Structural Elucidation of the Nigerones, Four New Naphthopyrones from Cultures of *Aspergillus niger*

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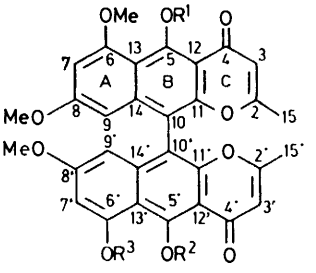
The isolation and structure of four new pigments, nigerone (1a), 6′-*O*-demethylnigerone (1b), isonigerone (2), and heminigerone (3), are described. A study of the chiroptical properties of the dimers indicates the *S*-configuration.

In our continuing studies on toxigenic food-borne fungi, several isolates of *Aspergillus niger* V. Tiegh were investigated. A strain (MRC 278) was isolated from Mozambican ground nuts and cultivated on sterilized yellow maize kernels for 21 days at 25 °C. The mouldered material was highly toxic to both ducklings and rats. The toxic principles were removed by prolonged extraction with chloroform–methanol. The crude extract was subjected to solvent partition and the fractions were biologically evaluated. The major pigment was designated nigerone (1a), whereas the minor pigments were characterized as 6′-*O*-demethylnigerone (1b), isonigerone (2), and heminigerone (3). Compounds (1a) and (1b) have the unusual C(10)–C(10′) coupling. A comparison of the circular dichroism data of (1a) and (2) with those of model compounds indicated the *S*-absolute configuration. An unusual case of rotational isomerism was observed in the study of the acetate-derivatives of (1a). These new pigments are related to a group of yellow pigments, derivatives of naphthopyrone, which occur as monomers [*e.g.* rubrofusarin (4) [1] and flavasperone (5) [2]] or as dimers [*e.g.* aurasperone A (6) [3] and aurasperone C [4]].

The five pigments [(1a), (1b), (2), (3), and (5)] do not contribute to the toxigenicity of the fungal culture of *A. niger* (MRC 278). The known toxins from *A. niger*, *e.g.* oxalic acid [5] and malformin C,[6] were not detected in cultures of this particular strain.

Nigerone (1a); $C_{32}H_{26}O_{10}$ [M^+, 570 (field desorption mass spectroscopy)], $\nu_{max.}$ 1 654 cm^{-1} (γ-pyrone), possesses ^1H and ^{13}C n.m.r. spectral characteristics (Tables 1 and 2) compatible with the proposed structure. The ^1H and ^{13}C n.m.r. spectra of (1a) show the presence of 13 protons and 16 carbon atoms, respectively; the compound is, therefore, dimeric. The well defined peaks and relative simplicity of the spectra indicate that the two halves of the molecule are identical. An AB pattern at δ 6.42 (7-H) and 6.06 (9-H) (*J* 2.5 Hz) is attributed to the *meta*-orientated aromatic protons. The position of coupling C(10)–C(10′) in (1a) was further substantiated by (a) the lack of an appreciable downfield shift of the aromatic protons in 5,5′-*OO*′-diacetylnigerone (1c) (such a shift is characteristic of a proton *para* to a phenolic hydroxy-group [7]) and (b) the methoxy-proton signal upfield shifts (>0.40 p.p.m.) for methoxy-groups *ortho* to an aromatic proton obtained upon change of solvent from chloroform to benzene.[8] The signals at δ 4.05 [6- and 6′-methoxy-groups] and 3.49 (8- and 8′-methoxy-groups) shifted upfield by 0.58 and 0.46 p.p.m., respectively, upon change of solvent from chloroform to benzene. The utility of the technique was shown by repeating the experiment on 5-*O*-methylnigerone (1f). In (1f) the methoxy-groups located *ortho* to aromatic protons showed upfield shifts whereas the methoxy-group signal at C(5) shifted downfield by 0.13 p.p.m.



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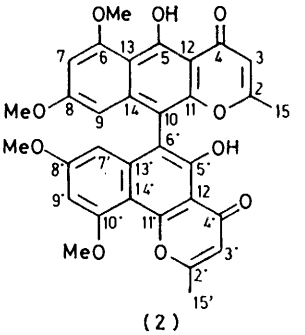
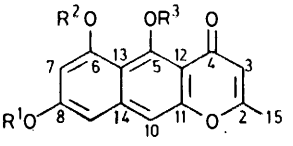
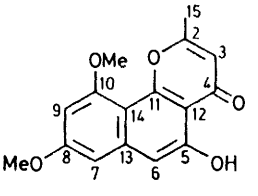


(2)

a; $R^1 = R^2 = H$; $R^3 = Me$
b; $R^1 = R^2 = R^3 = H$
c; $R^1 = R^2 = Ac$; $R^3 = Me$
d; $R^1 = Ac$; $R^2 = H$; $R^3 = Me$
e; $R^1 = R^2 = R^3 = Me$
f; $R^1 = R^3 = Me$; $R^2 = H$



