The Biosynthesis of Fungal Metabolites. Part VI.[1] Structures and Biosynthesis of Some Minor Metabolites from Variant Strains of *Aspergillus variecolor*

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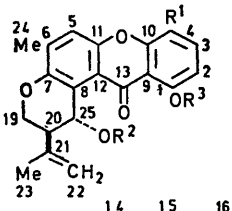
New minor metabolites from variant strains of *A. variecolor* are shown to be (1*R*,2*S*)-8-[(2*S*)-2,3-dihydroxy-3-methylbutyl]-2,3-dihydro-1,11-dihydroxy-2-isopropenyl-5-methyl- (III), (1*R*,2*S*)-2,3-dihydro-1,11-dihydroxy-8-[(2*S*)-2-hydroxy-3-methoxy-3-methylbutyl]-2-isopropenyl-5-methyl- (IV), 1,2-*trans*-1-acetoxy-8-(2,3-epoxy-1-hydroxy-3-methylbutyl)-2,3-dihydro-11-hydroxy-2-isopropenyl-5-methyl- (V), 1,2-*trans*-1-acetoxy-8-(2,3-epoxy-1-methoxy-3-methylbutyl)-2,3-dihydro-11-hydroxy-2-isopropenyl-5-methyl- (VI), and 1,2-*cis*-2,3-dihydro-1,11-dihydroxy-2-isopropenyl-5-methyl-10-(3-methylbut-2-enyl)-pyrano[3,2-*a*]xanthen-12(1*H*)-one (X), 1,10-dihydroxy-6-methoxy-8-methyl-2-(3-methylbut-2-enyl)-7-(3-methylbut-2-enyloxy)-dibenz[*b.e*]oxepin-11(6*H*)-one (XVIII), and 1,12a-dihydro-6,8-dihydroxy-1-(1-hydroxy-1-methylethyl)-4-methyl-9-(2-hydroxy-3-methylbut-3-enyl)[1]benzopyrano[4,5-*bc*][1]benzoxepin-7(2*H*)-one (XXI). A biogenetic relationship is suggested which explains the co-occurrence of these compounds with other metabolites of known structures.

In fungal metabolism it is sometimes possible to obtain an insight into the biosynthesis of the major secondary metabolites by examining the co-occurrence of minor metabolites, which may either be biogenetic intermediates or derived from them on 'shunt' pathways. Having already established the structures of shamixanthone (I),[2] tajixanthone (II),[2] and variecoxanthones A—C, (XIII)—(XV), respectively,[1] and having studied the biogenetic origins of the individual carbon atoms in tajixanthone from [^{13}C]acetate,[3] we have now undertaken a study of the minor metabolites of nine variant strains of *A. variecolor* in an attempt to throw light on the biogenesis of these and the related compounds arugosins A—C, (XVI), XVII), and (XX), respectively.[4,5] During this work we have characterised seven new metabolites: tajixanthone hydrate (III) and methanolate (IV), 14-hydroxy- (V) and 14-methoxy-tajixanthone 25-acetate (VI), epi-isoshamixanthone (X), 25-*O*-methylarugosin A (XVIII), and arugosin D (XXI).†

The small quantities of these compounds available for investigation necessitated their identification by spectroscopic methods. However, since these substances are structurally related to other known compounds in the series, the use of these methods was entirely reliable. In particular, their u.v. spectra enabled them to be classified as xanthones, *i.e.* (III)—(VI) and (X), or dibenzoxepins of the arugosin type, *i.e.* (XVIII) and (XXI). Detailed comparisons (Table 1) of their ^{1}H n.m.r. spectra with those of shamixanthone (I), tajixanthone (II), and arugosin C (XVIII) then allowed confident structural assignments to be made.

