

EVIDENCE OF BIOSYNTHETIC SIMPLICITY IN THE FLAVONOID CHEMISTRY OF THE RICCIACEAE

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Key Word Index—*Riccia crystallina*; *Ricciocarpus natans*; Hepaticae; liverworts; simple flavone glycosides; chemotaxonomy.

Abstract—The major flavonoids in *Riccia crystallina* are naringenin and its 7-*O*-glucoside, apigenin 7-*O*-glucoside and apigenin 7-*O*-glucuronide and derivatives. *Ricciocarpus natans* is a rich source of luteolin 7,3'-di-*O*-glucuronide and also contains the 7-*O*-glucuronides of apigenin and luteolin and the 3'-*O*-glucuronide of luteolin. A parallel between the production of biosynthetically simple flavonoids and reduced morphology is evident among these liverworts.

INTRODUCTION

To date flavonoids have been reported to occur in six of the major families that comprise the order Marchantiales* (Hepaticae) [1]. Ricciaceae is one major family as yet uninvestigated and the current work establishes for the first time the presence of flavonoids in species of this family.† The flavonoid chemistry of the order Marchantiales, dominated by flavone glycosiduronic acids, is emerging as a most useful adjunct to traditional taxonomic and phylogenetic procedures.

DISCUSSION

Ricciocarpus natans

The genus *Ricciocarpus* is monotypic and its sole species *R. natans*, is commonly found in a free floating form. Its flavonoid chemistry, as revealed in the present investigation, is clearly consistent with its being a member of the order Marchantiales.

RN-3 (see Fig. 1) is the major flavonoid and was isolated in 0.6% yield from dried plant material. RN-1 and RN-2 were isolated in 0.05 and 0.15% yield respectively. On prolonged paper chromatography, RN-2 was resolved into two compounds RN-2a and RN-2b in the ratio of 3:1. Acid and/or enzyme hydrolyses of these four compounds yielded the aglycones which were identified by UV spectroscopy and chromatography. Thus RN-1

was shown to be an apigenin derivative and RN-2a, RN-2b and RN-3, luteolin derivatives.

The UV spectra of the glycosides (using appropriate shift reagents [2]) defined RN-1 as an apigenin 7-*O*-glycoside, RN-2a and RN-2b as luteolin 3'- and 7-*O*-glycosides respectively and RN-3 as a luteolin 7,3'-di-*O*-glycoside. In each case the sugar was identified as glucuronic acid by β -glucuronidase hydrolysis followed by paper chromatography of the product. The structures assigned to these compounds are therefore apigenin 7-*O*- β -D-glucuronide (RN-1), luteolin 3'- and 7-*O*- β -D-glucuronides (RN-2a and RN-2b) and luteolin 7,3'-di-*O*- β -D-glucuronide (RN-3). Confirmation was achieved in every case by direct comparison with

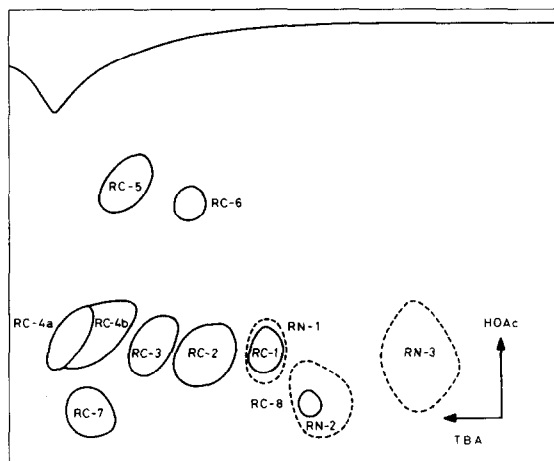


Fig. 1. The flavonoid constituents of *Riccia crystallina* (—) and *Ricciocarpus natans* (----).

* Classification according to Grolle, R. (1972) *J. Bryol.* 7, 201.

† A preliminary study by the authors has also established the presence of flavones in *Riccia bifurca*.

authentic materials from *Lunularia cruciata* [3] and *Marchantia polymorpha* [1].

Riccia crystallina

Riccia crystallina is a minute, morphologically simple liverwort which is classified in a genus, the species of which are reputed to possess the simplest kind of structure found in the order Marchantiales [4]. The plant material available was extracted with acetone–water and found to contain nine flavonoids (see Fig. 1). The percentages by weight of the major constituents were as follows: RC-1 (0.02%), RC-2 (0.15%), RC-3 (0.1%), RC-4a (0.12%), RC-4b (0.1%), RC-5 (0.35%) and RC-7 (0.05%).

Two of the major components, RC-4a and RC-5, comprised about 60% of the total flavonoids and possessed UV spectra typical of dihydroflavones [2]. Mild acid hydrolysis converted RC-5 to RC-4a which was shown to be identical with authentic 5,7,4'-trihydroxyflavanone (naringenin) by MS, PC and TLC. The hydrolysis was also carried out with β -glucosidase and yielded glucose as the only sugar, thus defining RC-5 as naringenin 7-*O*- β -D-glucoside. This constitutes the first isolation of a dihydroflavone from a liverwort and provides evidence that the biosynthetic sequence producing flavonoids in liverworts is the same as in higher plants.

