The Biosynthesis of Fungal Metabolites. Part IV.[1] Tajixanthone: ^{13}C Nuclear Magnetic Resonance Spectrum and Feedings with [1-^{13}C]- and [2-^{13}C]-Acetate

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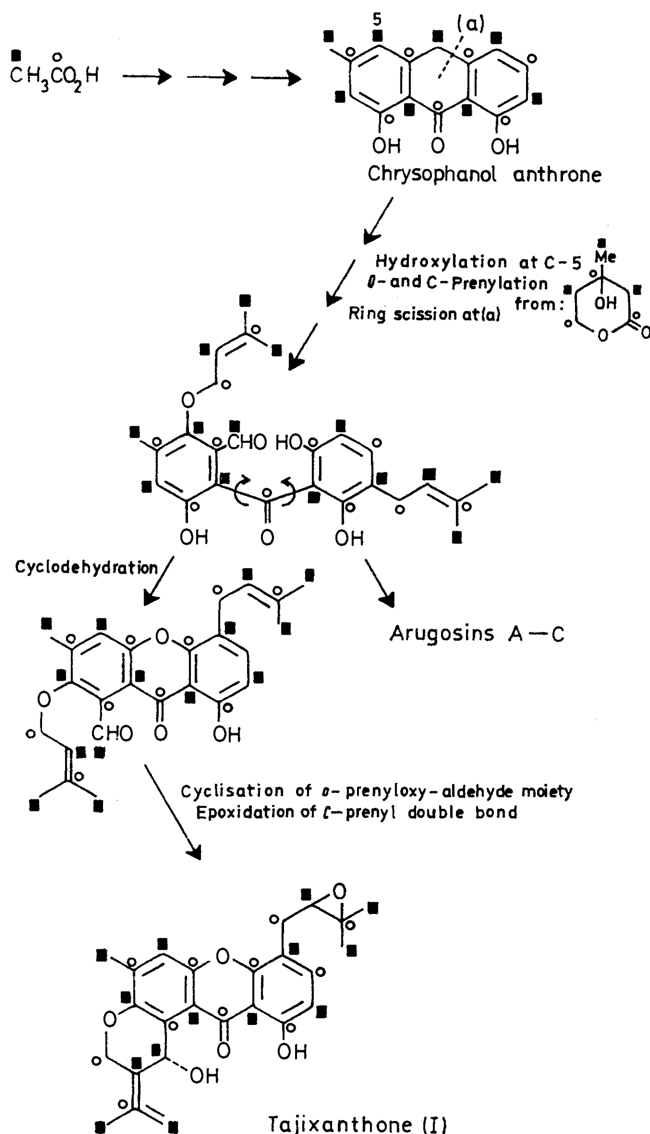
The ^{13}C N.m.r. spectra of tajixanthone (I) and related compounds (VI)—(XV) have been completely assigned Spectra of tajixanthone derived by incorporation of [1-^{13}C]- and [2-^{13}C]-acetate show patterns of enrichment consistent with a biosynthetic pathway in which the xanthone arises by ring scission of 1,8-dihydroxy-3-methyl-9-anthrone (chrysophanol anthrone), derived on a β-ketide pathway, with introduction of *C*- and *O*-prenyl units from mevalonate.

In a recent paper[1] we established the structures of tajixanthone (I) and shamixanthone (VI), metabolites of *Aspergillus variecolor*. Further studies[2] have shown that this organism contains a number of structurally related minor metabolites, together with the known compounds arugosins A (II) and B (III).[3] The structures of this group of compounds, together with arugosin C (IV),[4] suggest that they constitute a biogenetically related

[1] Part III, K. K. Chexal, C. Fouweather, J. S. E. Holker, T. J. Simpson, and K. Young, *J.C.S. Perkin I*, 1974, 1584.
[2] T. J. Simpson, unpublished observations.
[3] J. A. Ballantine, D. J. Francis, C. H. Hassall, and J. L. C. Wright, *J. Chem. Soc.* (*C*), 1970, 1175.
[4] J. A. Ballantine, V. Ferrito, C. H. Hassall, and M. L. Jenkins, *J.C.S. Perkin I*, 1973, 1825.

group: the xanthone system in tajixanthone (I) and shamixanthone (VI) could be formed by cyclodehydration of the *oo'*-dihydroxybenzophenone system present as a hemiacetal in arugosins A and B, the dihydropyran ring being formed by cyclisation of the *o*-prenyloxyaldehyde equivalent in (II) and (III). These proposals are summarised in the Scheme.