By these methods the structures of tajixanthone hydrate (III) and methanolate (IV) and 25-*O*-methylarugosin A (XVIII) became apparent and these were readily confirmed by interconversions with known compounds. Tajixanthone hydrate and methanolate were prepared from tajixanthone (II) by acid-catalysed hydrolysis and methanolysis respectively. The compounds obtained had specific rotations identical with those of the respective natural products (III) and (IV), and, since it would be expected that these reactions would



(I) $R^1 = CH_2 \cdot {}^{14}CH = {}^{15}C(Me)_2$, $R^2 = R^3 = H$

(II) $R^1 = CH_2 \cdot CH(O)C(Me)_2$, $R^2 = R^3 = H$

(III) $R^1 = CH_2 \cdot CH(OH) \cdot C(OH)Me_2$, $R^2 = R^3 = H$

(IV) $R^1 = CH_2 \cdot CH(OH) \cdot C(OMe)Me_2$, $R^2 = R^3 = H$

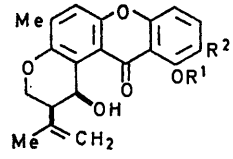
(V) $R^1 = CH(OH) \cdot CH(O)C(Me)_2$, $R^2 = Ac$, $R^3 = H$

(VI) $R^1 = CH(OMe) - CH(O)C(Me)_2$, $R^2 = Ac$, $R^3 = H$

(VII) $R^1 = CH_2 \cdot CH(O)C(Me)_2$, $R^2 = Ac$, $R^3 = Me$

(VIII) $R^1 = CH(OMe) \cdot CH(OH) \cdot C(OH)Me_2$, $R^2 = Ac$, $R^3 = Me$

(IX) $R^1 = CH(OMe) \cdot CHO$, $R^2 = Ac$, $R^3 = Me$



(X) (or mirror image) $R^1 = H$, $R^2 = CH_2 \cdot CH = CMe_2$

(XI) (or mirror image) $R^1 = Me$, $R^2 = CH_2 \cdot CH = CMe_2$

(XII) $R^1 = R^2 = H$

occur with retention of configuration at C-15, the relative and absolute stereochemistry of the natural products is established.

† The illustrated numbering of the carbon atoms in these compounds accords with that originally devised for arugosin C[5] and adopted by us in previous papers.[1-3]

[1] Part V, K. K. Chexal, J. S. E. Holker, T. J. Simpson, and K. Young, preceding paper.

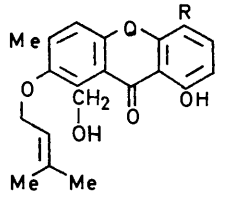
[2] K. K. Chexal, C. Fouweather, J. S. E. Holker, T. J. Simpson, and K. Young, *J.C.S. Perkin I*, 1974, 1584.

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

[4] J. A. Ballantine, D. J. Francis, C. H. Hassall, and J. L. C. Wright, *J. Chem. Soc.* (*C*), 1970, 1175.

[5] J. A. Ballantine, V. Ferrito, C. H. Hassall, and M. L. Jenkins, *J.C.S. Perkin I*, 1973, 1825.

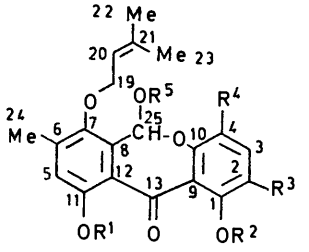
The methyl acetal, 25-*O*-methylarugosin A (XVIII), was prepared from the known racemic hemiacetal mixture of arugosins A and B [(XVI) and (XVII), respectively]



