Tetrahedron Vol. 47, No. 6, pp. 1095-1108, 1991
Printed in Great Britain

0040-4020/91 $3.00+.00
© 1991 Pergamon Press plc

THE QUINCYTE PIGMENTS: FOSSIL QUINONES IN AN EOCENE CLAY MINERAL

William G. Prowse[1], Katherine I. Arnot[1], Josef A. Recka[1], Ronald H. Thomson[2], James R. Maxwell*[1]
1 Organic Geochemistry Unit, Cantock's Close, University of Bristol, Bristol. BS8 1TS, England.
2 Department of Chemistry, University of Aberdeen, Merton Walk, Old Aberdeen. AB9 2UE, Scotland.

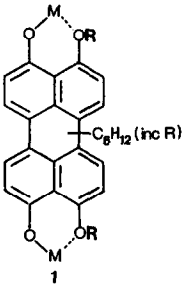
(*Received in UK* 1 *October* 1990)

Abstract - An unusual series of quinone pigments in a pink Eocene (*c.* 45x10^6 yr) sepiolite (quincyte, France) has been characterised by spectroscopy. The structure of the major pigment (*c.* 75% of total), 2,8-di-isopropyl-*peri*-xanthenoxanthene-4,10-quinone, was confirmed by synthesis. Spectroscopy revealed six minor related components with various oxygenated C-2 substituents (C_1-C_3) and, generally, a C-8 isopropyl group. Although the origin of the pigments is unknown, they (or their precursors) appear to have been biosynthesised by the acyl-malonate pathway.

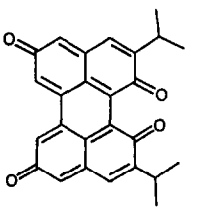
INTRODUCTION

Quincyte is the name given to the pink sepiolite (a fibrous clay mineral of the palygorskite group), which occurs as surface deposits (0.5-1.0 m) near Quincy sur Cher in the basin of Mehun sur Yèvre in Central France[1]. It is found (dispersed in limestone, 0.5-3% by weight)[2] only at the centre of the Berry Limestone formation. This was laid down by a large shallow Eocene lake, which frequently dried out in a sub-tropical climate[2,3,4]. The colour results from an organic pigment(s)[1], which can only be extracted into organic solvents following dissolution of the carbonate (HCl) and sepiolite phases (HF)[2,4]. Column chromatography on alumina of pigment isolates has produced either two[2] or three[4] pink fractions, whilst a further pink fraction could be obtained by treatment of the alumina with dilute HCl. In one study[4] the major chromatographic fraction was investigated by chemical and spectroscopic techniques and its structure tentatively assigned as an unusual perylene based complex (1). The authors proposed that the complexing moiety (M in 1) may have been part of the crystal structure of the sepiolite prior to HF treatment. In a later investigation the major pigment was obtained in sufficient purity for ^{1}H NMR studies, which revealed the presence of two equivalent isopropyl groups and three pairs of protons resonating at higher frequencies (δ = 6.5-8.1 ppm). Based on this and other spectroscopic data, a bisamphiquinone structure (2)[5] was proposed. However, the stability of such a compound, particularly to the extraction procedure, has been questioned and a *peri*-xanthenoxanthenequinone structure (3) was suggested on the basis of the literature data[6].

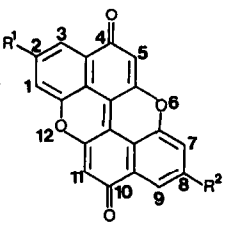
We report here the spectroscopic characterisation of a series of seven pigments from a sample of quincyte. Their structures are all based on the *peri*-xanthenoxanthenequinone skeleton, and the assignment of the major component has been confirmed by synthesis.



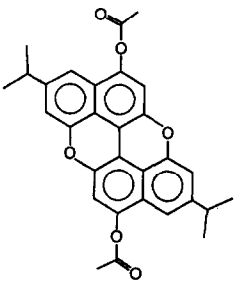
1



2



	R^1	R^2
3	^iPr	^iPr
4	CHO	^iPr
5	CH_3CO	^iPr
6	CH_3CO	CH_3CO
7	CH_3COCH_2	^iPr
8	$CH_3CH_2CH(OH)$	^iPr
9	$CH_3CH(OH)CH_2$	^iPr
10	H	H



11

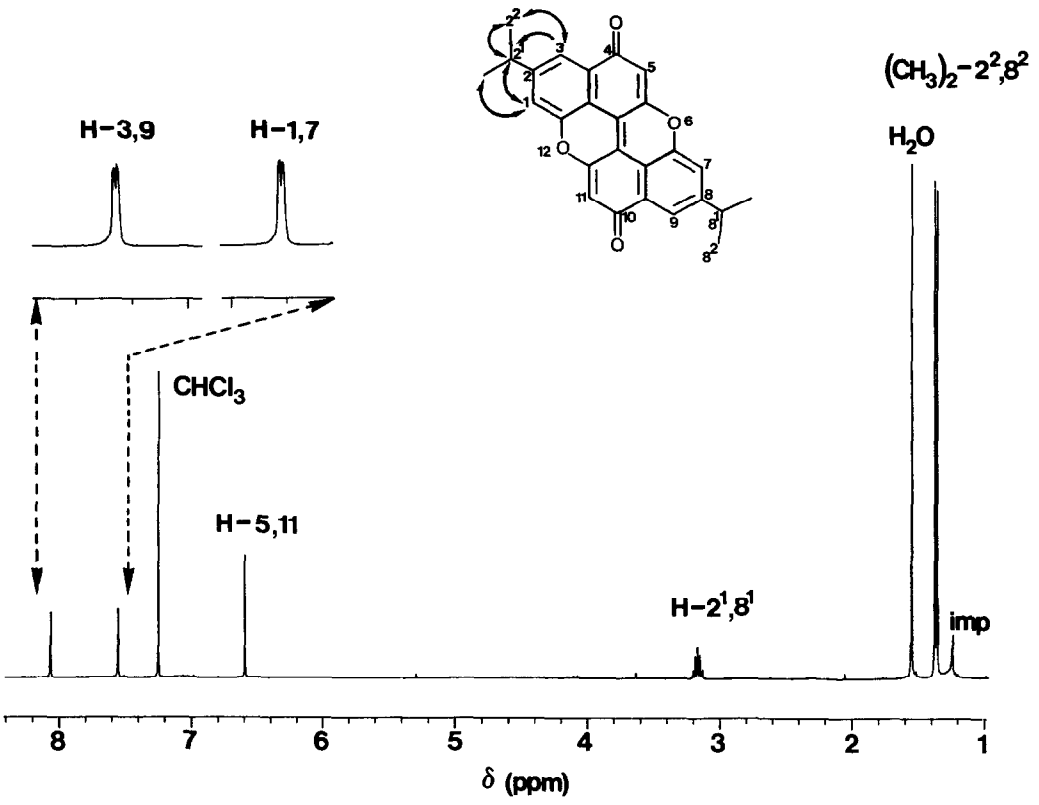
RESULTS

The crude pigment isolate was obtained according to the literature method[2,4]. Components were isolated by repetitive flash chromatography on silica and, where necessary, by preparative reversed phase HPLC. Seven compounds (**3-9**) were obtained in sufficient amount and purity for electronic absorption spectrophotometry, EI-MS and ^1H NMR studies. In addition, the least polar and by far the most abundant component (**3**, *c.* 75% of the total) was investigated by ^{13}C NMR.

