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SHORT COMMUNICATION

THE ISOLATION OF TWO ESTERS OF THE NAPHTHAQUINONE ALCOHOL, SHIKONIN, FROM THE SHRUB *JATROPHA GLANDULIFERA*

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Abstract—The major pigment responsible for the trunk and branch colour of *Jatropha glandulifera* (Roxb.) has been identified as 3,3-dimethylacrylylshikonin (I) which was isolated admixed with a small quantity of acetylshikonin (II).

THE HIGHLY coloured oil extracts of the trunk, branch and roots of the shrub *Jatropha glandulifera* (Roxb.) have been used for many years in West Pakistan as a balsam for the relief of rheumatic pains. The dried wood is widely available in the bazaars, where it is given the local name of "ratanjot". The two pigments responsible for the dark-red colour of the wood were isolated and then identified by a combination of spectroscopic and degradative techniques when the major pigment (0·68 per cent of the dry weight) was found to be 3,3-dimethylacrylylshikonin (I) and the minor pigment (0·066 per cent of the dry weight) acetylshikonin (II).

Shikonin (III) has been isolated previously from five members of the Boraginaceae, *Lithospermum erythrorhizon* (Sieb. & Zucc.),[1] *Macrotomia ugamensis* (Popov),[2] *Onosma caucasicum* (Levin),[3] *Echium rubrum* (Forsk.),[3] and *L. officinale* (L.) var. *erythrorhizon*[4] but the acetyl and 3,3-dimethylacrylyl esters of shikonin have only been obtained previously from *L. erythrorhizon*.[1, 5] This present isolation represents the first report of shikonin derivatives being found in a member of the Euphorbiaceae[6] and as the esters have been inadequately[4, 5] and in part erroneously[5] described in the literature, a full account of the characterization of these compounds is included.

The 1H NMR spectra of the esters are compared in Table 1 with the previously recorded spectrum of 3,3-dimethylacrylylshikonin and it can be seen that in the spectrum recorded by Morimoto *et al.*[5] that four proton absorptions are missing and two absorptions have been wrongly assigned.

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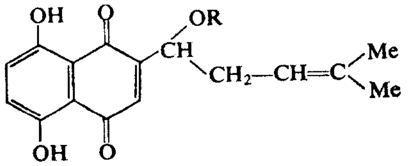
[2] A. S. ROMANOVA and A. I. BAN'KOVSKII, *Khim. Prirod. Soedinenii* 226 (1965).

[3] A. S. ROMANOVA, N. V. TAREEVA and A. I. BAN'KOVSKII, *Khim. Prirod. Soedinenii* 71 (1967).

[4] S. MIURA, *Syoyakugaku Zasshi* **17**, 45 (1963).

[5] I. MORIMOTO, T. KISHI, S. IKEGAMI and Y. HIRATA, *Tetrahedron Letters* 4737 (1965).

[6] C. MATHIS, in *Comparative Phytochemistry* (edited by T. SWAIN), pp. 262–267, Academic Press, London (1966).



