Phytochemistry, 1974, Vol 13, pp 1553 to 1555 Pergamon Press Printed in England

LUTEOLIN 3′,4′-DI-*O*-β-D-GLUCURONIDE AND LUTEOLIN 3′-*O*-β-D-GLUCURONIDE FROM *LUNULARIA CRUCIATA*

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(*Received* 21 *November* 1973)

Key Word Index—*Lunularia cruciata*, Hepaticae, liverworts, Marchantiales; luteolin 3′,4′-di-*O*-glucuronide, luteolin 3′-*O*-glucuronide, luteolin 4′-*O*-glucuronide

Abstract—Luteolin 3′,4′-di-*O*-β-D-glucuronide is the major flavonoid in the liverwort *Lunularia cruciata* It is accompanied by small amounts of luteolin 3′-*O*-β-D-glucuronide Both are new natural products and the former appears to be a unique example of a 3′,4′-diglycosylated flavonoid Luteolin 4′-*O*-β-D-glucuronide was isolated as a hydrolysis product of the diglucuronide

INTRODUCTION

THE OCCURRENCE of flavonoids in liverworts is now well established.[1] In particular, species of the order Marchantiales have been found to produce a remarkably diverse range of flavone types including mono- and di, *C*- and *O*-glycosides of aglycones such as apigenin,[1] acacetin,[2] chrysoeriol,[1] tricin[1] and 8-methoxyluteolin.[3] These flavonoids are likely to be of chemotaxonomic and phylogenetic value especially in view of the fact that phenotypic plasticity common in the Marchantiales, has posed taxonomic problems in the past when vegetative characters alone were available for taxonomy.[4] It was with this in mind that a further member of the Marchantiales, viz, *Lunularia cruciata*, was investigated.

Although flavonoids have not previously been isolated from *L. cruciata*, preliminary studies[5] suggested that they were present. Certainly, the phenylpropanoid–polymalonate pathway necessary for their production is operative in this liverwort as evidenced by the occurrence of the C_{15}-dihydrostilbene, lunularic acid, and other related compounds.[6]

RESULTS AND DISCUSSION

Two dimensional paper chromatography of a methanol–water extract of *L. cruciata* gametophyte tissue revealed the presence of two flavonoids, LC-1 and LC-2. These were subsequently isolated by large-scale PC in yields of 0·04 and 0·004% respectively.

Prolonged acid hydrolysis of both compounds gave the same aglycone, luteolin, which was identified by chromatographic, UV and mass spectral comparison with authentic material. Less vigorous acid hydrolysis of LC-1 gave two glycosides in equal proportions,

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[3] MARKHAM, K. R. (1972) *Phytochemistry* **11**, 2049
[4] CAMPBELL, E. O. (1971) *N. Z. J. Botany* **9**, 678
[5] MARKHAM, K. R., PORTER, L. J. and BREHM, B. G. (1969) *Phytochemistry* **8**, 2193
[6] PRYCE, R. J. (1972) *Phytochemistry* **11**, 1355, and references therein

one of which was LC-2 and the other, a new compound (LC-3) which appeared as a purple spot unaffected by NH_3 on a paper chromatogram (cf LC-2 which turned yellow in NH_3).

The UV spectra (Table 1) of LC-1, LC-2 and LC-3 indicate that all three possess free 5- and 7-hydroxyl groups but lack *ortho*-dihydroxyl groups in the B-ring. The free 5-hydroxyl group in each case is evidenced by the shift observed with $AlCl_3$/HCl, and the free 7-hydroxyl group by the bathochromic shift of band II with NaOAc. In NaOMe, LC-2 exhibited a 58 nm bathochromic shift of band I with an increase in intensity which is indicative of a free 4'-hydroxyl group. LC-2 must therefore be a 3'-glycosylated luteolin. LC-3 by the same criterion does not contain a free 4'-hydroxyl group and so must be a 4'-glycosylated luteolin. The relative R_f values of these two compounds are consistent with the proposed glycosylation patterns in that, as for the 3'- and 4'-glucosides of luteolin,[7] the 3'-glycoside (LC-2) is the less mobile in both solvents. Further confirmation of the glycosylation patterns of LC-2 and LC-3 was obtained when removal of the sugar by prolonged acid, or enzymatic, hydrolysis liberated, in both cases, the B-ring *ortho*-dihydroxyl system. It is thus clear that LC-1 is a 3',4'-diglycosylated luteolin. This glycosylation pattern must account for the unusual UV spectrum of LC-1 which, with band I absorption at 316 nm, is more typical of 5,7-dihydroxyflavone than of a luteolin derivative. Hypsochromic shifts in band I of flavone spectra have been observed previously when 5-, 7-, 3'- or 4'-glycosylation is present but the extent of these shifts is usually of the order of only 3–10 nm in monoglycosides and 14–16 nm in 7,4'-diglycosides.[3,8] In the present case, 3',4'-diglycosylation has produced a hypsochromic shift of almost 30 nm.

