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STUDIES ON FUNGAL METABOLITES—XXXII[1]

A RENEWED INVESTIGATION ON (−) FLAVOSKYRIN AND ITS ANALOGUES

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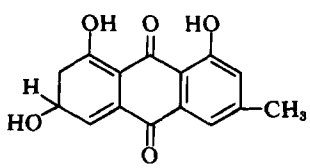
Abstract—1-Oxo-1,2,3,4-tetrahydroanthraquinone (**4a**), and its 8-hydroxy-(**4b**) and 8-hydroxy-6-methyl (**4c**) derivatives were dimerized to the compounds formulated as (**6a**), (**6b**) and (**6c**), respectively. The structure of **6a** was confirmed by X-ray crystallographic analysis.

By the analogy with these dimers and NMR spectral analysis, a revised structure (**7**) was proposed for (−) flavoskyrin, a yellow metabolite of *Penicillium islandicum* NRRL 1175. A biosynthetic scheme involving Diels-Alder type cyclo-addition ($\pi 4s + \pi 2s$) was proposed for (−) flavoskyrin.

(−) Flavoskyrin, m.p. 215°, $[\alpha]_D$-295° (dioxan), a yellow colouring matter of *Penicillium islandicum* Sopp NRRL 1175, was first isolated by Howard and Raistrick,[2] who represented it with a molecular formula, $C_{15}H_{12}O_5$, and found that it gave chrysophanol on dehydration. Our earlier investigation[3] showed that (−) flavoskyrin is not a quinonic compound, since it gave no positive colouration with magnesium acetate, but it is related closely with anthraquinones as it afforded chrysophanol and emodin by air oxidation in the presence of magnesium acetate.

The IR spectrum of (−) flavoskyrin showed a C=O absorption at 1715 cm^{-1} and a chelated C=O absorption at 1623 cm^{-1}. Thus structural formula (1) was proposed for (−) flavoskyrin, which would correspond to a monomeric half of the structural formula formerly proposed for rugulosin ((**15**) in the preceding paper[1]).



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Meanwhile, the former structural formulae of (−)flavoskyrin, (+)rugulosin, (−)luteoskyrin and (−)rubroskyrin were regarded to be supported by a series of model compounds originally synthesized by Zahn and Koch.[4]

On catalytic hydrogenation of 1-hydroxyanthraquinone followed by oxidation with $Pb(OAc)_4$ yielded 1-oxo-1,2,3,4-tetrahydroanthraquinone (= 3,4-dyhydro-1,9,10 (2H) anthracenetrione) (**4a**).

The quinonic compound (**4a**), in solution at room temperature, was converted into an isomeric non-quinonic compound for which Zahn and Koch proposed an enolic form (**5a**). The conversion is promoted by the addition of a drop of pyridine to the solution. This conversion of **4a** into **5a** was regarded as a model reaction of the transformation of quinonic (−)rubroskyrin into nonquinonic (−) luteoskyrin as formulated earlier (**17** → **16** in the preceding paper[1]).

The IR spectral data of **4a** and **5a** as well as some analogous synthetic compounds (**4b** and **5b**)[3b] seemed to rationalize the proposed structures. However, the recent revision of the structural formula of (+)rugulosin[1] made the structural formula (**1**) of (−)flavoskyrin unplausible, and re-investigation using an analogous compound, **4c**, newly prepared in addition to the previously described compounds (**4a** and **4b**) has made clear that the former concept should be revised.

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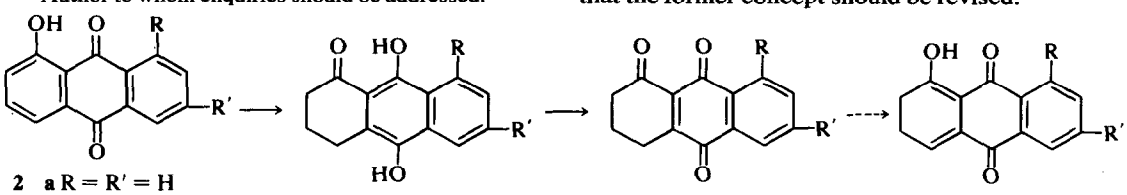


2 a R = R′ = H
b R = OH; R′ = H
c R = OH; R′ = CH_3

3a,b,c **4a,b,c** **5a,b**

Table 1. Mass and IR Spectra of the Non-quinonic Dimerization Products

Compounds	M+2	M⁺	m/e M−2	½M+2	½M	½M−2	ν_{max}^{IBr} C=O (cm⁻¹)
6a	454	452	450	228	226	224	1717, 1626
6b	486	484	482	244	242	240	1715, 1615
6c	514	512	510	258	256	254	1715, 1618

