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TWO SULPHATED ANTHRAQUINONE DERIVATIVES IN *RUMEX PULCHER*

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Key Word Index—*Rumex pulcher*; Polygonaceae; anthraquinone sulphates; emodin monoglucosidesulphate; emodin dianthrone diglucosidesulphate.

Recent studies have indicated that conjugates of phenolic compounds with inorganic sulphate occur regularly in nature, with especial frequency in water plants [1] and halophytes [2, 3]. The largest group of such conjugates are the flavonoids, since over 50 structures have been variously identified in some 200 species drawn from at least 20 different plant families [4]. In addition to the flavonoid sulphates, two other classes of phenolic compound have been found to occur with covalent sulphate attachment, namely betacyanins [5] and hydroxycinnamic acids [1, 6, 7]. We now wish to report the first identification of anthraquinone sulphates in plants, in various members of the Polygonaceae.

The very first report, in 1937, of flavonoid sulphates in nature was of persicarin (isorhamnetin 3-sulphate), which was found in the water pepperwort, *Polygonum hydropiper* L. [8]. The same compound was later reported in *P. thunbergii* [9] and *P. perfoliatum* [10]. In the course of determining whether flavonol sulphates occurred in other members of the Polygonaceae, we surveyed by paper electrophoresis leaf extracts of a range of *Polygonum*, *Rheum* and *Rumex* species and although sulphated flavonoids were clearly infrequent, a number of other mobile anionic components which were coloured in UV light became apparent. Two compounds with the colour reactions of anthraquinones and which occurred in considerable amount in the leaf of *Rumex pulcher* were chosen for study and purified. These two substances were eventually identified (see Experimental) as emodin 1 (or 8)-monoglucosidesulphate and the related emodin dianthrone diglucosidesulphate. Identification as sulphates were based on electrophoretic mobility and the detection of K^+ and HSO_4^- ions after acid hydrolysis. Identifications of the anthraquinone moieties were based on spectral and chromatographic properties and direct comparisons (UV, IR, MS) with authentic markers.

The occurrence of such emodin derivatives in *Rumex pulcher* is expectable, since a range of anthraquinones have been found to be widespread in this genus and in *Rheum* (11). In addition emodin 1 (or 8)-monoglucoside has been reported in *Polygonum cuspidatum* [12] and *Rhamnus palmatum* [13]. The failure of other workers to recognise the presence of sulphates in these plants is undoubtedly due to the instability of these conjugates, once they are isolated, since even on standing in methanol solution at room temperature, some hydrolysis of the *O*-sulphate link occurs within a few hours. That anthraquinone sulphates are widespread in Polygonaceae was evident when an electrophoretic survey showed the presence of similar constituents to the two emodin derivatives now reported in half of 27 *Rumex* species studied and also in some *Rheum* species. A number of other new phenolic conjugates are also present in these plants and these are under active investigation.

Table 1. R_f values and spectral properties of sulphated anthraquinones

Compounds	Colour in UV	R_f (×100) in‡ BAW	BEW	phenol	water	15% HOAc	Electrophoretic mobility*	Absorption spectrum (λ_{max}, nm) in MeOH	in Alkali	in $AlCl_3$
Emodin 1 (or 8)-glucoside sulphate	orange	31	37	44	51	54	1.46	225, 264, 414	257, 285†, 495	264†, 272, 298, 476
Emodin dianthrone diglucosidesulphate	dark red	22	25	15	71	66	2.29	232, 275, 345	230, 258, 388	265, 273†, 372†, 430
Emodin 1 (or 8)-glucoside	orange	73	64	82	16	30	0.0	232, 275, 418	230, 308, 495	227, 275, 310†, 484
Emodin dianthrone	red	94	95	96	0	0	0.0	230, 277, 362	225, 265, 392	233, 265†, 274†, 372†, 420
Emodin	orange	89	95	92	01	06	0.0	222, 253, 265, 284, 436	222, 252, 320, 520	258, 290, 305, 484, 510

*Electrophoretic mobility on the purified salt relative to quercetin 3-sulphate as 1.00.
†Shoulder
‡Solvent key: BAW = *n*-BuOH–HOAc–H_2O (4 :1 :5); BEW = *n*-BuOH–EtOH–H_2O (4 :1 :2.2); PhOH = PhOH–H_2O (3 :1).