(I) R = —CO—CH=CMe$_2$

(II) R = —COMe

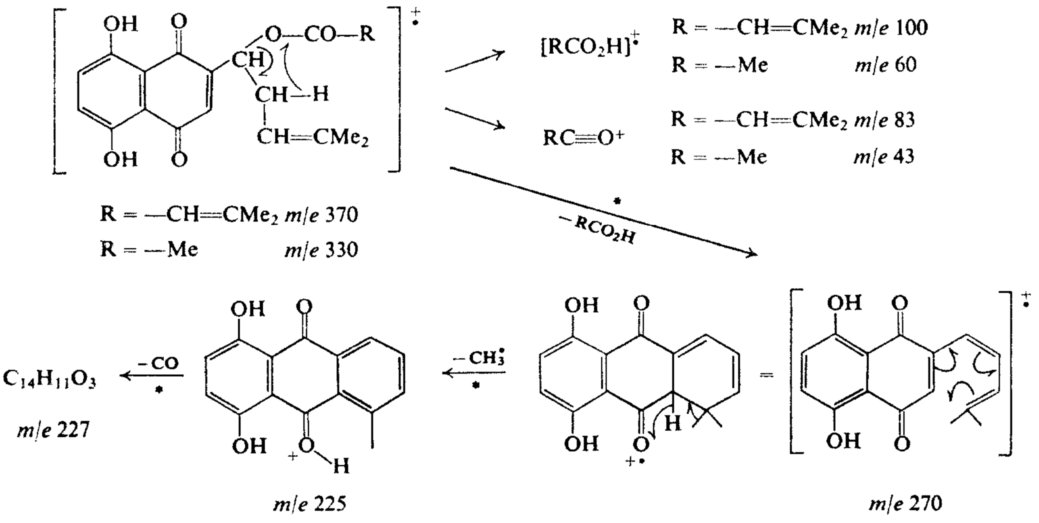
(III) R = —H

TABLE 1. NMR COMPARISON TABLE

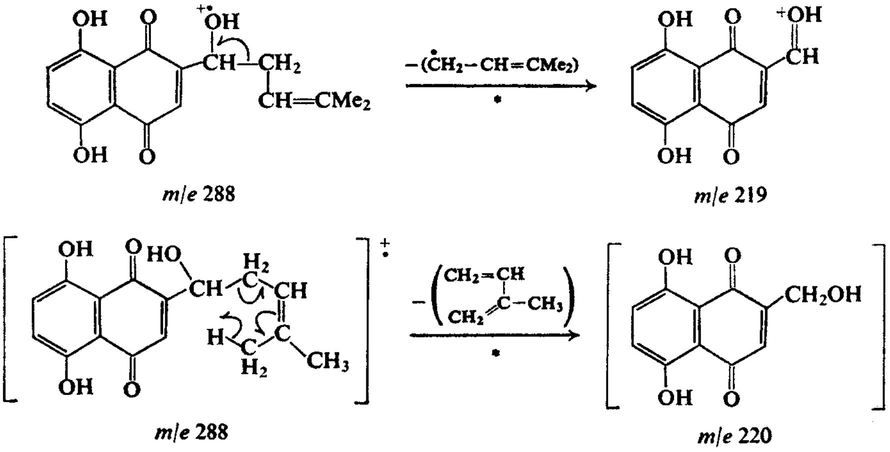
Acetylshikonin	Assignment	3,3-Dimethylacrylylshikonin		
τ (CDCl$_3$)*		This paper τ (CDCl$_3$)*	Morimoto *et al.*[5] τ (CDCl$_3$)*	
−2·5, 1H, s (D)	—OH	−2·50, 1H, s, (D)	—	?
−2·32, 1H, s (D)	—OH	−2·32, 1H, s, (D)	—	?
2·81, 2H, s	Ar-H	2·81, 2H, s	2·80, 2H	ArH
3·00, 1H, s	quinone H	3·00, 1H, s	2·89, 1H	ArH
3·93, 1H, t (J=7)	$\mathrm{Ar—}\overset{\mathrm{O^-}}{\overset{\vert}{\mathit{CH}}}\mathrm{—CH_2—}$	3·93, 1H, t (J=7)	3·95, 1H, t	olefin?
—	—CO—*CH*=CMe$_2$	4·18, 1H, s	4·2, 1H	olefin
4·82, 1H, bt	—CH$_2$—*CH*=CMe$_2$	4·82, 1H, bt	—	—?
7·42, 2H, bt	CH—*CH$_2$*—CH=	7·42, 2H, bt	7·4, 1H, t	vinyl?
—	—CO—CH=*CMe$_2$*	7·81, 3H, s	7·80, 3H, s	Me
7·85, 3H, s	—COCH$_3$	—	—	—
—	—CO—CH=*CMe$_2$*	8·04, 3H, s	8·03, 3H, s	Me
8·30, 3H, s	CH$_2$—CH=*CMe$_2$*	8·29, 3H, s	8·27, 3H, s	Me
8·40, 3H, s	CH$_2$—CH=*CMe$_2$*	8·39, 3H, s	8·37, 3H, s	Me

* s = singlet, t = triplet, b = broad, (D) = exchangeable with D$_2$O.

The mass spectra of the shikonin derivatives are very diagnostic as the major fragmentation processes are dominated by cleavages at the ester functions (Scheme I), whereas in the case of shikonin itself the major fragmentations arise from the normal fissions at the α-carbon atom to the alcohol function (Scheme II).



SCHEME I.



SCHEME II.

EXPERIMENTAL

All m.ps are uncorrected. Mass spectra were measured on an A.E.I. model MS9 mass spectrometer at 70 eV energy and a source temperature of 200°. R_f values refer to TLC on Kieselgel G (Merck) in benzene:ethyl acetate:acetic acid (85:10:5 v/v).

