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CHEMOTAXONOMY OF ANTHRAQUINONES IN *RUMEX*

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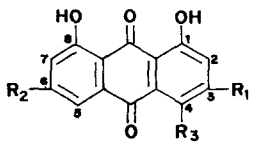
Abstract—The distribution of anthraquinones (aglycones, *O*-glycosides and *C*-glycosides) in all parts of the plant of 19 representative species of *Rumex* has been studied All species contained emodin, chrysophanol and physcion and in addition some contained aloe-emodin (side chain oxidized to the alcohol) and others rhein or rhein-like substances (side chain oxidized to the acid) Although there is no obvious correlation between type of anthraquinone present and taxonomic position based on morphological characters, it is possible to distinguish morphologically similar pairs of species by the type of anthraquinone in the root or fruit Methods of preparation and chromatographic and spectral data for a number of anthraquinones are given

INTRODUCTION

RUMEX is one of several genera which are characterized by the presence of anthraquinone derivatives and, during the last 100 years, several well-known ones have been isolated, e.g. chrysophanol (I) from *R. obtusifolius*, *R. palustris*;[1,2] emodin (II) and physcion (III) from *R. ecklonianus*, and *R. obtusifolius*[3,4] (for more detailed references, see Thomson[5]). Some of the compounds isolated were reported as unknown derivatives or chrysophanol of unusually low m.p.[6,7] but almost certainly they were mixtures of chrysophanol and physcion. These two derivatives are very difficult to separate and a mixture containing mainly chrysophanol would have the m.p. noted (178°) and have a UV spectrum similar to that of pure



	R_1	R_2	R_3
I - Chrysophanol	CH_3	H	H
II - Emodin	CH_3	OH	H
III - Physcion	CH_3	OCH_3	H
IV - Aloe-emodin	CH_2OH	H	H
V - Rhein	CO_2H	H	H
VI - Emodic acid	CO_2H	OH	H
VII - Catenarin	CH_3	OH	OH

chrysophanol. Other workers using paper chromatography reported the presence of chrysophanol, emodin[8] and rhein (V)[9] but their identifications were based on single systems and our own experience has shown that these systems are insufficient for identification.

Both Hegnauer[10] and Mathis[11] have dealt with the distribution of anthraquinones in the plant kingdom, including the Polygonales, and make reference to these in the genus

[1] E REIGAL, *Jahrest. Ph* **1**, 168 (1841)
[2] O HESSE, *J Chem Soc.* **78**, 40 (1900)
[3] F TUTIN and H. W B CLEWER, *J. Chem. Soc* **97**, 1 (1911).
[4] A TSCHIRCH and F. WEIL, *Arch Pharm* **250**, 20 (1912)
[5] R. H. THOMSON, *Naturally Occurring Quinones* (2nd Edition), pp. 367–535, Academic Press, London (1971)
[6] R L KHAZANOVICH, *Farmatsiya*, **2**, 30 (1942)
[7] G KURANO and T ISHIDA, *Ann. Rep Fac Pharm* (Kanazawa) **1**, 1 (1951).
[8] M. M. EL-KEIY, M S DARWISH and M. A MOUSTAFA, *J Pharm Sci.* (UAR) **5**, 209 (1965).
[9] K. TSUKIDA, *J Pharm Soc , Japan* **74**, 224, 386, 388; 394, 1090 (1954).
[10] R. HEGNAUER, *Planta Medica* **7**, 344 (1959)
[11] C MATHIS, *Comparative Phytochemistry* (edited by T. SWAIN), p. 261, Academic Press, New York (1966)

Rumex, but there are no recent reports of a systematic study. We have therefore examined these anthraquinones in relation to the taxonomy of the genus. The genus contains about 150 species, the British members falling into the three subgenera, *Acetosella*, *Acetosa* and