(3) $R^1 = R^2 = Me$; $R^3 = H$
(4) $R^1 = Me$; $R^2 = R^3 = H$



(5)

In (1a) the ^1H n.m.r. chemical shifts of 3-H (δ 6.00) and the 15-methyl group (δ 2.03) correspond well with the values observed in ustilaginoidin A hexamethyl

ether (7b) [9] and the hexa-acetate (7c).[9] The upfield shift of the 15-methyl proton signals in (1a) relative to (7b) and (7c) (0.14 and 0.22 p.p.m., respectively) may be attributed to increased shielding owing to the greater steric interactions between the two halves of the molecule which are associated with the change of the site of coupling from C(9)–C(9′) in the case of the ustilaginoidins to C(10)–C(10′) in (1a). For flavasperone (5) and heminigerone (3) the 15-methyl protons appear at δ 2.45 and 2.34, respectively. The shielding effect in the proximity of the site of coupling in (1a) is also evident from the chemical shift of the C(8),C(8′) methoxy-protons (δ 3.49) compared with that of the C(6),C(6′) methoxy-protons (δ 4.05) and the H-9,H-9′ protons which appear at δ 6.06.

Although the ^{1}H n.m.r. characteristics of nigerone (1a) are consonant with the proposed structure, the non-linear alternative (8) must also be considered.

TABLE 1

Chemical shifts (δ) in the ^{1}H n.m.r. spectra of compounds (1a), (1b), (1c), (1e), (1f), (2), (3), and (5) in deuteriochloroform at 40 °C *

Compound	3-H	5-OR	6-H	6-OMe	7-H	8-OMe	9-H	10-H	10-OMe
(1a)	6.00	15.32		4.05	6.42	3.49	6.06		
(1b)	5.99	15.54 [a]		4.10	6.53 [b]	3.53	6.11 [c]		
(1c)	5.95	2.53		3.97	6.47	3.45, 3.50	6.12		
(1e)	5.93	4.02 [a]		4.1 [a]	6.48	3.45	5.98		
(1f)	5.93 [d]	4.02 [a]		4.04 [a]	6.62 [b]	3.47	6.08		
(2)	5.98	15.30		4.02	6.45 [b]	3.48 [a]	6.07 [c]		
(3)	5.98	14.94		3.98 [a]	6.38	3.90 [a]	6.57	6.94	
(5)	6.23	12.93	6.80		6.53	3.94 [a]	6.35		3.91 [a]
Compound	**15-Me**	**3′-H**	**5′-OH**	**6′-OMe**	**7′-H**	**8′-OMe**	**9′-H**	**10′-OMe**	**15′-Me**
(1a)	2.03								
(1b)	2.03	5.87	15.77 [a]		6.49 [b]	3.65	6.23 [c]		2.07
(1c)	1.93, 1.99								
(1c)	1.90								
(1f)	1.91 [c]	5.98 [d]		4.09 [a]	6.43 [b]	3.47	6.08		1.99 [c]
(2)	2.04	6.35	13.14		6.41 [b]	3.52 [a]	6.17 [c]	4.02	2.56
(3)	2.34								
(5)	2.45								

* The coupling constant (*J*) between 7-H and 9-H, and between 7′-H and 9′-H, is 2.5 Hz throughout.
[a–d] These assignments may be interchanged.

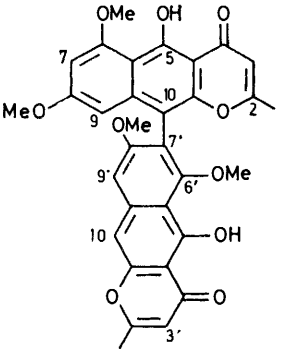
TABLE 2

^{13}C N.m.r. chemical shifts of compounds (1a), (1c), and (5)

Carbon atom	δ [a]/p.p.m		
	(1a)	(1c)	(5)
2	167.6 Sm	164.9	166.5 Sm
3	107.3 Dq	110.4	110.2 Dq
4	184.6 Ss	177.8	182.8 Ss
5	163.2 Sd	151.9	156.7 St
6	161.3 Sm [b]	161.2 [c]	105.7 Dt
7	97.3 Dd	99.3	98.0 Dt
8	161.9 Sm [b]	159.2 [c]	161.5 Sm [b]
9	96.6 Dd	95.8	97.0 Dd
10	105.5 Sd	113.6 [b]	159.1 Sm [b]
11	151.4 Ss	148.3	155.8 Ss
12	104.4 St	112.6	104.9 S
13	108.8 Sm	115.0 [b]	141.2 Ss
14	140.8 Ss	138.5	108.8 S
15	20.5 Qd	20.0	20.4 Qd
OMe	55.2 Qs	55.4	55.4 Qs
OMe	56.2 Qs	56.4	55.8 Qs
CH_3CO		169.7	
CH_3CO		21.2	

[a] Relative to internal Me_4Si. S or s = singlet, D or d = doublet, t = triplet, Q or q = quartet, m = multiplet (capital letters refer to the pattern arising from directly bonded protons, and lower-case letters to long-range ^{13}C–H coupling). [b,c] These assignments may be interchanged.