Table 2. NMR Spectra of the compounds, **6c** and **3c**. (δ)

	6c (in $CDCl_3$)	**3c** (in $(CD_3)_2CO$)
CH_3	2·02, 2·14	2·42
$-CH_2-$	2·0 ~ 3·0 (m)	2·6 ~ 3·1 (m)
Arom. H	6·25, 6·40 6·61, 6·98	6·69, 7·47
Phenolic and enolic OH	9·65, 11·35, 14·49, 15·81.	7·48, 9·81, 16·09

The mass spectral analysis has shown that the conversion of the quinonic compounds (**4a,b,c**) into non-quinonic compounds is not a simple enolization but a dimerization.

The UV spectrum of **6c** showed absorption maxima at 270, 306, 315, 330, 372, 417 and 434 nm, almost all of which except the absorption at 372 nm were also given by the hydroquinone type compound (**3c**) to reveal that the dimer (**6c**) involves the structure corresponding to **3c** as a monomeric moiety.

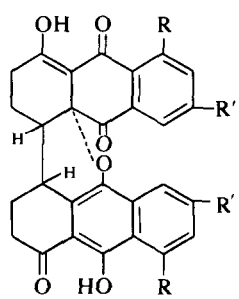
The NMR spectrum of **6c** gave the signals of 2 CH_3, 4 aromatic protons and 4 enolic or phenolic OH to reveal a dimeric structure consisting of unequivalent monomeric moieties. Comparing the NMR spectra of **6c** and **3c**, the OH signal at δ 7·48 of the latter was not observed in that of the former. This indicated that $C_{(9)}$-OH of **3c** must participate in the dimerization.

On the basis of these findings in reading the highest mass peak as the molecular ion (M^+), the dimeric compounds would be formulated with one of the structures (a) ~ (c).[5]

Of these formulae, c was ruled out by the absence of ketal OH signal in the NMR spectrum. The choice of a or b had no essential reason, but b seemed preferable, by the lack of evidence in the UV spectra for the presence of a rugulosin-like chromophore.

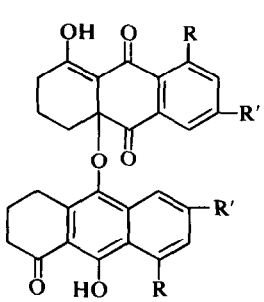
However, this concept has become doubtful, since the ESR measurements failed to prove the existence of radical states of **4a, b, c**, which must participate in the phenol oxidative coupling which would be involved in the formation of that type of dimers (a ~ c).

Conclusive evidence for the alternative formulations (**6a, b, c**) for the dimers has been provided by the X-ray crystallography of **6a***, which revealed the presence of a C—C linkage besides an oxygen bridge combining the two monomeric moieties. Accordingly, the highest mass peaks of the dimeric compounds (**6a, b, c**) must represent M+2. The appearance of such strong M+2 peak was observed in some benzoquinone derivatives.[6,7]



		Mol. wt.
6a R = R′ = H	$C_{28}H_{20}O_6$	452
6b R = OH, R′ = H	$C_{28}H_{20}O_8$	484
6c R = OH, R′ = CH_3	$C_{30}H_{24}O_8$	512

*The full detail and experiments of the X-ray crystallographic studies of the dimerized product of 1-oxo-1,2,3,4-tetrahydroanthraquinone are described in a separate paper (U. Sankawa and Y. Iitaka).



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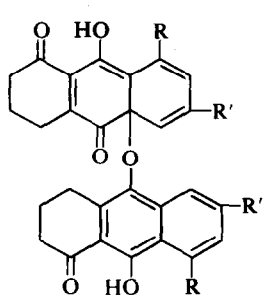

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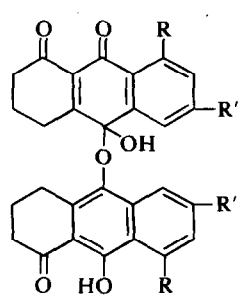