(XIII) R = H

(XIV) R = $CH_2 \cdot CH{=}CMe_2$

(XV) R = $CH_2 \cdot CH(O)CMe_2$

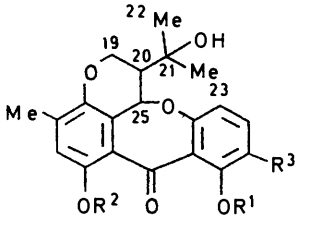


(XVI) $R^1 = R^2 = R^4 = R^5 = H$, $R^3 = CH_2 \cdot CH{=}CMe_2$

(XVII) $R^1 = R^2 = R^3 = R^5 = H$, $R^4 = CH_2 \cdot CH{=}CMe_2$

(XVIII) $R^1 = R^2 = R^4 = H$, $R^3 = CH_2 \cdot CH{=}CMe_2$, $R^5 = Me$

(XIX) $R^1 = R^2 = R^5 = Me$, $R^3 = CH_2 \cdot CH{=}CMe_2$, $R^4 = H$



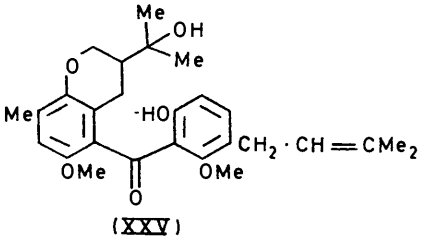
(XX) $R^1 = R^2 = H$, $R^3 = CH_2 \cdot CH{=}CMe_2$

(XXI) $R^1 = R^2 = H$, $R^3 = CH_2 \cdot CH(OH) \cdot CMe{:}CH_2$

(XXII) $R^1 = R^2 = Me$, $R^3 = CH_2 \cdot CH{=}CMe_2$

(XXIII) $R^1 = R^2 = H$, $R^3 = CH_2 \cdot CH_2 \cdot CHMe_2$

(XXIV) $R^1 = R^2 = Me$, $R^3 = CH_2 \cdot CH{=}CMe_2$



(XXV)

with methanol and hydrochloric acid, giving an orange gum identical with the natural product. The hemiacetals arugosins A and B exist as a 2 : 1 equilibrium mixture [4] but, on formation of the methyl acetal, only one isomer is obtained. Structure (XVIII) is assigned to this on the basis of the following evidence. Methylation with methyl iodide and potassium carbonate in acetone gave the dimethyl ether (XIX) in which the introduced *O*-methyl groups have ^1H chemical shifts of τ 6·20 and 6·24 in $CDCl_3$ and 6·08 and 6·72 in C_6D_6. The upfield shift of only one of these two signals in C_6D_6 indicates that only one of the two introduced methoxy-groups has a free *ortho*-position.[2,6] Since this must be the 11-methoxy-group, that at the 1-position must be flanked by two *ortho*-substituents, *i.e.* the *C*-prenyl residue must be at C-2. We also observe similar chemical shift differences with arugosin C dimethyl ether [5] (XXIV), *i.e.* τ_{OMe} 6·19 and 6·27 in $CDCl_3$, and 6·13 and 6·65 in C_6D_6. Presumably the formation of only one acetal from the equilibrium mixture of arugosins A and B is due to the increase in steric crowding on replacing the C-25 hydroxy-group by OMe, sufficient to displace the equilibrium towards isomer (XVIII) in which there is no steric interaction between the C-25 methoxy- and *C*-prenyl groups. The methyl acetal (XVIII) does not appear to be an artefact formed by using methanol in the isolation of the natural product, since when this was replaced by ethanol we were unable to detect the corresponding ethyl acetal.

The original tentative assignment of structure (XVI) to the predominant isomer, arugosin A,[4] in the equilibrium mixture of A and B is supported by the following evidence. Although the ^1H n.m.r. spectrum of the arugosin A and B mixture in $CDCl_3$ shows the 14-protons of both isomers with the same chemical shift, τ 6·74 (J 8 Hz); in C_6D_6 the signals separate and occur at τ 6·54 and 6·70 (J 8 Hz in each case) in the ratio 2 : 1, respectively. Since the methyl acetal (XVIII) has this signal at 6·54 in C_6D_6, then the predominant isomer in the arugosin A and B mixture must also have the *C*-prenyl group at C-2.

In the original publication on arugosin C (XX),[5] it was reported that the small quantity of material prevented confirmation of the structure by conventional degradative techniques. Evidence for the presence of the benzyl ether grouping at C-25 has now been obtained by reductive fission of the C(25)–O bond. Thus, although in attempted hydrogenolysis experiments arugosin C gave only the dihydro-derivative (XXIII), fission of the dimethyl ether (XXII) occurred on reduction with sodium in liquid ammonia giving in low yield a compound which contains both a chelated phenolic and a tertiary hydroxy-group (τ −2·62 and 8·70, respectively). Hence, this compound is the benzophenone (XXV) and correspondingly, arugosin C must be (XX).

