Robert A. Welch Foundation (Grant F-130). AGRN and PR express their thanks to the Principal of their Institute for encouragement, JC and MLB to Dr. Chari for unpublished ^{13}C-NMR data and to J. Favre-Bonvin for GLC and MS.

REFERENCES

1. Mabry, T. J. and Behnke, H. D. (1976) *Plant System. Evol.* 126, 1
2. Mears, J. A. (1976) *Chem. Plant Taxonomy Newsletter* **25**, 13.
3. Willis, J. C. (1957) *A Dictionary of the Flowering Plants and Ferns*. Cambridge University Press, London.
4. Bannerji, A. and Chintalwar, G. J. (1971) *Indian J. Chem.* **9**, 1029.
5. Harborne, J. B. (1975) in *The Flavonoids* (Harborne, J. B., Mabry, T. J. and Mabry, H. eds). Chapman & Hall, London.
6. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids*. Springer, New York.
7. Markham, K. R. and Mabry, T. J. (1975) in *The Flavonoids* (Harborne, J. B., Mabry, T. J. and Mabry, H. eds). Chapman & Hall, London.
8. Koeppen, B. H. and Roux, D. G. (1965) *Biochem. J.* **97**, 444.
9. Bouillant, M. L., Favre-Bonvin, J. and Chopin, J. (1975) *Phytochemistry* **14**, 2267.
10. Chopin, J., Durix, A. and Bouillant, M. L. (1966) *Tetrahedron Letters* 3657.
11. Chopin, J. and Bouillant, M. L. (1970) *Compt. Rend.* **270**, 331.
12. Chopin, J., Biol, M. C. and Bouillant, M. L. (1972) *Compt. Rend.* **274**, 1840.
13. Komatsu, M., Tomimori, T. and Ito, M. (1967) *Chem. Pharm. Bull.* **15**, 263.
14. Asen, S. and Jurd, L. (1967) *Phytochemistry* **6**, 577.
15. Wagner, H., Chari, V. M. and Sonnenbichler, J. (1976) *Tetrahedron Letters* 1799.
16. Chari, V. M., personal communication.
17. Hostettmann, K. and Jacot-Guillarmod, A. (1976) *Helv. Chim. Acta* **59**, 1584.
18. Bouillant, M. L., Favre-Bonvin, J. and Chopin, J. (1975) *Comptes rendus de l'Assemblée annuelle du Groupe Polyphenols* p 232. Gargnano sul Garda.
19. Besset, A., Bouillant, M. L. and Chopin, J. (1977) *Compt. Rend* **284**, 1007.
20. Chopin, J., Dellamonica, G., Bouillant, M. L., Besset, A., Popovici, G. and Weissenböck, G. (1977) *Phytochemistry* **16**, 2041.
21. Weissenböck, G., Fleing, I. and Ruppel, H. G. (1972) *Z. Naturforsch.* **276**, 1216.