SCHEME

Proposed pathway to metabolites from acetate *via* chrysophano anthrone

It has been suggested [5] that the arugosins are biogenetically derived from an anthraquinone, such as islandicin (V), through oxidative cleavage of the quinone ring, by a pathway similar to those established for the biosynthesis of the benzophenone sulochrin from questin [6] and the ergot pigments from emodin.[7] A similar pathway has also been suggested for the biosynthesis of the fungal xanthone sterigmatocystin.[8] In the Scheme, we suggest that the actual precursor of the *A. variecolor* pigments may be chrysophanol anthrone, the probable precursor of chrysophanol [9] and islandicin.

In the present study we set out to obtain biogenetic evidence for the above proposals. Since it had been established that fungal anthraquinones, *e.g.* islandicin (V), are derived on the β-ketide pathway,[10] it appeared that incorporations of acetate into tajixanthone would provide such evidence. However, in view of the predicted difficulties of degrading the xanthone system in tajixanthone, necessary to establish labelling patterns from ^{14}C-labelled precursors, it was decided to use [1-^{13}C]- and [2-^{13}C]-acetate and to establish the specificity of incorporations by ^{13}C n.m.r. spectroscopy. Hence, the investigation required (i) the establishment of ^{13}C assignments for tajixanthone and (ii) studies to determine the necessary conditions for suitable incorporations of precursors into the pigment.

Assignments of ^{13}C *Resonances.*—The assignments were made by comparisons between resonances of tajixanthone (I) and the derived compounds (VI)—(XV), by use of off-resonance decoupling to determine the number of attached protons at each carbon atom, and by carrying out lanthanide-induced shift (LIS) studies on compounds (I), (VI), (VII), and (VIII), and were confirmed by comparison with literature values.[11-13] The assignments of resonances due to sp^3-hybridised carbon atoms, with chemical shifts less than 80 p.p.m. downfield from tetramethylsilane, were straightforward and the chemical shifts are listed in Table 1.

The assignment of resonances due to sp^2-hybridised atoms ($\delta_C > 100$ p.p.m.) was more difficult. The xanthone carbonyl (C-13) resonance in each compound is assigned in the range 177—184 p.p.m., and the high-field shift of this resonance on methylation of the 1-hydroxy-group is consistent with a loss of intramolecular hydrogen bonding.[14,15] The resonance due to the ester carbonyl group (C-20) in compound (XV) was identified by its typical shift of 168·1 p.p.m., and the αβ-unsaturated carbonyl (C-21) resonances of compounds (XIII) and (XIV) were also typical.

Resonances associated with protonated olefinic and aromatic carbon atoms were identified by their high

[5] C. H. Hassall, 'Genetics in Industrial Microorganisms,' eds. Z. Vaněk, Z. Hošťálek, and J. Cudlin, Academia, Prague, 1973, p. 297.

[6] R. F. Curtis, C. H. Hassall, and D. R. Parry, *J.C.S. Perkin I*, 1972, 240.

[7] B. Franck, F. Hüper, D. Gröger, and D. Erge, *Chem. Ber.*, 1968, **101**, 1954; D. Gröger, D. Erge, B. Franck, U. Ohnsorge, H. Flasch, and F. Hüper, *ibid.*, p. 1970.

[8] J. S. E. Holker and S. A. Kagal, *Chem. Comm.*, 1968, 1574.

[9] B. H. Howard and H. Raistrick, *Biochem. J.*, 1950, **46**, 49.

[10] S. Gatenbeck, *Acta Chem. Scand.*, 1960, **14**, 296.

[11] G. C. Levy and G. L. Nelson, 'Carbon-13 Nuclear Magnetic Resonance for Organic Chemists,' Wiley-Interscience, New York, 1972, and references cited therein.

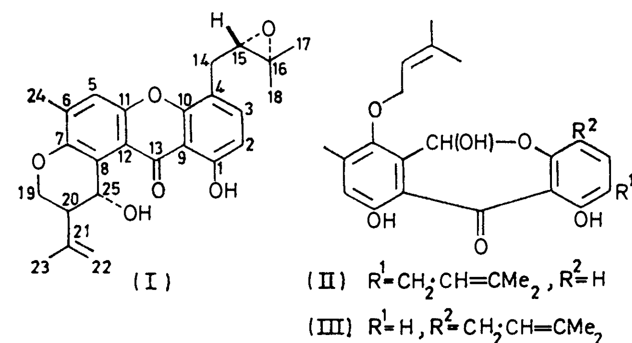
[12] J. B. Stothers, 'Carbon-13 NMR Spectroscopy,' Academic Press, New York, 1972, and references cited therein.

