Myricetin Glycosides in *Lysimachia punctata*

Though myricetin was noted in *Lysimachia punctata* L. (Primulaceae) hydrolysates[1], a detailed study of the corresponding glycosides had not yet been made. In the present paper, myricetin 3-arabinoside, myricetin 3-rhamnoside and two other myricetin 3-glycosides are reported as constituents of *L. punctata*.

Material and methods. A weight of 107 g fresh whole plant (obtained from the University of Liverpool Botanical Garden, Ness) was extracted for 20 min with 60 ml boiling 95% ethanol and allowed to stand overnight at room temperature. The extract was filtered, concentrated, washed twice with petroleum ether (40–60 °C) and chromatographed. The main bands were cut out, eluted with 70% ethanol and purified by successive chromatography on Whatman 3MM paper in BAW (butanol-acetic acid-water: 4:1:5), 5% HOAc (5% acetic acid) and water, as descending solvents. Aliquots of the 4 band eluates were examined by acid and enzymic hydrolyses as well as H_2O_2 oxidation which preferentially removed the sugar attached in the 3 position. The eluates were hydrolysed for 30 min with *N* HCl at 100 °C; the aglycones were extracted with ethyl acetate (3 times) and chromatographed on Whatman No. 1 paper in BAW, BEW (butanol-ethanol-water: 4:1:2.2), PhOH (water-saturated phenol) and Forestal (concentrated HCl-acetic acid-water: 3:30:10). The H_2O_2 oxidation was carried out for 2 and 4 h at room temperature and enzymic hydrolyses for 1, 2, 4, 8 and 24 h at 37 °C in acetate buffer, pH 5.0, using a β-glucosidase/flavonoid ratio of about 1 mg/ml. On enzymic hydrolysis and oxidative cleavage, the sugars were identified by paper chromatography in BEW, PhOH and BBPW (butanol-benzene-pyridine-water: 5:1:3:3) (Table II). Standard compounds were run with every chromatogram. The identities of myricetin 3-glycosides were confirmed by UV spectral analysis in the presence of diagnostic reagents.

Results and discussion. Two-dimensional paper chromatography of *L. punctata* extract showed the presence of 4 major flavonoids having the dark brown colour under UV-light typical of flavonoid glycosides. The flavonoids were successively purified by paper chromatography. The following bands were eluted:

Rf 0.40–0.47 (BAW) { 0.12–0.22 (5% HOAc)
0.24–0.32 (5% HOAc)

Rf 0.54–0.69 (BAW) 0.08–0.51 (5% HOAc) { 0.05–0.19 (H_2O)
0.22–0.36 (H_2O)

In the acid hydrolysates, myricetin was the only aglycone present (Table I). The sugars obtained either by oxidation or acid hydrolysis were identified as arabinose, rhamnose and glucose (Table II), but hydrolysis with β-glucosidase failed to give any sugar from both glucosides. However,

[1] E. C. BATE-SMITH, J. Linn. Soc. *58*, 95 (1962).

Table I. Rf values (× 100) of *L. punctata* aglycones and glycosides

	BAW	BEW	PhOH	Fr[a]	15%HOAc	5% HOAc	H_2O
Aglycones							
Rf 40–47 (BAW), 12–22 (5% HOAc)	51	38	05	26			
Rf 40–47 (BAW), 24–32 (5% HOAc)	54	35	04	23			
Rf 54–69 (BAW), 08–51 (5% HOAc), 05–15 (H_2O)	53	40	06	24			
Rf 54–69 (BAW), 08–51 (5% HOAc), 22–36 (H_2O)	53	39	08	26			
Myricetin	54	42	06	26			
Glycosides							
Myricetin 3-arabinoside	64	68	31	41	24		07
Myricetin 3-rhamnoside	65	65	25	70	38	30	15
Myricetin 3-rhamnoside + Marker		66	26	71	41	32	16
Myricetin 3-rhamnoside (Marker)		65	26	71	39	32	16
Myricetin 3-glucoside	43	57	17	67	28	12	06
Myricetin 3-glucoside + Marker	–	58	17	67	28	12	06
Myricetin 3-glucoside (tea)	47[b]	58	16	67	28	12	06

[a] Fr, Forestal. Other abbreviations in text. [b] Literature.

Table II. R_g values of the *L. punctata* sugars liberated by peroxide oxidation (H_2O_2) and acid hydrolysis (HCl)

	BBPW		PhOH		BEW	
	H_2O_2	HCl	H_2O_2	HCl	H_2O_2	HCl
Rf 40–47 (BAW), 12–22 (5% HOAc)	0.98	0.99	0.99	0.99	1.06	0.97
Rf 40–47 (BAW), 24–32 (5% HOAc)	0.90	0.98	0.99	0.99	0.90	0.95
Rf 54–69 (BAW), 08–51 (5% HOAc), 05–19 (H_2O)	1.03	1.17	1.40	1.40	1.06	
Rf 54–69 (BAW), 08–51 (5% HOAc), 22–36 (H_2O)	1.70	–	1.60	–	1.79	
Galactose	0.80	0.84	1.06	1.10	0.90	0.89
Glucose	1.00	1.00	1.00	1.00	1.00	1.00
Arabinose	1.10	1.18	1.40	1.40	1.10	1.20
Xylose	1.30	1.40	1.20	1.20	1.30	1.30
Rahmnose	1.70	1.80	1.60	1.60	1.80	1.80
Rutinose	0.70	0.70	0.95	0.90	0.68	0.60

one of them showed identical Rf values in 7 solvents when co-chromatographed with myricetin 3-glucoside isolated from tea (Table I) and, therefore, was tentatively identified as myricetin 3-glucoside. The other unidentified glucoside has Rf values higher than monoglucosides and its position in the chromatograms suggests a diglucoside, probably 3-gentiobioside or 3-sophoroside. The 2 other glycosides were examined by standard procedures and identified as myricetin 3-arabinoside, only reported once before[2] in plants, and the more common myricetin 3-rhamnoside (Table I). The occurrence of myricetin 3-arabinoside in *L. punctata* (Primulaceae) has chemotaxonomic interest since it was previously reported[2] in *Vaccinium macrocarpon* (Ericaceae). Both families belong to orders with such fairly close affinities that many authors placed them in the series pentacyclic Gamopetalae[3].

