^{13}C Nuclear Magnetic Resonance Spectra and Biosynthetic Studies of Xanthomegnin and Related Pigments from *Aspergillus sulphureus* and *melleus*

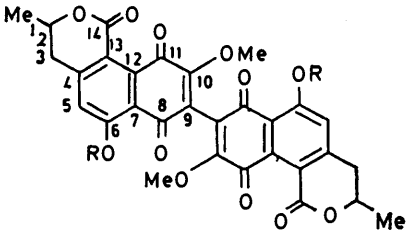
By **Thomas J. Simpson,*** Research School of Chemistry, Australian National University, P.O. Box 4, Canberra, A.C.T. 2600, Australia

The ^{13}C n.m.r. spectra of xanthomegnin (1) and related compounds [(2)—(5), (7), and (9)] have been assigned. Spectra of xanthomegnin and viomellein biosynthesised from singly and doubly ^{13}C-labelled acetate confirm the polyketide origin of these metabolites and provide additional evidence for the structures proposed for the pigments.

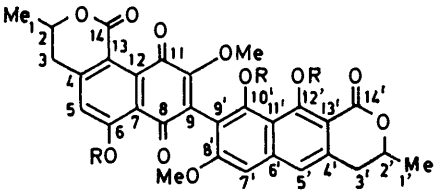
THE isolation of the fungal pigments xanthomegnin (1), viomellein (4), rubrosulphin (6), and viopurpurin (8) from the mycelium of *Aspergillus sulphureus* and *A. melleus* was described recently.[1] Xanthomegnin had previously been isolated from *Trichophyton* spp. and assigned the dimeric naphthoquinone structure (1).[2] The naphthoquinone–naphthalene structures for the remaining pigments were proposed on the basis of chemical and spectroscopic evidence. ^{13}C N.m.r. structural studies of xanthomegnin and its related metabolites and biosynthetic studies in *A. melleus* are now reported which provide additional evidence for their structure and indicate their mode of biogenesis.

These metabolites are closely related to other groups of fungal pigments, *e.g.* the dimeric naphthoquinone aurofusarin,[3] fuscofusarin,[4] which has a naphthoquinone–naphthalene structure, and the dimeric naphthalenes vioxanthin,[5] viriditoxin,[6] and flavomannin.[7] Owing to the highly substituted nature of these structures, ^1H n.m.r. is of limited applicability, but ^{13}C n.m.r. was expected to prove a useful method of structure elucidation in this area. The ^{13}C n.m.r. spectrum of viriditoxin has been reported [8] but only partially assigned.

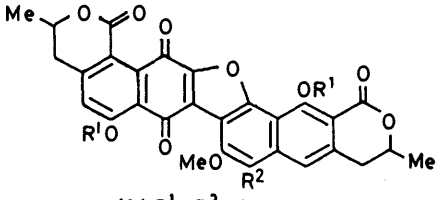
Assignments of ^{13}C *Resonances.*—The ^{13}C n.m.r. spectra of xanthomegnin (1), xanthomegnin acetate (3), viomellein (4), and the methyl ethers [(2), (5), (7), and (9)] of xanthomegnin, viomellein, rubrosulphin, and viopurpurin, respectively, are summarised in Table 1. The assignments are based on comparisons between resonances in the proton noise-decoupled (p.n.d.) spectra, use of single frequency off-resonance-decoupled (s.f.o.r.d.) spectra to determine the number of attached protons on each carbon atom,[9] and comparisons with model compounds. The substituent-induced shifts tabulated by Wells *et al.*[10] are useful in indicating the expected chemical shifts in the naphthalene portions of the molecules. Detailed analysis of the fully proton-coupled spectra, making use of D_2O exchange and low-power selective proton decoupling to identify the origin of long-range couplings,[11] is of particular utility.