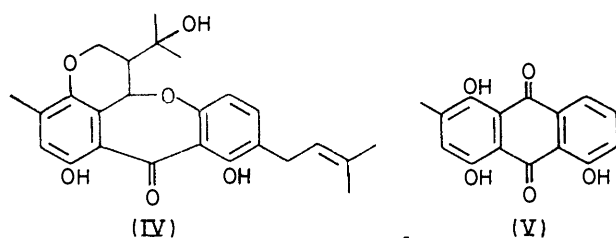
[13] L. F. Johnson and W. C. Jankowski, 'Carbon-13 NMR Spectra,' Wiley-Interscience, New York, 1972.

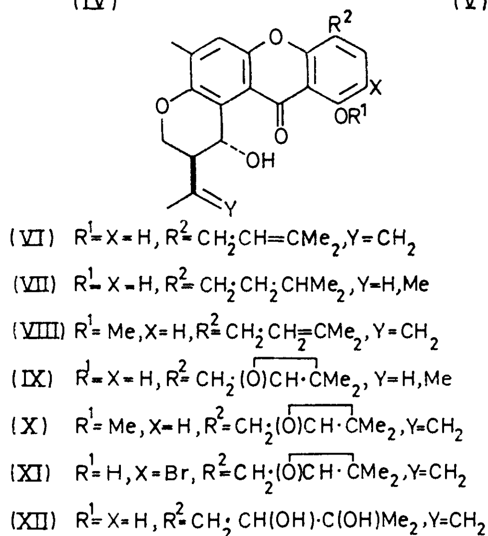
[14] P. C. Lauterbur, *Ann. New York Acad. Sci.*, 1958, **70**, 841.

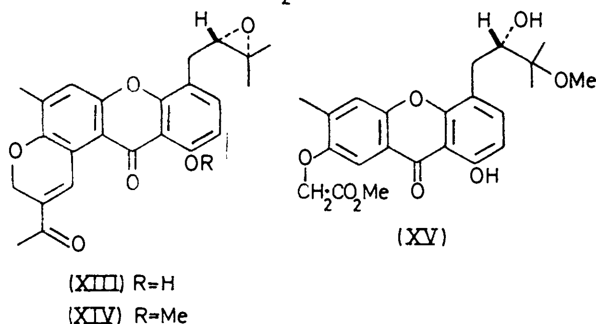
[15] G. E. Maciel, and G. B. Savitsky, *J. Phys. Chem.*, 1964, **68**, 437.

intensities in comparison with those of the quaternary carbon atoms, and assignments were confirmed by off-resonance decoupling. The C-22 methylene resonance









was thus assigned in compounds (I), (VI), (VIII), and (X)—(XII); the absence of this resonance in the spectra of dihydrotajixanthone (IX) and tetrahydroshamixanthone (VII) provided confirmatory evidence. The similar lack of the >C= resonance at 142·0 p.p.m. in the spectra of compounds (IX) and (VII) enabled the assignment of the C-21 signals. By similar reasoning the C-15 and C-16 olefinic resonances of shamixanthone (VI) were assigned.

The assignment of the signal due to the protonated aromatic carbon atom (C-5) in tajixanthone (I) was based on comparison with the 2-bromo-derivative (XI) in which this was the only unaffected protonated aromatic carbon atom. The signals due to the two remaining aromatic CH systems in tajixanthone are at 109·7 and 136·4 p.p.m.; the corresponding resonance for the 2-bromo-derivative is at 139·0 p.p.m. Since the substituent chemical shift (SCS) effects of a bromo-substituent [11,12] on an aromatic ring are expected to be *ca.* −6 p.p.m. at the attached carbon atom and *ca.* +3 p.p.m. at the *ortho*-positions, C-3 must correspond to the 136·4 and 139·0 p.p.m. signals and C-2 in (I) to the resonance at 109·7 p.p.m. By comparison C-2 in (XI) is assigned the signal at 101·9 p.p.m.