Resumen. Miricetina-3-ramnósido y miricetina-3- arabinósido se identificaron en *L. punctata* (Primulacea). Otros dos glicósidos parecen ser miricetina-3-glucósido y miricetina-3-soforósido o 3-gentiobiósido.

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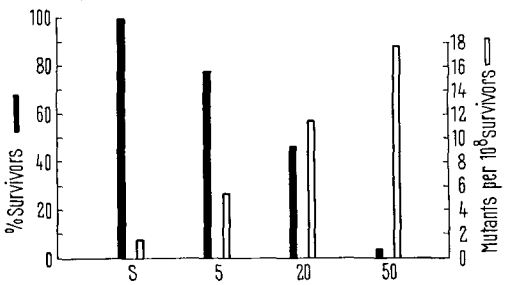
[2] O. Puski and F. J. Francis, J. Food Sci. *32*, 527 (1967).
[3] This work was carried out at the Botany Department of the University of Liverpool while the author was a recipient of a Fundación J. March fellowship. Thanks are due to Prof. B. Casaseca, Botany Department, University of Salamanca, Spain, for valuable information, to Dr. J. B. Harborne for the facilities given and to the Fundación J. March for financial support.

Griseofulvin Resistance in Dermatophytes

By introducing the antifungal antibiotic griseofulvin (GF), a decisive change in the therapy of dermatomycoses was brought about. However, from the history of chemotherapy, it is known that the efficacy of every antibiotic is inhibited by the occurrence of resistant cultures[1]. For this reason, the question of the further perspective of applying GF has been systematically studied from this aspect[2,3]. In this brief communication the knowledge concerning the frequency and properties of GF-resistant mutants is summed up.

Two compatible monsporic strains 155 and Z of the dermatophyte *Microsporum gypseum*[4] were used in these experiments. Z is the wild type with typical cinnamon-brown colony, 155 is a spontaneous mutant with cream colony (*cre*). GF-solution in dimethylformamide was added to Sabouraud dextrose agar. The resulting concentration in orientation experiments amounted to 10–30 µg GF/ml, in quantitative experiments to 50 µg GF/ml. On this selective medium, a spore suspension of a standard concentration was pipetted and after cultivation for 7 days all colonies grown were isolated. After 4 transfers on medium without GF, the sensitivity of the colonies to GF was evaluated by the mycelial growth test[5]. Besides these spontaneous mutants, others were prepared by means of UV-radiation. The procedure with UV was described in a previous communication[6].



Frequency of griseofulvin resistant mutants. S, spontaneous mutants; 5, 20, 50, UV-induced mutants (time of irradiation in sec).

From the macroconidial strain Z, no mutants could be obtained; from the spores of the microconidial strain 155, 13 spontaneous and 134 UV-induced mutants were isolated. Loci for resistance to GF were designated *grf*. The frequency of the mutants is shown in the Figure. Each value represents the mean obtained in at least 3 experiments. The frequency of spontaneous mutants varied at about 1.5×10^{-8}. By using UV the frequency of the

[1] R. J. Schnitzer and E. Grunberg, *Drug Resistance of Microorganisms* (Academic Press, New York 1957).
[2] K. Lenhart, Čslká Derm. *42*, 30 (1967).
[3] K. Lenhart, Mycopath. Mycol. appl. *36*, 150 (1968).
[4] N. Hejtmánková-Uhrová and M. Hejtmánek, Mycopath. Mycol. appl. *25*, 183 (1965).
[5] K. Lenhart, Mykosen *11*, 195 (1968).
[6] K. Lenhart, Z. allg. Mikrobiol. *5*, 222 (1965).

Table 1. Results of crossing between several GF-resistant mutants (*cre grf*) and sensitive wild strain Z (*cre*$^+$ *grf*$^+$)

Mutants in crossing	Locus for resistance	N_i	*cre*$^+$ *grf*	*cre*$^+$ *grf*$^+$	*cre* *grf*	*cre* *grf*$^+$	χ^2 for 1:1:1:1	P
VIII/1	*grf*-1	156	41	33	37	45	2.05	0.50–0.60
X/2	*grf*-1	146	31	41	35	39	1.7	0.60–0.70
X/3	*grf*-1	173	48	39	44	42	1.0	0.80
X/5	*grf*-1	194	45	52	40	57	3.5	0.30–0.40
X/8	*grf*-1	240	62	54	69	55	2.4	0.40–0.50
IX/1	*grf*-1	176	43	38	49	46	1.5	0.60–0.70
XI/2	*grf*-1	128	34	28	35	31	0.9	0.80–0.90
XI/3	*grf*-2	154	0	73	81	0	(1:1) (0.42)	0.50–0.60

N_i, total number of colonies isolated and tested.