a) Analysis of 3

The electronic absorption spectrum was identical to that reported previously[2,4,5] for the major pigment and showed a marked similarity to that of *peri*-xanthenoxanthene-4,10-quinone (**10**)[7]. The EI mass spectrum was dominated by ions at m/z 396 ($M^{+\cdot}$, base peak), 381 and 365. Accurate mass measurements confirmed[4,5] the molecular formula as $C_{26}H_{20}O_4$ (measured 396.1362; $C_{26}H_{20}O_4$ requires 396.1360) and, along with EI-MS-MS parent-daughter experiments, showed that the two major fragment ions were the β-cleavage ion $[M\text{-}^{\cdot}CH_3]^+$ and $[M\text{-}^{\cdot}CH_3\text{-}CH_4]^+$ respectively (parent ions of m/z 365 being both m/z 396 and 381).

The ^1H NMR spectrum (Fig. 1) is similar to that reported previously, its simplicity suggesting a symmetrical structure[5]. Nuclear Overhauser effect (nOe) difference spectroscopy (results summarised by arrows in Fig. 1) revealed that the two equivalent isopropyl groups (3.17 ppm, 2H and 1.37 ppm, 12H) are flanked by aromatic *meta* protons (doublets, 8.06 ppm 2H and 7.56 ppm 2H), whilst irradiation of the two proton singlet (6.60 ppm) gave no detectable enhancements. These results were substantiated by the observation of a fine unresolved coupling of the two isopropyl CH protons to the aromatic *meta* protons, decoupling of the CH signal causing considerable sharpening of the two broad doublets. Clearly, these results are inconsistent with **2**[5], but would be expected for **3**[6]. Given this structure the aromatic proton environments may be differentiated; the protons *peri* to the quinone carbonyl (H-3, H-9) resonate at higher



Proton	δ (ppm)	Multiplicity[a]
H-3,9	8.06	bd (1.1, 7.56; <0.4, 3.17)
H-1,7	7.56	bd (1.1, 8.06; <0.4, 3.17)
H-5,11	6.60	s
$H\text{-}2^1,8^1$	3.17	sept (6.9, 1.37)
$CH_3\text{-}2^2,8^2$	1.37	d (6.9, 3.17)

Fig. 1. 400 MHz ^1H NMR spectrum of 3 (isolated from quincyte) recorded in $CDCl_3$. Arrows indicate nOe enhancements observed, imp = impurity. a = J Hz, δ coupled nuclei.

frequency due to the electric field effect and magnetic anisotropy of the carbonyl. The ^{13}C NMR spectrum (Fig. 2) shows only 12 carbon environments. The presence of a lone quinone carbonyl resonance (183.43 ppm) is again only consistent with structure **3** of those proposed previously. A ^{13}C-^{1}H heteronuclear COSY experiment assigned the five protonated carbon environments leaving six quaternary carbon environments unassigned.

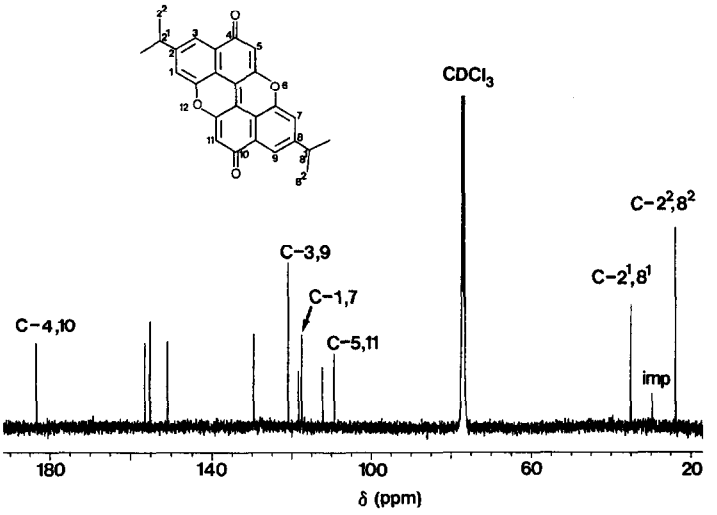


Fig. 2. 100 MHz ^{13}C NMR spectrum of **3** (isolated from quincyte) recorded in $CDCl_3$, imp = impurity.

δ (ppm)	Carbon	δ (ppm)	Carbon
183.43	C-4,10	118.27	
156.38		117.50	C-1,7
155.17		112.22	
150.95		109.30	C-5,11
129.61		35.09	C-2^1,8^1
120.91	C-3,9	23.79	C-2^2,8^2

The presence of two equivalent quinone carbonyls was confirmed by formation of the diacetate (**11**) on reductive acetylation. Spectroscopic analysis of the product was consistent with **11** and the data extend those obtained previously[4]. The quinone was regenerated simply by hydrolysis and oxidation using methanolic K_2CO_3, the failure to achieve this latter transformation in one of the earlier studies[4] being a reason for the structural mis-assignment.

b) Synthesis of **3**

The spectroscopic and chemical data above provide strong evidence for a *peri*-xanthenoxanthene-quinone structure (**3**) for the major pigment. To confirm the assignment **3** was synthesised by the route in Fig. 3, all of the intermediates being characterised by spectroscopy, including full ^{1}H NMR and partial ^{13}C NMR (by ^{13}C-^{1}H COSY) assignments. The scheme was based (*cf.* ref. 8) on oxidative phenolic coupling of a suitably substituted naphthol (**14**). This was produced from 6-bromo-2-naphthol (**12**) by a Grignard reaction[9,10] using the protected naphthol **13** and acetone, followed by de-protection. Oxidative coupling of **14** using the cupric complex of pyridine[11,12] produced the expected C-2,2' dimer (**15**). Further coupling

using Ag_2O in toluene[8] gave the di-isopropyl-*peri*-xanthenoxanthene (**16**) in low yield, along with an equal amount of a related species (**17**). After reversed phase HPLC separation of **16** and **17** the required quinone (**3**) was produced by oxidation of **16** with periodic acid[13,14]. As expected, the quinone was spectroscopically identical in all respects to the major quincyte pigment and co-eluted with it on reversed phase HPLC (5μ Hypersil ODS, 10% H_2O in methanol, 1 ml min^{-1}, R_T = 20 min).