Differentiation between these isomers is possible by a consideration of the u.v. absorption spectrum of (1a) and the chemical shift of the C(5),C(5′) phenolic hydroxy-protons. The u.v. spectrum of the angularly fused flavasperone (5) [2] is fundamentally different from that of the linear compound rubrofusarin (4)[1] (Table 3), and as the u.v. spectrum of (1a) is practically superimposable on that of rubrofusarin (4), the linear compound is indicated. The ^{1}H n.m.r. data (Table 1) show



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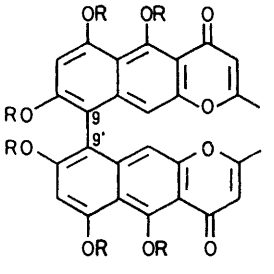
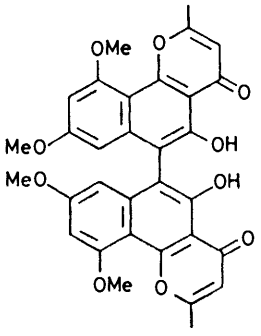


(7)

a; R = H
b; R = Me
c; R = Ac

that the resonance position of the C(5) hydroxy-proton is also diagnostic of the linear and non-linear isomers. For the linear compounds the hydroxy-proton is more strongly hydrogen-bonded to the carbonyl group and resonates around δ 15, whereas in the non-linear compounds the hydrogen bonding is weaker, with the

hydroxy-proton appearing around δ 13. A comparison of the ^{13}C resonance of C(4) in nigerone (δ_C 184.6 p.p.m.) and flavasperone (δ_C 182.8 p.p.m.) substantiates the relatively weaker hydrogen bonding to the carbonyl oxygen of flavasperone, a non-linear compound. Chelation of an anthraquinonoidal carbonyl oxygen with a



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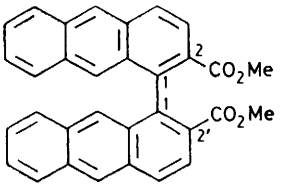


(9)

phenolic proton leads to a downfield shift.[10] However, a physical explanation for this difference in strength of the chelation of the 4-carbonyl oxygen with the phenolic proton in these linear and non-linear systems is not clear.

The assignments of the natural abundance ^{13}C n.m.r. data of (1a), derived from proton-noise-decoupled, single-frequency off-resonance decoupled, and coupled spectra, and deuterium exchange experiments are collated in Table 2. The chemical shifts of the atoms constituting the γ-pyrone comform with the values reported for related compounds.[11,12] The low solubility of the pigments in deuteriochloroform and the long relaxation times of the quarternary carbon atoms

TABLE 3

U.v. absorption spectra (in methanol) of compounds (1a), (1b), (2), (3), (4), and (5)

Compound	$\lambda_{max.}$/nm; log ε in parentheses
(1a)	226 (4.71); 278 (4.92); 407 (4.15)
(1b)	226 (4.60); 278 (4.80); 408 (4.00)
(2)	228 (4.70); 248 (4.75); 279 (4.82); 390 (3.98)
(3)	225 (4.40); 242 (4.51); 247 (4.50); 277 (4.53); 376 (3.65)
(4)	225 (4.45); 278 (4.68); 406 (3.74)
(5)	241 (4.65); 282 (4.41); 370 (3.66)

necessitated the addition of tris(dipivaloylmethanato)-gadolinium [Gd(dpm)$_3$] [13] to solutions of the pigments to obtain acceptable spectra. In the ^1H coupled ^{13}C n.m.r. spectrum of (1a) no clearly defined $^{>1}J$(C,H) coupling was observed either for C(4) or C(14), although both resonances were slightly broadened. The same effect was observed for 1,4-naphthoquinones by Höfle [10] and was also apparent in the spectrum of (5) (see below).

Nigerone (1a) does not react readily with diazomethane or with acetic anhydride–pyridine. A mixture of 5-*O*-methylnigerone (1f), $C_{33}H_{28}O_{10}$, and 5,5′-*O*,*O*′-dimethylnigerone (1e), $C_{34}H_{30}O_{10}$, was obtained upon methylation of nigerone by the method of Hakomori.[14] In the dimethoxy-compound (1e) the C(5),C(5′), and C(6),C(6′) methoxy-protons appear as singlets at δ 4.10 and 4.02 whereas the C(8),C(8′) methoxy-protons appear at δ 3.45. The diacetate (1c), $C_{36}H_{30}O_{12}$, was obtained upon treatment of nigerone with acetic anhydride–perchloric acid. The diacetate (1c) is very labile and hydrolyses to the monoacetate (1d) and nigerone (1a) during careful work up.

