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BEHAVIOUR OF 7-METHYLJUGLONE AND TWO RELATED NAPHTHOQUINONES ON SILICA GEL EXPOSED TO AIR

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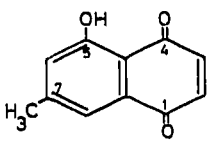
SUMMARY

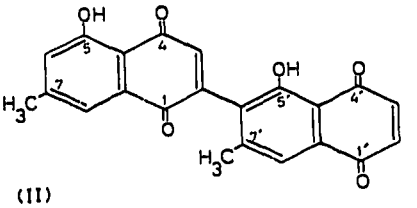
The simple naphthoquinone 7-methyljuglone is a frequently encountered constituent of species of *Ebenaceae* and tends to undergo conversion reactions when adsorbed on silica gel exposed to air. One of the transformation products is methylnaphthazarin, an oxygenated 7-methyljuglone, and four others, *i.e.*, mamegakinone, biramentaceone, rotundiquinone and neodiospyrin, are products of oxidative dimerisation. Under the same experimental conditions, diospyrin and isodiospyrin (the most frequently encountered 7-methyljuglone dimers of the *Ebenaceae*) show no detectable amounts of transformation products.

INTRODUCTION

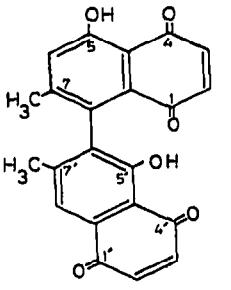
The naphthoquinone 7-methyljuglone (I) and several of its dimers, *e.g.*, diospyrin (II) and isodiospyrin (III), as well as related tri- and tetrameric compounds, have been isolated from *Ebenaceae* (ebony wood) species[1]. A few of these naphthoquinones exhibit instability and undergo transformations during the extraction and purification processes inherent in a phytochemical investigation. The use of methanol as extraction solvent induces the formation of several transformation products from I (ref. 2). For the separation of naphthoquinones in a mixture and the purification of the individual components, column and thin-layer chromatography (TLC) are gener-



(I)



(II)



(III)

ally used. Column chromatography, with aluminium oxide as adsorbent and methanol as eluent, induces transformation reactions with I and III, but with silica gel and benzene these compounds exhibit no changes[3]. Several substances have been reported to show instability when adsorbed on layers and exposed to air, *e.g.*, substances of the pyrazolinone type[4] and 2-aryl-1,3-indanediones[5]. With this in mind, we investigated the behaviour of three frequently encountered *Ebenaceae* naphthoquinones, *i.e.*, monomeric I and its asymmetric dimers II and III, when adsorbed on layers of silica gel.

MATERIALS AND METHODS

Chromatography

The naphthoquinones used were 7-methyljuglone (IA) isolated from *Ebenaceae* species[6] and 7-methyljuglone (IB) synthesised as described by Cooke and Dowd[7], and diospyrin (II) and isodiospyrin (III) isolated from *Ebenaceae* species[6].

Silica gel-coated plates were used for the TLC and for preparative TLC (PTLC); these were Kieselgel 60 F_{254}, DC-Fertigplatten (E. Merck, Darmstadt, G.F.R.), with a layer thickness of 0.25 mm. Solutions of the naphthoquinones in chloroform were applied with a glass micropipette to the layers, and the chromatograms were developed with chloroform or one of the other solvent systems mentioned in the text, in saturated glass tanks. As several naphthoquinones are known to undergo light-induced transformations[8], the experimental procedures were performed at room temperature (*ca.* 18°) under dim light, and the development tanks were wrapped in black covers.

peri-Hydroxynaphthoquinones of the I type have deep orange colours that are easily detected in daylight; on treatment with alkali, the wavelength of max. absorption is shifted from *ca.* 440 nm to *ca.* 550 nm. Even colourless substances can be detected as absorbing spots on a fluorescent background in 254-nm radiation or as fluorescent spots on a dark background in 366-nm radiation. Known naphthoquinones are identified by comparing spectral data and by comparative TLC with authentic reference materials.

Structure elucidation of new compounds

The initial comparative TLC data are often insufficient for identifying the new compound, and elucidation of its structure is based on interpretation of spectral data. Spectrometric analyses are performed on the following instruments. Absorption spectra (UV and visible regions) of solutions in chloroform are recorded on a Perkin-Elmer 402 spectrophotometer; IR absorption spectra of thin dry films of the compounds on a sodium chloride disc are recorded on a Unicam SP1000 spectrophotometer; Fourier-transform NMR (100 MHz) spectra of solutions in deuterated chloroform are recorded on a Varian XL-100 (12″ wg) spectrometer [1000 pulses per sample are required, and chemical-shift values (δ ppm) relative to tetramethylsilane as internal reference are recorded]; and mass spectra are recorded with a Varian CH5 single focusing mass spectrometer.