The nine >C= resonances of the aromatic rings present the greatest assignment difficulties. The use of additivity of SCS effects on aromatic rings requires caution in extrapolating from single ring to multi-ring aromatic systems, but does provide the following groupings: the four oxygen-substituted atoms at positions 1, 7, 10, and 11 correspond to the four signals always observed in the 148—160 p.p.m. range, and the carbon atoms at positions 4, 6, 8, 9, and 12 correspond to the remaining five resonances in the range 108—138 p.p.m. Comparisons of these latter five shifts in compounds (I) and (XIII), where the only change is in the function attached to C-8, show that the values of 108·7 and 114·8 p.p.m. in (I) are not altered significantly in (XIII). These two shifts must correspond to C-4 and C-9, which are the furthest from the point of change. Comparison of compounds (I), (VI), and (XII), in which the C-4 substituent is varied, shows that the 108·7 p.p.m. resonance is unchanged and so this may be assigned to C-9 (*meta* to the point of change). The 114·8 p.p.m. signal in (I), becoming 118·4 in (VI) and 115·9 p.p.m. in (XII), can then be assigned to C-4. The assignment of these resonances in the other derivatives then follows by comparison and has been confirmed by LIS studies for compound (VIII) (see Table 2) and by observing the SCS effect of 2-bromination in (XI).

Comparison of the spectra of compound (VI) and the derived methyl ether (VIII) shows that two of the four signals due to oxygen-bearing aromatic carbon atoms are significantly different, whereas the other two are relatively unaffected. The former two must be due to C-1 and C-10 and the latter to C-7 and C-11. LIS Studies (Table 2) of compounds (VI) and (VII) show a large down-field shift of the C-25 signal due to co-ordination of Eu(fod)$_3$ at the secondary hydroxy-group whereas in the methyl ether (VIII) this signal is relatively unaffected and the site of co-ordination is the oxygen atom at C-1. The LIS of 13·3 and 3·6 p.p.m. (downfield) in the signals at 157·9 and 153·9 p.p.m., respectively, for compound (VIII) establish that these signals are due to C-1 and

C-10, respectively. These latter assignments can then be applied to the other molecules in conjunction with SCS effects of 2-bromination [compound (VII)] and comparison of shifts.

For the remaining five resonances, associated with carbon atoms 6, 7, 8, 11, and 12, comparisons between chemical shifts of compounds (I), (VI), and (XIII) and their respective methyl ethers (VIII), (X), and (XIV) allow self-consistent groupings of shifts across the series but do not permit unambiguous assignments. However, the LIS effects in shamixanthone (VI) clearly define the C-8 and C-12 signals as a pair in the high-field region of the $\geq$C= band, leaving only the 137·6 p.p.m. resonance which must therefore be assigned to C-6. Distinction between signals due to C-7 and C-11 is based on comparison of compounds with structural changes at C-8. Thus, comparison between tajixanthone (I) and its methyl ether (X) on the one hand with compound (XIII) and its methyl ether (XIV) on the other, shows larger changes in chemical shift for one of the oxygen-bearing carbon atoms than the other. Since C-11 is *meta* to the point of change, it should be less affected than C-7, and hence the resonances for tajixanthone are assigned as those at 151·4 and 149·0 p.p.m., respectively.

TABLE 1

^{13}C Chemical shifts of a series of tajixanthone derivatives (in p.p.m. downfield from internal Me_4Si for $CDCl_3$ solutions; multiplicities are indicated for compounds for which off-resonance decoupled spectra have been obtained)