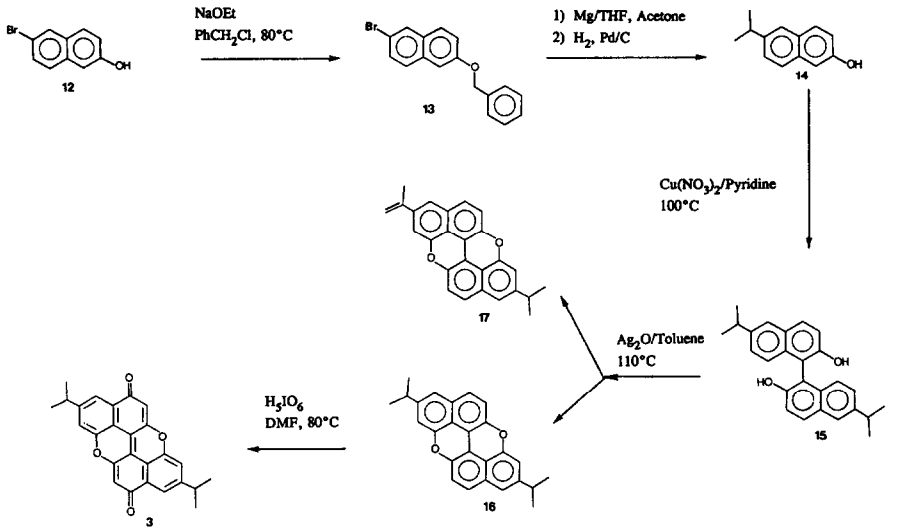


Fig. 3. Synthetic route to **3**

c) Minor quincyte components

Six minor components (**4-9**) were also shown to be derivatives of *peri*-xanthenoxanthene-quinone, with a C-8 isopropyl substituent (apart from **6**) and oxygenated C_1-C_3 substituents at C-2. Electronic absorption spectra of **7-9** were virtually identical to that of **3**. Those of **4-6**, with a conjugated carbonyl, show the expected bathochromic shifts of up to 13 nm in the major visible bands, and an absorption at *c.* 330 nm replacing the two at 302 and 317 nm in the spectrum of **3**. The presence of UV absorbing impurities in some of the compounds resulted in a rising background below *c.* 350 nm, and may lead to an over-estimate of the relative intensity of short wavelength absorptions. EI mass spectra show significant molecular ions (40-100%) and even-electron fragment ions arising predominantly from either β-cleavage processes (*e.g.* loss of $^{\cdot}CH_3$ from isopropyl and methyl ketone substituents; loss of $^{\cdot}C_2H_5$ from alcohol side-chain in **8**), or McLafferty-type rearrangements involving the oxygenated substituents (*e.g.* $[M\text{-}CH_2{=}CHOH]^{+\cdot}$ m/z 368 for **9**, and $[M\text{-}CH_2{=}CO]^{+\cdot}$ m/z 368 for **7**).

^1H NMR spectra were assigned using a combination of decoupling experiments and nOe difference spectroscopy (to distinguish the aromatic protons at C-1,3,7,9). For example, in the spectrum of **5** (Fig. 4) the *meta* protons flanking the methyl ketone (H-3 and H-1) resonate at higher frequencies than those flanking the isopropyl (H-7 and H-9), as a result of the electron withdrawing effect and magnetic anisotropy of the C=O bond in the C-2 substituent. These assignments were confirmed by nOe studies; however, inspection of the aromatic proton resonances (Fig. 4 expansion) allows an immediate distinction to be made between H-7 and H-9, which appear as broad doublets due to the fine coupling to H-8[1], and the sharp doublets of H-1

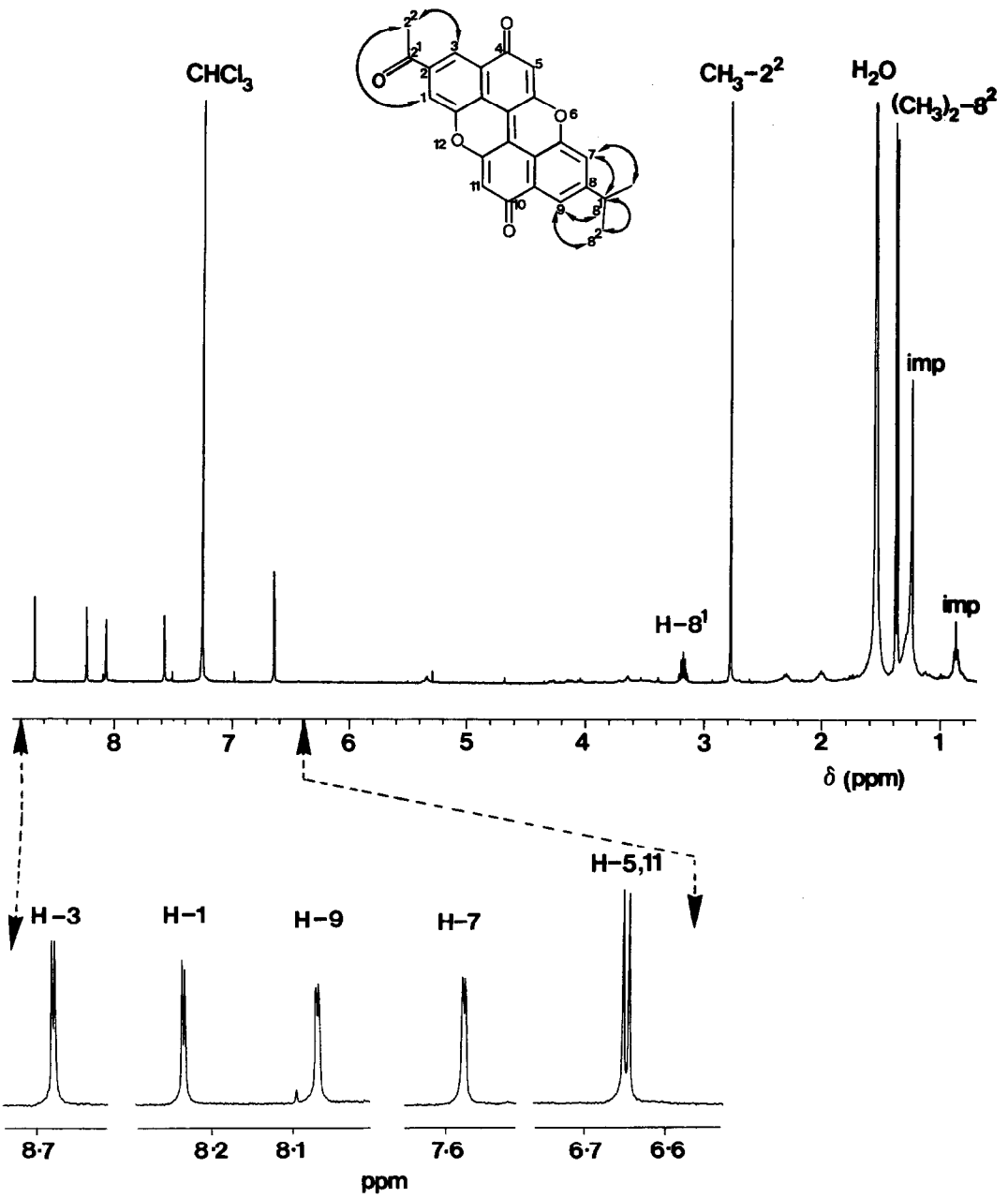
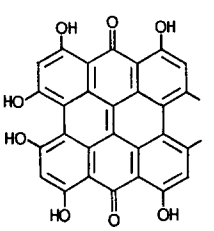