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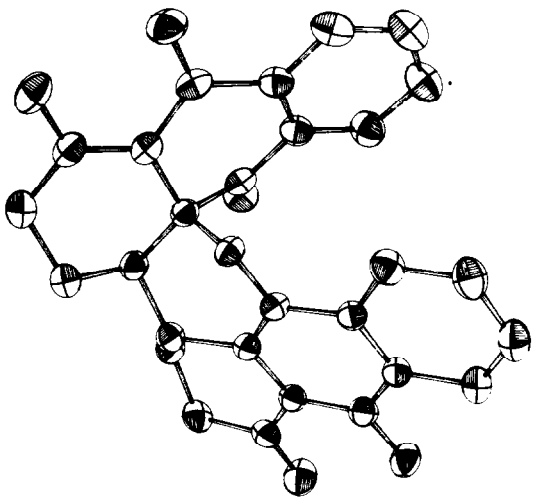


Fig 1. A perspective drawing of the structure (**6a**) of the dimerization product of 1-oxo-1,2,3,4-tetrahydroanthraquinone (**4a**) drawn by the plotter programme ORTEP.

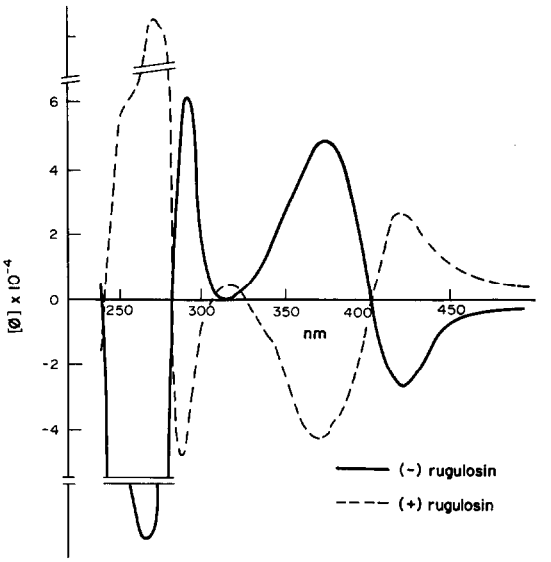


Fig 2. The ORD curves of (+) rugulosin isolated from *P. brunneum* and (−) rugulosin derived from (−) flavoskyrin.

The extremely higher shift of the signals of the bezenoid ring proton can be explained by the proximity (3·03 ~ 4·37 Å) of the aromatic H atoms and the centre of the benzenoid ring to the other moiety.

The higher frequencies of IR absorption of C=O (1715 1717 cm^{-1}) are also rationalized by the twisted state of the C=O at $C_{(9)}$, which was actually demonstrated by the X-ray analysis (twisted angle from the plane of benzene ring system: 30°34′).

Meanwhile, (−)flavoskyrin was treated with $SOCl_2$ and pyridine to form (+)dianhydrorugulosin and chrysophanol. The former showed an ORD curve which is the reverse of (−)dianhydrorugulosin derived from (+)rugulosin. Moreover, it has been found that (−)flavoskyrin on treatment with pyridine affords (−)rugulosin and dianhydrorugulosin.

Thus (−)flavoskyrin must be a dimer analogous to the synthetic model compounds (**6a, b, c**). The mass spectrum of (−)flavoskyrin gave M—H_2O peak at *m/e* 526 as the highest peak to indicate the molecular formula, $C_{30}H_{24}O_{10}$.

The UV spectral curve of (−)flavoskyrin is almost superimposable with that given by a model compound (**6c**) and suggests the presence of the same chromophore.

The NMR spectrum of (−)flavoskyrin showed an unequivalent dimeric nature indicating the presence of 2 methyls, 4 aromatic ring protons, 4 enolic or phenolic hydroxyls. The multiplet signal of —CH_2-coupled with multiplet of the proton attached to the carbon bearing a hydroxyl indicated that the C—C linkage connecting two monomeric moieties is present between the 1, and 1′ positions.

Table 3. NMR Spectrum of (−)Flavoskyrin (in d_8-Dioxan) (δ)

CH_3	1·96, 2·10
—CH_2—CH—	2·5 ~ 3·0 (m)
H	
—CH_2—C	4·06 (m), 4·30 (m)
OH	4·60 (d), 4·90 (d)
Arom. H	6·13, 6·31, 6·55, 6·96
Phenolic and enolic OH	9·50, 11·21, 14·13, 15·76

In comparison of the NMR, UV and IR spectral data, as well as the X-ray crystallographic result of a model compound (**6a**), (−)flavoskyrin could be represented most favourably by the following structure (**7**).

The dimerization of 1-oxo-1,2,3,4-tetrahydroanthraquinone derivatives (**6a, b, c**) has now been elucidated by a Diels-Alder type cycloaddition ($\pi 4s + \pi 2s$) with exo approach of the monomers.