Carbon †	(I)	(VI)	(VII)	(VIII)	(IX)	(X)	(XI)	(XII)	(XIII)	(XIV)	(XV)
1	152·3	152·1	152·1	157·9	152·3	158·6	156·0	152·4	152·3	158·6	152·3
2	109·7d	109·3d	109·1d	104·3	109·7	104·7d	101·9	109·5d	109·9	104·7	109·3d
3	136·4d	135·8d	135·9d	133·5	136·3	134·3d	139·0	137·7d	136·5	134·3	137·7d
4	114·8	118·4	119·5	121·2	114·8	117·9	116·1 *	115·9	114·9	117·9	116·8
5	118·7d	118·7d	118·6d	118·3	118·6	118·3d	118·8	118·7d	121·2	120·8	119·2d
6	137·8	137·6	137·5	135·7	137·8	135·9	138·6	137·9	135·9	134·1	137·7
7	149·0	148·8	149·2	148·4	149·4	148·6	149·3	149·0	150·4	149·8	153·0
8	120·6	120·5	120·4	120·6	120·6	120·8	120·6	120·4	117·6	117·9	104·0d
9	108·7	108·7	108·7	112·2	108·9	112·3	109·3	108·7	108·9	112·4	108·1
10	159·7	159·2	159·1	153·9	159·9	154·1	156·0	159·5	159·9	154·1	159·7
11	151·4	151·5	151·4	149·9	151·4	149·9	151·4	151·3	151·1	150·3	150·5
12	116·4	116·4	116·7	119·0	116·8	119·1	116·7 *	116·3	113·4	115·8	118·2
13	183·6	183·6	183·6	179·2	183·6	179·0	183·0	183·5	183·0	177.3	181·0
14	28·5t	27·4t	26·6t	27·7	28·5	28·9t	28·4	31·9t	28·4	28·8	31·4t
15	63·0d	121·3d	39·2t	121·2	63·1	63·0d	62·9	77·4d	63·1	62·9	76·2d
16	58·5	132·6	27·7d	132·7	58·5	58·5	58·5	72·6	58·5	58·5	77·0
17 *	19·0q	17·9q	22·4q	17·8	19·0	19·0q	19·1	23·6q	19·0	19·0	19·2q
18 *	24·7q	25·7q	22·4q	25·6	24·7	24·7q	24·7	26·3q	24·7	24·7	20·8q
19	64·3t	64·4t	63·6t	64·0	63·6	64·0t	64·4	64·3t	63·1	62·9	65·3t
20	44·8d	44·9d	44·8d	44·6	44·9	44·6d	44·8	44·6d	130·5	130·0	168·1
21	142·0	142·1	25·5d	142·1	25·5	142·1	141·9	141·9	195·6	196·0	
22	111·8t	111·8t	21·2q *	111·6	21·2 *	111·6t	111·9	111·8t			
23	22·4q	22·5q	20·4q *	22·5	20·4 *	22·5q	22·4	22·4q	25·2	25·3	
24	17·3q	17·3q	17·2q	17·1	17·3	17·2q	17·5	17·3q	16·7	16·5	17·2q
25	63·0d	63·0d	62·4d	62·6	62·4	62·6d	62·9	62·9d	132·0	133·1	
1-OMe				56·1		56·3q				56·2	
OMe											49·1q
OMe											52·1q

* Assignments may be reversed. † For numbering system see formula (I).

No unambiguous distinction between signals due to C-8 and C-12 is possible, and the assignments of these in Table 1 are based on their different biogenetic origins as revealed in the enrichment studies.

Incorporation Studies and Discussion.—In order to obtain incorporations of ^{13}C-labelled precursors sufficiently large to be readily observed in ^{13}C n.m.r. spectra it is necessary to use much larger quantities of precursor than are usual in equivalent ^{14}C studies for which radioactivity is used as an assay method. In the present investigation the amounts of [1-^{13}C]- and [2-^{13}C]-acetate necessary for the feedings were calculated by determining the overall dilution of ^{14}C label in experiments with [1-^{14}C]acetate. Under our culture conditions [1] the required enrichment was obtained by using 1·0 g of acetate for every 25 g of sucrose in the medium.

The major disadvantages of loading the biological system with relatively large quantities of acetate precursor are: (i) the possible metabolic effects on the organism and (ii) the much greater opportunity for randomisation of label from [2-^{13}C]acetate *via* the tricarboxylic acid cycle than would occur in ^{14}C studies with small amounts of precursor. In the present investigation the yields of tajixanthone were not materially affected by the acetate additions and it is therefore unlikely that any major metabolic changes occur.

From the ^{13}C n.m.r. spectra of the [1-^{13}C]- and [2-^{13}C]-acetate-derived samples of tajixanthone the ^{13}C enrichments at individual positions were calculated as follows: If x, y, and z are the observed intensities of individual resonances for corresponding carbon atoms in the natural abundance spectra and those of the [1-^{13}C]- and [2-^{13}C]-acetate-derived samples, respectively, then intensities normalised to the natural abundance spectrum are given by $Y = (\bar{x}/\bar{y}')y$ for the [1-^{13}C]acetate-derived sample and $Z = (\bar{x}/\bar{z}')z$ for the [2-^{13}C]acetate-derived sample, where y' and z' are the resonance intensities for the expected unlabelled atoms in the [1-^{13}C]- and [2-^{13}C]acetate-derived samples respectively. This is a valid operation since all resonances in the spectrum of each enriched

