Phytochemistry, Vol 22, No 1, pp 143–146, 1983
Printed in Great Britain

0031-9422/83/010143-04$03 00/0

TAXONOMICALLY SIGNIFICANT 8-HYDROXYFLAVONE GLUCURONIDES FROM THE MARCHANTIALEAN LIVERWORT, *BUCEGIA ROMANICA*

KENNETH R MARKHAM and RUDIGER MUES*

Chemistry Division, DSIR, Petone, New Zealand, *FB 16, FR Botanik, Universität des Saarlandes, D-6600 Saarbrucken, West Germany

(*Received* 20 *May* 1982)

Key Word Index—*Bucegia romanica*, Marchantiaceae, Hepaticae, chemotaxonomy, flavone glucuronides, 8-hydroxyapigenin, 8-hydroxyluteolin, 5,7-dihydroxy-8,4′-dimethoxyflavone 7-*O*-*β*-D-glucuronide, bucegin

Abstract—The monotypic genus, *Bucegia*, was not investigated in an earlier comparative flavonoid study of the Marchantiaceae In the present communication *Bucegia romanica* is shown to elaborate solely a range of 8-hydroxyapigenin and 8-hydroxyluteolin glucuronides with variable levels of methylation Such flavonoid biochemistry is unique within this family and the taxonomic implications of this are discussed One new flavonoid was identified as 5,7-dihydroxy-8,4′-dimethoxyflavone (bucegin) 7-*O*-*β*-D-glucuronide

INTRODUCTION

In a recent comprehensive survey of flavonoid distribution in the liverwort family Marchantiaceae, the only genus not investigated was the monotypic genus *Bucegia* [1] Although the flavonoid survey provided the basis upon which taxonomic and phylogenetic relationships within the family were defined, cognizance was also taken of available morphological data In the absence of flavonoid data, *Bucegia* was tentatively aligned with the morphologically similar *Neohodgsonia* In the present communication the major flavonoid types found in *Bucegia* are defined and the taxonomic and phylogenetic significance of the findings is interpreted in terms of the criteria set out in the earlier survey

RESULTS AND DISCUSSION

Although three specimens of *Bucegia romanica* from different localities were studied, only one was sufficient for detailed flavonoid analysis This originated from the Tatry mountains area of southern Poland A 2D-PC of the methanol–water extract from this sample revealed a flavonoid pattern consisting of one major, two minor and two trace flavonoid glycoside spots (see Fig 1)

The major component, **1**, appeared as a deep purple spot which was unchanged by ammonia vapour when viewed in UV light (366 nm) The absorption spectrum was apigenin-like but possessed a prominent shoulder at 294 nm, a feature associated with some 8-hydroxyapigenins [2, 3] The compound was unaffected by *β*-glucuronidase but prolonged hydrolysis with trifluoroacetic acid (TFA) yielded an aglycone, **1a**, together with a mixture of glucuronic acid and glucuronolactone The aglycone had an absorption spectrum similar to that of the glycoside and with M^+ at 314 appeared to be a dihydroxydimethoxyflavone Accompanying the M^+ in the mass spectrum were A- and B-ring derived fragments at *m/z* 167 $[A - 15]^+$ and 132 $[B_1]^+$, 133 $[B_1 + H]^+$, 135 $[B_2]^+$ which require [4] that one of the methoxyls be sited on the B-ring and two hydroxyls plus one methoxyl be sited on the A-ring The sodium methoxide, sodium acetate and aluminium chloride–hydrochloric acid induced shifts in the spectrum (of the glycoside) indicate that the 5- and 7-hydroxyl groups are free and that the 4′-hydroxyl is substituted This evidence is suggestive of a 6- or 8-methoxylated 5,7-dihydroxy-4′-methoxyflavone formulation for **1a**, and the 8-methoxyl isomer is favoured by the following (1) Compound **1a** did not cochromatograph with pectolinarigenin (5,7-dihydroxy-6,4′-dimethoxyflavone) (2) In the mass spectrum the $[M - Me]^+$ ion is the base peak (cf 6-methoxyflavones in which M^+ is the base peak [5]) and $[M - 18]^+$ is smaller than 10% (cf 6-methoxyflavones in which $[M - 18]^+$ is greater than 10% [5]) (3) Wessely–Moser rearrangement of **1a** in hydrochloric acid isomerizes it in part to the thermodynamically more stable isomer, pectolinarigenin, an isomerization characteristic of 8- but not 6-oxygenated flavones [6] (4) The aluminium chloride–hydrochloric acid induced shift in band I of glycoside **1** at + 65 nm is typical of 8- (but not 6-)methoxylated flavones (see ref [7] and the Experimental) The structure of **1a** is, therefore, defined as 5,7-dihydroxy-8,4′-dimethoxyflavone This is the first report of its occurrence as a natural product† and we propose the name bucegin for this aglycone Since absorption data indicate that the 7-hydroxyl is substituted in glycoside **1**, and since the chromatographic mobility of the glycoside (in TBA, water and 15% acetic acid) can only be that of a mono-*O*-glucuronide [8], the structure 5,7-dihydroxy-8,4′-dimethoxyflavone 7-*O*-*β*-D-glucuronide (bucegin 7-*O*-glucuronide) is assigned to glycoside **1** The