TABLE 1 PHYSICAL CONSTANTS OF THE ANTHRAQUINONES REFERRED TO IN THE TEXT

Compound	M p	R_f (× 100) System I	II	IV	UV_{max} (in nm) in MeOH	in N NaOH	IR spectra (cm^{-1})
Emodin	257–8°	52	62	18	223 (4 56)	237	1630 C=O chelated
					254 (4 25)		1670 C=O free
					267 (4 24)	285	1570 C=C arom
					290 (4 36)		2990 —CH—
					440 (4 09)	520	3400 —CH groups
Chrysophanol	197–8°	76	81	53	225 (4 57)	236	1628 C=O chelated
					258 (4 33)		1670 C=O free
					279 (4 01)	282	1570 C=C
					288 (4 07)		2890 —CH—
					432 (4 08)	502	3400 —CH groups
Physcion	205°	75	80	42	226 (4 45)	240	1625 C=O chelated
					255 (4 22)		1668 C=O free
					267 (4 25)	305	1570 C=C
					288 (4 22)		3400 —OH groups
					440 (4 02)	510	
Aloe-emodin	225°	36	45	52	225 (4 59)	236 (4 51)	1630 C=O chelated
					258 (4 36)		1670 C=O free
					279 (4 03)	282 (4 05)	1570 C=C
					287 (4 03)		2890 —CH—
					430 (4 03)	500 (3 96)	3400 —OH groups
Rhein	330°	24	43	3	230 (4 57)	243 (4 36)	1630 C=O chelated
							1670 C=O free
					260 (4 34)	280 (4 04)	1705 COOH
							1570 C=H
					432 (4 07)	508 (3 99)	3400 —OH groups
Emodic acid	310°	18	26	0	227	237	1625 C=O chelated
					252		1670 C=O free
					274	284	1570 C=C
					290	525	1705 COOH
					444		3400 —OH groups
Rhein like I	280–2°	9	17	0	288 (4 62)	240 (4 67)	1625 C=O chelated
					257 (4 48)	285 (4 28)	1670 C=O free
					280 (4 10)	515 (3 98)	1570 C=C
					432 (4 06)		1710 COOH
							3400 —OH groups
Rhein like II	305°	3	9	0	227 (4 71)	238 (4 63)	1620 C=O chelated
					255 (4 52)		1675 C=O free
					267 (4 51)	300 (4 40)	1570 C=C
					290 (4 56)		1715 COOH
					440 (4 25)	530 (4 24)	3400 —OH groups

Log ε values in brackets

TABLE 2. DISTRIBUTION OF ANTHRAQUINONES IN *Rumex*

Rumex Species	Aloe-Emodin				Rhein				Rhein Like I				Rhein like II			
	rt	st	lf	fr	rt	st	lf	fr	rt	st	lf	rf	rt	st	lf	fr
R hydrolapathum Huds. (La)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
R scutatus L (At)	(+)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
R altissimus Wood (La)	—	—	—	—	—	—	—	—	+	—	—	—	—	—	—	—
R crispus L (La) var *arvensis*	—	—	—	—	—	—	—	—	+	-	—	—	—	—	—	—
var *littoreus* (*sensu* Lousley[13])	—	—	—	—	—	—	—	—	+	—	—	—	—	—	—	—
R stenophyllus (La) Ledeb	—	—	—	—	-	—	—	—	+	—	—	—	—	—	—	—
R arifolius (At)	—	—	—	(+)	—	—	—	—	—	—	—	+	—	—	—	—
R. patientia L (La)	(+)	—	—	—	—	—	—	—	+	—	—	—	—	—	—	—
R confertus Will (La)	+	—	—	—	—	—	—	—	+	—	—	—	—	—	—	—
R sanguineus L (La) var *viridis*	—	—	—	—	—	—	—	—	+	—	—	—	—	—	—	—
var *purpureus*	—	—	—	—	—	—	—	—	+	—	+	—	—	—	—	—
R brownii Campd (La)	—	—	—	—	—	—	—	—	+	—	—	+	—	—	—	—
R pulcher L. (La)	—	—	—	—	—	—	—	—	(+)	(+)	—	+	—	—	—	—
R acetosa L (At)	(+)	—	—	(+)	—	—	—	—	+	—	—	+	—	—	—	—
R. conglumeratus (La) Murr.	(+)	(+)	(+)	(+)	—	(+)	(+)	(+)	—	(+)	—	—	—	—	—	—
R. acetosella agg (At) (subss *tenuifolius* (Wallr) Love	(+)	—	—	(+)	—	—	—	—	+	—	—	+	—	—	—	—
R. nepalensis (La) Sprengel	(+)	—	—	+	—	—	—	—	+	(+)	+	+	—	—	—	—
R maritimus L (La)	—	—	—	+	—	—	—	—	+	(+)	+	+	+	—	—	+
R. alpinus L (La)	—	—	—	+	—	—	—	—	+	—	+	+	+	—	—	+
R palustris Sm (La)	+	(+)	(+)	(+)	(+)	(+)	(+)	(+)	+	—	(+)	+	—	—	—	—
R. obtusifolius L (La) forma *trigranis*	(+)	—	—	+	—	—	—	—	+	(+)	+	+	+	—	—	+
forma *purpureus* (sensu Lousley[13])	(+)	—	—	+	—	—	—	—	+	(+)	+	+	+	—	—	+