The ^1H n.m.r. spectrum of arugosin D shows that it is closely related to arugosin C, the only differences being associated with the five carbon atoms of the *C*-prenyl residue, *cf.* (XX) and (XXI) in Table 1. However, since very small amounts of arugosin D were available for in-

[6] A. Pelter, R. Warren, K. K. Chexal, B. K. Handa, and W. Rahman, *Tetrahedron*, 1971, **27**, 1625, and references cited therein.

vestigation, an accumulated spectrum only was obtained and although this strongly suggested that the compound contained a 2-hydroxy-3-methylbut-3-enyl residue, the couplings were not sufficiently well resolved to confirm this feature. However, this residue was also present in D and compound (XXVII) show similar direct losses of C_4H_7O, supported by the requisite metastable ions. Since this loss has not been observed in any other compound in the series, it presumably arises from this new structural feature which must therefore be present in

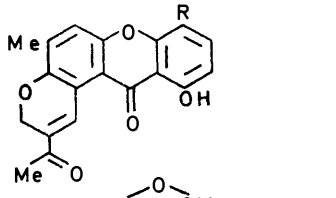
TABLE 1

^{1}H N.m.r. chemical shifts of metabolites in $CDCl_3$, including values for the known compounds (I), (II), and (XX) to facilitate comparisons [couplings in Hz where the values are different, or additional to those in (I), (II), and (XX)]

Proton(s)	(I)	(II)	(III)	(IV)	(V)	(VI)	(X)	(XVIII)	(XX)	(XXI)
1-OH	−2·58	−2·60	−2·51	−2·58	−2·96	−2·03	−2·86	−0·83 or −2·43	−0·65 or −3·84	−0·60 or −3·88
2	3·31	3·31	3·31	3·26	3·20	3·09				
3	2·60	2·61	2·61	2·53	2·22	2·31	2·57	2·71	2·76	2·70
5	2·75	2·74	2·82	2·98	2·73	2·72	2·80	3·14	3·20	3·20
				6·89						
			6·85	7·32	4·90	5·40				
14	6·65	6·97					6·65	6·65	6·70	*ca.* 7·10
			7·30	($J_{14.14}$ 14; $J_{14.15}$ 2) and 9)	($J_{14.15}$ 7)	($J_{14.15}$ 7)				
15	4·71	6·97	6·32	6·24	6·90	6·84	4·74	4·68	4·68	*ca.* 5·65
17 and 18	8·24	8·57	8·62	8·67	8·52	8·70	8·25	8·12	8·25	8·20
	8·27	8·69	8·67	8·74	8·70	8·77		8·18	8·28	{5·00 5·16
					5·44	5·49	5·57			
					5·69	5·71	5·74		5·67	
19	5·64	5·62	5·65	5·63				5·67	5·87	*ca.* 5·7
					($J_{19.19}$ 11; $J_{19.20}$ 2 and 3)	($J_{19.19}$ 11; $J_{19.20}$ 2 and 3)	$J_{19.19}$ 10; $J_{19.20}$ 5·5 and 10)			
20	7·29	7·30	7·25	7·25	7·22	7·27	7·50	4·48	7·70	*ca.* 7·58
22	5·42	5·42	5·42	5·20			5·25			
	5·24	5·22	5·46	5·41	5·20	5·23	4·98	{8·26	{8·70	{8·70
23	8·24	8·19	8·19	8·15	8·10	8·11	8·02	8·31	8·75	8·76
24	7·69	7·69	7·70	7·65	7·65	7·66	7·70	7·66	7·78	7·80
25	4·61	4·60	4·66	4·60	3·08	3·09	4·55 ($J_{20.25}$ 3·6)	3·63	4·97	4·90
Others	4·91 (25-OH)	4·89 (25-OH)	5·02 (25-OH) *ca.* 7·7 (15- and 16-OH's)	5·00 (25-OH) 7·56 (15-OH) 6·71 (16-OMe)	7·25 (14-OH) 7·93 (25-OAc)	6·66 (14-OMe) 7·94 (25-OAc)	3·21 (4-H) 5·45 (25-OH) ($J_{25.25OH}$ 3·5)	3·50 (4-H) −2·43 or −0·83 (11-OH) 6·66 (25-OMe)	3·60 (4-H) −3·84 or −0·65 (11-OH) *ca.* 8·5 (21-OH)	3·58 (4-H) −3·88 or −0·60 (25-OH) †