An interesting feature was observed at 40 °C in the ^1H n.m.r. spectra of both the monoacetate (1d) and the diacetate (1c), namely the doubling of signals. In the spectrum of (1d) the protons constituting one half of the molecule exhibited the expected coupling pattern, whereas protons belonging to the other half were affected, *e.g.* 9′-H (broad unresolved doublet), 8′-OMe (two singlets), and 15-Me (two singlets). From the observed chemical shifts it appears that the acetate carbonyl group exerts an anisotropic effect not on the protons of the half of the molecule on which it is located but on the protons (9′-H, 8′-OMe and 15′-Me) of the adjoining half. In the spectrum of the diacetate (1c) the resonances belonging to 3-H,3′-H (δ 5.95); 6-OMe,6′-OMe (δ 3.97); and 7-H,7′-H (δ 6.47, $J_{7,9}$ 2.5 Hz) remained unchanged at 40 and 55 °C; however, the doubling effect was observed on 8-OMe,8′-OMe (δ 3.45 and 3.50); 9-H,9′-H (δ 6.12, broad unresolved signal), and 15-Me,-15′-Me (δ 1.93 and 1.99). These observations prompted a variable-temperature study of the ^1H n.m.r. spectrum of (1c). The same phenomenon was observed at −40 °C; however, the difference in the chemical shift of the 'doublets' was accentuated, *e.g.* 15-Me,15′-Me resonated at δ 1.95 and 2.07. On raising the temperature to 60 °C, the signals attributed to 8-OMe,8′-OMe and 15-Me,15′-Me coalesced to single lines, whereas at 80 °C all the signals of (1c) appear as lines with coupling patterns as in (1a). The same sample of the diacetate (1c) was subsequently cooled to 40 °C and the doubling was again observed. The ^1H n.m.r. spectrum of nigerone (1a) showed no signs of doubling at 40 or −40 °C, although at −40 °C a change in the chemical shift of 3-H,3′-H and 9-H,9′-H to δ 6.07 and 6.00, respectively, was observed. This observation might indicate a change in the interplanar angle between the two halves of (1a). The ^1H n.m.r. spectra of the 5,5′-*OO*′-dimethylnigerone (1e) or the monomethyl derivative (1f) showed no doubling of peaks. From the foregoing data and from inspection of molecular models it appears that this effect is associated with the presence of the acetoxy-group(s) in (1c) and (1d), which leads to conformational isomerism below 55 °C. This isomerism is most likely due to steric hindrance which inhibits the rotation of the acetoxy-group(s). The protons of the C(15),C(15′) methyl group, the 8,8′-methoxy protons, and the C(9),C(9′) protons are thus influenced by the anisotropic effect of the acetate carbonyl group. The chemical shift of carbon atoms depends critically upon the electronic state of the nucleus and is hardly affected by the anisotropy of neighbouring groups. The ^{13}C n.m.r. signals of (1c) assigned to C(9),C(9′) (δ_C 95.8 p.p.m.), C(15),C(15′) (δ_C 20.0 p.p.m.), and 8-OMe,8′-OMe (δ_C 55.4 p.p.m.),

therefore, showed only broadening with a corresponding loss of amplitude.

Acetylation shifts for carbon atoms constituting ring B of (1c) (Table 2) were of a similar magnitude to those observed for the corresponding carbon atoms in *O*-acetyldihydrosterigmatocystin.[15]

The structure of 6′-*O*-demethylnigerone (1b), $C_{31}H_{24}O_{10}$, was evident from inspection of its spectroscopic parameters, particularly ^{1}H n.m.r. characteristics (Table 1). The compound consists of dissimilar halves with the chemical shifts in the one half being equivalent to those of (1a). Two methoxy-proton signals (δ 3.53 and 3.65) and one methoxy-proton signal (δ 4.10) indicated the presence of a phenolic hydroxy-group at C(6′) in (1b). The hydrogen-bonded hydroxy-proton resonances (δ 15.54 and 15.77 p.p.m.) and the u.v. spectral data of (1b) established that both halves of the molecule existed in the linaer form.

Another minor pigment, isomeric with nigerone (1a), was characterized as isonigerone (2). The ^{1}H n.m.r. resonances of the C(5),C(5′) hydroxy-protons (δ 15.30 and 13.14) of (2) suggest that one half of the molecule exists in the linear form (δ 15.30), whereas the other half exists as the angularly fused form (δ 13.14). This supposition was supported by the u.v. spectrum of (2) (Table 3) which was markedly different from those of nigerone (1a) and of (1b). The presence of two pairs of *meta*-coupled protons confirms the point of coupling of the two molecular halves.

The monomer, heminigerone (3), $C_{16}H_{14}O_5$, was characterized on the basis of its u.v. and ^{1}H n.m.r. spectral data. The major difference between the spectral data of (1a) and (3) can be attributed to the ring current effects in the case of the dimer (1a). The 10-H resonates as a singlet (δ 6.94). The u.v. spectrum of (3) and the chemical shift of the hydroxy-proton (δ 14.94) confirmed its linear structure.

