ment turns to orange–yellow when fumed with ammonia. By heating with aqueous HCl, it was converted to naringenin. By comparison with a synthetic sample, the pigment was identified as 2',4',6',4-tetrahydroxychalcone. In some other inbred lines of *Petunia* the chalcone is also present. It gives a yellow colour to the pollen or, together with anthocyanin, a green colour. If no chalcone is present, the colour of the pollen is white or blue.

The presence of a yellow pigment in pollen of *Petunia* was reported earlier [1–4]. Our genetical experiments have shown that the pigment is formed when a gene called W by Müller [1] is homozygous recessive. Literature data do suggest that 2',4',6',4-tetrahyroxychalcone is a common pigment in pollen. The pigment itself has been found in *Tulipa* [5]; naringenin, easily produced from the chalcone by HCl treatment, has been found in pollen extracts, after hydrolysis, of many angiosperms [16].

EXPERIMENTAL

Anthers of *ca* 1000 flowers were extracted in MeOH + HCl 0·1%. The yellow pigment was purified by PC in BAW, HOAc–HCl–H_2O (30:3:10) and HOAc 5%. Identification was by direct R_f and spectral comparison with a synthetic sample of 2',4',6',4-tetrahydroxychalcone [7]. R_f's were detd. by TLC on cellulose and polyamide. Spectra were measured in MeOH ± the usual shift reagents. After treatment with 2 N HCl 30 min 100°, it gave naringenin, identified in a similar manner.

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TRICETIN, DIOSMETIN AND LUTEOLIN SULPHATES IN LEAVES OF *LACHENALIA UNIFOLIA*

CHRISTINE A. WILLIAMS and JEFFREY B. HARBORNE
Plant Science Laboratories, The University, Reading
and
TREVOR S. CROSBY
Department of Plant Sciences, The University, Leeds, England

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Key Word Index—*Lachenalia unifolia*; *L. unifolia var. wrightii*: Liliaceae; flavone sulphates; tricetin, diosmetin and luteolin 3'-sulphates and 7,3'-disulphates; chemical races.

During the course of a leaf flavonoid survey of the genus *Lachenalia* (Liliaceae), the aglycones tricetin, diosmetin and luteolin were identified in conjugation with sulphate in *L. unifolia* Jacquin WFB/26/59. Neither tricetin nor flavone sulphates were found in eight other *Lachenalia* species examined. Since tricetin (5,7,3',4',5'-pentahydroxyflavone) has previously been reported only twice as a plant constituent [1,2] and flavonoid sulphates have been detected in only one other member of the Liliaceae, *Bellevalia flexuosa* [3], also a member of the tribe Scilleae [4], a more detailed chemical study of this plant was undertaken.

Six flavone sulphates were isolated from leaves of the above-mentioned accession of *L. unifolia*: two tricetin, two luteolin and two diosmetin derivatives. The luteolin and diosmetin conjugates were identified by standard procedures and co-chromatography with synthetic samples as their respective 3'-sulphates and 7,3'-disulphates. The tricetin derivatives were provisionally identified by R_f comparison and spectrophotometric data as the corresponding 3'- sulphate and 7,3'- disulphate but no authentic markers were available for comparison. (Table 1).

Three other accessions received under the name of *L. unifolia* were also examined: WFB/20/59, K/1781/70 and K/1381/71. These three accessions, all from the Darling Flower Reserve, were found to differ from the Killarney accession WFB/26/59 in the absence of the diosmetin sulphates and the presence of two unidentified apigenin conjugates. Thus there are at least two chemovars within the species *L. unifolia* and the chemical evidence appears to agree with morphological evidence in that the Killarney accession (having pedicels *ca.* 9 mm long, perianth *ca.* 13 mm long and stamens reaching the tip of the perianth) is attributable to the type variety of the species, whereas the accessions K/1781/70 and K/1381/71 from the Darling Flower Reserve (having pedicels respectively 6–7 and 7–8 mm long, and both having a perianth *ca.* 9–10 mm long and stamens slightly exserted) are both attributable to *L. unifolia var. wrightii* Baker [5]. It has not so far proved possible to separate *L. unifolia* and its *var. wrightii* on purely vegetative morphology but,

Table 1. R_f and electrophoretic mobility of flavone sulphates of *Lachenalia unifolia*

Flavone sulphate	R_f (× 100) in					Colour in UV/+ NH_3	Electrophoretic mobility*
	BAW	BEW	PhOH	15% HOAc	H_2O		
Tricetin							
3′-sulphate	30	22	05	10	04	DK/Y	0·24
7-sulphate	29	15	05	07	03	DK/Y	0·20
7,3′-disulphate	07	05	0	23	28	DK/Y	1·70
Luteolin							
3′-sulphate	39	42	28	18	14	DK/Y	0·48
7-sulphate	35	38	24	16	15	DK/Y	0·60
7,3′-disulphate	12	15	05	44	51	DK/Y	2·70
Diosmetin							
3′-sulphate	41	42	48	30	30	DK/DK	0·50
7-sulphate	42	45	47	33	36	DK/DK	0·55
7,3′-disulphate	20	28	18	49	61	DK/DK	4·0

* Relative to quercetin 3- sulphate, run at pH 2·2 for 2 hr at 400V/cm on Whatman 3 mm paper. Key: DK = dark, Y = yellow.

while Miss Barker's collection from the Darling Flora Reserve WFB/20/59 has not yet produced flowers despite many years of cultivation, it is possible that this also is the *var. wrightii* since it was collected at the same locality as the two Kirstenbosch accessions.