---

† A claim that cirsitakaogenin possesses this structure is refuted in Markham, K R (1983) *Phytochemistry* **22**, 316 Further, **1a** is chromatographically very distinct from cirsitakaogenin (see Experimental)

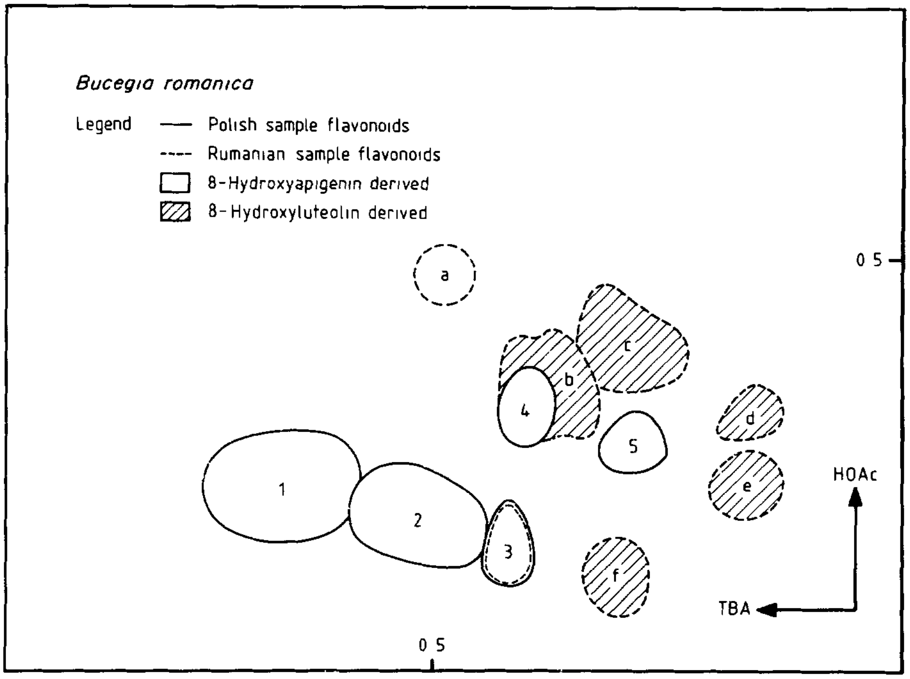

Fig 1 2D-PC flavonoid patterns of *Bucegia romanica* samples originating from Poland and Rumania

failure of β-glucuronidase to hydrolyse this glycoside contrasts with its action on glycosides **2** and **3** and is considered to be due to steric interference by the 8-methoxyl group

Both the minor flavonoid glycosides of this plant were cleaved by β-glucuronidase, had a high mobility on cellulose TLC in water and gave absorption spectra in methanol similar to those of glycoside **1** Glycoside **2** was distinguished from **1** by the effect of sodium acetate on the absorption spectrum which signified the presence of a free 7-hydroxyl group Sodium acetate also had the same effect on the spectrum of **3**, but with this glycoside the sodium methoxide shift suggested that the 4′-hydroxyl was also free The probable constitutions of these glycosides, 5,7,8-trihydroxy-4′-methoxyflavone (takakin) 8-*O*-β-D-glucuronide for **2**, and 5,7,8,4′-tetrahydroxyflavone (8-hydroxyapigenin) 8-*O*-β-D-glucuronide for **3**, were confirmed by cochromatography with authentic material from *Takakia lepidozioides* [9]