Eventually, an enolized form of tetrahydroemodin (**1**) which was once proposed for the structure of flavoskyrin has now been considered as a monomeric precursor which could be dimerized by the Diels-Alder condensation to form (−) flavoskyrin (**7**).

It is noted that in the biosynthesis dimerization could also be performed by a Diels-Alder reaction other than phenol oxidative coupling.

A few examples of such a type of reaction have been suggested.[8,9]

The biosynthetic experiments of (−)flavoskyrin

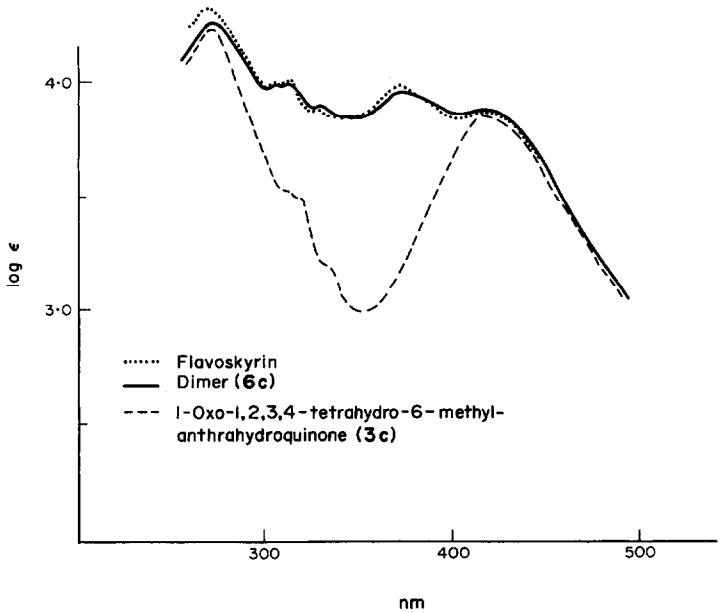
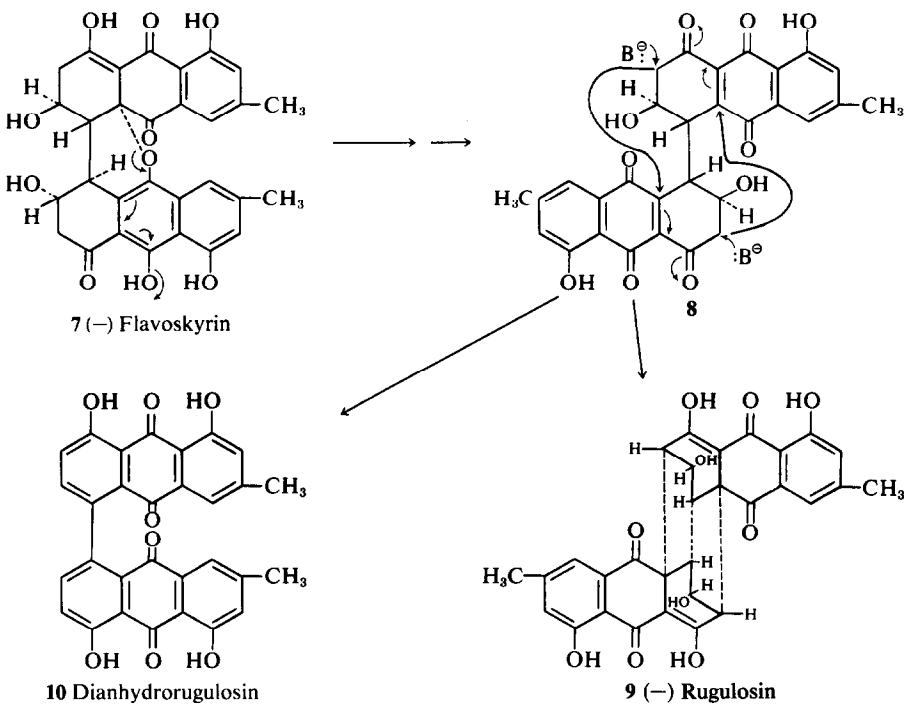


Fig 3. The UV spectra of (−) flavoskyrin and the dimeric compound (**6c**) in comparison with 1-oxo-1,2,3,4-tetrahydroanthrahydroquinone (**3c**) (in dioxan).