Preparation of methyl ether derivatives

The more soluble methyl ethers of *peri*-hydroxynaphthoquinones are prepared

by refluxing a solution of the parent compound in chloroform with excess amounts of iodomethane and freshly prepared silver oxide for 2–3 h.

RESULTS

Diospyrin (II) and isodiospyrin (III)

With a time lapse of 30 min between sample application and development of the chromatogram, II shows no detectable transformation products other than a faint spot on the application line. Under similar conditions, III shows no detectable amount of its known symmetrical dimer, bisisodiospyrin[1]. However, the brown spot on the application line is more distinct than that found with II.

TLC of 7-methyljuglone (I)

Under identical circumstances, the natural (IA) and synthetic (IB) naphthoquinones produce identical chromatographic patterns. The number of spots obtained with TLC of pure I is dependent on the time lapse between sample application and development of the chromatogram.

Development of the chromatogram within 1 min after sample application is completed produces the single spot for I and a faint pale-brown colour at the application line.

With a 10-min interval between sample application and development, during which time the plates are kept in complete darkness, the chromatographic pattern contains at least four spots: a large I spot with the highest R_F value, a broad brown zone at the application line and, between these, two small orange spots. With longer periods between sample application and development, the intensities of the two orange

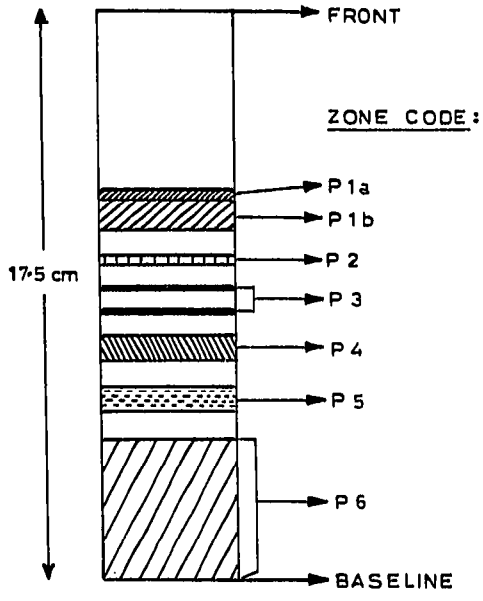


Fig. 1. Chromatographic pattern of transformed 7-methyljuglone.

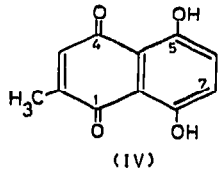
spots increase, as does that of the brown zone: with periods longer than 30 min, the spot at the application line becomes very dark brown and broad, whereas the intensities of the orange spots hardly change.

PTLC of transformed (I)

In order to obtain and identify some of the transformation products of I, PTLC is performed on silica gel plates. The same plates are used as for TLC, and the chloroform solution of I is applied over the whole length of the baseline. A period of 30 min is allowed to elapse for the transformation reaction(s) to take place, and the chromatograms are then developed with chloroform. The chromatographic pattern (see Fig. 1) is more complex than that obtained with TLC, probably because the concentrations of the minor products are too small to show up in TLC.

Directly after development (within 2 min) all zones on each chromatogram are separately scraped from the plates into containers with chloroform. The silica gel is removed by filtration, and, after evaporation of the solvent, six fractions are obtained in a concentration ratio of *ca.* 60:3:2:10:10:15; these fractions are coded as follows (see Fig. 1):

P1 —orange-red, containing residual I;
P2 —green-blue;
P3 —green-blue, a combination of two zones, each at low concentration;
P4 and P5 —orange;
P6 —dark-brown complex mixture.



(IV)

Transformation fraction P1. The TLC pattern of this fraction shows one broad orange zone with the front portion a deeper orange-red. Continuous PTLC of this fraction with chloroform–*n*-hexane (4:6) as solvent gives two zones, P1a (*ca.* 1 mg, red), which was identified by TLC and UV–visible spectrophotometry as methylnaphthazarin (IV), a naphthoquinone isolated from several *Ebenaceae* species, and P1b (orange) residual unchanged I.

Transformation fraction P2. The TLC, NMR and mass-spectrometric (MS) data show this fraction (*ca.* 3 mg) to be a pure substance. Its spectral values are as follows.

UV–visible: $\lambda_{max.}$ 250, 279, 315, 432 and 586 nm

IR: $\nu_{max.}$ 1615 (shoulder), 1605 and 1585 cm^{-1}

Fourier-transform NMR: δ 12.04 s; 11.89 s; 11.60 s; 7.60 br; 7.04 br; 6.89 s; 2.52 s and 2.38 s; on inspection, the relative intensities of the peaks are found to be in the ratio 1:1:1:2:2:1:3:3, respectively.