One of the trace components observed, (**4**), is thought to be an 8-*O*-glycosylglucuronide of 8-hydroxyapigenin since it was converted to **3** plus 8-hydroxyapigenin on TFA treatment, and possessed absorption spectra and R_f values (relative to those of **3**) consistent with this formulation The other trace flavonoid, **5**, was available in insufficient quantity to be typed

Two further small samples of *Bucegia* were also surveyed, one from Rumania and the other from Czechoslovakia, but the size of these samples precluded detailed chemical studies The 2D-PC display of flavonoids in the Czechoslovakian sample revealed only three flavonoid glycosides, **1**–**3**, the 'diglycosides' of the Polish sample being absent The Rumanian sample produced a pattern which contained only one minor flavonoid, **3**, in common with the other two samples (see Fig 1) One other minor glycoside, **a**, produced **3** on partial hydrolysis with hydrochloric acid and is considered to be an 8-*O*-glycosylglucuronide of 8-hydroxyapigenin since it gave the same set of absorption spectra as did **3** On partial hydrolysis with hydrochloric acid, the other dominant 'diglycosides', **b**, **c** and **e** all gave compound **f** Compound **f** was identified as 8-hydroxyluteolin 8-*O*-glucuronide from its R_f values and appearance on PC, its absorption spectra and by direct comparison with authentic material from *Marchantia berteroana* [2] Since the absorption spectra of diglycosides **b** and **c** are identical with those of **f**, these compounds are considered to be 8-*O*-glycosylglucuronides of 8-hydroxyluteolin Diglycoside **e** gave absorption spectra which indicate that the 7-hydroxyl is substituted as well as the 8-hydroxyl and accordingly, **e** is considered to be a 7-*O*-glycoside of 8-hydroxyluteolin 8-*O*-glucuronide The limited data available for the minor glycoside, **d**, suggest that it too may be of the same type

It follows from the results above that the monotypic genus *Bucegia*, as presently circumscribed, cannot be defined precisely by its flavonoids beyond the observation that it elaborates solely a range of 8-hydroxyapigenin and 8-hydroxyluteolin glucuronides with variable levels of methylation and additional glycosylation This rather broad phytochemical categorization is none-the-less unique for a species of the Marchantiaceae The only other species in this family known to produce 8-hydroxyflavones at all is *Marchantia berteroana* [2] which produces them, together with other flavonoids, only in the sexual branches and never in the vegetative thallus [10]

Current botanical opinion [1, 11] favours the taxonomic placement of *Bucegia* close to *Neohodgsonia* and indeed Schuster [11] places the two genera in their own subfamily within the Marchantiaceae Comparative flavonoid biochemistry, however, does not support this view since the flavonoids of *Neohodgsonia* (apigenin-7-*O*-glucuronide and luteolin-7,-3 and 7,3′-glucuronides) are of a type commonly encountered in this family [1] and thus contrast markedly with those of *Bucegia* Further, the flavonoid 'score' [1] for the Polish *Bucegia* computes at + 2 0, and as such indicates a high level of biochemical advancement relative to *Neohodgsonia* which scored only − 0 2 [1] On these grounds *Bucegia* alone appears to qualify for subfamilial separation from the remainder of the Marchantiaceae

It is thus concluded that our earlier positioning of *Bucegia* and *Neohodgsonia* together as a distinct group on the taxonomic tree, based on morphological grounds, must be modified such that *Bucegia* is separated from all other Marchantiaceae genera In this context it is of interest to note that our proposal (made in the same publication) for the separation of *Wiesnerella* and *Monoselenium* from the Marchantiaceae, which was based primarily on their distinctive flavonoid biochemistry, has subsequently been fully supported by Schuster [11]

EXPERIMENTAL

Plant material Bucegia romanica Radian Samples were collected from three sites (1) Tatry mountains, Mala Laka valley, e 1570 m, south Poland (Jerzy Szweykowski, 8 Sept 1981), herbarium sample MPN 18566, (2) Belianske Tatry, Berg Zaolnel Jatky, el 2000 m, Czechoslovakia (J Vana, 5 Sept 1967), (3) Karpaten Oberhalb der Malaesti-Hutte im Bucegi-Gebirge in Richtung Omul auf Erde, Rumania (M Siegel, 22 Aug 1978)