Fig. 4. 400 MHz ^{1}H NMR spectrum of **5** recorded in $CDCl_3$. Expansion shows high frequency proton resonances. Arrows indicate nOe enhancements observed, imp = impurity.

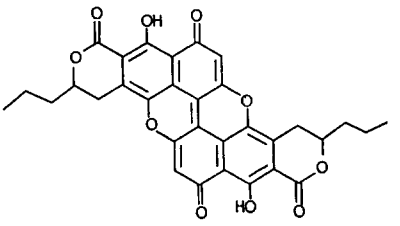
and H-3 (no fine coupling). Although H-5 and H-11 are just resolved as two singlets, absolute assignment of these isolated protons was not possible. Quantitative analysis of nOe data suggests that the molecule prefers the conformation depicted in Fig. 4, with the C=O groups of the quinone and methyl ketone in an "opposed arrangement", presumably minimising electrostatic interactions. Thus, irradiation of CH_3-22 resulted in a 6% enhancement of H-1 compared to a 15% enhancement of H-3. ^{1}H NMR data and assignments for **5** and the other minor pigments are presented in Table 1, nOe enhancements being summarised for **4** and **6-9** by arrows on the structures.

DISCUSSION AND CONCLUSIONS

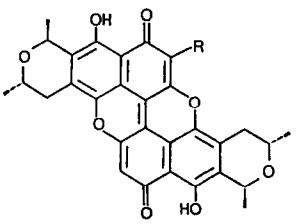
The major pigment (**3**) responsible for the colour of quincyte has been assigned by spectroscopy and total synthesis and is as predicted by one of us previously[6]. A further six minor components were characterised by electronic absorption spectrophotometry, EI-MS and ^{1}H NMR, and all were based on the same *peri*-xanthenoxanthenequinone skeleton with a variety of oxygenated C-2 substituents. The possibility that these represent artifacts of the isolation procedure appears unlikely; for example, the major alcohol (**9**) has the hydroxy β to the aromatic nucleus whereas, in a chemical sense, side-chain oxidation would be expected to occur at the activated α position. It is noteworthy that **8** and **9**, containing C_3 substituents found in natural anthraquinones[6], survived treatment in hydrofluoric acid. Owing to lack of material it was not possible to check this with the isolated compounds, and for the same reason measurement of optical rotations was precluded. The inextractable nature of the pigments prior to dissolution of the sepiolite may be explained by their physical entrapment and/or chemical interaction with the clay matrix. The sepiolite structure contains longitudinal channels (5.6 x 11.0 Å cross section) which enable it to act as a molecular sieve[15], and also has a number of active absorption sites[16].



18



19



20

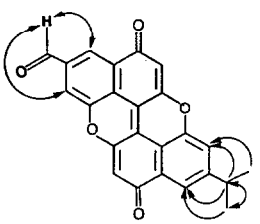
R= H, OMe

The biological origin of the pigments is, however, open to question. Reports of sedimentary pigments, other than the widespread tetrapyrroles derived from chlorophylls[17], are rare. The only other sedimentary polycyclic quinones reported are the fringelites[18] found in a Jurassic fossil crinoid (*Apiocrinus* sp.). One of these pigments (**18**) is identical to hypericin, orginally found in *Hypericum* plants[19], although similar compounds have now been found in living crinoids[20]. In the case of the quincyte pigments no such biological link is apparent, although there are reports of *peri*-xanthenoxanthenequinones in other organisms. The green pigment xylindein (**19**) gives rise to the colour of rotting wood infected with the fungus *Chlorociboria aeruginosa*[8,21,22], and the root bark of *Ventilago calyculata* contains small amounts of two green/blue compounds ventilein A and B (**20**)[23]. Presumably the quincyte quinones arise from an unidentified precursor(s) in an organism whose contemporary counterparts have not been examined for

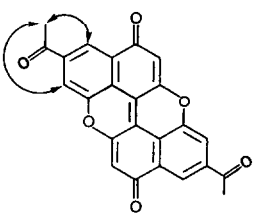
Table 1: ^{1}H NMR data for compounds **4-9** recorded in $CDCl_3$. Chemical shift (ppm), multiplicity, coupling constant (Hz). s = singlet, d = doublet, t = triplet, sept = septet, m = multiplet, b = broad.

Proton	4	5	6	7	8	9
H-3	8.64 d (1.3)	8.68 d (1.3)	8.67 d (1.5)	7.97 bd (1.4)	8.11 bs	8.01 bd (1.4)
H-1	8.13 d (1.3)	8.23 d (1.3)	8.25 d (1.5)	7.52 bd (1.4)	7.76 bs	7.58 bd (1.4)
H-9	8.07 bd (1.4)	8.07 bd (1.3)	8.67 d (1.5)	8.07 bd (1.4)	8.07 bs	8.01 bd (1.4)
H-7	7.58 bd (1.4)	7.58 bd (1.3)	8.25 d (1.5)	7.56 bd (1.4)	7.57 bs	7.54 bd (1.4)
H-5 or 11	6.66 s	6.65 s	6.70 s	6.61 s	6.61 s	6.56 s
H-11 or 5	6.66 s	6.64 s	6.70 s	6.61 s	6.61 s	6.53 s
H-8^1	3.18 sept (7.1)	3.18 sept (7.1)		3.17 sept (7.0)	3.18 sept (7.0)	3.17 sept (7.0)
$(CH_3)_2$-8^2	1.38 d (7.1)	1.37 d (7.1)		1.37 d (7.0)	1.38 d (7.0)	1.38 d (7.0)
H-2^1	10.20 s					
CH_3-2^2		2.77 s	2.78 s			
CH_3-8^2			2.78 s			
CH_2-2^1				3.98 s		
CH_3-2^3				2.27 s	0.99 t (7.3)	1.35 d (6.2)
H-2^1					4.89 m	3.03 m (13.6,4.3)
H-2$^{1'}$						2.95 m (13.6,8.2)
OH					2.11 d (3.5)	not obs.
CH_2-2^2					1.89 m	
H-2^2						4.21 m