MS: the more important fragmentation ions are at *m/e* 746 (M^+), 585, 583 and 571.

The molecular formula, $C_{44}H_{26}O_{12}$, agrees with a molecular ion at *m/e* 746; this formula suggests a tetrameric structure. One natural tetramer of I with a molec-

ular weight of 746 is known, *i.e.*, bisisodiospyrin[1]. However, the other spectral data of P2 are not in accordance with the expected characteristics of tetrameric I.

The green-blue colour of this product is the first indication of its dissimilarity to normal I polymers; this is also apparent from the UV–visible spectrum, which has a peak in the longer-wavelength region (at 586 nm) and shows multiple bands in the region 240–320 nm, but the absorption at 432 nm is reminiscent of the benzenoid absorption of *peri*-hydroxynaphthoquinones. The carbonyl absorption in the IR spectrum is also shifted to lower frequencies than are normal for *peri*-hydroxynaphthoquinones. The NMR spectrum is relatively simple, suggesting a symmetrical structure; this is supported by the two singlet signals (δ 2.38 and 2.52) for methyl groups. However, the three *peri*-hydroxy-group signals (δ 12.04, 11.89 and 11.60) of equal intensity do not support this hypothesis if the I structure is regarded as the unit. Thus, the available spectral data are inadequate to permit any structure to be assigned to this transformation product.

Transformation fraction P3. With PTLC and chloroform–*n*-hexane (7:3) as solvent system, the two green-blue coloured components (*ca.* 2 mg) are separated, and they each give UV–visible spectra similar to that of P2.

Transformation fraction P4. The initial NMR data of this fraction (*ca.* 10 mg) suggest it to be a mixture, but it is impossible to separate the constituents by PTLC. With continuous PTLC and chloroform–*n*-hexane (7:3) as solvent, a broad zone is obtained of which the front portion (A) gives a NMR spectrum differing from those of the middle (B) and bottom (C) portions, thereby confirming the composite nature of this fraction. PTLC of the methylation products of P4 yields three zones for naphthoquinone methyl ethers; these zones (designated, in order of decreasing R_f value) P4A, P4B and P4C, are in a concentration ratio of *ca.* 2:1:1, and their spectral data are as follows.

UV-visible —P4A methyl ether: $\lambda_{max.}$ 254 and 412 nm; P4B methyl ether: $\lambda_{max.}$ 253, 272 (shoulder) and 412 nm; and P4C methyl ether: $\lambda_{max.}$ 252, 274 (shoulder) and 412 nm.

Fourier-transform NMR: see Table I.

MS (only the more important fragmentation ions above *m/e* 370 are given) —P4A methyl ether: *m/e* (*I*%): 402 (M^+, 81), 387 (23), 386 (28); 385 (100) and 373 (8);

TABLE I

NMR SPECTRA OF FRACTION P4 CONSTITUENTS

On inspection, the relative intensities of the peaks are found to be in accordance with the numbers of protons assigned here.

Assignment	*P4A*	*P4B*	*P4C*	*Dimethyl ethers of*		
				P4A	*P4B*	*P4C*
2 + 2'H	6.99 s	6.99 s (1H)	—	6.89 s	6.90 s (1H)	—
3 + 3'H	—	7.02 s (1H)	7.02 s	—	6.92 s (1H)	6.94 s
5 + 5'OH	11.71 s	11.71 s (1H)	—	3.96 s	3.97 s (3H)	—
or OMe	—	11.81 s (1H)	11.81 s	—	4.01 s (3H)	4.01 s
6 + 6'H	7.13 br	7.13 br	7.13 br	7.10 br	7.12 br	7.13 br
7 + 7'Me	2.46 s	2.46 s	2.46 s	2.49 s	2.49 s	2.49 s
8 + 8'H	7.50 br	7.50 br	7.50 br	7.57 br	7.57 br	7.59 br

P4B methyl ether; *m/e* (*I*%): 402 (M^+, 100), 387 (32), 386 (9), 385 (27) and 373 (9); P4C methyl ether, *m/e* (*I*%): 402 (M^+, 89), 387 (21), 386 (29), 385 (100) and 373 (11).

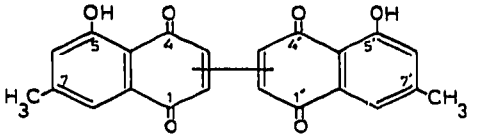
The light absorption and molecular-ion mass (*m/e* 402) show these three substances to be dimethyl ether derivatives of three different dimeric methyljuglones. The NMR spectra (see Table I) support I as structural unit, and the simplicity of the P4A and P4C spectra (parents and methyl ethers), suggests that they are symmetrical dimers.

The NMR spectrum of P4A [the bottom (C) portion of the PTLC parent zone] is identical to that of mamegakinone[1] (V), a known I dimer from *Ebenaceae* species, and the NMR and TLC data of the dimethyl ether is identical to that of mamegakinone dimethyl ether.