The ^{13}C n.m.r. spectral parameters of flavasperone (5) are collated in Table 2. The major chemical shift differences of corresponding carbon atoms in compounds (5) and (1a) occur for carbon atoms C(5) and C(11). These carbon atoms are involved in the formation of the γ-pyrone on transformation from the angular to the linear form.

The co-occurrence of nigerone (1a) and isonigerone (2) with heminigerone (3) and flavasperone (5) [(3) and (5) are the polyketide-derived building units of (1a) and (2)] in cultures of *A. niger* is biosynthetically significant. It indicates that the phenol oxidative coupling of these pre-formed monomers leads to the dimeric pigments. The C(10)–C(10′) linkage of the naphthopyrone moieties in (1a) and (1b) is unique. The C(9)–C(9′) coupling as in the ustilaginoidins,[9] the C(10–C(7′) coupling as in the aurasperones,[3,4] or the C(7)–C(7′) as in the aurofusarins [9] is more common.

The Chiroptical characteristics of Nigerone (1a) *and of Isonigerone* (2).—Iridoskyrin [9] and the ustilaginoidins [9,16] are the first examples reported among natural products showing optical activity arising only from restricted rotation about a C–C linkage. The molecular rotation, [φ], and/or differential dichroic absorption data, Δε, of nigerone, isonigerone, ustilaginoidin A, and some reference compounds are given in Tables 4 and 5.

TABLE 4

Molecular rotations [φ] of some biaryls

Compound	Reported configuration	Molecular rotation [φ]
(1a)		$[\phi]_{550}$ −1.4 × 10³, $[\phi]_{441}$ −12.9 × 10³, $[\phi]_{395}$ 0, $[\phi]_{378}$ 6.9 × 10³, $[\phi]_{365}$ 0, $[\phi]_{337}$ −22.1 × 10³, $[\phi]_{291}$ −100.5 × 10³, $[\phi]_{284}$ 0, $[\phi]_{274.5}$ 305 × 10³, $[\phi]_{265}$ 0, $[\phi]_{256}$ −153 × 10³, $[\phi]_{240}$ 0
(7a) [16,*]	*R*	$[\phi]_{530}$ −3 × 10³, $[\phi]_{485}$ 0, $[\phi]_{470}$ 1.5 × 10³, $[\phi]_{460}$ 0, $[\phi]_{400}$ −12.8 × 10³, $[\phi]_{380}$ 0, $[\phi]_{370}$ 4.6 × 10³, $[\phi]_{360}$ 0, $[\phi]_{315}$ −750 × 10³, $[\phi]_{305}$ 0, $[\phi]_{280}$ 1 350 × 10³, $[\phi]_{265}$ 0
(9) [17,*]	*R*	$[\phi]_{450}$ 0, $[\phi]_{403}$ 32 × 10³, $[\phi]_{385}$ 0, $[\phi]_{262}$ −1 200 × 10³, $[\phi]_{260}$ 0, $[\phi]_{255}$ 2 400 × 10³, $[\phi]_{240}$ 0
(10) [16,*]	*R*	$[\phi]_{520}$ 0, $[\phi]_{360}$ 22.5 × 10³, $[\phi]_{335}$ 0, $[\phi]_{285}$ −355 × 10³, $[\phi]_{275}$ 0, $[\phi]_{280}$ 1 350 × 10³, $[\phi]_{265}$ 0

* The rotational values were estimated from graphs reported in the literature.

Shibata and Ogihara [16] concluded from a comparison of the o.r.d. Cotton effects of the ustilaginoidins and related compounds [*e.g.* 2,2′,4,4′,5,5′,7,7′-octamethoxy-6,6′-diacetyl-1,1′-binaphthalene (10), derived from (7b)] with those of bianthryls [17] and of bridged binaphthyls [18] (Table 4) that the ustilaginoidins possess the *R*-configuration. The conclusions of Shibata and Ogihara [16] were apparently based mainly on the positive Cotton effects at higher wavelengths (*ca.* 400 nm) of the ustilaginoidins. It is of importance to note that the *R*-1,1′-binaphthalene (10) had o.r.d. characteristics very similar to those of the ustilaginoidins. Mislow *et al.*[19] found that the c.d. curves of biaryls and binaphthyls correspond to the signs of the related o.r.d. curves which reflect the chirality of the chromophore.