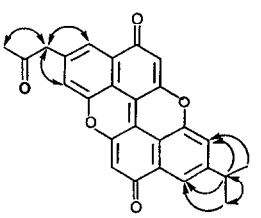
Arrows indicate nOe enhancements observed



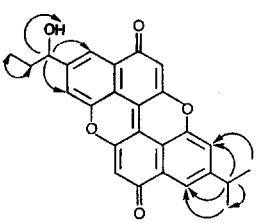
4



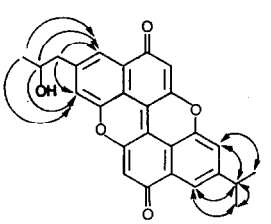
6



7



8



9

pigment content. Given the depositional conditions, and the fact that extended quinones commonly occur in fungi[19], a fungal origin seems plausible.

Most natural quinones, particularly those elaborated by micro-organisms, are thought to arise by the acetate-malonate pathway[19]. The symmetrical structure of one of the minor pigments (**6**) suggests a biosynthesis *via* oxidative phenolic coupling of a substituted naphthol, formed by folding and condensation of a polyketide chain derived from six acetate units. The remaining pigments are closely related to **6** and may arise by way of secondary modifications such as reduction, dehydration, C-methylation, *etc.* On the other hand, the presence of isopropyl substituents in the major pigment **3**, and in **4**, **5**, **7**, **8**, and **9** could suggest a terpenoid origin[19].

EXPERIMENTAL

General

Analytical and preparative HPLC were performed using a Spectra Physics SP8000 ternary solvent delivery system fitted with a Rheodyne 7125 injector. Detection was achieved by a LDC Spectromonitor III variable wavelength detector, monitoring at 520 nm. Conditions and columns employed are given where appropriate. EI mass spectra were acquired on a Finnigan TSQ70 spectrometer using a direct insertion probe whose temperature was raised ballistically to *c.* 350°C. An ionisation voltage of 70 eV and emission current of 200 μA were used, and the spectrometer scanned from m/z 50 to 500 in 3 s. Tandem MS data were obtained with a collision gas pressure of 0.6 mTorr of argon in the second quadrupole region and a collision energy of 30eV. Electronic absorption spectra were obtained using a Perkin-Elmer 555 spectrophotometer with a scan speed of 120 nm min^{-1}, a slit width of 2 nm and cells of 1 cm path length. NMR spectra were recorded on a Jeol GX400 instrument operated at ambient temperature. Proton spectra were obtained using 32K data points, with a pulse width of *c.* 60°, and a typical pulse delay of 0.5 s. The FID was zero filled and processed using a Gaussian window function, giving a digital resolution of 0.2 Hz (coupling constants accurate to $\pm$ 0.4 Hz). Carbon-13 spectra were acquired using 16K data points, with a pulse width of *c.* 30°, and a typical pulse delay of 2.5 s. The FID was zero filled and processed using a Gaussian window function. All spectra were referenced to the chemical shift of the solvent. Heteronuclear shift correlation experiments (^{13}C-^{1}H) were recorded with 2048 data points in the F2 dimension and 256 data points in the F1 dimension. The spectra were zero filled once in the F1 dimension and processed using a Sine-bell window.

Isolation of pigments

The rock (5 kg) was broken into small pieces (< 1 cm^3, hammer) and milled to a fine powder (TEMA mill, < 30 s). Treatment with HCl (10 M, 20 l) yielded a flocculent red-brown precipitate, which was centrifuged (20°C, 8000 rpm, 5 min) and the yellow/green supernatant decanted. The red-brown gelatinous residue was washed with water (4 l) and recovered by centrifugation, rinsed with acetone and dried at ambient temperature. Treatment of this residue (170 g, in two portions) with HF (20%, 500 ml, 48 h) followed by centrifugation (3000 rpm, 5 min) yielded a brown solid (*c.* 20 g), which was washed with water until neutral (4 x 200 ml). Extraction with acetone (4 x 100 ml) by sonication (10 min) and centrifugation (3000 rpm, 5 min) yielded a red supernatant, which was decanted and the solvent removed under vacuum to give a dark red extract (*c.* 0.6 g).

Initial fractionation was achieved by flash chromatography on silica. The extract was adsorbed on to silica (*c.* 6 g), loaded on to the column (5 x 45 cm; prepacked using a 3% acetone/CH_2Cl_2 solvent mixture) and eluted with solvents of increasing polarity; seven fractions

Solvent conditions in initial fractionation of crude quincyte pigments

Elution solvent	Eluant colour	Label
3% Acetone/CH_2Cl_2	Yellow	S1
	Orange	S2
	Orange/pink	S3
10% Acetone/CH_2Cl_2	Orange/pink	S4
Acetone	Orange	S5
Methanol	Brown	S6
	Brown	S7

Fraction S2 (*c.* 10 mg) was relatively insoluble in organic solvents and was purified by repetitive flash chromatography on silica (isocratic elution with 3% acetone/CH_2Cl_2) to give a single component (**3**, 4.5 mg). Fractions S3 and S4 were submitted to preparative reversed phase HPLC (Spherisorb S5-ODS2, 250 x 10 mm: S3, 10% H_20 in methanol; S4, 100% methanol; 3 ml min^{-1}), yielding a total of four components **4-7** (50-250 μg). Fraction S5 was separated into two components by flash chromatography on silica using gradient elution. The compounds were further purified by flash chromatography using suitable isocratic elution conditions (10% acetone/CH_2Cl_2 - **8**, *c.* 100 μg; 20% acetone/CH_2Cl_2 - **9**, *c.* 1 mg).

Spectroscopic data on **3-9**

i) EI-MS; m/z (%)

3: 396 (100), 381 (75), 366 (22), 365 (28), 338 (8), 322 (4). **4**: 382 (94), 381 (16), 367 (100), 353 (8), 338 (10), 324 (10). **5**: 396 (100), 381 (94), 368 (4), 353 (17), 338 (33), 310 (7). **6**: 396 (100), 381 (82), 368 (15), 353 (54), 338 (12), 310 (28). **7**: 410 (46), 368 (100), 353 (32), 352 (34), 339 (6), 310 (7). **8**: 412 (60), 394 (31), 383 (100), 368 (32), 354 (17), 339 (23). **9**: 412 (43), 381 (12), 368 (100), 353 (48), 339 (6), 310 (4).

ii) Electronic absorption spectra recorded in CH_2Cl_2; nm (rel. int.).