Extraction and Purification of the Pigments

Finely milled trunk and branch sections of *Jatropha glandulifera* (75·0 g) were extracted with hot pentane in a soxhlet until the fresh extracts were no longer pigmented (*ca.* 4 hr). The solvent was removed to yield a viscous red oil (9·0 mg), which was subjected to gradient elution on a column of acid-washed silica gel (200–300 mesh). Early fractions (petroleum ether 60–80°), contained crude 3,3-dimethylacrylylshikonin (I) which was purified by further chromatography and repeated crystallization from pentane to yield analytically pure red prisms (510 mg), m.p. 114–115° (lit. m.p.[5] 113°); R_f 0·72 (red spot); (Found: C, 68·4; H, 5·9; M, by mass spectrometry, 370·1426±0·0015. Calc. for $C_{21}H_{22}O_6$: C, 68·1; H, 6·0 per cent; M, 370·1415); $[\alpha]_D^{20}$ +105° (c = 1·18 in $CHCl_3$);* ν_{max} (nujol) included 1710 (ester), 1635, 1617 (CO) cm^{-1}; λ_{max} (EtOH) 278, 335, 458f, 488, 521 and 562 nm. (Log ε 3·84, 2·91, 3·61, 3·18, 3·86 and 3·64); λ_{max} (EtOH/OH$^-$) 310, 586 and 624 nm. (Log ε 3·76, 4·08 and 4·16.) The major peaks in the mass spectrum were as follows:

m/e	370	272	271	270	255	229	227	204	187	145	100	83	69	60	57	55	41	39
A%	<1	15	13	68	38	13	9	9	19	8	27	100	21	10	8	16	21	15

Later fractions from the chromatograph of the natural extract (petroleum ether/benzene, 90:10) contained crude acetylshikonin (II) which was purified by further chromatography and repeated crystallization from pentane to furnish analytically pure red prisms (49·9 mg), m.p. 85–86° (lit.[1] m.p. 85–86°); R_f 0·67 (red spot); (Found: C, 65·8; H, 5·8; M, by mass spectrometry, 330·1107±0·0015. Calc. for $C_{18}H_{18}O_6$: C, 65·4; H, 5·5 per cent; M, 330·1103); $[\alpha]_D^{20}$ +26° (C = 1·75 in $CHCl_3$);* ν_{max} (nujol) included 1740 (ester), and 1618 (CO) cm^{-1}; λ_{max} (EtOH) 278, 335, 458f, 488, 521 and 562 nm. (Log ε 3·82, 2·79, 3·61, 3·82, 3·86 and 3·64); λ_{max} (EtOH/OH$^-$) 310, 586 and 624 nm. (Log ε 3·76, 4·07 and 4·16.) The major peaks observed in the mass spectrum were as follows:

m/e	330	288	272	271	270	256	255	254	229	228	227	220	219	204	108	91	69	60	45	43	41	39
A%	2	1	16	17	100	10	60	9	16	10	11	23	39	10	11	8	42	19	21	76	27	10

No free shikonin was present in the natural extracts.

Alkaline Hydrolysis of the Pigments

The major pigment (172 mg) was shaken with 100 ml 2 N NaOH at 20° for 15 hr, after which time the blue solution was filtered, cooled in ice and acidified with dil. H_2SO_4. The red suspension was extracted with ether (2 × 100 ml), which was then extracted with sat. $NaHCO_3$ (200 ml), from which the acidic hydrolysis

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* Some difficulty was experienced in measuring the optical rotations due to low transmission through the highly coloured solutions at the NaD wavelength. However, as a higher wavelength source was not available the NaD values are quoted.

product was isolated by acidification and ether extraction. Removal of the ether at 0° followed by sublimation at 40°/15 mm yielded 3,3-dimethylacrylic acid (24·6 mg) as colourless plates, m.p. and mixed m.p. with a commercial sample 69–70°. The i.r. spectrum of the degradation acid was identical in all respects to that of 3,3-dimethylacrylic acid.

The phenolic hydrolysis product was obtained as a red solid by evaporation of the ether solution remaining after bicarbonate extraction. Repeated crystallization from ether–pentane yielded shikonin as red prisms (74·9 mg). R_f 0·43 (red spot), m.p. 148 (lit.[4] m.p. 147°). (Found: C, 67·1; H, 5·4; M, by mass spectrometry, 288·0996 ± 0·0014. Calc. for $C_{16}H_{16}O_5$: C, 66·7; H, 5·6 per cent; M, 288·0998.) $[\alpha]_D^{20}$ +119° (C = 1·94 in C_6H_6);* ν_{max} (nujol) included, 3240 (OH) and 1610 (CO) cm^{-1}; λ_{max} ($CHCl_3$), 278, 458f, 491, 523 and 564 nm. (Log ϵ 3·81, 3·54, 3·76, 3·81, and 3·59.)

The major peaks observed in the mass spectrum were as follows:

m/e	288	286	272	271	270	255	221	220	219	218	191	190	189	163	70	69	41
A%	4	2	2	2	7	5	13	100	60	7	7	7	4	3	7	50	27

Alkaline hydrolysis of the minor pigment under identical conditions also yielded shikonin.

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