The letters in brackets after each species indicate the sub-genera, Al = *acetosella*, At = *acetosa*; La = *lapathum* (or *rumex*)

rt = root, lf = leaf, fr = fruit; + significant amounts, (+) traces, — absent Note. All species contain chrysophanol, emodin and physcion in all parts

Rumex (or *Lapathum*).[12] We chose 19 species for study to include members from these three subgenera, certain species which are difficult to distinguish from one another, and one or two species in which anthraquinones had not previously been reported. In view of the faulty identification already referred to, we decided to base our identification on R_fs in several solvents, followed by elution and subsequent determination of UV and IR spectra and m p. For these purposes, we found it necessary to prepare a number of pure anthraquinones for reference purposes and we include the methods devised to do this.

Anthraquinone derivatives usually occur in higher plants as free aglycones in the

[12] A R CLAPHAM, T. A TUTIN and E F WARBURG *Flora of the British Isles* (2nd Edition), Cambridge University Press, London (1962)

[13] J E LOUSLEY Notes on the British Rumices *Rep. Bot Soc and Exch Club* 118, 547 (1938)

quinone form or as *O*- or *C*-glycosides with the aglycone normally in a reduced form (—CH_2 at position 10 instead of C=O) To simplify the work, we decided to convert all the compounds to the basic aglycone in the quinone form (positions 9 and 10 in the keto form) and to study particularly the substituents at the relatively stable positions 1, 3, 6 and 8. However, the initial state of occurrence (free, *O*- or *C*- glycoside) was noted. In order to be as comprehensive as possible, each part of the plant (root, stem, leaf and fruit) was studied separately

RESULTS

All species examined contained chrysophanol (I), emodin (II) and physcion (III) in all parts of the plant and almost always in all three forms, free, *O*- and *C*- glycosides. Quite surprisingly, rhein (V) was only occasionally found and then in trace quantities. However, two derivatives which, like rhein, possessed carboxylic groups, were isolated In some chromatographic systems, they had identical R_fs to rhein, and no doubt were the substances reported as rhein by Tsukida.[9] Our two new substances differed from rhein and from another common carboxylic acid derivative, emodic acid (VI) in several specially devised TLC systems and by spectral characters (Table 1).

The new compounds were never found as free aglycones or *O*-glycosides, only as *C*-glycosides Apart from this fact, the form in which the anthraquinone occurred in the plant seemed to have no taxonomic significance. The results (Table 2) therefore omit reference to the form of occurrence. Table 2 also omits the common fact that all species contained chrysophanol, emodin and physcion, but summarizes the distribution of the remaining anthraquinones, aloe-emodin (IV), rhein, rhein-like I and rhein-like II in all parts of the plants of 19 species of *Rumex* (for a more detailed account, see El-Muhtadi [14]

DISCUSSION

All the species examined contained anthraquinone derivatives and this, therefore, justifies the association of *Rumex* with *Rheum*.[15] Species of the latter, however, frequently contain rhein, which only rarely occurs in the *Rumex* species examined. From a practical point of view, the results in Table 2 show that the root and fruit are the best organs for testing for anthraquinone derivatives as the latter usually occur there in larger amounts.