sample are normalised by the same factor; the prior assumption of which carbon atoms are not expected to be enriched does not affect the overall operation. The method merely allows the spectrum of each enriched sample to be compared directly with the natural abundance spectrum, with automatic compensation for any randomisation of [2-^{13}C]acetate label. The enrichment for each carbon atom is given by $1{\cdot}1(Y - Z)/Z$ for [1-^{13}C]acetate-enriched positions and $1{\cdot}1(Z - Y)/Y$ for [2-^{13}C]acetate-enriched positions.

TABLE 2

^{13}C Resonance shifts induced by addition of $Eu(fod)_3$ [in p.p.m. downfield; the values quoted are the sum from four experiments with 1 : 20, 1 : 10, 3 : 20, and 1 : 5 ratios of $Eu(fod)_3$ to compound, *i.e.* the effective $Eu(fod)_3$-to-compound ratio in each case is 1 : 2]

Carbon	(I)	(VI)	(VII)	(VIII)
1	1·8	1·1	1·3	13·3
2	1·6	0·7	1·0	5·5
3	1·4	1·1	1·0	2·9
4	1·1	0·3	0·5	2·0
5	1·9	2·2	1·6	−0·5
6	2·0	2·3	2·0	2·2
7	2·7	2·8	2·4	1·0
8	4·0	4·3	4·1	2·0
9	1·6	2·0	1·0	6·8
10	2·0	1·4	1·1	3·6
11	2·2	2·4	1·9	0·6
12	6·0	7·6	6·4	1·0
13	4·3	4·8	4·6	4·1
14	2·8	0·4	0·4	1·1
15	6·3	0·0	0·1	−0·3
16	6·3	0·3	0·1	0·8
17	1·8	0·0	0·3	0·4
18	2·3	0·0	0·3	0·3
19	1·8	1·9	1·8	0·3
20	0·4	0·0	0·5	−0·2
21	0·9	0·5	1·4	0·1
22	1·7	1·4	1·0	0·2
23	0·4	0·2	0·5	0·0
24	0·7	0·8	0·9	0·5
25	13·2	16·6	14·7	1·0
OMe				19·3

The results thus calculated from the data in Table 3 are summarised in Table 4, and indicate that each carbon atom in tajixanthone is derived from either C-1 or C-2 of acetate. The wide range of values for each label is due to the relatively low enrichment obtained and the limited number of instrumental plot data points. Although individual differences have no biogenetic significance, it is clear that overall enrichment at positions derived from C-1 of acetate is larger than at those derived from C-2, despite the fact that the feedings of the two labelled precursors were carried out under identical conditions. This is probably a result of randomisation of C-2 label which would lead to an overall increase in ^{13}C abundance at ‘ unlabelled ’ positions, thereby reducing the apparent enrichment at labelled positions.

Since the coincidence of the C-15 and C-25 resonances at 63·0 p.p.m. in the spectrum of tajixanthone might have cast doubt on the origin of these two atoms, the spectrum of the metabolite obtained from the [2-^{13}C]-acetate experiment was redetermined after addition of a lanthanide shift reagent, which caused separation of the two resonances (Table 2). The spectrum clearly indicated that the two positions were enriched to approximately the same extent.

TABLE 3

Computer-listed relative intensities of individual signals in the ^{13}C n.m.r. spectra of tajixanthone

		Observed intensities ($\times 10^5$)		
Carbon	Signal (p.p.m.)	Natural	[2-^{13}C]-Acetate-derived	[1-^{13}C]-Acetate-derived
1	152·3	26	23	60
2	109·7	145	145	83
3	136·4	159	93	207
4	114·8	70	88	50
5	118·7	154	153	102
6	137·8	57	31	87
7	149·0	35	58	36
8	120·6	51	36	95
9	108·7	32	46	30
10	159·7	68	49	72
11	151·4	38	39	94
12	116·4	27	34	19
13	183·6	36	46	103
14	28·5	139	63	173
15	63·0	207 *	208 *	129 *
16	58·5	51	44	71
17	19·0	75	74	33
18	24·7	82	71	42
19	64·3	132	80	198
20	44·8	152	133	78
21	142·0	88	50	96
22	111·8	129	155	84
23	22·4	83	75	47
24	17·3	91	78	40
25	63·0	207 *	208 *	129 *

* Overall enrichment for C-15 and C-25.

