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ANTHRAQUINONE PIGMENTS FROM *XANTHORIA PARIETINA* (L.)*

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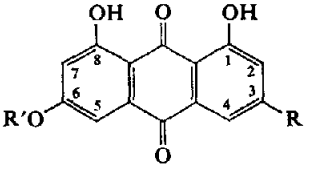
Abstract—Physcion, fallacinol, fallacinal, parietinic acid and emodin have been isolated from extracts of *Xanthoria parietina*. A much higher concentration of total anthraquinones was found in *X. parietina* var. *aureola* which contains, besides these five pigments, small amounts of citreorosein and emodic acid.

INTRODUCTION

ANTHRAQUINONE pigments of *Xanthoria* species have been studied by several investigators. The wall lichen *X. parietina* (L.) Beltram has been found to contain a yellow pigment[1] which was identified as 1,8-dihydroxy-6-methoxy-3-methylanthraquinone (physcion, I).[2] This pigment was isolated also from *X. fallax* (Hepp.) Arn. (= *X. substellaris* Wain.),[3] *X. elegans* (Lnk.) Th. Fr. (= *Caloplaca elegans* (Lnk.))[4, 5] and many other lichens. In 1956, Murakami[6] isolated fallacinol (II) and fallacinal (III) from *X. fallax* and at a later date, Eschrich[7] showed the presence in *X. parietina* of small amounts of parietinic acid (IV).



(I)	$R=R'=CH_3$		(physcion)
(II)	$R=CH_2OH$,	$R'=CH_3$	(fallacinol)
(III)	$R=CHO$	$R'=CH_3$	(fallacinal)
(IV)	$R=COOH$,	$R'=CH_3$	(parietinic acid)
(V)	$R=CH_3$	$R'=H$	(emodin)
(VI)	$R=CH_2OH$,	$R'=H$	(citreorosein)
(VII)	$R=COOH$,	$R'=H$	(emodic acid)
(VIII)	$R=CH_2OCOCH_3$,	$R'=CH_3$	(fallacinol monoacetate)
(IX)	$R=CH{=}CH{-}CO{-}CH_3$,	$R'=CH_3$	

These four anthraquinones, when arranged according to the state of oxidation of the side chain, show a complete sequence from physcion to parietinic acid. It was of interest,

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[2] R. EDER and F. HAUSER, *Helv. Chim. Acta* **8**, 126 (1925).
[3] M. ASANO and Y. ARATA, *J. Pharm. Soc. Japan* **60**, 521 (1940).
[4] S. NEELAKANTAN and T. R. SESHADRI, *J. Sci. Ind. Res.* **11** B, 126 (1952).
[5] W. STEGLICH, W. LOSEL and W. REININGER, *Tetrahedron Letters*, 4719 (1967).
[6] T. MURAKAMI, *Pharm. Bull. Japan* **4**, 298 (1956).
[7] W. ESCHRICH, *Biochem. Z.* **330**, 73 (1958).

therefore, to determine whether *X. parietina*, which was already known to contain the two latter pigments, also contains fallacinol and fallacinal.

The present paper describes the separation and identification of these and other acetone soluble anthraquinones from *X. parietina* and *X. parietina* var. *aureola*. The compounds were isolated chromatographically and identified by chromatographic comparisons (see Table 1), and by their u.v., i.r., NMR and mass spectra (see Experimental).

TABLE 1. THE R_f VALUES OF ANTHRAQUINONES FROM *X. parietina* ON THIN LAYER CHROMATOGRAPHY

Anthraquinone	R_f* on silica-gel	R_f† on polyamide	R_f† on acetylated polyamide
Physcion	0·88	0·80	0·60
Fallacinal	0·76	0·80	0·60
Emodin	0·39	0·36	0·30
Fallacinol	0·35	0·67	0·55
Parietinic acid	0·35	0·36	0·30
Unknown pigment	0·28	0·95	0·86
Citreorosein	0·13	0·30	0·22
Emodic acid	0·10	0·08	0·08

* Solvent system: benzene:ethyl acetate:acetic acid (18:1:1 v/v).
† Solvent system: acetone:acetic acid (9:1 v/v).

It must be noted that, as previously experienced by other workers in the field of natural polyhydroxyanthraquinones,[8,9] the isolation of individual anthraquinones in a pure form proved to be rather difficult. Many successive chromatographic separations were often necessary to obtain a pure compound and severe losses could not be avoided. Furthermore, due to possible mutual contamination, the amounts of individual anthraquinones present in *X. parietina* and *X. parietina* var. *aureola*, estimated as described in the Experimental Section and reported in Table 2, must be considered approximate.