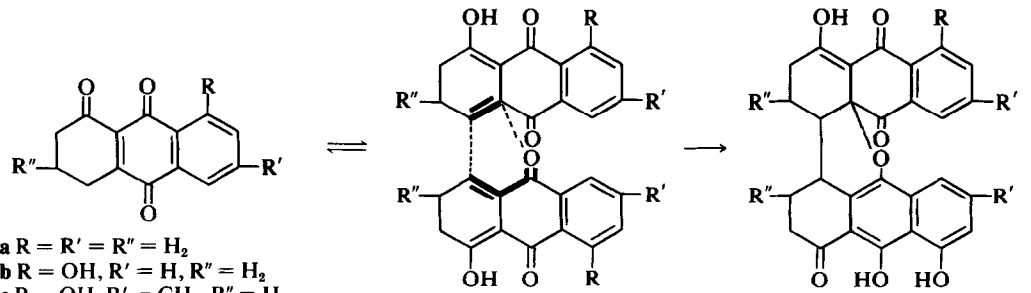


and other fungal anthraquinoid colouring matters are now in progress.

EXPERIMENTAL

The physical constants of the new compounds described in the present paper were measured as mentioned in the preceding paper of this series.[1]

Isolation of pigments of Penicillium islandicum Sopp NRRL 1175. *Penicillium islandicum* was cultivated stationarily on Czapek-Dox media for 16 days at 29°. The dried mycelia (120 g) were pulverized and extracted first with n-hexane and then with acetone. Yellow brown ppts separated on concentration and were recrystallized from dioxane to obtain (−)flavoskyrin. The filtrate was chro-



4a R = R′ = R″ = H_2
4b R = OH, R′ = H, R″ = H_2
4c R = OH, R′ = CH_3, R″ = H_2
4d R = OH, R′ = CH_3, R″: —H / OH

6a R = R′ = R″ = H
6b R = OH, R′ = H, R″ = H_2
6c R = OH, R′ = CH_3, R″ = H_2
7 R = OH, R′ = CH_3, R″: —H / OH

CHART 1

matographed over silica gel impregnated with 0·5 N oxalic acid eluting with benzene and benzene-acetone (9:1) subsequently to separate chrysophanol and (+) dianhydrorugulosin first and then emodin and (+)auroskyrin, which were identified by TLC and IR spectra. Eluting with benzene-acetone (7:1), (+)skyrin, (−) rugulosin, (−)flavoskyrin, erythroskyrine were separated successively. The ppts formed on concentration of the eluate were recrystallized from dioxan or EtOAc to obtain (−)flavoskyrin, and from acetone ω-hydroxyemodin.

(−)Flavoskyrin, yellow crystals, m.p. 215° (dec) (from dioxan or EtOAc), $[\alpha]_D$-295° (dioxan) (Found: C, 66·22; H, 4·79. $C_{30}H_{24}O_{10}$ requires; C, 66·17; H, 4·44%); UV λ_{max}^{dioxan} nm (log ε): 267 (4·64), 303 (4·04), 312 (4·04), 328 (3·92), 368 (4·01), 414 (3·89), 433 (sh) (3·85); IR ν_{max}^{KBr} cm^{-1}: 1715, 1623, 1590; ORD (in dioxan) nm (φ): 262 ($3·18 \times 10^5$), 276 (0), 285 ($-1·34 \times 10^5$), 340 ($-2·58 \times 10^4$), 355 ($-3·58 \times 10^4$), 380 (0), 390 ($+1·45 \times 10^4$), 415 (0), 499 ($-1·45 \times 10^4$).

(−)*Rugulosin.* A yellow pigment was isolated from the acetonic extracts of the mycelia by an active carbon column chromatography eluting with acetone. The crude pigment contaminated with oily substances was chromatographed again on a column of silica gel impregnated with 0·5 N oxalic acid. The ppts obtained from the concentrated eluate was recrystallized from acetone and yielded yellow crystals, $C_{30}H_{22}O_{10}$, m.p. 290° (dec), $[\alpha]_D$-490°, which were assigned to (−)rugulosin, since the yellow pigment revealed the same R_f value on TLC superimposable IR spectral absorptions with those of (+)rugulosin, while its ORD curve was entirely the reverse of (+)rugulosin.

(−)Rugulosin was reported to be isolated from the culture of *Myrothecium vercaria* (Alb. et Schw.) Ditmer ex. Fr.).