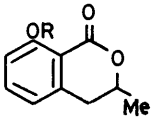
(1) R = H
(2) R = Me
(3) R = Ac



(4) R = H
(5) R = Me



(6) $R^1 = R^2 = H$
(7) R^1 = Me, R^2 = H
(8) R^1 = H, R^2 = OH
(9) R^1 = Me, R^2 = OMe



(10) R = H
(11) R = Me

Only fourteen resonances are observed in the normal p.n.d. spectrum of xanthomegnin. The multiplicities

* *Present address:* Department of Organic Chemistry, The Robert Robinson Laboratories, P.O. Box 147, Liverpool L69 3BX.

[1] R. C. Durley, J. MacMillan, T. J. Simpson, A. T. Glen, and W. B. Turner, *J.C.S. Perkin I*, 1975, 163.
[2] G. Just, W. C. Day, and F. Blank, *Canad. J. Chem.*, 1963, **41**, 74.
[3] P. M. Baker and J. C. Roberts, *J. Chem. Soc.* (*C*), 1966, 2234.
[4] T. Takeda, E. Morishita, and S. Shibata, *Chem. and Pharm. Bull.* (*Japan*), 1968, **16**, 2213.
[5] A. S. Ng, G. Just, and F. Blank, *Canad. J. Chem.*, 1969, **47**, 1223.
[6] D. Weisleder and E. B. Lillehoj, *Tetrahedron Letters*, 1971, 4705.
[7] J. Atherton, B. W. Bycroft, J. C. Roberts, P. Roffey, and M. E. Wilcox, *J. Chem. Soc.* (*C*), 1968, 2560.
[8] J. Jiu and S. Mizuba, *J. Antibiotics*, 1974, **27**, 760.
[9] J. B. Stothers, 'Carbon-13 NMR Spectroscopy,' Academic Press, New York, 1972.
[10] P. R. Wells, D. P. Arnold, and D. Doddrell, *J.C.S. Perkin II*, 1974, 1745.
[11] K. G. R. Pachler, P. S. Steyn, R. Vleggaar, and P. L. Wessells, *J.C.S. Chem. Comm.*, 1975, 355.

observed in the s.f.o.r.d. spectrum readily allow the assignment of C-1, C-2, C-3, C-5, and OCH_3; the remaining carbon atoms all give singlets. The quinone carbonyl resonances at 186.0 and 179.8 p.p.m. are assigned to C-8 and C-11 respectively, the C-8 signal appearing at lower field owing to chelation with the C-6 OH,[9] whereas the C-11 signal appears at higher field than in 1,4-naphthoquinone (184.6 p.p.m.)[12] owing to the shielding effect of the C-10 OMe. In 2-methoxy-1,4-naphthoquinone the C-1 carbonyl resonance appears at 180.0 p.p.m.[13] On removal of the chelation, the C-8 carbonyl resonance moves upfield to 180.8 and 179.8 p.p.m. in the methyl ether (2) and the acetate (3), respectively. In addition, in the fully-coupled spectrum of (1), the C-8 resonance appears as a sharp singlet, whereas C-11 gives a doublet, J 5 Hz, due to a five-bond coupling to H-5 (low-power irradiation at the frequency of H-5 causes collapse of the resonance to a sharp singlet). This long-range coupling is valuable in distinguishing the quinone carbonyl resonances in all the compounds. The resonances at 157.9, 162.2, and 162.7 p.p.m. are assigned to C-10, C-14, and C-6, respectively: in the fully-coupled spectrum the broad resonance at 157.9 p.p.m. collapses to a sharp singlet on irradiation at the frequency of the methoxy-protons (τ 5.85); the resonance at 162.7 p.p.m. appears as a doublet, J 5 Hz, owing to coupling to the C-6 OH, which collapses to a singlet on addition of D_2O with a small upfield deuterium isotope shift of 0.2 p.p.m.; and the 162.2 p.p.m. resonance appears as a singlet which greatly increases in intensity on irradiation at the frequency of the C-2 proton (τ 5.39). The remaining resonances at 148.1, 134.7, 117.5, and 114.8 p.p.m. are assigned to C-4, C-12, C-13, and C-7, respectively. In the fully coupled spectrum the 148.1 p.p.m. resonance appears as a broad unresolved signal which collapses to a sharp singlet on irradiation at the frequency of the C-3 benzylic protons (τ 6.96). This irradiation also causes the broad satellites of the C-5 doublet ($^1J_{C-H}$ 168 Hz) to sharpen, and the broad resonance at 117.5 p.p.m. to collapse to a doublet, J 4 Hz, the residual splitting being due to a three-bond coupling to H-5. Addition of D_2O causes the triplet at 114.8 p.p.m. to change to a doublet, J 7 Hz, owing to removal of the three-bond coupling to the chelated phenolic proton. The remaining three-bond coupling to H-5 is removed by irradiation at τ 2.51. In di-*O*-methylxanthomegnin (2), the C-7 resonance has moved downfield to 124.0 p.p.m., owing to removal of chelation.[9] The remaining resonance at 134.7 p.p.m. is a sharp singlet in the fully coupled spectrum as anticipated for C-12; in 1,4-naphthoquinone, the corresponding carbon atom resonates at 131.7 p.p.m. Under normal conditions of spectral determination the C-9 resonance of xanthomegnin cannot be observed owing to its very long relaxation time. However, on addition of the relaxation agent, $Cr(acac)_3$,[14] the C-9 resonance appears at 123.0 p.p.m. In the spectra of the remaining compounds, this resonance is visible, but usually as a very weak singlet, which aids its assignment. In viriditoxin, the signal for the equivalent carbon atom at the dimer linkage was not observed.[8] Similar analysis of the ^{13}C n.m.r. spectra of the dimethyl ether (2) and the diacetate (3) readily allows the assignments given in Table 1.