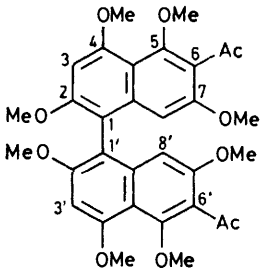
It is evident from the c.d. data (Table 5) that nigerone (1a) and isonigerone (2) are chromophorically related substances; the high values of [φ] and Δε reflect the

TABLE 5

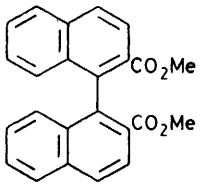
Differential dichroic absorption (Δε) of some biaryls

Compound	Reported configuration	Δε
(1a)		$\Delta\varepsilon_{480}$ 0, $\Delta\varepsilon_{432}$ −5.05, $\Delta\varepsilon_{393}$ 0, $\Delta\varepsilon_{373}$ 4.0, $\Delta\varepsilon_{343}$ 5.70, $\Delta\varepsilon_{327}$ 0, $\Delta\varepsilon_{320}$ −1.04, $\Delta\varepsilon_{312}$ 0, $\Delta\varepsilon_{303}$ 2.61, $\Delta\varepsilon_{298}$ 0, $\Delta\varepsilon_{282}$ −73.13, $\Delta\varepsilon_{275.5}$ 0, $\Delta\varepsilon_{267}$ 70.9, $\Delta\varepsilon_{254}$ 0, $\Delta\varepsilon_{250}$ −17.4, $\Delta\varepsilon_{240}$ −6.53
(2)		$\Delta\varepsilon_{470}$ 0, $\Delta\varepsilon_{403}$ −1.87, $\Delta\varepsilon_{382}$ 0, $\Delta\varepsilon_{361}$ 1.32, $\Delta\varepsilon_{350}$ 1.2, $\Delta\varepsilon_{343}$ 1.49, $\Delta\varepsilon_{325}$ 0, $\Delta\varepsilon_{288}$ −33, $\Delta\varepsilon_{282}$ 0, $\Delta\varepsilon_{273}$ 70.24, $\Delta\varepsilon_{259}$ 0, $\Delta\varepsilon_{251}$ −51.24, $\Delta\varepsilon_{245}$ −31.66
(11) [19]	*S*	$\Delta\varepsilon_{368}$ 1.82, $\Delta\varepsilon_{326}$ −3.58, $\Delta\varepsilon_{305}$ −1.52, $\Delta\varepsilon_{288}$ −21.42, $\Delta\varepsilon_{245}$ +158.8, $\Delta\varepsilon_{240}$ +139.4

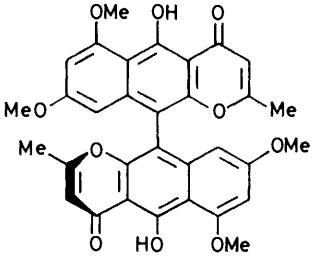
inherent dissymmetry of the complex chromophores. A difficulty arises in the correlation of the rotational data and the absolute configuration of (1a) and (2). Nigerone exhibits a negative o.r.d. ($[\phi]_{441}$ − 12.9 × 10³) and c.d.



(10)

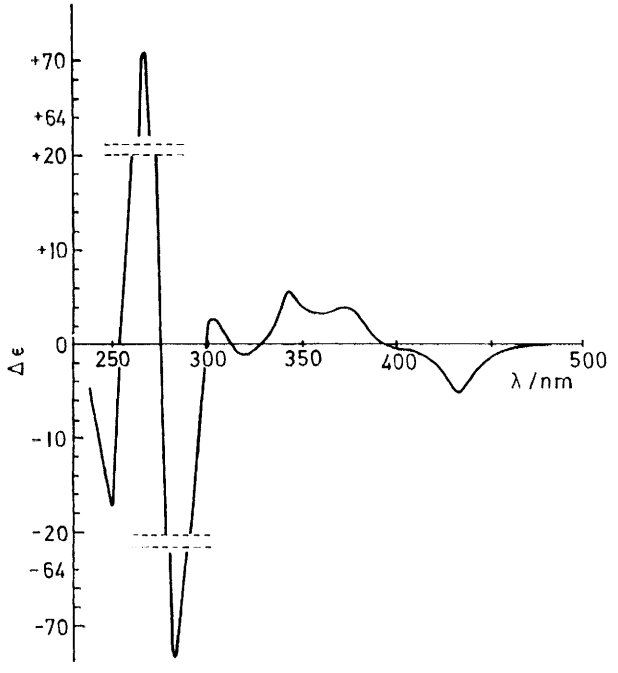


(11)



(12)

($\Delta\varepsilon_{432}$ — 5.05) (Figure) Cotton effect in the long wavelength regions; this characteristic would indicate the *S*-configuration (12) in a comparison with the ustilaginoidins [16] and the bianthryl, *e.g.* (*R*)-(—)-2,2′-dimethoxycarbonyl-1,1′-bianthryl (9).[17] Nigerone (1a) shows strong Cotton effects below 300 nm, *e.g.* $[\phi]_{291}$ — 100.5 × 10^3, $[\phi]_{274.5}$ 305 × 10^3, $\Delta\varepsilon_{282}$ — 73.1 and $\Delta\varepsilon_{267}$ + 70.9, which would again suggest the *S*-configuration in comparison with the c.d. data of, *e.g.*, (*S*)-2,2′-dimethoxycarbonyl-1,1′-binaphthalene (11) ($\Delta\varepsilon_{288}$ — 21.4 and $\Delta\varepsilon_{245}$ 158.8).[19] The contradiction arises as bianthryl,[17] the binaphthalene (10) ($[\phi]_{285}$ — 335 × 10^3 and $[\phi]_{280}$ 1 350 × 10^3), and the ustilaginoidins with the *R*-configuration exhibit o.r.d. Cotton effects of similar sign in this region. An unambiguous assignment of the absolute configuration of the ustilaginoidins and nigerone will require a further concerted study.



