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ACACETIN 7-*O*-RHAMNOSYL-GALACTURONIDE FROM *REBOULIA HEMISPHERICA*

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INTRODUCTION

THE OCCURRENCE of flavonoids in liverworts has only recently been firmly established. All flavonoid glycosides isolated to date from this source have been *C*-glycosides or their derivatives,[1,2] the only indication of the occurrence of *O*-glycosides being the identification of the mixed *O*- and *C*- glycoside, isovitexin 7-*O*-glucoside in *Porella platyphylla*.[1,3] We now report the isolation, from the liverwort *Reboulia hemispherica*, of the previously unknown flavone *O*-glycoside acacetin 7-*O*-rhamnosyl-galacturonide, together with an *O*-glycoside of an acacetin 8-*C*-glycoside.

RESULTS AND DISCUSSION

Two chromatographically similar compounds, *RH*1 and *RH*2, were isolated from the MeOH–H_2O extract of *Reboulia hemispherica* and the major one, *RH*1, was separated in pure form by repeated recrystallization of the mixture.

The UV absorption spectrum of *RH*1 (λ_{max} 270 and 323 nm) suggested that it was a flavone of the acacetin (5,7-dihydroxy-4′-methoxyflavone) type, and shifts induced in the spectrum by the addition of $AlCl_3$ and NaOAc indicated[4] that the 5-hydroxyl group was free and that the 7- and 4′-hydroxyl groups were substituted. Confirmation of the acacetin-type structure was obtained from the PMR spectrum which revealed a three proton signal at 3·87 ppm (–OCH_3), a pair of two proton doublets ($J = 8$ Hz) at 8·01 and 7·11 ppm

[1] E. NILSSON, *Acta Chem. Scand.* **23**, 2910 (1969); N. A. TJUKAVKINA, V. BENESOVA and V. HEROUT, *Coll. Czech. Chem. Commun.* **35**, 1306 (1970).

[2] K. R. MARKHAM, L. J. PORTER and B. G. BREHM, *Phytochem.* **8**, 2193 (1969).

[3] However, an *O*-glycosidically linked flavone-polysaccharide compound has recently been isolated from *Monoclea forsteri*. K. R. MARKHAM, *Phytochem.* **11**, 2047 (1972).

[4] T. J. MABRY, K. R. MARKHAM and M. B. THOMAS, *The Systematic Identification of Flavonoids*, Springer, Heidelberg–New York (1970).

(H-2'6' and H-3'5', respectively), a singlet at 6·92 ppm (H-3) and two broad one proton singlets at 6·80 and 6·35 ppm (H-8 and H-6, respectively).

The R_f values of *RH*1, together with the presence in the PMR spectrum of two signals attributable to *C*-1 sugar protons (5·08 and 4·63 ppm), suggested that *RH*1 was a diglycoside. This was confirmed by complete acid hydrolysis which produced two sugars, galacturonic acid and rhamnose, identified by both GLC and PC. Partial hydrolysis yielded a monoglycoside which contained galacturonic acid as the only sugar, and since the UV absorption data for this monoglycoside were identical with those for *RH*1, it is evident that *RH*1 is a rhamnosylgalacturonide derivative. The aglycone of *RH*1 was identified as acacetin by MS and TLC comparison with authentic material.

The minor flavonoid constituent *RH*2 was not obtained in pure form. It co-chromatographed with *RH*1 on paper and, on acid hydrolysis, produced a monoglycoside which co-chromatographed with the monoglycoside from *RH*1. Unlike the *RH*1 monoglycoside however, it behaved like an 8-*C*-glycoside in that it isomerized on further acid treatment and did not produce an aglycone. The isomer so produced was completely free of *RH*1 products and possessed UV absorption characteristics similar to those of cytisoside (acacetin 8-*C*-glucoside). This information suggests that the isomer is an acacetin 6-*C*-glycoside and therefore that *RH*2 is a mono-*O*-glycoside of an acacetin 8-*C*-glycoside.

The identification of acacetin 7-*O*-rhamnosyl-galacturonide in *Reboulia hemispherica* is only the second example of a flavone glycoside which has been isolated from a liverwort and completely identified. It is also of interest that these data confirm that plants of the class Hepaticae possess the biosynthetic capability to methylate and to *O*-glycosylate the basic flavonoid nucleus.

EXPERIMENTAL

A voucher specimen of *Reboulia hemispherica* has been deposited in the University of Texas at Austin Herbarium (Averett 444). PMR spectroscopy was carried out in d^6-DMSO on a Varian DA601 spectrometer fitted with a Varian C1024 time-averaging computer. PCs were run on Whatman 3MM paper using *t*-BuOH–HOAc–H_2O, 3:1:1 (TBA) or *n*-BuOH–HOAc–H_2O, 4:1:5 (BAW) and HOAc (2 or 15%). TLC was performed on polyamide plates using MeOH–H_2O–HOAc, 18:1:1.

Extraction procedure and isolation of RH1. *Reboulia hemispherica* gametophyte tissue (100 g) was pulverized with MeOH–H_2O (1:1); the mixture was allowed to stand for 2 days. Purification of the extract by polyamide[4] column chromatography yielded a paper chromatographically pure, R_f 0·41 (TBA), 0·57 (15% HOAc), 0·42 (BAW), 0·19 (2% HOAc), white solid (0·02 g). Repeated crystallization of this material from MeOH–H_2O yielded TLC pure *RH*1 (R_f 0·32) as white crystals, m.p. 219–224°, together with mother liquors rich in *RH*2 (R_f 0·26). *RH*1 had λ_{max} nm (MeOH) 269, 323; (NaOMe) 293, 373; ($AlCl_3$ and $AlCl_3$ HCl) 277, 298, 341, 378; and no shifts in NaOAc or NaOAc–H_3BO_3.

Hydrolysis of RH1. Using 5% aq. HCl/100°/2 hr, *RH*1 produced an aglycone and small amounts of a monoglycoside. Sugars were identified as rhamnose and galacturonic acid by PC and GLC[4] (the latter was used in particular to distinguish galacturonic acid from glucuronic acid). The aglycone was indistinguishable from acacetin by TLC, PC [R_f 0·88 (TBA), 0·10 (15% HOAc)], UV and MS [M^+ (100%) 284, M^+-CO 256, M^+-$COCH_3$ 241)].

Monoglycoside material was isolated by PC, R_f 0·45 (TBA), 0·29 (15% HOAc) and on further acid or enzyme (β-glucuronidase, Koch-Light) hydrolysis yielded acacetin plus galacturonic acid (PC and GLC).

Hydrolysis of RH2. Mother liquors rich in *RH*2, R_f 0·41 (TBA), 0·57 (15% HOAc), 0·42 (BAW), 0·19 (2% HOAc), were hydrolysed as above. Two monoglycosides were produced, (i) the major, R_f 0·45 (TBA), 0·29 (15% HOAc) and (ii) the minor, R_f 0·67 (TBA), 0·32 (15% HOAc), which were interconvertible under acid conditions and which did not hydrolyse further. The minor isomer had λ_{max} nm (MeOH) 268, 323; (NaOMe) 276, 295sh, 322sh, 370 [acacetin 8-*C*-glucoside had λ_{max} nm (MeOH) 269, 323; (NaOMe) 278, 298sh, 367)]. The major monoglycoside did not co-chromatograph (on paper) with cytisoside.

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