3: 520 (100), 482 (70), 450 (29), 302 (22), 317 (24), 257 (92), 227 (65). **4**: 529 (100), 495 (83), 461 (35), 329 (53), 255 (110). **5**: 527 (100), 490 (77), 459 (37), 327 (46), 255 (90). **6**: 532 (100), 492 (77), 458 (40), 330 (45). **7**: 520 (100), 482 (73), 450 (34), 317 (37), 303 (37), 251 (105), 227 (110). **8**: 521 (100), 483 (71), 452 (30), 318 (27), 303 (31), 251 (105), 227 (87). **9**: 520 (100), 482 (71), 452 (30), 317 (26), 303 (28), 250 (102), 225 (109).

Reductive acetylation of **3**

Acetic anhydride (0.3 ml), glacial acetic acid (0.2 ml), sodium acetate (10 mg) and zinc dust (40 mg) were added to **3** (*c.* 1 mg). The mixture was heated at reflux (1 h), diluted with water (10 ml) and the resulting emulsion extracted with CH_2Cl_2 (3 x 10 ml). The organic extract was washed with saturated $NaHCO_3$ solution (3 x 10 ml) and water (3 x 10 ml), the solvent removed under reduced pressure and the product (**11**) purified by flash chromatography, eluting isocratically with CH_2Cl_2.

^1H NMR, δ ppm ($CDCl_3$): 6.83 (bd, 1.2 Hz, H-3,9), 6.78 (s, H-5,11), 6.44 (bd, 1.2 Hz, H-1,7), 2.88 (sept, 6.9 Hz, H-2^1,8^1), 2.42 (s, CH_3COO), 1.25 (d, 6.9 Hz, $(CH_3)_2$-2^2,8^2). MS, m/z (%): 482 (33), 440 (30), 398 (100), 381 (16), 366 (10), 365 (10), 339 (6). Abs. Spec., nm (rel. int.): 446 (100), 418 (75), 395 (33), 329 (46).

Hydrolysis and oxidation of 11

A solution of **11** (*c.* 1 mg) in CH_2Cl_2 (1 ml) was added to methanolic K_2CO_3 (20 mg K_2CO_3 in 5 ml of methanol), sonicated (5 min) and left at room temperature until the reaction was judged to be complete by TLC (*c.* 2-3 h). Solvent was removed under reduced pressure and the product (**3**) dissolved in CH_2Cl_2 (5 ml) and washed with water (3 x 5 ml). Purification of the crude product was achieved by isocratic flash chromatography with 3% acetone in CH_2Cl_2.

2-Benzyloxy-6-bromo-naphthalene **13**

6-Bromo-2-naphthol (**12**, 20 g, 90 mmol) was added to a solution of Na (2.1 g, 90 mmol) in dry ethanol (500 ml). The mixture was brought to reflux, benzyl chloride (11.4 g, 90 mmol) added, and the resulting mixture maintained at reflux for 6 h. The solution was cooled and solvent removed under reduced pressure. The crude product was recrystallised twice from ethanol to give **13** (15.8 g, 51 mmol, 56%).

Mpt. 110.5-111.5°C. ^{1}H NMR, δ ppm ($CDCl_3$): 7.92 (d, 1.5 Hz, H-5), 7.67 (d, 8.9 Hz, H-4), 7.59 (d, 8.6 Hz, H-8), 7.50 (dd, 8.6 Hz, 1.5 Hz, H-7), 7.48-7.32 (m, 5H), 7.25 (dd, 8.9 Hz, 2.5 hz, H-3), 7.18 (d, 2.5 Hz, H-1), 5.17 (s, -O-C$\underline{H}_2$-C_6H_5). ^{13}C NMR, δ ppm ($CDCl_3$): 156.99, 136.58, 132.95, 130.08, 129.60 (C-7 + C-5), 128.62, 128.53 (C-4), 128.40 (C-8), 128.09, 127.53, 120.05 (C-3), 117.15, 107.12 (C-1), 70.05 (-O-CH_2-C_6H_5). MS, m/z (%): 314,312 ($M^{+\cdot}$, 40); 195,193 (10); 114 (20); 91 (100); 65 (30). IR, ν cm^{-1} (CCl_4): 3067, 3034 (C-H); 1629, 1588, 1503, 1454 (C=C). Accurate mass: $C_{17}H_{13}O^{79}Br$ obs. 312.0150 calc. 312.0150.

6-Isopropyl-2-naphthol **14**

All glassware was oven-dried and flushed with N_2. Dry THF (3 ml) was added to Mg turnings (1.4 g, 58 mmol, 1.1 eq.) along with a few crystals of I_2. The mixture was stirred for 10 min, producing an orange colour, and an aliquot (1/10th) of **13** added (total; 15.5 g, 50 mmol, in 150 ml dry THF). The mixture was brought to reflux (decolourising the solution), the remainder of **13** added and reflux maintained for 45 min, resulting in a dull yellow solution. On addition of acetone (3.4 g, 58 mmol, 1.1 eq., HPLC grade) the solution decolourised and was stirred for 30 min. Addition of excess NH_4Cl solution (saturated, 100 ml) resulted in a vigorous effervesence; the cloudy mixture was diluted with water (100 ml) and extracted with CH_2Cl_2 (3 x 50 ml). TLC (20% EtOAc in hexane as developing agent) revealed two spots (R_f *c.* 0.7, *c.* 0.3). The mixture was transferred to toluene (100 ml), perchloric acid (60%, 1 ml) added and the solution refluxed in a Dean-Stark apparatus for 1 h, the solvent then being removed under reduced pressure. TLC revealed only one major fast running diffuse spot which was purified by crude flash chromatography, eluting with 20% EtOAc in hexane. The purified product was taken up in ethyl acetate (50 ml), Pd-C 10% (100 mg) was added and the mixture shaken in a Parr apparatus (H_2, 30 psi) for 5 days. Filtration and removal of solvent under reduced pressure yielded a clear oil. TLC (20% EtOAc in hexane developer) showed two poorly resolved fast running spots (R_f *c.* 0.7) and two poorly resolved slow running spots (R_f *c.* 0.3). The product was fractionated by flash chromatography (eluting with 20% EtOAc/hexane) to give an early eluting clear oil (2 g) and two white crystalline components: 6-bromo-2-naphthol (**12**, 2.2 g, 10 mmol) and the required product 6-isopropyl-2-naphthol (**14**, 3.5 g, 19 mmol, 38%), which was recrystallised twice from hexane.