The c.d. spectrum of nigerone (1a) in dioxan

The optical rotation of nigerone (1a) is associated with the restricted rotation (atropisomerism) about the C(10)–C(10′) bond. The heating of nigerone under reflux in acetic acid for 4 h (samples taken every hour) caused only a slight reduction (*ca.* 1%) in its $[\alpha]_D^{20}$ value (—244° in acetic acid); rotation about the C(10)–C(10′) bond of (1a) is therefore virtually inhibited. A high-energy maximum (severe steric hindrance) must make it difficult for the enantiomers to racemise. Aurasperone A (6), a biaryl with a C(10)–C(7′) coupling, is stable at room temperature; however, it was readily racemised when boiled in acetic acid ($t_{\frac{1}{2}}$ 1.9 h).[4] It is of importance to note that the restricted rotation is much weaker in the case of biquinonoidal compounds, *e.g.* aurofusarin,[9] where the C–C linkage is at the 7,7′-position with adjacent methoxy-groups and quinonoidal carbonyl groups which would not restrict rotation, and for the pigments viomellein and xanthomegnin which racemise during isolation.[20]

EXPERIMENTAL

M.p.s were determined on a Kofler hot-stage apparatus. U.v. absorptions were measured for solutions in methanol on a Unicam SP 800 spectrometer. I.r. spectra were recorded on a Perkin-Elmer 237 spectrometer for solutions in chloroform. Mass spectra were taken on an AEI MS 9 double-focusing spectrometer. ^{13}C and ^1H N.m.r. spectra were recorded on a Varian CFT-20 spectrometer and a Varian EM-390 spectrometer, respectively. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. C.d. and o.r.d. spectra were measured for solutions in dioxan (JASCO J-20 spectropolarimeter). T.l.c. separations were carried out on Merck pre-coated silica plates (thickness 0.25 and 2 mm). For column chromatography Merck silica gel, particle size 0.063—0.200 mm, was used.

Isolation of Metabolites from Maize Meal infected with A. niger (MRC 278).—Dried, milled, mouldy maize (10 kg) was extracted with chloroform–methanol (1 : 1 v/v) for 48 h and the solvent was removed under reduced pressure to yield a dark brown gum (1.6 kg) which was partitioned between hexane and 90% methanol, and the methanol extract was evaporated to dryness. The resultant brown gum was partitioned between chloroform and water and the chloroform extract was evaporated to dryness to yield a dark residue (180 g) which was separated by chromatography on silica gel (1 kg). Elution with hexane–ethyl acetate (4 : 1 v/v) removed all the fatty material, and further elution with chloroform–acetone (9 : 1 v/v) gave a mixture of the crude pigments as an orange gum (1.7 g). Separation was achieved by p.l.c. in a variety of solvent systems, *e.g.* chloroform–acetone (9 : 1 v/v), chloroform–methanol (97 : 3 v/v), hexane–ethyl acetate (1 : 1 v/v), and benzene–acetone (4 : 1 v/v). The metabolites (1a), (1b), (2), (3), and (5) were crystallised from chloroform–methanol.

Nigerone: 5,5′-Dihydroxy-6,6′,8,8′-tetramethoxy-2,2′-dimethyl-10,10′-bi-(4H-naphtho[2,3-b]pyran)-4,4′-dione (1a)

(321 mg), orange needles, m.p. >330 °C, had $[\alpha]_D^{20}$ −287.7° (*c* 1.00 in $CHCl_3$); $\nu_{max.}$ 1 652, 1 618, 1 591, 1 412, 1 171, and 1 128 cm^{-1} (Found: C, 63.7, H, 4.7%; M^+ 570.149. $C_{32}H_{26}O_{10}$·CH_3OH requires C, 63.8; H, 4.4%; *M*, 570.152).

6′-O-*Demethylnigerone:* 5,5′,6′-*Trihydroxy*-6,8,8′-*trimethoxy*-2,2′-*dimethyl*-10,10′-*bis*-(4H-*naphtho*[2,3-b]*pyran*)-4,4′-*dione* (1b) (8 mg), orange powder, m.p. >330 °C, had $[\alpha]_D^{20}$ −235.7° (*c* 0.14 in $CHCl_3$); $\nu_{max.}$ 1 651, 1 615, 1 590, 1 410, 1 170, and 1 123 cm^{-1} (Found: M^+, 556.139. $C_{31}H_{24}O_{10}$ requires *M*, 556.137).