Isolation procedure Each sample was hand-picked clean, washed and dried to give 1 5 g (sample 1), 0 2 g (sample 2) and 0 4 g (sample 3), dry wt Each sample was ground and extracted overnight (twice) with Me_2CO–H_2O (1 1) Flavonoids were isolated by 2D-PC on Whatman 3MM paper in *t*-BuOH–HOAc–H_2O (3 1 1) (TBA) and 15 % HOAc (HOAc), the spots being eluted with H_2O Compound **1** was further purified by elution through a column of Sephadex LH-20 with MeOH

Hydrolysis conditions (1) *β-Glucuronidase* (Sigma) the sample in H_2O was left at 20° with the enzyme for 45 min Under these conditions luteolin 7-*O*-glucuronide was completely hydrolysed Compounds unaffected by this treatment were treated overnight with the enzyme to confirm the initial observation (2) *TFA* the sample was heated with 1N TFA in a steam-bath for 3 hr (for complete hydrolysis without Wessely–Moser rearrangement) or for 30 min (for partial hydrolysis) (3) *HCl* for production of 6-oxygenated aglycones, the glycoside was heated with 1 5 N HCl at 100° for 3 hr, but for partial hydrolysis 1 hr was sufficient

Cochromatography Chromatographic comparisons of glycosides with authentic samples were carried out on Schleicher and Schull F1440 TLC cellulose plates in 15 % HOAc, 40 % HOAc, TBA and H_2O, and for flavonoids **2** and **3** additionally in BAW and MeOH–HOAc–H_2O (9 1 1) (on Schleicher and Schull A1700 polyamide) For comparison of aglycones, especially pectolinarigenin, cochromatography was carried out on cellulose TLC in TBA and C_6H_6–HOAc–H_2O (125 73 3) and better on Schleicher and Schull silica F-1500 TLC plates in $CHCl_3$–MeOH (46 3) and toluene–Me_2CO (9 1) Sugars were cochromatographed on cellulose in EtOAc–pyridine–HOAc–H_2O (36 36 7 21) Spray reagents $AlCl_3$ (5 % in MeOH) and Naturstoffreagenz A (0 5 % in MeOH), ('NA' ex C Roth) for flavonoids, and *p*-anisidine HCl for sugars (cf ref [4])

Absorption spectra Spectra were measured and analysed according to procedures outlined in ref [4]

*5,7-Dihydroxy-8,4′-dimethoxyflavone 7-O-β-*D*-glucuronide,* **1** Physical data R_f 0 64 (TBA), 0 21 (HOAc), 0 67 (H_2O), UV$\lambda_{\max}$ nm (MeOH) 273, 294 sh, 320, 350 sh, (NaOMe) 283 inc *, 378 dec * (NaOAc and NaOAc–H_3BO_3) 272, 294 sh, 320, 350 sh, ($AlCl_3$) 276, 308, 348, 385, ($AlCl_3$–HCl) 270, 305, 337, 385 *Aglycone* (ex TFA treatment) R_f (**1a**, pectolinarigenin) 0 69, 0 84 (SiO_2, $CHCl_3$–MeOH, 46 3), 0 27, 0 33 (SiO_2, toluene–Me_2CO, 9 1) In this last solvent, cirsitakaogenin had a R_f of 0 18 on the same TLC plate UV$\lambda_{\max}$ nm (MeOH) 274, 295 sh, 321, 350 sh (NaOMe) 283 inc, 295 sh, 360 dec, (NaOAc) 280 inc, 300 sh, 346 sh, dec [Pectolinarigenin UV$\lambda_{\max}$ nm (MeOH) 274, 328, (NaOAc) 274 inc, 296 sh, 363 dec, ($AlCl_3$–HCl) 295, 350] MS 314 $[M]^+$ (59 6), 300 (18 9), 299 $[M-Me]^+$ (100), 296 (5 1), 286 $[M-CO]^+$ (4 8), 282 (15 3), 167 (A_1 from base peak, 14 6 %), 139 $[A_1-Me-CO]^+$ (31 1), 135 $[B_2]^+$ (19 %), 133 $[B_1+H]^+$ (28 1), 132 $[B_1]^+$ (8 6)