TABLE 4

Excess ^{13}C abundance (%) at individual positions in [2-^{13}C]- and [1-^{13}C]-acetate-derived samples of tajixanthone (I)

Carbon	Signal (p.p.m.)	[2-^{13}C]	[1-^{13}C]
1	152·3		2·1
2	109·7	0·6	
3	136·4		1·5
4	114·8	0·6	
5	118·7	0·4	
6	137·8		1·9
7	149·0	0·5	
8	120·6		2·3
9	108·7	0·3	
10	159·7		0·7
11	151·4		1·9
12	116·4	0·6	
13	183·6		1·7
14	28·5		2·2
15	63·0	0·6 *	
16	58·5		0·8
17	19·0	1·1	
18	24·7	0·5	
19	64·3		2·0
20	44·8	0·5	
21	142·0		1·4
22	111·8	0·7	
23	22·4	0·5	
24	17·3	0·9	
25	63·0	0·6 *	

* Overall enrichment for C-15 and C-25.

The observed labelling pattern in tajixanthone is identical with that predicted in the Scheme, which shows the derivation of the xanthone system from an anthrone or anthraquinone precursor with introduction of two

prenyl units from mevalonate. In particular, the derivation of C-25 from the methyl group of acetate strongly suggests that this atom corresponds to the methylene group of the anthrone system in the precursor. Furthermore, the formation of the 20,25-bond in tajixanthone by linkage between two carbon atoms derived from the methyl groups of acetate is consistent with the proposed cyclisation of the *o*-prenyloxyaldehyde system in the precursor xanthone.

EXPERIMENTAL

The isolation of tajixanthone (I) from cultures of *A. variecolor* and the preparation of all derived compounds except (XI) and (XII) were effected as previously described.[1] All compounds were crystallised to constant m.p.

^{13}C *N.m.r. Determinations*.—The ^{13}C spectra were obtained for samples in acid-free deuteriochloroform ($\leqslant$0·3M depending on sample availability and solubility) with Me_4Si (2—5%) as internal reference, with a Varian XL-100-15FT spectrometer operating at 25·197 MHz; 12 mm tubes were used, with 5 mm coaxial tubes containing D_2O to provide the locking signal. In the case of ^{13}C-enriched samples of tajixanthone, 5 mm tubes were used, because of the small quantities available, and $CDCl_3$ was used to provide the locking signal. Sweep widths of 5120 Hz with 2048 plot data points were used throughout, to give chemical shift values accurate to within $\pm$2·5 Hz, *i.e.* $\pm$0·1 p.p.m. A pulse width of 30 μs corresponding to a ' tilt angle ' of the nuclear magnetisation vector of *ca.* 20° was used throughout and the computer data memory size (4096 addresses) limited the data acquisition time to 0·4 s. Both proton noise-decoupled and single-frequency off-resonance decoupled spectra were obtained to assist in determining the number of protons attached to each carbon atom. Tris-(1,1,1,2,2,3,3-heptafluoro-7,7-dimethyloctane-4,6-dionato)-europium(III) [$Eu(fod)_3$] was used as shift reagent and in each case spectra were run with the following molar ratios of $Eu(fod)_3$ to compound: 1 : 20, 1 : 10, 3 : 20, and 1 : 5.

^{14}C-*Radiochemical Assays*.—^{14}C-labelled tajixanthone was crystallised to constant molar radioactivity, which was determined with a Packard 3003 Tricarb Scintillation Spectrometer. Counting efficiencies were measured with [^{14}C]hexadecane as internal standard and using ' aqueous ' scintillator solution, prepared from 5-(biphenyl-4-yl)-2-(4-t-butylphenyl)-1,3,4-oxadiazole (Butyl-PBD) (10 g) in toluene (500 ml) and methanol (500 ml). The samples for counting were decolourised by reduction *in situ* with diborane, as previously described for rubropunctatin derivatives.[16]

Incorporations of Sodium [1-^{14}C]*Acetate*.—Preliminary experiments on cultures of *A. variecolor* (I.M.I. strain 112543) grown in static culture as previously described [1] showed that tajixanthone production commenced on the sixth day after inoculation from a spore suspension and reached a maximum on the fourteenth day. Furthermore, feedings of [1-^{14}C]acetate showed that maximum incorporation into tajixanthone occurred when the label was introduced 5 days after inoculation and the compound was isolated from 14-day cultures.