EXPERIMENTAL

The two anthraquinone sulphates were separated from leaf extracts of *Rumex pulcher* by electrophoresis on Whatman No. 3 paper at 400 V/cm for 2 hr in acetate formate buffer, pH 2.2. They were purified by standard PC procedures as quickly as possible to avoid loss of material due to hydrolytic breakdown. The spectral and R_f properties are recorded in Table 1. Emodin 1 (or 8)-glucosidesulphate was identified on the basis of the above properties and the fact that very mild acid hydrolysis gave emodin 1 (or 8)-glycoside, identified by direct comparison with an authentic sample. Location of the sulphate group on the glucose residue follows from the absorption spectral properties and from the fact that the IR spectrum of the sulphate was practically identical to that of the glucoside. Complete acid hydrolysis gave glucose, bisulphate, potassium and emodin, which was identified by comparison (IR, UV, MS, co-PC and co-TLC) with an authentic sample. Emodin dianthrone diglucosidesulphate was identified by similar procedures. On acid hydrolysis, it gave glucose, bisulphate, potassium and emodin dianthrone, which was identified by comparison with an authentic specimen. Location of the glucose and sulphate residues was based on the fact that on standing in methanolic solution, it gave rise to emodin 1 (or 8)-glucoside and emodin as a result of aerial oxidation. Emodin and its dianthrone were identified by PC (see Table 1) and also by TLC on Si gel in C_6H_6–EtOAc–HOAc (15 :4 :1) and in EtOAc–toluene–HOAc (49 :50 :1) and on polyamide in MeOH–C_6H_6 (4 :1).

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6,8-DIHYDROXY-3-HYDROXYMETHYLISOCOUMARIN, AND OTHER PHENOLIC METABOLITES OF *CERATOCYSTIS MINOR*

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The fungus *Ceratocytis minor* (Hedgc.) Hunt is generally introduced into the phloem and xylem of southern pine trees during attack by the southern pine beetle *Dendrotonus frontalis* Zimmerman, and development of *C. minor* in the xylem is considered to be central to the death of beetle-attacked trees [1]. There are close parallels in both the cause and symptoms of this disease to those of the Dutch Elm disease in which bark beetles introduce the fungus *Ceratocystis ulmi* (Buism.) C. Moreau, into the xylem of Elms [2, 3]. Claydon *et al.* [4] showed that phenolic C-10 acids or their dihydroisocoumarin tautomers were produced in highest yields by the most virulent strains of *C. ulmi* and compounds of the isocoumarin class are known for their biological activity on plant growth [5–8]. The similarities in these two important tree diseases prompted us to examine the phenolic metabolites of *C. minor*.

3 major phenolic metabolites of *C. minor* grown on malt extract were indicated by PC and TLC of EtOAc extracts of culture filtrates. The spectral properties of the major phenolic compound **1** indicated a 6,8-dihydroxy-hydroxymeth-y-lisocoumarin. The vinyl proton (δ6.83, 1H, *s*) appeared further down-field than that reported for 3-Me substituted isocoumarins [5–10]. This shift would be expected because of the hydroxymethyl group (cf δ6.6 for the α-vinyl proton of *trans*-cinnamyl alcohol with 6.36 for *trans*-isoeugenol models [11]). The PMR shift for the vinyl proton supports a 3-hydroxymethyl substitution since a vinyl proton adjacent to the lactone would be expected to be much further down-field (cf δ7.75 for vinylacetate [12]). Based on the above evidence, we have concluded that **1** is a new isocoumarin 6,8-dihydroxy-3-hydroxymethyl-1H-2-benzopyran-1-one.

Compound **2** occurred in much smaller quantities than **1**. Comparison of mp and spectral properties of **2**