There is no obvious correlation between the basic type of anthraquinone and the classification of this genus on morphological grounds. However, the type of anthraquinone sometimes serves as an additional character in identification Thus *R stenophyllus* and *R. obtusifolius* are very similar but phytochemical examination of the fruits or roots would show that *obtusifolius* contains aloe-emodin and rhein-like I and rhein-like II, these do not occur in *stenophyllus* Other similar pairs which can be distinguished readily by the same means are *R. sanguineus* and *R. conglumeratus* and *R maritimus* and *R palustris* In fact in all these pairs, phytochemical differences could be easily detected by examining the roots only; this may be a significant advantage when ripe fruits are not available

The results also show that all the species examined contain emodin (II), chrysophanol (I) and physcion (III) and this is consistent with the fact that the acetate-malonate biosynthetic route is involved;[14,16] emodin would be first formed and the other compounds

[14] F J. EL-MUHTADI, *Chemotaxonomic and Biosynthetic Studies in certain* Rumex *species* Ph D Thesis, Univ of London (1969)
[15] G BENTHAM and J D. HOOKER, *Genera Plantarum*, Vol 3, p 100, L Reeve, London (1883)
[16] E LEISTNER and M H ZENK, *Chem Commun* 210 (1969).

readily derived from it. In addition, certain species have the ability to oxidize the methyl group at position 3 to form the alcohol (aloe-emodin (IV)) and even to form the acid (rhein (V) and rhein-like compounds). The species in Table 2 are arranged to bring out this interesting fact. Further work on this genus may indeed show that all species have these basic anthraquinones, but only a limited number have the ability to oxidize the side chain at position 3.

EXPERIMENTAL

Materials. Most of the species were collected in the vicinity of London, others were grown from seed in our botanical garden in Enfield, and some were obtained from the Royal Botanical Gardens, Kew, and from the Chelsea Physic Garden In all instances, the material was authenticated by comparison with flora descriptions, herbarium material at the Linnean Society of London and advice from acknowledged experts (see Acknowledgements) Herbarium specimens of all samples are deposited in the Museum of the Pharmaceutical Society, University of Bradford, England

Preparation of reference anthraquinones. A convenient starting material is pharmaceutical aloin, but as this may be a variable material, it is wiser to prepare barbaloin from it by crystallization from water then methanol [17-18]

Aloe-emodin. (IV) Barbaloin (6 g), hydrated $FeCl_3$ (200 g) are refluxed in a mixture of 1 2 l HCl and 2 l. H_2O for 4 hr at 100° The mixture is then cooled, diluted with 2 l H_2O and allowed to stand overnight The granular precipitate is collected, washed with water till free from iron and acid, and dried at 110° *in vacuo*. The dry product can be crystallized from toluene to yield crystals or sublimed at 110° *in vacuo* to yield lemon-yellow crystals (Yield, 2–2·3 g Theoretical 3 1 g) Calc for $C_{15}H_{10}O_5$ C, 66 67, H, 3 70, Found: C, 66 42; H, 3 56%

Rhein (V) Aloe-emodin (1 g) and Cr_2O_3 (3 g) are dissolved in 100 ml HOAc and refluxed at 100° for 3 hr The mixture is then cooled, diluted with 1 5 l H_2O and kept overnight at 4° The yellow–orange crystals are washed with water till free from chromic acid, and dried at 100° *in vacuo* (Yield, 0 58 g, Theoretical 1 05 g) Calc for $C_{15}H_8O_6$ C, 63 38, H, 2 82, Found C, 63 57, H, 3 83%

Chrysophanol (I). A mixture of aloe-emodin (0 5 g), red P (0 5 g), HI s g 1 94 (20 ml) and HOAc (100 ml) is boiled under reflux (105°) for 3 hr The mixture is then cooled, diluted with 1 5 l H_2O and left to stand overnight The yellow precipitate of chrysophanol anthrone and unchanged phosphorus is collected and thoroughly washed, dissolved in N NaOH and gently warmed to promote oxidation to chrysophanol (deep red colour). After filtering, the filtrate is acidified (HCl) and left at 4° overnight The residue is collected, washed thoroughly, dried at 105° *in vacuo*, then purified by sublimation at 110° *in vacuo* (Yield 0 2 g Theoretical 0 47 g) Calc for $C_{15}H_{10}O_5$ C, 70 87, H, 3 94, Found C, 70 23, H, 3 88%