TABLE 1

^{13}C Chemical shifts of xanthomegnin and related metabolites (in p.p.m. downfield from internal Me_4Si for $CDCl_3$ solutions: multiplicities are indicated for compounds for which s.f.o.r.d. spectra have been obtained)

Carbon	(1)	(2)	(3)	(4)	(5)	(7)	(9)
1	20.6q	20.5q	20.5q	20.7q	20.7	20.8q	20.5
2	74.4d	74.3d	74.5d	74.1d	74.3	74.2d	74.8
3	36.1t	36.4t	35.8t	36.3t	36.4	36.5t	36.4
4	148.1	146.9	146.2	147.9	146.4	147.1	147.1
5	116.8d	120.9d	123.2d	116.4d	120.8	120.4d	120.8
6	162.7	162.3	151.3	162.8	162.3	162.7	162.9
7	114.8	124.0	124.0	114.8	124.8	124.8	125.3
8	186.0	180.8	179.8	188.3	182.2	176.8	177.0
9	123.0	126.2	123.7	123.6	127.2	126.2	126.4
10	157.9	156.4	156.7	158.2	156.4	150.8	151.6
11	179.8	180.2	179.8	180.1	180.7	172.5	172.5
12	134.7	136.0	135.3	134.4	136.1	136.1	136.5
13	117.5	125.2	132.7	117.6	125.3	125.6	125.8
14	162.2	160.4	159.7	162.4	160.4	160.1	160.5
1′				20.7q	20.7	20.8q	20.5
2′				76.5d	74.3	74.2d	74.8
3′				34.6t	36.4	36.5t	36.4
4′				134.0	136.9	138.6	138.7
5′				116.0d	120.8	119.7d	115.8
6′				140.5	140.5	138.9	133.9
7′				97.8d	102.2	101.9d	145.1 [a]
8′				160.1	156.3 [a]	155.1	146.2 [a]
9′				99.9	117.5 [b]	113.3 [a]	116.8 [b]
10′				161.3	162.7	154.9	149.9
11′				105.1	117.1 [b]	111.7 [a]	115.6 [b]
12′				155.3	157.9 [a]	159.5	159.5
13′				107.9	113.9	113.9	114.0
14′				171.2	160.6	161.7	161.7
10-OMe	61.4q	60.9q	61.9q	60.3q	60.3		
6′-OMe		63.4q			63.5	63.4q	63.6
8′-OMe				55.9q	55.9	55.8q	62.1 [c]
10′-OMe					63.5		
12′-OMe					62.9	62.7q	63.1
7′ OMe							61.6 [c]
CH_3CO			20.9q				
CH_3CO			168.7				

[a–c] Assignments may be reversed.