TABLE 1. PROPERTIES OF LUTEOLIN GLYCOSIDES OF *Lamium album*

	LC-1	LC-2	LC-3
Spot colour (UV + NH_3)	Dark	Yellow–green	Dark
R_f (TBA)	0·27	0·44	0·53
R_f (15% HOAc)	0·42	0·10	0·19
Spectral maxima (nm) in			
MeOH	267, 316	267,286 *sh*, 333	266, 286 *sh*, 329
NaOMe	272, 300 *sh*, 341	272, 327 *sh*, 391	265, 293 *sh*, 371
NaOAc	272, 303 *sh* 340 *sh*	270, 387	269, 366
NaOAc/H_3BO_3	267, 315	267, 334	266, 327
$AlCl_3$	278, 289 *sh*, 328, 378 *sh*	274, 292 *sh*, 343, 377	273, 290 *sh*, 320 *sh*, 350, 385 *sh*
$AlCl_3$/HCl	278, 289 *sh*, 328, 378 *sh*	274, 292 *sh*, 343, 377	270, 285 *sh*, 320 *sh*, 340, 378 *sh*

Both LC-2 and LC-3 appear to be monoglycosides on the basis of their R_f values (Table 1). LC-3 for example has R_f values of 0·53 (TBA) and 0·19 (HOAc) which are very close to those previously reported for the 4'-galacturonide of 6-methoxyluteolin (0·50, 0·23),[3] but differ markedly from those of the 7,4'-digalacturonide (0·40, 0·54). Further, no flavonoid-containing intermediate was observed during the course of any of the conversions of LC-2 and LC-3 to their aglycone; and in view of the variety of hydrolyses involved this is taken to exclude the presence of disaccharides in these compounds.

The sugar moiety in each of these glycosides was suspected to be a uronic acid since all three were relatively resistant to acid hydrolysis. This was confirmed by hydrolysis with

[7] HARBORNE J. B. (1967) *Phytochemistry* **6**, 1569.

[8] HARBORNE J. B. (1967) *Comparative Biochemistry of the Flavonoids*, pp. 40–46. Academic Press, London.

β-glucuronidase and pectinase (polygalacturonidase). β-Glucuronidase was found to convert LC-1 solely to luteolin whereas pectinase treatment for the same time period gave only small quantities of luteolin and a 50% conversion to LC-2 (but not LC-3). Both LC-2 and LC-3 were completely converted to luteolin with β-glucuronidase but only partially converted with pectinase. The specificity of these enzymes is thus not sufficiently great to allow distinction of galacturonides from glucuronides and for this reason the sugars produced from all of the above hydrolyses were analysed by paper chromatography. In all cases glucuronic acid (as distinct from galacturonic acid) was produced. The stereochemistry of the sugar-aglycone linkage is defined by the β-glucuronidase hydrolyses[9] and thus LC-1 is luteolin 3′,4′-di-*O*-β-D-glucuronide, LC-2, luteolin 3′-*O*-β-D-glucuronide and LC-3, luteolin 4′-*O*-β-D-glucuronide.

Luteolin 3′,4′-di-*O*-β-D-glucuronide and luteolin 3′-*O*-β-D-glucuronide from *L. cruciata* are new natural products. The former compound is the only known example of a 3′,4′-diglycosylated flavonoid. Flavone glucuronides and galacturonides have been isolated from other members of the Marchantiales[1–3] (with one possible exception[10]), although methylation which was observed in *Monoclea*, *Marchantia* and *Reboulia* is noticeably absent in the present case. To permit full evaluation of the chemotaxonomic significance of these results, further studies of Marchantiales species are currently under way

EXPERIMENTAL

A voucher specimen of *Lunularia cruciata* has been deposited with Massey University, Palmerston North (MPN 8574) Paper chromatograms were run on Whatman 3 MM using *t*-BuOH-HOAc-H_2O, 3 1 1 (TBA) and 15% HOAc MS were determined on an AEI MS-30 spectrometer UV spectra were measured as described in Ref 11

Isolation procedure. Air-dried *L cruciata* gametophyte tissue (27 g) was blended in 30% aqueous MeOH (300 ml) and left overnight The extract was filtered and washed with $CHCl_3$ It was then applied to paper sheets, and following a 1-D run in HOAc, the bands containing LC-1 and LC-2 were cut out and eluted with 30% aqueous MeOH Elution gave LC-1 (10 mg), and crude LC-2 Further purification of the latter by 1-D PC in TBA followed by extraction of the product from water into *sec*-BuOH, gave pure LC-2 (*ca* 1 mg)

LC-1 and its hydrolysis products UV spectra and PC properties are as listed in Table 1 Hydrolyses of LC-1 were carried out under the following conditions, product analysis being by 2-D PC (i) HCl (5%) at 100° for 1 5 hr, products LC-2, LC-3 (in equal proportions) and the aglycone (ii) HCl (5%) at 100° for 4 hr, product largely the aglycone, (iii) β-glucuronidase (ex marine mollusc) in distilled H_2O overnight, product aglycone (100% conversion), (iv) Pectinase (ex *A niger*) in distilled H_2O overnight, products LC-2 (ca 50% conversion), LC-3 (absent), aglycone (*ca* 5%), unchanged LC-1 (*ca* 50% unchanged) The LC-2 isolated from (i) and (iv) above exhibited the same spectral and PC properties as did the naturally occurring LC-2 (Table 1) It also co-chromatographed with the naturally occurring material on acid washed silica TLC (EtOAc–Pyr–H_2O–MeOH, 80 12 10 5, R_f 0 15) and polyamide TLC (H_2O–HOAc–butanone–EtOH–acetylacetone, 13 4 3·3 1, R_f 0 16) Hydrolyses of LC-2 and LC-3 using acid as in (i) and (ii) above yielded the aglycone as the sole flavonoid-containing product Both enzymes converted LC-2 and LC-3 into the aglycone overnight, but pectinase hydrolysed only 50% of the starting material in each case

The aglycone, isolated from hydrolysis of LC-1 by (i) and (iii) above was compared directly (TLC, UV and mass spectra) with authentic luteolin The sugars were identified by PC using standard procedures

Acknowledgements—We are indebted to Dr Ella O Campbell, Massey University, Palmerston North, for the plant material and Mr G J Down of Chemistry Division, DSIR, for the MS

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