Luteolin and diosmetin 3′- sulphates and tricetin 3′- sulphate and 7,3′- disulphate are reported here for the first time as plant constituents. Tricetin is reported for the first time as a constituent of the monocotyledons.

EXPERIMENTAL

Plant sources. Fresh plant material was removed from the collection of *Lachenalia* maintained under glass in the experimental gardens of the Department of Plant Sciences, University of Leeds. Details of origin of the accessions referred to in this paper are as follows: WFB/20/59 Grown from seed collected by W. F. Barker in 1959 from bulbs she collected in that year at the Darling Flora Reserve, Malmesbury Div., South Africa. WFB/26/59 Grown from seed collected by W. F. Barker in 1959 from bulbs she collected in that year at Killarney, Cape Div., South Africa. K/1781/70 Grown from seed of Kirstenbosch accession no. 1781/70: Darling Flower Reserve, Nat. Grid Ref. 3318 AD; growing in sandy clayish soil; collected by National Botanic Gardens expedition. K/1381/71 Grown from seed of Kirstenbosch accession no. 1381/71: Darling Flower Reserve, indigenous, Nat. Grid Ref. 3318 AD; collected by National Botanic Garden expedition, 7/12/1971. The first two accessions were sent in 1960 by Miss W. F. Barker, at that time Curator of the Compton Herbarium, Kirstenbosch, and the latter two were supplied by The Director, National Botanic Gardens, Kirstenbosch in 1971 and 1972 respectively. Our grateful thanks are expressed for their assistance. Chromosomal and other studies have been carried out on all these plants, the results of which will be published separately. Voucher specimens of all the plants will be retained at Leeds for the time being and deposited in one of the national herbaria later.

Flavonoid identification. Leaf material was extracted with hot 80% MeOH and flavonoid sulphates isolated and purified on Whatman No 3 mm paper using standard solvents. Known sulphates were identified on the basis of R_f, UV spectral analysis, electrophoretic mobility in 2·5% formic acid–7·5% HOAc (1:1) pH 2·2 buffer for 2 hr at 400 V, acid hydrolysis to aglycone and sulphate and by direct comparison with authentic samples [6]. There was difficulty in the case of diosmetin 7,3′- disulphate in obtaining exactly identical R_f data for natural and synthetic specimens but this was found to be due to some impurities in the synthetic sample. K^+ and HSO_4^- were detected after acid hydrolysis by TLC using the method previously described [6].

Luteolin 3′- sulphate. Chromatographic and electrophoretic data are given in Table 1. Acid hydrolysis gave luteolin and sulphate only and the compound co-chromatographed with synthetic luteolin 3′- sulphate [7] in five solvents. The absence of a borate shift, the dark to yellow colour (in UV + NH_3) and the ready breakdown to luteolin on PC in 15% HOAc confirm that the sulphate is attached at the 3′- position.

Diosmetin 3′- sulphate. Chromatographic and electrophoretic data are given in Table 1. Acid hydrolysis yielded diosmetin and sulphate and the compound readily broke down to give diosmetin on PC in 15% HOAc and co-chromatographed with synthetic diosmetin 3′- sulphate in five solvents.

Tricetin 3′- sulphate and 7,3′- disulphate. Chromatographic and electrophoretic data are given in Table 1. Acid hydrolysis yielded tricetin and sulphate only. Complete spectral data is not available for tricetin 3′- sulphate but chromatographic data suggest a monosulphate and break down on PC in 15% HOAc to tricetin suggests that the sulphate is attached at the 3′- position. Spectral data (λ_{max}) for tricetin 7,3′- disulphate are as follows: MeOH 255, 267, 348, + NaOAc 255, 267, 350; + H_3BO_3 252, 369. The lack of NaOAc shift indicates that there is a sulphate molecule in the 7 position. Chromatographic data suggest the compound is a disulphate and its dark to yellow colour (in UV + NH_3) suggests that the second sulphate molecule is attached at the 3′- position. A sulphate at the 4′- position would give a dark absorbing compound in UV not changing with NH_3. Authentic tricetin was obtained by demethylation of a natural specimen of tricin isolated from *Medicago*.

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