Other flavonoids (*Polish sample*) **2** R_f 0 53 (TBA) 0 16 (HOAc), 0 48 (H_2O), UV$\lambda_{\max}$ nm (MeOH) 273, 292 sh, 320, 350 sh, (NaOMe) 282 inc, 310 sh, 360 dec **3** R_f 0 40 (TBA), 0 13 (HOAc), 0 43 (H_2O), UV$\lambda_{\max}$ nm (MeOH) 272, 290 sh, 325, 350 sh, (NaOMe) 280, 325 sh, 392 inc **4** R_f 0 37 (TBA), 0 30 (HOAc), 0 64 (H_2O), UV$\lambda_{\max}$ nm (MeOH) 272, 295 sh, 324, 350 sh, (NaOMe) 280, 325, 394 inc **5** R_f 0 25 (TBA), 0 27 (HOAc), 0 54 (H_2O); UV$\lambda_{\max}$ nm (MeOH) 275 *br*, 325, (NaOMe) 276, 392 nm

Flavonoids ex Rumanian sample Chromatographic data (R_fs in TBA, HOAc and H_2O) **a**, 0 45, 0 5, 0 45, **b**, 0 35, 0 35, 0 57, **c**, 0 26, 0 41, 0 53, **d**, 0 14, 0 3, 0 45, **e**, 0 14, 0 2, 0 42, **f**, 0 30, 0 09, 0 42 All spots appeared dark purple in UV light (366 nm) and all turned olive in NH_3 With NA, **a** gave a yellow-green fluorescence in UV and all others gave an orange fluorescence Absorption data **a** UV$\lambda_{\max}$ nm (MeOH) 272, 294 sh, 327, 350 sh, (NaOMe) 279, 325, 397 inc **b**, **c** and **f** UV $\lambda_{\max}$ nm (MeOH) 259, 272, 295 sh, 356, (NaOMe) 274, 295 sh, 320, 418 inc, ($AlCl_3$) 267, 274 sh, 303 sh, 360 sh, 414, ($AlCl_3$–HCl) 265, 300 sh, 360, 391 **e** UV $\lambda_{\max}$ nm (MeOH) 259, 272 sh, 290 sh, 355, (NaOMe) 270, 302 sh, 405 inc, (NaOAc) 260, 272 sh, 370, (NaOAc–H_3BO_3) 261, 272 sh, 375

Acknowledgements—We are indebted to Dr Jerzy Szweykowski of the Biological Institute, Adam Mickiewicz University, Poznan, Poland for the supply of the Polish *Bucegia*, to Dr S Huneck, Institut fur Biochemie der Pflanzen Halle, Halle, East Germany for the Czechoslovakian and Rumanian samples, to Miss C Sheppard of the Chemistry Division, DSIR, for the MS determination, and to Professor N Morita of Toyama Medical and Pharmaceutical University for an authentic sample of cirsitakaogenin

REFERENCES

1 Campbell, E O, Markham, K R, Moore, N A, Porter, L J and Wallace, J W (1979) *J Hattori Bot Lab* **45**, 185

2 Markham, K R and Porter, L J (1975) *Phytochemistry* **14**, 1093

3 Markham, K R (1977) *Phytochemistry* **16**, 617

4 Markham, K R (1982) *Techniques of Flavonoid Identification* Academic Press, London

5 Goudard, M, Favre-Bonvin, J, Lebreton, P and Chopin, J (1978) *Phytochemistry* **17**, 145

6 Seshadri, T R (1962) in *The Chemistry of Flavonoid Compounds* (Geissman, T A, ed) p 184 Pergamon Press, Oxford

7 Markham, K R and Mabry, T J (1975) in *The Flavonoids* (Harborne, J B, Mabry, T J and Mabry, H, eds) p 60 Chapman & Hall, London

8 Markham, K R and Porter, L J (1978) in *Progress in Phytochemistry* (Reinhold, L, Harborne, J B and Swain, T, eds) Vol 5, p 234 Pergamon Press, Oxford

---

*Abbreviations dec, decrease in intensity, inc, increase in intensity

9 Markham, K R and Porter, L J (1979) *Phytochemistry*, **18**, 611

10 Markham, K R, Moore, N A and Porter, L J (1978) *Phytochemistry* **17**, 911

11 Schuster, R M (1979) in *Bryophyte Systematics* (Clark, G C S and Duckett, J G, eds) Proceedings of an International Symposium held at the University College of North Wales, Bangor Academic Press, London