Compounds RC-1, 2, 3 and 4b all gave UV absorption data which defined them as 7-substituted apigenins [2]. On acid hydrolysis all were converted to some extent to RC-7 which was chromatographically (PC, TLC) and spectroscopically (UV) identical with apigenin. PC analysis of the acid hydrolysis products revealed that RC-2 had been completely hydrolysed to apigenin whereas RC-3 and RC-4b had been partly hydrolysed to apigenin and partly converted to RC-1. RC-2 was thus behaving as a typical apigenin glycoside and was shown to be apigenin 7-*O*- β -D-glucoside by hydrolysis with β -glucosidase. RC-3 and RC-4b, on the other hand, are clearly acid-labile derivatives or isomers of the acid resistant RC-1 which was identified as apigenin 7-*O*- β -D-glucuronide by enzyme hydrolysis and direct comparison (PC, UV) with authentic material from *Marchantia polymorpha* [1]. Although RC-1 was apparently converted into RC-3 by brief CH_2N_2 treatment (PC evidence), IR spectroscopy failed to confirm the presence of an ester group in RC-3. In fact both

RC-3 and RC-4b exhibited carbonyl absorption near 1720 cm^{-1} as did RC-1. The small amount of material available precluded further study of this intriguing problem.

Traces of 3',4'-dioxxygenated flavonoids may also occur in this plant. RC-8, a faint dark spot which turned yellow in NH_3 , cochromatographed with RN-2 and is thought to be a luteolin glycoside, and RC-6, has a dihydroflavone UV spectrum and R_f values relative to naringenin 7-*O*-glucoside (RC-5) consistent with its being an eriodictyol glycoside.

General comments

The two representatives of the Ricciaceae studied in the present investigation fit the established pattern for members of the order Marchantiales [1] in that they both possess flavone glycosiduronic acids which to date have not been isolated from liverworts of any other order. A feature of both species is the presence of predominantly simple flavonoids. This is particularly marked in *Riccia crystallina* in which 60% (by wt.) of the flavonoids are dihydroflavones with the most basic oxygenation pattern, and these are known to be the precursors of all other flavonoids in higher plants. Flavones with the basic 5,7,4'-oxygenation pattern comprise the remaining 40% and these are nominally only one step removed biosynthetically from the dihydroflavones. This biochemical simplicity is paralleled by the morphological features upon which the family classification is based. The family Ricciaceae is characterized by very much reduced features [5] and *Riccia* in particular has been described [4,6] as possessing the most reduced sporophytes in the order. *Riccia* is considered to be a reduced rather than a primitive Marchantiale plant and is possibly derived from the evolutionary antecedents of *Marchantia* by a process of simplification [7]. In this context it is of interest that the flavonoid chemistry of *Ricciocarpus* is very closely related to that of *Marchantia polymorpha*, in that all compounds identified in the former species are also major constituents of the latter.

EXPERIMENTAL

Voucher specimens of *Riccia crystallina*, *Riccia bifurca*, and *Ricciocarpus natans* have been deposited with Massey University, Palmerston North (MPN 8578, 8579 and 17002 respectively). PC was carried out on Whatman 3MM paper using *t*-BuOH–HOAc– H_2O (3:1:1), (TBA) and 15% HOAc as sol-

vents. MS were determined by direct insertion on an AEI MS-30 spectrometer. UV spectra were measured as described in Ref. 2.

Isolation procedure. *Riccia crystallina* (0.2 g dry wt) was macerated with mortar and pestle and extracted at room temp. with acetone:H₂O, 1:1 (50 ml) overnight. *Ricciocarpus natans* (56 g wet wt or 1.5 g dry wt) was treated in the same manner. Individual compounds from both species were isolated by 2-D PC (TBA, HOAc) and the amounts present estimated by UV spectroscopy (Band I). RN-2a and RN-2b were separated by 1-D PC, over-run in HOAc overnight.

Physical data. PC and UV absorption data for compounds RN-1, RN-2a, RN-2b, RN-3 and RC-1 are essentially the same as those reported earlier [1]. *R_f*s for unreported compounds (TBA, HOAc): RC-1 (0.52, 0.20), RC-2 (0.63, 0.20), RC-3 (0.74, 0.23), RC-4b (0.85, 0.28), RC-5 (0.81, 0.66), RC-6 (0.70, 0.60), RC-8 (0.43, 0.10). UV absorption data previously unreported: RC-2, RC-3, RC-4b as for RC-1; RC-5 λ_{\max} (MeOH) 282, 324, λ_{\max} (NaOMe) 241 sh, 282, 415 nm after 10 min (identical to authentic naringin). MS of RC-5: *M/e* 272 (*M*⁺, 88%), 271 (*M*⁺-1, 44%), 179 (30%), 153 (100%), 120 (75%).

Hydrolysis procedures. Enzyme hydrolyses with β -glucosidase, β -glucuronidase and pectinase were all carried out at 37° in a pH 5 acetate buffer for 2 hr. Subsequent sugar analyses were by PC in either EtOAc-Pyr-H₂O (12:5:4) or *n*-BuOH-Pyr-H₂O (2:2:1) with glucose, glucuronic acid and galacturonic acid as reference compounds. Aglycones were compared

with authentic samples by PC (TBA or HOAc), and by TLC on SiO₂ (6% MeOH in CHCl₃) or Avicel F 1440 (Bz-HOAc-H₂O, 125:72:3), followed by UV spectroscopy. Acid hydrolyses were carried out in 2N aq. HCl at 100° for 1.5 hr.

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