Twenty-nine resonances are resolved in the p.n.d. ^{13}C n.m.r. spectrum of viomellein (4), and the fifteen resonances due to the naphthoquinone moiety are readily assigned by comparison with those of xanthomegnin. In both viomellein and tri-*O*-methylviomellein (5), substitution of C-9 by a naphthalene rather than a naphthoquinone unit causes a *ca.* 2 p.p.m. downfield shift of the C-8 signal. The C-1 and C-1′ resonances are coincident, but not those of C-2 and C-2′, and of C-3 and C-3′. The C-14′ lactone carbonyl resonance at 171.2 p.p.m. moves upfield to 160.6 p.p.m. on methylation

[12] L. F. Johnson and W. C. Jankowski, 'Carbon-13 Nuclear Magnetic Resonance Spectroscopy. A Collection of Assigned, Coded and Indexed Spectra,' Wiley–Interscience, New York, 1972.

[13] T. J. Simpson, unpublished observations.

[14] O. A. Gansow, A. R. Burke, and G. N. La Mar, *J.C.S. Chem. Comm.*, 1972, 456.

[to (5)], with concomitant shifts of the C-13′ resonance from 107.9 to 113.9 p.p.m., and the C-2′ and C-3′ resonances become coincident with those of C-2 and C-3. These chemical shifts and their behaviour on methylation are almost identical with those observed [13] in mellein (10) and *O*-methylmellein (11), and are due to removal of the chelation and resultant slight conformational changes in the lactone ring. The aromatic protonated carbon resonances at 116.0 and 97.8 p.p.m. are readily identified from the s.f.o.r.d. spectrum of viomellein and are assigned to C-5′ and C-7′, respectively, the C-7′ signal being at higher field owing to the extra shielding from the C-8′ OMe. The resonances at 155.3, 160.1, and 161.3 p.p.m. are assigned to C-12′, C-8′, and C-10′, respectively: in the fully-coupled spectrum the broad resonance at 160.1 p.p.m. sharpens to a singlet on irradiation at the OCH_3 frequency; the resonance at 155.3 p.p.m. appears as a doublet, J 3 Hz, collapsing to a singlet on addition of D_2O; the 161.3 p.p.m. resonance appears as a sharp singlet. Wehrli has shown that only strongly chelated phenolic protons normally couple to the phenolic carbon atom.[15] The resonances at 134.0 and 140.5 p.p.m. can be assigned to C-4′ and C-6′, respectively, on the basis of calculated chemical shifts (136 and 140 p.p.m., respectively),[10] and these assignments are confirmed in the fully-coupled spectrum where the broad resonance at 134.0 p.p.m. sharpens on irradiation at the frequency of the benzylic C-3′ protons; the C-6′ resonance appears as a sharp singlet, showing no coupling as expected. The remaining resonances at 99.9 and 105.1 p.p.m. are assigned to C-9′ and C-11′ (calculated shifts 98 and 112 p.p.m.), respectively. No confirmation could be obtained in the fully-coupled spectrum, but in the p.n.d. ^{13}C n.m.r. spectrum of [1,2-^{13}C]acetate-enriched viomellein (see below) a ^{13}C–^{13}C coupling is apparent between C-11′ and C-12′. The assignment of the ^{13}C n.m.r. spectrum of tri-*O*-methylviomellein (5) follows from comparison with that of di-*O*-methylxanthomegnin and similar considerations, as above, of the fully-coupled spectrum. The large downfield shifts of the C-9′ and C-11′ signals are difficult to account for by simple change of substituent effects, but are almost certainly due to changes in hydrogen bonding and steric effects which are difficult to quantify as yet.