The NMR spectrum of P4C [the top (A) portion of the PTLC parent zone] is identical to that of biramentaceone[1] (VI), a known I dimer from *Droseraceae* and *Ebenaceae* species.

The middle (B) portion of the PTLC parent zone gives the NMR signals that might be expected from an equimolar mixture of V and VI. However, the methyl ether mixture of P4 gives three distinct zones, of which the NMR spectrum of the middle zone (P4B dimethyl ether) again resembles that of an equimolar mixture of the two symmetrical isomers. Only structure VII, with a 2,3′-bond, can account for the slight difference in chemical shifts of the two quinonoid protons in parent and dimethyl ether, as well as the slight difference in chemical shifts of the two *peri*-hydroxy-group protons and the two methoxy-groups in parent and dimethyl ether, respectively, while the magnetic equivalence of the protons in the rest of the molecule is retained. This structure also explains the resemblance of its NMR data to those of equimolar concentrations of V and VI.

Compound VII has been isolated from the roots of *Diospyros rotundifolia*, and the name rotundiquinone has been proposed for it[9]. One of the reaction products of I with 7-methylnaphthalene-1,4,5-triol in pyridine–ethanol was claimed by Brockmann and Laatsch[10] to have the same 2,3′-bis(I) structure.



(V): 3,3′–bond = mamegakinone

(VI): 2,2′– bond = biramentaceone

(VII): 2,3′– bond = rotundiquinone

Transformation fraction P5. The spectral data of this orange fraction (*ca.* 10 mg) show it to be a pure substance. The methyl ether was also prepared. The relevant spectral data are as follows.

UV–visible —parent: $\lambda_{max.}$ 254, 293 (shoulder) and 435 nm; methyl ether: $\lambda_{max.}$ 254, 282 (shoulder) and 404 nm.

Fourier-transform NMR: see Table II.

MS (only the more important fragmentation ions above *m/e* 300 are given) —methyl ether: *m/e* (*I*%):402 (M^+, 100), 387 (35) and 373 (38).

TABLE II

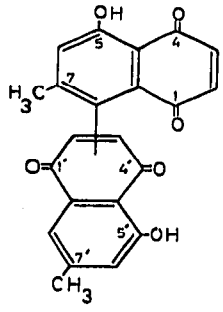
NMR SPECTRA OF FRACTION P5 AND ITS METHYL ETHER

On inspection, the relative intensities of the peaks are found to be in accordance with the numbers of protons assigned here. Coupling constants are in Hz.

Assignment	*P5*	*P5 methyl ether*
2H	6.79 d* (J = 10)	6.67 d* (J = 10)
3H	6.93 d* (J = 10)	6.79 d* (J = 10)
2′ or 3′H	6.62 s	6.53 s
5OH or OMe	11.75 s*	4.04 s*
5′OH or OMe	12.28 s*	3.96 s*
6H	?	7.25 s
6′H	7.12 br	7.13 br
7Me	2.30 s*	2.33 s*
7′Me	2.47 s*	2.51 s*
8′H	7.53 br	7.65 br

* Tentative assignments.

The light absorption of parent and methyl ether, and the molecular-ion mass of the methyl ether at *m/e* 402, support a bis(methyljuglone) structure. The NMR spectrum suggests that I is the structural unit. Signals for three quinonoid protons, of which two are split into an AB quartet (parent: δ 6.79 d + 6.93 d) show the one unit to be linked via its C-8 atom to the C-2 or C-3 atom of the other unit. Such a I dimer has been described, *viz.*, neodiospyrin[9] (VIII). With this structure in mind, a signal for the 6H is lacking in the NMR spectrum of the parent compound (see Table II); it is possible that this signal is obscured by the prominent peak of the residual chloroform at δ = 7.26. In the methyl ether derivative, this 6H signal becomes visible (δ = 7.25) next to the chloroform peak. The UV–visible and NMR data of P5 and its methyl ether are consistent with the proposed structure for VIII, and the data for the methyl ether compare well with published data for this compound.



(VIII)

Transformation fraction P6. This dark-brown fraction is a complex mixture, probably of polymeric condensation products of high molecular weight, and was not further investigated.

CONCLUSIONS

Conversion reactions induced when compounds adsorbed on silica gel are

exposed to air may seriously influence the results of qualitative and quantitative analyses. In consequence of our findings, the exposure of naphthoquinone extracts to air should be limited during TLC or PTLC, especially in the presence of I. In phytochemical experiments where IV, V, VI, VII and VIII are found in the presence of substantial amounts of I, the origin of the first five components is questionable; these components should not necessarily be regarded as natural plant metabolites, as they could also be experimental artefacts.

ACKNOWLEDGEMENTS

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