Mpt. 113.5-114.0°C; literature[24], 111.5-112.5°C. ^{1}H NMR, δ ppm ($CDCl_3$): 7.70 (d, 8.6 Hz, H-4), 7.63 (d, 8.6 Hz, H-8), 7.57 (bs, H-5), 7.36 (dd, 1.5 Hz, 8.6 Hz, H-7), 7.12 (d, 2.5 Hz, H-1), 7.07 (dd, 2.5 Hz, 8.6 Hz, H-3), 4.90 (s, OH), 3.04 (sept, 7.0 Hz, H-6^1), 1.33 (d, 7.0 Hz, $(CH_3)_2$-6^2). ^{13}C NMR, δ ppm ($CDCl_3$): 152.61 (C-2)*, 144.08 (C-6)*, 133.03 (C-8a)*, 129.44 (C-4), 129.09 (C-4a)*, 126.42 (C-7), 126.33 (C-8), 123.95 (C-5), 117.57 (C-3), 109.36 (C-1), 33.95 (C-6^1), 23.92 (C-6^2) * tentative literature comparison[25]. MS, m/z (%): 186 ($M^{+\cdot}$, 70), 171 (100), 153 (15), 144 (10), 143 (10), 128 (15). IR, ν cm^{-1} (CCl_4): 3610 (O-H); 3060 (Ar-H); 2962 (C-H); 1637, 1610, 1510, 1484 (C=C); 1384, 1367 (CH_3 deformation). Accurate mass: $C_{13}H_{14}O$ obs. 186.1045 calc. 186.1044.

6,6'-Di-isopropyl-2,2'-di-hydroxy-1,1'-binaphthyl **15**

To a solution of $[Cu(NO_3)_2]_2.5H_20$ (2.7 g, 14 mmol) in water (100 ml) was added pyridine (4 g, 60 mmol, forming a deep blue solution of the cupric-pyridine complex), and a solution of **14** (1.5 g, 8.1 mmol) in acetone (10 ml). The solution was refluxed (5 min) and allowed to cool. The dark brown reaction mixture was shaken with HCl (2 N, 100 ml, forming a pale yellow solution) and extracted with CH_2Cl_2 (2 x 50 ml). Solvent was removed under reduced pressure and the crude product purified by flash chromatography (eluting with 20% EtOAc in hexane) and recrystallisation from hexane, to give **15** as a white crystalline solid (0.5 g, 1.4 mmol, 35%).

Mpt. 162.5-163.5°C. ^{1}H NMR, δ ppm ($CDCl_3$): 7.93 (d, 8.9 Hz, H-4,4'), 7.70 (bs, H-5,5'), 7.36 (d, 8.9 Hz, H-3,3'), 7.22 (dd, 1.6 Hz, 8.9 Hz, H-7,7'), 7.12 (d, 8.9Hz, H-8,8'), 4.98 (s, OH), 3.03 (sept, 6.7 Hz, H-6^1,6^1'), 2 x 1.31 (d, 6.7 Hz, CH_3-6^2,6^2'). ^{13}C NMR, δ ppm ($CDCl_3$): 152.09 (C-2,2')*, 144.44 (C-6,6')*, 131.80 (C-8a,8a')*, 130.98 (C-4,4'), 129.57 (C-4a,4a')*, 127.32 (C-7,7'), 124.66 (C-5,5'), 124.22 (C-8,8'), 117.57 (C-3,3'), 110.76 (C-1,1')*, 33.86 (C-6^1,6^1'), 23.95 + 23.86 (C-6^2,6^2') * tentative. MS, m/z (%): 370 (M$^{+\cdot}$, 100), 355 (60), 185 (10), 170 (20). IR, ν cm^{-1} (CCl_4): 3530 (O-H); 3060 (Ar-H); 2963 (C-H); 1628, 1600, 1508, 1475 (C=C); 1382, 1367 (CH_3 deformation). Accurate mass: $C_{26}H_{26}O_2$ obs. 370.1933 calc. 370.1931

2,8-Di-isopropyl-*peri*-xanthenoxanthene **16**

Ag_2O (1 g, excess) was added to a solution of **15** (400 mg, 1.08 mmol) in toluene (40 ml) and the mixture refluxed for 15 min. The solution was cooled, filtered and the solvent removed under reduced pressure. TLC (10% EtOAc in hexane developer) revealed a yellow fluorescent spot at the solvent front and a large amount of polar material on the baseline. Flash chromatography eluting with 2% EtOAc in hexane afforded a yellow oil (40 mg, 0.1 mmol, 10%) which was separated into two yellow crystalline compounds by preparative reversed phase HPLC (Spherisorb S5-ODS2, 250 x 10 mm, 40% acetone in methanol, 3 ml min^{-1}). The early eluting component (R_T = 15 min) was the desired product **16** (20 mg, 5%).

Mpt. 179-182°C. ^{1}H NMR, δ ppm ($CDCl_3$): 7.41 (d, 9.0 Hz, H-4,10), 7.08 (bs, H-3,9), 6.99 (d, 9.0 Hz, H-5,11), 6.69 (bs, H-1,7), 2.92 (sept, 7.0 Hz, H-2^1,8^1), 1.27 (d, 7.0 Hz, $(CH_3)_2$-2^2,8^2). ^{13}C NMR, δ ppm ($CDCl_3$): 152.60, 148.41, 143.83, 131.25, 125.88, 120.03, 117.25, 116.50, 111.33, 108.09, 34.54 (C-2^1,8^1), 23.77 (C-2^2,8^2). MS, m/z (%): 366 (M$^{+\cdot}$, 100), 351 (25), 350 (15), 335 (10), 334 (10), 308 (15), 282 (5), 183 (10), 168 (25). IR, ν cm^{-1} (CCl_4): 3170 (Ar-H); 2962 (C-H); 1636, 1603 (C=C). Abs. spec.; λ_{max} nm (CH_2Cl_2): 444, 415, 393, 372, 326, 312, 228 ε mol^{-1} l cm^{-1}: 17020, 13020, 5750, 2970, 7510, 4720, 93260. Accurate mass: $C_{26}H_{22}O_2$ obs. 366.1620 calc. 366.1617.

