Phytochemistry, 1976, Vol. 15, p 348. Pergamon Press. Printed in England

ISOPONCIMARIN: NEW COUMARIN FROM *PONCIRUS TRIFOLIATA**

A. GUIOTTO, P. RODIGHIERO, U. QUINTILY and G. PASTORINI

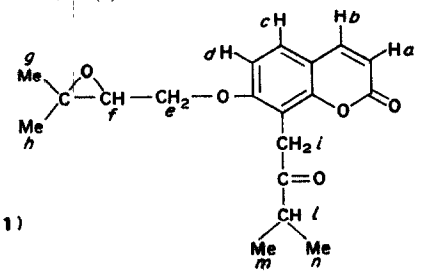
Istituto di Chimica Farmaceutica dell'Universita'di Padova, via Marzolo 5, 35100 Padova, Italy

(*Received* 28 *July* 1975)

Key Word Index—*Poncirus trifoliata*; Rutaceae; new coumarin; isoponcimarin.

Plant. Poncirus trifoliata L. (Botanical Institute of University Padua Herbarium) *Source.* Padua surroundings. *Previous work.* Essential oil [1], bitter principles [2], coumarins and furocoumarins from seeds [2–5] unripe fruits [6] and roots [7].

In an earlier communication [6] the isolation of a 7-*O*, 8-*C*-diepoxyisoprenylcoumarin, poncimarin, was reported from unripe fruits of the above plant. We now report the isolation from the petrol extract of the same material of a new unknown coumarin, isoponcimarin. From chemical and spectral evidence isoponcimarin is 7-(3′-methyl-2′,3′-epoxybutyloxy)-8-(3″-methyl-2″-oxobutyl)coumarin (**1**).



(1)

The extract was chromatographed on a silica gel column eluting with a mixture of solvents of slowly increasing in polarity; EtOAc–C_6H_6 (1:1; v) fractions gave a crystalline compound, which after recrystallization from *n*-hexane had mp 85° and gave a single blue-fluorescent spot in UV light in several TLC systems.

The elemental analysis of isoponcimarin agrees with a molecular formula $C_{19}H_{22}O_5$, molecular weight 333·3 (calcd. 330·37; osmometric method; $CHCl_3$) and $[\alpha]_{465}^{20}$ − 6·94 ($CHCl_3$; $c = 3·75$). UV absorption spectrum, characteristic of a 7-alkoxycoumarin chromophore, is virtually identical with that of poncimarin [6] and it is not affected by addition of alkali, indicating the absence of a free phenolic hydroxyl group [(95% EtOH) λ_{max} nm (log. ϵ) 217 (4·16, (sh); 255 (3·63); 320 (4·20)].

The H^1-NMR spectrum of isoponcimarin (60 Mc; $CDCl_3$; TMS internal standard) confirmed the presence of a 7,8-disubstituted coumarin system showing doublets at δ 6·23 (1 H; J 9·5 Hz) and δ 7·64 (1 H; J 9·5 Hz), assigned to C_3 and C_4 protons *a* and *b* respectively and doublets at δ 6·87 (1 H; J 8·6 Hz) and δ 7·38 (1 H; J 8·6 Hz) assigned to C_6 and C_5 *ortho* benzenic protons *d* and *c* respectively. A sharp singlet at δ 4·07 (2 H) due to the benzylic protons *i*, because of the combined diamagnetic effects of the aromatic nucleus and contiguous carbonyl group [8]. An incompletely resolved septet at δ 2·86 (1 H) assigned to the methyne proton *l* and a doublet at δ 1·23 (6 H; J 7 Hz) for the terminal methyl groups *m* and *n*. A four line signal of an AB_2 system centered at δ 4·19 (2 H) assigned to methylene *e* and a triplet at δ 3·08 (1 H) due to the epoxide proton *f*. Two singlets at δ 1·35 and δ 1·37 (3 H each) for the methyls *g* and *h*. Decoupling experiments are in agreement with the attributions. In HOAc solution isoponcimarin gave on addition of conc. H_2SO_4 a mixture of 3 compounds, the major of which was identified as the corresponding diketo product arising from isomerization of epoxy group. However, isoponcimarin is not an artifact from poncimarin, since the latter compound did not undergo isomerization under the condition of isolation and both coumarins are present in the original petrol extract of the plant material as indicated by TLC.

* Part 4 in the series, Coumarin and furocoumarin from *Poncirus trifoliata*. For part 3 see A. Guiotto *et al.* (1975) *Z. Naturforsch.* **30 c**, 420.

REFERENCES

1. Scora, R. W., England, A. B. and Bitters, W. P. (1966) *Phytochemistry* **5**, 1139.
2. Dreyer, D. L. (1965) *J. Org. Chem.* **30**, 749.
3. Dreyer, D. L. (1966) *Phytochemistry* **5**, 367.
4. Weinstein B., Craig, A. R., Fuller, L. W., Jung-Bu Kang and McBreen, S. A. (1972) *Phytochemistry* **11**, 1530.
5. Guiotto, A., Rodighiero, P. and Fornasiero, U. (1973) *Z. Naturforsch.* **28c**, 260; (1974) *Z. Naturforsch.* **29c**, 201.
6. Guiotto, A., Rodighiero, P. and Quintily, U. (1975) *Z. Naturforsch.* **30c**, 420.
7. Tomimatsu, T., Hashimoto, M., Shingu, T. and Tori, K. (1972) *Tetrahedron* **28**, 2003.
8. Stanley, W. L., Waiss, Jr. , A. C., Lundin, R. E. and Vannier, S. H. (1965) *Tetrahedron* **21**, 89.

Phytochemistry, 1976, Vol. 15, pp 348–349 Pergamon Press. Printed in England.

4,2′,4′,6′-TETRAHYDROXYCHALCONE IN POLLEN OF *PETUNIA HYBRIDA*

P. DE VLAMING* and K. F. F. KHO†

* Institute of Genetics, University of Amsterdam; † Department of Plant Physiology, University of Amsterdam, The Netherlands

(*Received* 5 *May* 1975)

Key Word Index—*Petunia hybrida*; Solanaceae; pollen; flavonoids; chalcones.

In pollen of the inbred line V11 of *Petunia hybrida*, maintained in the collection of the Institute of Genetics, University of Amsterdam, a yellow pigment has been found. On paper chromatograms the colour of this pig-

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.

REFERENCES

1. Müller, I. (1958) *Z. Vererbungslehre* **89**, 246.
2. Mosig, G. (1960) *Z. Vererbungslehre* **91**, 164.
3. Meynet, J., Cornu, A. and Paynot, M. (1971) *Ann. Amélioration Plantes* **21**, 103.
4. Wiering, H. (1974) *Genen Phaenen* **17**, 117.
5. Quast, L. and Wiermann, R. (1973) *Experientia* **29**, 1165.
6. Wiermann, R. (1968) *Ber. Deut. Botan. Ges.* **81**, 3.
7. Moustafa, E. and Wong, E. (1967) *Phytochemistry* **6**, 625.

Phytochemistry, 1976, Vol. 15, pp. 349–350. Pergamon Press. Printed in England.

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

(*Received* 18 *August* 1975)

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,