Emodin (and chrysophanol) II (*and* I) These can be prepared from chrysarobin by a modification of the method of Gardner [19] Chrysarobin (30 g) is dissolved in 400 ml HOAc and reduced by boiling under reflux with $SnCl_2$ (22 g), Sn (20 g) and 250 ml HCl for 6 hr The mixture is then cooled, diluted with 1 l H_2O and kept overnight in the refrigerator The precipitate is collected, washed, demethylated by boiling in 350 ml HOAc and 250 ml HBr under reflux for 8 hr The mixture is kept overnight, filtered, the residue well washed and dried at 105° *in vacuo* The residue consists mainly of chrysophanol and emodin anthrones and these are separated by chromatography (silica gel G, slurried with 0 01 N NaOH, dried at 110° for 1 hr solvent benzene –EtOAc, 72 25) The appropriate fractions are eluted, dissolved in N NaOH and warmed to oxidize to the quinone (bright red) After acidification, they are allowed to stand overnight and the yellow precipitate collected, washed and dried and re-crystallized as for the other compounds Calc for $C_{15}H_{10}O_5 \cdot$ C, 66 67, H, 3 70, Found C, 66 77, H, 3 51%

Emodin (from catenarin, VII) Catenarin (2 g), HOAc (200 ml), HI, s g 1 94 (20 ml), red P (1 g) were refluxed at 105° for 3 hr The mixture was then cooled, diluted with 1 5 l H_2O and allowed to stand overnight. The precipitate was collected, washed and dissolved in N NaOH with warming to form the quinone (bright red) The solution was filtered, acidified (HCl) and kept in the refrigerator overnight The precipitate was washed, dried *in vacuo* at 110° for 0 5 hr, and further purified by sublimation *in vacuo* at 110°. (Yield, 1 g, theoretical 1 9 g)

Physcion (III). Coarsely powdered *Xanthoria parietina* (a lichen) was exhaustively extracted with ether; the latter extracted with N NaOH, and the resulting red solution acidified with HCl and kept in a refrigerator overnight The brownish yellow precipitate was collected, washed, dried and crystallized from EtOH as yellow crystals. (Yield 0 45 g) Calc for $C_{16}H_{12}O_5 \cdot$ C, 67 60, H, 4 21, Found C, 67 71, H, 4 26%

[17] J. E Hay and L J Haynes, *J Chem Soc.* 3141 (1956)
[18] J W. Fairbairn and S Simic, *J Pharm Pharmacol* **15**, 325 (1963).
[19] J H. Gardner, *J Am Pharm Ass* **28**, 143–144 (1939).

Emodic acid (VI) Emodin (0 5 g), HOAc (50 ml), Ac_2O (50 ml) and pyridine (a few drops) are refluxed at 100° for 2 hr After cooling, the mixture is poured into ice-water, left overnight and the yellow precipitate of emodin triacetate collected, washed and dried The methyl group is then oxidized by dissolving the acetate in HOAc (100 ml) and Cr_2O_3 (1 g) and heating at 70° for 1 5 hr After cooling at 4° overnight, the yellow precipitate is collected and saponified by heating in N NaOH (25 ml) at 100° for 2 hr After cooling, the mixture is acidified, extracted with ether and the latter re-extracted with N $NaHCO_3$ to separate from unchanged emodin The bicarbonate solution is acidified, extracted with ether and the latter solution allowed to evaporate at room temperature till yellow crystals of emodic acid are formed (Yield 0 15 g)

TLC Systems

Systems I, II *and* III Plates, silica gel (25 g) in 0 01 N NaOH (50 ml), 0 25 mm thick Mobile phase with increasing amount of acid, as follows I, benzene–EtOAc–HOAc (75 24 1), II, same solvents (75 20·5), III, same solvents (7 2 1) *System IV* Plates, polyamide 0 25 mm Mobile phase, MeOH–benzene (80 20) [20]

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[20] H WAGNER and H. P. HORHAMMER, JR, *Deutsch Apothek-Zeitung* **108,** 633 (1968)

Key Word Index—*Rumex*, Polygonaceae, anthraquinones, chemotaxonomy, emodin chrysophanol, physcion