TABLE 2. THE QUANTITATIVE DISTRIBUTION OF ANTHRAQUINONES IN *X. parietina*. THE VALUES GIVEN ARE A PERCENTAGE OF THE TOTAL ANTHRAQUINONES

Pigment	*X. parietina*	*X. parietina* var. *aureola*
Physcion	94·5	46·5
Fallacinal	1·8	27·3
Emodin	1·7	0·5
Fallacinol	2·0	25·7
Parietinic acid	< 0·1	< 0·2
Unknown pigment	—	< 0·1
Citreorosein	—	< 0·1
Emodic acid	—	< 0·1
Total anthraquinones (mg/g)	0·44	5·8

During this investigation two apparently new constituents were isolated in very small amounts, which were identified by mass spectra and comparison with synthetic samples and

[8] I. R. C. BICK and C. RHEE, *Biochem. J.* **98**, 112 (1966).
[9] A. W. K. CHAIN and W. D. CROW, *Australian J. Chem.* **19**, 1701 (1966).

found to be fallacinol monoacetate (VIII) and 1,8-dihydroxy-6-methoxy-3-(3-oxo-1-buten-1-yl) anthraquinone (IX).

The possibility was considered that these compounds were artifacts formed during the extraction, arising respectively from fallacinol by esterification of the primary alcoholic group and from fallacinal by acid catalysed condensation with acetone. Neither of these compounds was detectable when a sample of *X. parietina* var. *aureola* was extracted with methanol and the extract examined by chromatography.

CONCLUSIONS

Previously the only anthraquinone pigments reported to occur in *X. parietina* have been physcion (I) and parietinic acid (IV). In our present study, also fallacinol (II) and fallacinal (III) have been isolated from this plant material, and this gives a complete sequence of compounds differing only in the state of oxidation of the side chain.

The finding of emodin (V), which had never been formerly reported in lichens, is of particular interest since all the isolated anthraquinones appear to be derived from this substance by methylation and oxidation of the side chain.

Also citreorosein (VI) and emodic acid (VII), isolated in small amounts from *X. parietina* var. *aureola*, are biosynthetically related to emodin, from which they derive by oxidation of the side chain. These results show that in *X. parietina* var. *aureola* emodin oxidation can take place not only *via* physcion, as in *X. parietina*, but also without previous methylation of the hydroxyl group at position 6.

EXPERIMENTAL

Melting points were measured on a Kofler block and are uncorrected. The u.v. spectra were taken in ethanol solution on a Zeiss RPQ 20 A spectrophotometer and the i.r. spectra on a Perkin-Elmer Model 137 Infracord instrument in KBr pellets or in nujol mull.

The NMR spectra were recorded on a Varian A-60 spectrophotometer, with tetramethylsilane as internal reference. The mass spectra were measured on a Hitachi RMU6D instrument, using a direct inlet system to the source. TLC on silica-gel (Kieselgel G, Merck) was carried out by using benzene:ethyl acetate:acetic acid (18:1:1) as solvent system.

Plates of polyamide (Merck) and acetylated polyamide (MN-Polyamide AC, Machery and Nagel) were developed with acetone:acetic acid (9:1).

Plant Material

Samples of *X. parietina* var. *aureola* were collected from volcanic rocks near Aci Trezza (Catania) from November 1966 to June 1967. No significant differences were observed in the composition of the anthraquinone fraction of the various samples. *X. parietina* growing on spruce bark was collected on Mount Celado (Trento) in the summer 1963; its phycobiont has been shown to be *Trebouxia albulescens* G. De Nic. and G. Di Ben.,[10] in this differing from all the other specimens of *X. parietina* so far examined, whose phycobiont is *T. decolorans* Ahm.[11]

Isolation of Pigments

Air-dried material (100 g) was washed with water, homogenized in acetone with an Ultraturrax blender and extracted several times at 45–50° with acetone:acetic acid (99:1 v/v) until no more colour was extracted. The combined extracts were evaporated *in vacuo* at 40° to a heavy brown syrup which after being repeatedly shaken with light petroleum yielded a powdery solid. The petrol extract, containing sterols, carotenoids, chlorophylls and only minor amounts of anthraquinones (mainly physcion), were discarded. The red-brown residue was dissolved in hot methanol and the clear solution allowed to stand overnight at 0° and the precipitate collected.

[10] M. Giudici de Nicola and G. Di Benedetto, *Boll. Ist. Univ. Catania* **3**, 22 (1962).
[11] R. Tomaselli, *Atti Ist. Bot. Univ. Pavia* **1**, 1 (1965).