The later eluting product (R_T = 17.5 min) was identified as 2-(1-methylethenyl)-8-isopropyl-*peri*-xanthenoxanthene (**17**). ^{1}H NMR, δ ppm ($CDCl_3$): 7.49 (d, 8.9 Hz, H-4), 7.43 (d, 9.2 Hz, H-10), 7.33 (d, 1.2 Hz, H-3), 7.10 (bs, H-9), 7.03 (d, 8.9 Hz, H-5), 7.01 (d, 9.2 Hz, H-11), 6.95 (d, 1.2 Hz, H-1), 6.71 (d, 1.2 Hz, H-7), 5.50 (bs, H-2^2), 5.17 (bt, H-2^2), 2.92 (sept, 7.0 Hz, H-8^1), 2.18 (bs, CH_3-2^2), 1.27 (d, 7.0 Hz, $(CH_3)_2$-8^2). MS, m/z (%): 364 (M$^{+\cdot}$, 100), 349 (25), 348 (20), 322 (10), 306 (5), 282 (5), 182 (5), 174 (10). Abs. spec., λ_{max} nm (CH_2Cl_2): 442, 414, 390, 330, 316, 262, 238 (rel. int. 100, 77, 36, 40, 25, 298, 360).

2,8-Di-isopropyl-*peri*-xanthenoxanthene-4,10-quinone **3**

Periodic acid (50%, 0.03 ml, 0.2 mmol) was added to a solution of **16** (18 mg, 0.05 mmol) in DMF (10 ml). The solution was heated at 80°C for 15 min, poured into water (30 ml) and extracted with CH_2Cl_2 (3 x 15 ml). The combined extracts were washed with water (3 x 20 ml) to remove DMF and the solvent removed under reduced pressure. The product was separated from polar impurities by flash chromatography (eluting with 2% acetone in CH_2Cl_2) to give **3** as a red solid (4.2 mg, 0.01 mmol, 20%)

Mpt. > 300°C. ^{1}H NMR, δ ppm ($CDCl_3$): 8.07 (bd, 1.2 Hz, H-3,9), 7.56 (bd, 1.2 Hz, H-1,7), 6.60 (s, H-5,11), 3.17 (sept, 7.0 Hz, H-21,81), 1.37 (d, 7.0 Hz, $(CH_3)_2$-22,82). ^{13}C NMR, δ ppm ($CDCl_3$): 183.40 (C-4,10), 156.39, 155.19, 150.99, 129.67, 120.91 (C-3,9), 118.29, 117.47 (C-1,7), 112.26, 109.32 (C-5,11), 35.11 (C-21,81), 23.78 (C-22,82). MS, m/z (%): 396 ($M^{+\cdot}$, 100), 381 (65), 366 (15), 365 (25), 339 (6), 338 (7), 322 (4), 310 (2), 198 (3), 183 (7). IR, ν cm^{-1} (CCl_4): 2966, 2929 (C-H); 1645, 1609, 1587, 1566; 1325, 1313 (CH_3 deformation); 1185; 855. Abs. spec.; λ_{max} nm (CH_2Cl_2): 519, 482, 450, 316, 300, 249, 225 ε mol^{-1} l cm^{-1}: 42200, 29200, 12400, 9900, 10000, 38300, 33400. Accurate mass: $C_{26}H_{20}O_4$ obs. 396.1362 calc. 396.1360.

ACKNOWLEDGEMENTS

We thank the SERC for a studentship (W.G.P.), the NERC for MS facilities (GR3/2951 & GR3/3758) and Dr C-J Guillemin for a sample of the quincyte containing limestone. We also thank Mr J.F. Carter for skilled mass spectrometric technical assistance.

REFERENCES

1. Berthier, M.P. *Annales des Mines* **1825,** *10*, 272-273.
2. Louis, M.; Guillemin, C.J.; Goni, J.C.; Ragot, J.P. In *"Advances in Organic Geochemistry 1968"*; Schenck, P.A.; Havenaar, I., Eds; Pergamon: London, 1969, pp. 553-566.
3. Jodot, P. *C. R. Sommaire Séances Soc. Géol. France* **1947,** 327-329.
4. Watts, C.D.; Simoneit, B.R.; Maxwell, J.R.; Ragot, J.P. In *"Advances in Organic Geochemistry 1975"*; Campos, R.; Goñi, J., Eds; ENADIMSA: Madrid, 1975, pp. 223-235.
5. Treibs, A.; Wilhelm, R.; Jacob, K. *Liebigs Ann. Chem.* **1981,** 842-848.
6. Thomson R.H. *"Naturally occurring quinones III. Recent advances"*; Chapman and Hall: London and New York, 1987.
7. Blackburn, G.M.; Cameron, D.W; Chan, H.W.-S. *J. Chem. Soc. (C)* **1966,** 1836-1842.
8. Blackburn, G.M.; Ekong, D.E.U.; Neilson, A.H.; Lord Todd. *Chimia* **1965,** *19*, 208-212.
9. Gilman, H.; St John, N.B.; Schulze, F. *Org. Syn. Coll. Vol.* **1943,** *2*, 425-427.
10. Whitmore, F.C.; Loder, D.J. *Org. Syn. Coll. Vol.* **1943,** *2*, 282-283.
11. Feringa, B.; Wynberg, H. *Tet. Lett.* **1977,** *18*, 4447-4450; and references therein.
12. Hovorka, M.; Günterová, J.; Závada, J. *Tet. Lett.* **1990,** *31*, 413-416.
13. Fatiadi, A.J. *Synthesis* **1974,** 229-272.
14. Fatiadi, A.J. *Chem. Commun.* **1967,** 1087-1088.
15. Robertson, R.H.S. *Chem. and Ind.* **1957,** 1492-1495.
16. Alvarez A. In *"Palygorskite-Sepiolite. Occurrences, Genesis and Uses"*; Singer, A.; Galan, E., Eds; Elsevier: Amsterdam, 1984, pp. 253-287.
17. Reviewed by: Chicarelli, M.I.; Kaur, S.; Maxwell, J.R. *Metal Complexes in Fossil Fuels. American Chemical Society Symposium* **1987,** *344*, 40-67.
18. Blumer, M. *Geochim. Cosmochim. Acta.* **1962,** *26*, 225-227.
19. Thomson, R.H. *"Naturally occurring quinones"*; Academic press: London and New York, 1971.
20. Rideout, J.A.; Smith, N.B.; Sutherland, M.D. *Experienta* **1979,** *35*, 1273-1274.
21. Blackburn, G.M.; Neilson, A.H.; Lord Todd. *Proc. Chem. Soc.* **1962,** 327-328.
22. Edwards, R.L. Kale, N. *Tetrahedron* **1965,** *21*, 2095-2107.

23. Hanumaiah, T.; Marshall, D.S.; Rao, B.K.; Rao, J.U.M.; Rao, K.V.J.; Thomson, R.H. *Phytochem.* **1985,** *24*, 2669-2672.
24. Stork, G.; Schulenberg, J.W. *J. Am. Chem. Soc.* **1962,** *84*, 284-292.
25. Hansen, P.E. *Org. Magn. Reson.* **1979,** *12*, 109-142.