On formation of the furanoid ring in di-*O*-methylrubrosulphin (7), only the C-8—11 and C-8′—11′ resonances move significantly relative to those in tri-*O*-methylviomellein. The quinone carbonyl resonances both move upfield from 182.2 and 180.7 to 176.8 and 172.5 p.p.m., respectively. The C-10 and C-10′ resonances also move upfield, to 150.8 and 154.9 p.p.m., respectively, and can be distinguished from the COMe resonances by their lack of coupling in the fully-coupled spectrum, and from each other by the further upfield shift of C-10′ in tri-*O*-methylviopurpurin (9) due to shielding by the C-7′ OMe.

The structure of viopurpurin (8), a 7′-hydroxy-derivative of rubrosulphin, was based on the ^1H n.m.r. shift of the 5′-proton.[1] The ^{13}C n.m.r. spectrum of tri-*O*-methylviopurpurin (9) is entirely consistent with this. The C-7′ and C-8′ resonances at 101.9 and 155.1 p.p.m. in di-*O*-methylrubrosulphin are replaced by resonances due to *ortho*-methoxy-substituted carbon atoms at 145.1 and 146.2 p.p.m. In addition the C-6′, C-10′, and C-5′ signals move upfield owing to extra shielding by the introduced methoxy-group in an *ortho*-, *para*-, or *peri*-position, respectively. Thus the ^{13}C n.m.r. data provide strong confirmatory evidence for the structure (8) proposed for viopurpurin.

^{13}C *Enrichment Studies.*—The amounts of [^{13}C]acetate necessary for feedings were found by determining the dilution of ^{14}C label in experiments with [1-^{14}C]acetate. These conditions were optimised for study of metabolites found in the culture medium,[16] but they also give high enrichment of the mycelial pigments. Accordingly, *A. melleus* was grown in the presence of sodium [1-^{13}C]- and [1,2-^{13}C]-acetate (0.8 and 0.5 g l^{-1}, respectively) and the enriched xanthomegnin and viomellein were isolated. The enhancements observed in the p.n.d. ^{13}C n.m.r. spectra of the [1-^{13}C]acetate derived samples are summarised in Table 2. The enrichment of labelled sites is high so that only the enriched resonances are visible, seven for xanthomegnin and fourteen for viomellein, clearly showing the labelling of alternate carbons anticipated for a polyketide origin.

TABLE 2

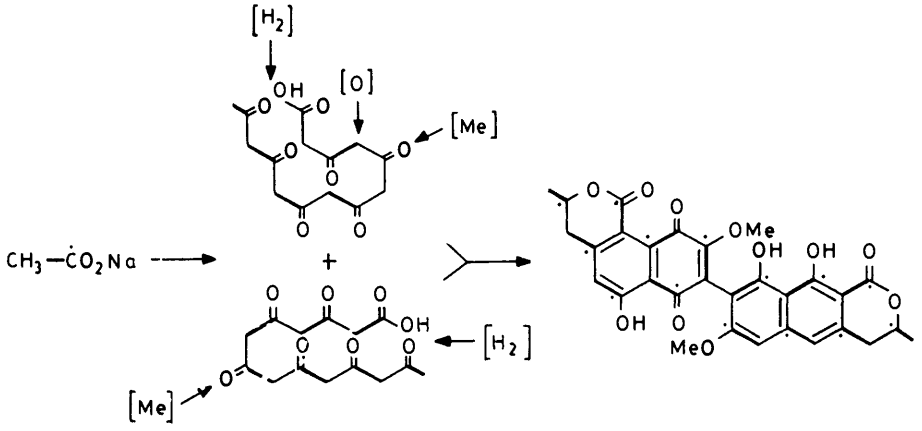
^{13}C N.m.r. spectra of [1-^{13}C]acetate-derived xanthomegnin and viomellein; couplings observed in [1,2-^{13}C]acetate-derived viomellein.