An aliquot of this crude mixture of anthraquinones was dissolved in benzene and chromatographed on a 3·5 × 50 cm column of acid-washed silica-gel. The column was eluted with benzene containing increasing amounts of ethyl acetate followed by ethyl acetate: acetic acid (99:1 v/v). Column fractions were monitored by TLC on silica-gel, polyamide and acetylated polyamide; partially resolved anthraquinones were further separated by repeated preparative TLC on silica-gel.

Fallacinol and parietinic acid, which migrate as a single band on silica-gel plates, were separated by TLC on acetylated polyamide. The R_f of the individual pigments are given in Table 1, in order of increasing absorption on silica-gel.

In order to obtain some minor components (parietinic acid (IV), emodic acid (VII), citreorosein (VI)), not easily isolated by the above procedure, another aliquot of the crude mixture of anthraquinones was dissolved in ethyl acetate and the solution was extracted three times with an equal volume of aq. 1 per cent $NaHCO_3$. The aqueous layers were combined, re-extracted with 5 × ethyl acetate and acidified to pH 2 with N HCl. The anthraquinones were extracted with ether, the ether extract washed with water, dried (Na_2SO_4), filtered and evaporated. Preparative TLC on silica-gel of the residue gave parietinic acid, emodic acid, citreorosein and another anthraquinone which, on account of the very small quantity, could not be obtained in a pure form and was not further investigated.

Identification of Pigments

Physcion. Had m.p. 205–207° alone and in admixture with synthetic physcion; on acetylation with acetic anhydride and fused sodium acetate it gave a diacetate, m.p. 185–186°, undepressed upon admixture with physcion diacetate. The NMR spectrum in $CDCl_3$ of the diacetate showed δ: 2·40 (6H,2 CH_3COO—), 2·45 (3H,CH_3—), 3·92 (3H,CH_3O—), 6·85 (1H, doublet J = 3 c/s, H-7), 7·17 (1H, broad, half-width 4 c/s, H-2), 7·62 (1H, doublet J = 3 c/s, H-5), and 7·95 (1H, broad, half-width 4 c/s, H-4).

Fallacinol. After recrystallization from aqueous acetic acid had m.p. 234–235° unchanged by admixture with an authentic sample. On acetylation it gave a triacetate, m.p. 192–193°, whose NMR spectrum ($CDCl_3$) showed δ: 2·15 (3H, CH_3COO—, aliphatic acetate), 2·42 (6H, $2CH_3$ COO—, aromatic acetate), 3·94 (3H, CH_3O—), 5·20 (2H, Ar—CH_2—O—), 6·88 (1H, doublet J = 3 c/s, H-7), 7·35 (1H, broad, half width 4 c/s, H-2), 7·65 (1H, doublet J = 3 c/s, H-5), 8·15 (1H, broad, half-width 4 c/s, H-4).

Fallacinal. This pigment, recrystallized from acetic acid, had m.p. 250–251° (lit.[6] 251–252°). The visible spectrum had an absorption maximum at 434 nm, indicating the presence of two α-OH groups.[12,13] I.r. bands at 1625 cm^{-1} (intense; bonded CO) and at 1675 cm^{-1} (weak; unbonded CO) permitted assignment of the 1,8-arrangement of hydroxyl groups. A third CO absorption (1720 cm^{-1}) suggested the presence of an CHO group. Mass spectrum of the pigment showed a molecular ion at $m/e = 298$, which was the most abundant ion in the spectrum. A peak at mass 297 (confirmed by the metastable peak at $m^* = 296$) is attributed to loss of hydrogen of the formyl group, one at mass 269 (metastable peak at $m^* = 243{\cdot}5$) to successive elimination of CO. From the above data it was deduced that the isolated pigment was fallacinal. The identification was confirmed as follows: in alkaline solution the pigment (5 mg in 1 ml of 25 per cent KOH in 50 per cent ethanol overnight at room temp.) underwent the Cannizzaro reaction giving fallacinol and parietinic acid, which were separated by TLC and identified by spectral and chromatographic comparison with appropriate authentic samples.

Parietinic acid. U.v. and i.r. spectra of this pigment, m.p. *ca.* 300°, were superimposable with those of an authentic sample of parietinic acid. Comparison of the chromatographic properties of the natural and synthetic pigment confirmed the identification.

Emodin. This pigment, m.p. 254–255° unchanged by admixture with an authentic sample, was soluble in Na_2CO_3 (β-hydroxyl group) and showed u.v. λ_{max} at 437 nm (two α-hydroxyl groups) and i.r. bands at 1630 cm^{-1} (bonded CO) and 1680 cm^{-1} (unbonded CO). Its identity with emodin followed from a comparison of its spectral and chromatographic properties with those of an authentic specimen.

