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THE TAXONOMIC POSITION OF *SPHAEROCARPOS* AND *RIELLA* AS INDICATED BY THEIR FLAVONOID CHEMISTRY

KENNETH R. MARKHAM and LAWRENCE J. PORTER

Chemistry Division, DSIR, Petone, New Zealand

and

NORTON G. MILLER*

Department of Botany, University of North Carolina, Chapel Hill 27514 U.S.A.

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Abstract—The major flavonoid glycosides of *Sphaerocarpos texanus* are luteolin 7-*O*-glucuronide and 7,4'-di-*O*-glucuronide. *Riella americana* and *R. affinis* both contain apigenin, chrysoeriol and luteolin 7-*O*-glucuronides but *R. americana* additionally contains luteolin 3'-*O*-glucuronide. This finding supports the inclusion of Sphaerocarpaceae and Riellaceae in the order Marchantiales rather than their separation into another order.

INTRODUCTION

Previous studies of the flavonoid chemistry of a wide variety of liverworts from the orders Marchantiales [1,2], Jungermanniales [3–5], Metzgeriales [6,7] and Monocleales [8] have established that flavone glycosiduronic acids occur exclusively in members of the order Marchantiales. Species representative of all major families in this order have without exception contained apigenin and/or luteolin glycosiduronic acids or their derivatives. In contrast, flavonoids appear to occur only sporadically in the orders Jungermanniales and Metzgeriales and usually as *C*-glycosyl derivatives.

Liverworts of the families Sphaerocarpaceae and Riellaceae have together, variously been placed in the orders Sphaerocarpales [9–11], Jungermanniales [12] and Marchantiales [13,14]. The flavonoid chemistry of species within these small families is thus of potential value in determining their taxonomic status. A study of the species, *Sphaerocarpus texanus*, *Riella americana* and *Riella affinis*, representing both families, is reported here.

RESULTS AND DISCUSSION

Two dimensional PC of the Me_2CO–H_2O extract of *Sphaerocarpos* gametophyte tissue revealed the presence of 2 compounds, ST-1 and ST-2, both resembling in appearance and R_f values, compounds previously encountered in species of the order Marchantiales.

Compound ST-1 is paper chromatographically identical with luteolin 7,4'-di-*O*-glucuronide (ex *Marchantia polymorpha* [1] and quite distinct from luteolin 7,4'-di-*O*-galacturonide (ex *Marchantia berteroana* [15]). With β-glucuronidase it hydrolysed completely to luteolin and on acid hydrolysis it was converted into a mixture of luteolin and ST-2. Compound ST-2 was PC and TLC identical with authentic luteolin 7-*O*-glucuronide from both *M. polymorpha* and *Ricciocarpus natans* [16] and when treated with β-glucuronidase was hydrolysed completely to luteolin. *Sphaerocarpus* thus contains luteolin 7-*O*-glucuronide and luteolin 7,4'-di-*O*-glucuronide as its major flavonoids.

Two dimensional PCs of the Me_2CO–H_2O extracts of *Riella americana* (male plant)† and *R. affinis*, demonstrated the presence of four flavonoid glycosides, RA-1, RA-2, RA-3 and RA-4 in the former and three, RA-1, RA-2 and RA-3 in the latter. Compounds RA-1, 2 and 3 from *R. americana* were shown to be identical with those from *R. affinis* by combinations of 2D PC, TLC and UV spectroscopy (low levels of RA-3 in both species precluded the use of UV spectroscopy with this compound). All four glycosides were cleaved by β-glucuronidase to produce, in addition to glucuronic acid, the aglycones, apigenin (from RA-1), luteolin (from RA-3 and 4) and chrysoeriol (from RA-2). These were identified by cochromatography (TLC, PC) with authentic samples.

UV spectroscopy (see Experimental) defined the sites of glycosylation in RA-1 and 2 as the 7-hydroxyl group and in RA-4 as the 3'-hydroxyl group. Thus RA-1 is assigned the structure apigenin 7-*O*-glucuronide, RA-2 the structure chrysoeriol 7-*O*-glucuronide and RA-4 the structure luteolin 3'-*O*-glucuronide. The paper chromatographic behaviour of RA-3 (i.e. relative R_f values and distinctive appearance in UV/NH_3) suggests that it too is 7-*O*-glycosylated, that is, luteolin 7-*O*-glucuronide. In each case confirmation of structure was obtained by cochromatography (TLC, 3 solvents) with the authentic flavone glucuronide previously isolated from *Marchantia*

* Present address: Herbaria and Dept. of Biology, Harvard University, Cambridge, MA 02138, U.S.A.

† A small sample of the female plant did not appear to contain component RA-4. *R. affinis* is dioecious. Plants from culture were chemically identical with those from natural sources.

polymorpha [1], *Ricciocarpus natans* [16] or *Marchantia foliacea* [17].

The flavone glycoside isolated in this study, from both *Riella* and *Sphaerocarpos* are all compounds which in the liverworts occur exclusively in members of the order Marchantiales. The earlier assignment of Sphaerocarpaceae (and Riellaceae) to the order Jungermanniales is therefore not supported by this evidence, nor is their separation from the Marchantiales into a distinct order. This conclusion, of course, takes no account of the weight of morphological evidence which has previously been cited in botanical papers on this problem. However, our findings do support the views of Grolle, who in his recently published classification of the Hepaticae [14] has eliminated the order Sphaerocarpales and placed the Riellaceae and the Sphaerocarpaceae in the order Marchantials.

EXPERIMENTAL

A voucher specimen of *Sphaerocarpos texanus* Aust. (Miller 7755, coll. near Chapel Hill) has been deposited in the Farlow Herbarium of Harvard University. Specimens of *Riella americana* and *R. affinis*, cultured by Professor H. C. Bold, are held in the University of Texas Herbarium, Austin, Texas.

Experimental details are essentially as outlined in previous papers (see Ref. [1]). All compounds were isolated by 2D PC on Whatmans 3 MM paper in the solvents *t*-BuOH–HOAc–H_2O, 3:1:1 (TBA) and 15% HOAc. H_2O (alone) was used to distinguish glucuronides from glucosides. TLC was performed on plastic-backed cellulose sheets with the solvents, H_2O, TBA, 15% HOAc and 30% HOAc (for glycosides) and with C_6H_6–HOAc–H_2O 125:72:3 (for aglycone identification). Enzyme hydrolyses were carried out with Koch-Light β-glucuronidase (16 h, dist H_2O) and the product analysed, where possible, by PC with EtOAc–Pyr–H_2O (12:5:4) as solvent. Acid hydrolysis conditions were: MeOH–3N HCl (1:1), reflux, 75 min. PC characteristics (R_f TBA, R_f 15% HOAc, spot colour in UV/NH_3) of the various components are as follows: ST-1 (0·19, 0·38, purple); ST-2 (0·42, 0·14, yellow); RA-1 (0·52, 0·22, yellow–green); RA-2 (0·44, 0·16, lemon); RA-3 (0·40, 0·14, yellow); RA-4 (0·47, 0·08, deep yellow- green). UV spectral data for the *Riella* compounds are as follows: RA-1, λ_{max} (MeOH) 268, 330 nm, λ_{max} (NaOMe) 268, 390 nm; RA-2, λ_{max} (MeOH) 251, 267, 346 nm, λ_{max} ($AlCl_3$ and $AlCl_3$/HCl) 275, 295sh, 317sh, 363, 380sh nm; RA-4, λ_{max} (MeOH) 267, 290sh, 340 nm, λ_{max} (NaOMe) 274, 324, 390 nm.

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