	δ_C		$^1J(^{13}C–^{13}C)$/Hz
Carbon	Xanthomegnin	Viomellein	Viomellein
1			40
2	74.4	74.1	40
3			40
4	148.0	147.9	41
5			64
6	162.7	162.9	64
7			
8	185.8	188.2	58
9			
10	158.0	158.4	62
11			60
12	134.8	134.5	
13			
14	162.2	162.6	68
1′			40
2′		76.5	
3′			42
4′		134.0	40
5′			57
6′		140.6	56
7′			71
8′		160.3	
9′			70
10′		161.4	
11′			79
12′		155.5	77
13′			68
14′		171.2	67

The yields of pigment in the [1,2-^{13}C]acetate experiment were low; only viomellein was isolated in sufficient

[15] F. W. Wehrli, *J.C.S. Chem. Comm.*, 1975, 663.
[16] T. J. Simpson, *Tetrahedron Letters*, 1975, 175.

amount for ^{13}C spectral determination, and owing to the poor signal-to-noise ratio obtained and to overlapping of signals it was not possible to resolve all the ^{13}C–^{13}C couplings. However, the results (Table 2) clearly indicate that viomellein is derived from fourteen intact acetate units, arranged as shown in the Scheme, suggesting formation of viomellein from two units, arising from alternate foldings of a common heptaketide chain, followed by introduction of C_1 units and oxidative coupling. Subsequent ring closure and hydroxylation would give rise to rubrosulphin and viopurpurin. The order



SCHEME Incorporation of acetate into viomellein

and timing of these operations is not clear, though in this respect it is noteworthy that viomellein is readily converted into rubrosulphin *in vitro*.[1]

Coupling of two napthoquinone moieties would give rise to xanthomegnin. In the structural assignment of xanthomegnin,[2] and hence of the remaining pigments, the 10-position for the methoxy-group was chosen in accord with the acetate hypothesis, and was not established from chemical evidence. The above spectral and biosynthetic evidence confirms this assignment, as the methoxy-group is shown to be adjacent to the C-11 carbonyl and on a carbon atom derived from a carboxy-group of acetate, as required by the acetate hypothesis.

EXPERIMENTAL

The isolation of pigments from cultures of *A. melleus* and *A. sulphureus* and the preparation of all derived compounds were effected as previously described.[1]

^{13}C *N.m.r. Determinations.*—The ^{13}C n.m.r. spectra were obtained for samples in acid-free deuteriochloroform with tetramethylsilane as internal reference. Proton noise-decoupled spectra, single frequency off-resonance decoupled spectra, and the spectra of ^{13}C enriched samples were obtained with a Varian XL100-15FT spectrometer operating at 25.197 MHz as previously described;[17] additional p.n.d. spectra and fully proton-coupled spectra were determined with a JEOL JNM FX-60 spectrometer operating at 15.04 MHz. Fully coupled spectra were determined under GATED-1 decoupling conditions, to retain nuclear Overhauser effects,[18] by using a 0.5 kHz noise modulated proton irradiating frequency of 57 dB. For specific decoupling experiments a continuous wave irradiating frequency of 17 dB was used. Trisacetylacetonatochromium [$Cr(acac)_3$; 0.1M] was used as relaxation agent.

Incorporations of Sodium [1-^{13}C]- *and* [1,2-^{13}C]-*Acetate.*—To each of two culture vessels containing a 7-day growth of *A. melleus* was added 90% [1-^{13}C]acetate (400 mg) or 90% [1,2-^{13}C]acetate (250 mg). After a further 4 days growth the mycelium was harvested and the pigments were isolated {viomellein (26 mg) and xanthomegnin (24 mg) from [1-^{13}C] acetate and viomellein (20 mg) from [1,2-^{13}C]acetate feedings, after purification}.

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[17] J. S. E. Holker, R. D. Lapper, and T. J. Simpson, *J.C.S. Perkin I*, 1974, 2135.

[18] R. Freeman and H. D. W. Hill, *J. Magnetic Resonance*, 1971, **5**, 278.