Citreorosein. This anthraquinone had m.p. 287–288° alone or on admixture with an authentic sample. In the mass spectrum, in addition to the main parent peak at mass 286, there was an intense peak at m/e 257 ($M^+ - 29$), confirmed by the metastable peak at $m^* = 231{\cdot}1$, which can be attributed to the loss of H plus CO from the molecular ion.

The u.v. and i.r. spectra of the pigment and that of citreorosein were identical.

Emodic acid. This pigment, m.p. > 300°, showed a molecular ion at $m/e = 300$ and peaks at 283 ($M^+ - 17$) and 255 ($M^+ - 45$) formed from the parent ion by loss of OH and COOH, respectively. Its spectral and chromatographic properties were identical to those of a synthetic sample.

Fallacinol monoacetate. Mass spectrum of this compound showed a base peak at $m/e = 43$ (CH_3CO^+), a molecular ion at $m/e = 342$ and significant peaks at $m/e = 300$ ($M^+ - 42$), which corresponds to the loss of $CH_2{=}{=}CO$, and at 271 ($300^+ - 29$). Transitions $342^+ \longrightarrow 300^+ + 42$ and $300^+ \longrightarrow 271^+ + 29$ were confirmed by the occurrence of intense metastable peaks at $m^* = 262{\cdot}5$ and 245·2, respectively. Spectral and chromatographic properties of the compound were found to be identical to those of a synthetic sample.

[12] L. H. BRIGGS, G. A. NICHOLLS and R. M. PETERSON, *J. Chem. Soc.* 1718 (1952).
[13] J. H. BIRKINSHAW, *Biochem. J.* **59**, 485 (1955).

1,8-*Dihydroxy*-6-*methoxy*-3-(3-*oxo*-1-*buten*-1-*yl*)*anthraquinone*. The molecular ion of this substance occurred at mass 338 ($C_{19}H_{14}O_6$ requires mol. wt. 338). An intense peak observed at $m/e=323$ (M^+-15) and confirmed by the metastable peak at $m^*=308{\cdot}5$ is due to the loss of a methyl group. The successive elimination of CO originates the ion at mass 295. This compound was indistinguishable in spectral and chromatographic properties from an authentic specimen obtained from fallacinal by condensation with acetone in the presence of acetic acid.

Authentic Pigments

Emodin was purchased from Fluka AG (Buchs, Switzerland). Physcion was obtained from emodin by diazomethane. Fallacinol was synthesized from physcion diacetate by N-bromosuccinimide bromination followed by reaction of ω-bromophyscion diacetate with sodium acetate in acetic anhydride and final hydrolysis of the triacetate.[6] Parietinic acid was prepared according to Eschrich[7] from physcion diacetate by chromic acid oxidation and hydrolysis. Citreorosein was synthesized from emodin triacetate according to a procedure[14] similar to that used for the preparation of fallacinol. Emodic acid was prepared by chromic acid oxidation of emodin triacetate followed by hydrolysis.[15] *Fallacinol monoacetate* (VIII) was synthesized as follows: a solution of fallacinol (5 mg) in acetic acid (2 ml) was refluxed for 2 hr. Excess acetic acid was removed *in vacuo* and the crude product purified by TLC on silica-gel. Recrystallized from benzene it had m.p. 195–196°. R_f on silica-gel 0·64; on polyamide 0·95; on acetylated polyamide 0·80. (Found: mol. wt. from mass spectrum 342. $C_{18}H_{14}O_7$ requires 342).

1,8-*Dihydroxy*-6-*methoxy*-3-(3-*oxo*-1-*buten*-1-*yl*)*anthraquinone* (*IX*). A Solution of fallacinal (2 mg) in acetone (5 ml) and acetic acid (0·5 ml) was left for 7 days at room temp. After evaporation of the solvents, the crude product was purified by TLC on silica-gel: m.p. 261–262°. R_f on silica-gel 0·54; on polyamide 0·73; on acetylated polyamide 0·52. (Found: mol. wt. from mass spectrum 338. $C_{19}H_{14}O_6$ requires 338).

Quantitative Determination of Anthraquinones

The amounts of individual anthraquinones were estimated by extracting with methanol samples (10 g) of plant material. The extracts after concentration *in vacuo* were streaked on silica-gel plates, the plates were developed with solvent, the various bands scraped off, eluted and restreaked separately on another plate. The individual pigments were eluted and their amount determined by u.v. spectrophotometry, measuring E_{max} and comparing with $E^{1\,cm}_{1\%}$ values at λ_{max} for pure pigments.

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[15] O. Fischer and H. Gross, *J. Prakt. Chem.* **84**, 369 (1911).