When sodium [1-^{14}C]acetate (1 g; 0·60 μCi mmol^{-1}) was added to a culture vessel containing a 5-day growth of the organism on Czapek-Dox medium (500 ml; 5% sucrose) and the mycelium was harvested after a further 9 days, tajixanthone (*ca.* 20 mg; 0·13 μCi mmol^{-1}) was obtained. This corresponds to a dilution factor for the label of 4·6. On this basis it would be anticipated that equivalent feedings of [1-^{13}C]acetate (90%) would give tajixanthone with an excess of 1·8% of ^{13}C-label over natural abundance at each labelled position (assuming a total of 11 labelled positions in the molecule).

Incorporations of Sodium [1-^{13}C]- *and* [2-^{13}C]-*Acetate*.—To each of two culture vessels containing a 5-day growth of *A. variecolor* was added [1-^{13}C]- or [2-^{13}C]-acetate (1 g; 92·1 and 90·2% enriched, respectively). After a further 9 days the mycelium was harvested and the tajixanthone isolated (24 mg from [1-^{13}C]-acetate and 20 mg from [2-^{13}C]-acetate feedings, after purification). These samples were used for ^{13}C n.m.r. studies.

2-*Bromotajixanthone* (XI) (with K. YOUNG).—A solution of tajixanthone (100 mg) and *N*-bromosuccinimide (50 mg, 1·2 mol. equiv.) in carbon tetrachloride (30 ml) was heated under reflux for 4 h. The precipitated succinimide was filtered off and the filtrate evaporated to dryness. The residue was purified by t.l.c. [silica gel GF (Merck)] with ether–benzene (5 : 95 v/v) as developing solvent. The band of R_F 0·40 was separated and crystallised from ethanol–chloroform to give fine yellow needles (84 mg) of 2-*bromotajixanthone*, m.p. 181—183°, $\nu_{max.}$ (KBr) 3480, 1639, 1598, 1570, 1475, and 1240 cm^{-1}, $\lambda_{max.}$ 245, 254, 272sh, 278, 297sh, and 392 nm (log ε 3·95, 4·25, 4·42, 4·47, 4·01, and 3·28), τ ($CDCl_3$) −2·17 (1-OH, s, exchangeable), 2·29 (3-H, s), 2·73 (5-H, s), and 5·23 (25-OH, d, exchangeable) with other resonances as in tajixanthone [1] (Found: M^+, 502·082/500·081; C, 59·2; H, 5·0%. $C_{25}H_{25}BrO_6$ requires *M*, 502·082/500·081; C, 59·9; H, 5·0%).

Tajixanthone ' Hydrate ' (XII) (with K. YOUNG).—Tajixanthone (50 mg) in dioxan (24 ml) and oxalic acid dihydrate (6 mg) in water (6 ml) were mixed and heated under reflux for 15 h. After dilution with water (100 ml), the product was isolated in ethyl acetate (3 × 25 ml); the solution washed with water, dried (Na_2SO_4), and evaporated. The residue was purified by t.l.c. [Silica Gel GF (Merck)] with ether–benzene (20 : 8 v/v) as developing solvent. The band of R_F 0·10 was separated and crystallised from methanol to give yellow needles (38 mg) of *tajixanthone ' hydrate '*, m.p. 195—196°, $[\alpha]_D$ −71·5° (*c* 2·3 in $CHCl_3$), $\nu_{max.}$ (KBr) 3495br, 1645, 1600, 1580, 1475, 1245, and 1050 cm^{-1}, $\lambda_{max.}$ 241, 258, 270sh, 274, 297, and 393 nm (log ε 3·48, 3·47, 4·58, 4·61, 4·11, and 3·94), τ ($CDCl_3$) −2·51 (1-OH, s, exchangeable), 6·32 (15-H, unresolved m), 6·85 (14-H, unresolved m), *ca.* 7·3 (14-H, unresolved m), and *ca.* 7·7 (15- and 16-OH, exchangeable), with other resonances as in tajixanthone [1] (Found: C, 67·9; H, 6·4. $C_{25}H_{28}O_7$ requires C, 68·2; H, 6·4%).

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[16] J. R. Hadfield, J. S. E. Holker, and D. N. Stanway, *J. Chem. Soc.* (*C*), 1967, 751.

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