Transformation of (−)flavoskyrin into (−)rugulosin. A soln of (−)flavoskyrin (30 mg) dissolved in pyridine (4 ml) was allowed to stand at room temp. After 19 hr, the mixture was poured into water and extracted with ether. The ethereal layer was washed with 10% HCl to remove pyridine, and then washed with water and dried. The residue obtained on evaporation of the solvent was separated by preparative TLC on a plate of silica gel impregnated with 0·5 N oxalic acid using benzene-acetone (4:1) as the developing solvent to obtain 4 separated bands. An orange red pigment isolated from the top band was identified with dianhydrorugulosin by IR (KBr) spectra, yield: 7 mg (23%); a yellow pigment obtained from the second band showing a yellow fluorescence under UV illumination was assigned to (−)rugulosin by the comparison of IR spectra with (+)rugulosin and the measurements of ORD, yield: 7 mg (23%). The other two yellow bands which gave no fluorescence and no colouration with $Mg(OAc)_2$ were not identified.

Transformation of (−)flavoskyrin into (+)dianhydrorugulosin. To a soln of (−)flavoskyrin (3 mg) dissolved in pyridine (1 ml) was added with $SOCl_2$. The mixture was neutralized with 1 N HCl and then extracted with ether. After evaporation of the solvent, the residue was chromatographed over silicic acid using benzene as the solvent to separate chrysophanol and (+)dianhydrorugulosin. The IR spectrum (in $CHCl_3$) of (+)dianhydrorugulosin was superimposable with that of (−)dianhydrorugulosin derived from (+)rugulosin, while their ORD curves of are reverse of each other. ORD (in dioxan) nm (φ): 262 ($2·23 \times 10^5$) 270 (0), 282 ($-3·20 \times 10^4$), 444 ($-1·42 \times 10^5$), 477 (0), 500 ($6·07 \times 10^4$).

Catalytic reduction of chrysophanol (Formation of 1-oxo-1,2,3,3-tetrahydro-6-methylanthrahydroquinone (3c)). Chrysophanol (1 g) was suspended in EtOH (200 ml) and hydrogenated using PtO_2 (200 mg) as the catalyst. Two moles of H_2 were absorbed during 35 min to form a clear brownish soln. The filtrate separated from the catalyst was concentrated and allowed to stand at room temp. A brown coloured substance which separated out was recrystallized from acetone-EtOH to form yellow crystals, m.p. 196° (yield 700 mg); IR ν_{max}^{KBr} cm^{-1} 1630, 1615, 1597, 1580; UV λ_{max}^{dioxan} nm. (log ε) 270 (4·42) 304 (3·68), 314 (3·57), 332 (3·35), 424 (3·87). (Found: C, 69·89; H, 5·39. $C_{15}H_{14}O_4$ requires: C, 69·75; H, 5·46%).

1-Oxo-1,2,3,4-tetrahydro-6-methyl-8-hydroxyanthrahydroquinone (4c). To a solution of 3c (600 mg) dissolved in AcOH (10 ml) was added gradually a soln of Pb $(OAc)_4$ (1·2 g) in AcOH (15 ml) under stirring. After continuous stirring for 30 min, the orange coloured mixture was poured into ice water to obtain an orange ppt which were recrystallized from $CHCl_3$—MeOH. 1-Oxo-1,2,3,4-tetrahydro-6-methyl-8-hydroxyanthaquinone, m.p. 196°, thus obtained (yield 500 mg) is very unstable, and readily converted into a dimer (5c), when the soln is allowed to stand at room temp, IR_{max}^{KBr} cm^{-1}: 1965, 1663, 1625, 1585, 1573, UV λ_{max}^{dioxan} nm (log ε): 425 (3·52). (Found: C, 69·76; H, 4·63. $C_{15}H_{12}O_4$ requires: C, 70·30; H, 4·72%).

Dimerization of 4c (*Formation of* 6c). To a soln of 4c

(500 mg) dissolved in $CHCl_3$ was added a drop of pyridine, and the mixture was allowed to stand overnight. The residue obtained on evaporation of the solvent was recrystallized from $CHCl_3$—MeOH to give yellow crystals m.p. 208–211° (yield 320 mg); IR ν_{max}^{KBr} cm^{-1}: 1715, 1618, 1585, UV λ_{max}^{dioxan} nm (log ϵ): 270 (4·53), 306 (4·00), 315 (4·00), 330 (3·91), 372 (3·98), 417 (3·87), 434 (sh) (3·83). (Found C, 69·99; H, 4·79. $C_{30}H_{24}O_8$ requires: C, 70·31; H, 4·69%).

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