Isonigerone (2): 5-*Hydroxy*-6-(5-*hydroxy*-6,8-*dimethoxy*-2-*methyl*-4-*oxo*-4H-*naphtho*[2,3-b]*pyran*-10-*yl*)-8,10-*dimethoxy*-2-*methyl*-4H-*naphtho*[1,2-b]*pyran*-4-*one* (30 mg), orange solid, m.p. >330 °C had $[\alpha]_D^{20}$ −93.1° (*c* 0.74 in $CHCl_3$); $\nu_{max.}$ 1 659, 1 614, 1 588, 1 427, 1 389, 1 322, and 1 160 cm^{-1} (Found: M^+, 570.150. $C_{32}H_{26}O_{10}$ requires *M*, 570.152).

Heminigerone (3): 5-*Hydroxy*-6,8-*dimethoxy*-2-*methyl*-4H-*naphtho*[2,3-b]*pyran*-4-*one* (53 mg), yellow needles, had m.p. 178—180 °C; $\nu_{max.}$ 1 658, 1 621, 1 587, 1 428, 1 409, 1 379, and 1 162 cm^{-1} (Found: M^+ 286.094 4. $C_{16}H_{14}O_5$ requires *M*, 286.084 1).

Flavasperone (5): 5-*Hydroxy*-8,10-*dimethoxy*-2-*methyl*-4H-*naphtho*[1,2-b]*pyran*-4-*one* (205 mg), yellow needles, had m.p. 204 °C (lit.,[2] 203—204 °C); $\nu_{max.}$ 1 669, 1 621, 1 582, 1 460, 1 431, 1 380, and 1 324 cm^{-1} (Found: M^+, 286. Calc. for $C_{16}H_{14}O_3$: *M*, 286).

Acetylation of Nigerone (1a).—Nigerone (1a) (60 mg) in acetic anhydride (10 ml) was cooled to −70 °C. Perchloric acid (0.05 ml) was added and the solution was allowed to reach room temperature, before it was poured on ice (50 ml). After extraction with benzene (3 × 10 ml), the organic layer was dried (Na_2SO_4) and the solvent removed under reduced pressure. The mixture was purified by p.l.c. on silica with development in benzene–acetone (4 : 1 v/v). Standard elution of the three yellow bands gave the diacetate (1c) (23 mg) as a yellow glass, the monoacetate (1d) (7 mg), and nigerone (1a) (30 mg). The diacetate (1c) had $[\alpha]_D^{20}$ −116.6° (*c* 0.75 in $CHCl_3$); $\lambda_{max.}$ 227, 270, 334(s), 345, and 384 nm (log ε 4.94, 5.07, 4.07, 4.15 and 4.32); $\nu_{max.}$ 1 762, 1 652, 1 622, 1 598, 1 569, 1 357, and 1 171 cm^{-1} (M^+, 654. Calc. for $C_{36}H_{30}O_{12}$: *M*, 654). The monoacetate (1d) was obtained as a yellow foam. It had $\lambda_{max.}$ (dioxan) 267, 277.5, 325, 341, 370(sh), and 390 nm (log ε 4.78, 4.80, 3.71, 3.72, 3.93, and 4.02); $\nu_{max.}$ 1 765, 1 656, 1 620, and 1 595 cm^{-1} (M^+, 612. Calc. for $C_{34}H_{28}O_{11}$: *M*, 612). Nigerone (1a) was obtained as the major component. T.l.c. analysis of the benzene extract of the reaction mixture indicated the formation of the diacetate (1c) only. Compounds (1a) and (1d) are clearly derived from hydrolysis.

Permethylation of Nigerone (1a).—Sodium hydride (11 mg; 50% in oil) was stirred for 30 min with dry dimethyl sulphoxide (5 ml). Nigerone (50 mg) in dimethyl sulphoxide (5 ml) was added and the mixture stirred for 30 min. Methyl iodide (0.5 ml) in dimethyl sulphoxide (1.0 ml) was added to the mixture, which was stirred for 50 min, and then poured on ice and extracted into benzene. The product was separated by t.l.c. on silica with chloroform–methanol (97 : 3 v/v). Nigerone (1a) (30 mg) appeared at R_F 0.67 whereas 5-*O*-methylnigerone (1f) (11 mg) had an R_F of 0.50 in this system. Compound (1f) had m.p. 256—257 °C (from MeOH), $\lambda_{max.}$ (MeOH) 225, 275, 324, 342, and 392 nm (log ε 4.77, 4.97, 3.56, 3.54, and 4.16); $\nu_{max.}$ 1 655, 1 615, 1 590, 1 555, 1 412, and 1 172 cm^{-1} (M^+, 584. Calc. for $C_{33}H_{28}O_{10}$: *M*, 584). The minor product 5,5′-*OO*′-dimethylnigerone (1e) (1 mg) had an R_F of 0.35 and $\nu_{max.}$ 1 655, 1 620, 1 595, 1 555, 1 355, and 1 170 cm^{-1} (M^+, 598. Calc. for $C_{34}H_{30}O_{10}$: *M*, 598).

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