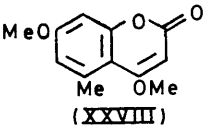
† 15- and 21-OH signals not seen in accumulated spectrum.

the compound (XXVII), which we obtained by isomerisation of the previously reported αβ-unsaturated ketone (XXVI) [2] by a trace of acetic acid in hot ethyl acetate,



(XXVI) R = $CH_2{\cdot}CH{-}CMe_2$ (O bridge)

(XXVII) R = $CH_2{\cdot}CH(OH)\ CMe:CH_2$



(XXVIII)

and in this case the ^{1}H n.m.r. spectrum clearly confirmed the structure, *i.e.* $\cdot CH_2{\cdot}CH(OH)\cdot$ shows an ABX pattern with τ_A 7·16, τ_B 6·88, τ_X 5·66 (J_{AB} 14, J_{AX} 5, J_{BX} 8 Hz) and the isopropenyl group has τ 5·10, 5·18 (methylene protons), and 8·14 (vinyl methyl). Since arugosin D has closely similar chemical shifts for this five-carbon residue, it also contains the 2-hydroxy-3-methylbut-3-enyl group. Furthermore the mass spectra of both arugosin D and compound (XXVII) show similar direct losses of C_4H_7O, supported by the requisite metastable ions. Since this loss has not been observed in any other compound in the series, it presumably arises from this new structural feature which must therefore be present in arugosin D. Hence, we propose structure (XXI) for this compound, in which, by analogy with arugosin C, the modified prenyl group is located at C-2 of the dibenzoxepin ring.

14-Hydroxy- (V) and 14-methoxy-tajixanthone 25-acetate (VI) are two further metabolites with modified *C*-prenyl residues. The 14-hydroxy-group in the former compound is indicated by the ^{1}H n.m.r. spectrum which shows a single proton at C-14, τ 4·90 ($J_{14.15}$ 7 Hz) (sharpening on addition of D_2O); the equivalent proton in the latter compound resonates at τ 5·40 ($J_{14.15}$ 7 Hz) with the methoxy-signal at τ 6·66. In both compounds the 15-, 17-, and 18-protons have chemical shifts closely similar to those in tajixanthone (II). The close similarities between the chemical shifts and couplings of the 19-, 20-, and 25-protons in both these new compounds and the known *O*-methyltajixanthone 25-acetate (VII) [2] confirm the presence of the 1,2-*trans*-1-acetoxy-2-isopropenyldihydropyran residue, although the absolute configuration in this residue and at C-14 has not been determined owing to shortage of material. The structure of the 2,3-epoxy-1-methoxy-3-methylbutyl residue in compound (VI) was

confirmed by acid-catalysed hydration leading to compound (VIII), containing the 2,3-dihydroxy-1-methoxy-3-methylbutyl residue. Oxidation of this with lead tetra-acetate gave the α-methoxy-aldehyde (IX).

Epi-isoshamixanthone (X) was isolated in minor amounts from one strain only of *A. variecolor* (IMI 53749). Its i.r., u.v., and ^{1}H n.m.r. spectra were closely similar to those of shamixanthone (I). Indeed the only marked differences occurred in the 19-, 20-, and 25-^{1}H couplings, *i.e.* $J_{19,19}$ 11, $J_{19,20}$ 5·5 and 10, and $J_{20,25}$ 3·6 Hz for (X). These values are identical with those for (±)-de-*C*-prenylepishamixanthone (XII), which has been shown to contain a 1,2-*cis*-1-hydroxy-2-isopropenyldihydropyran residue.[1] Hence, compound (X) must also have the corresponding *cis*-relationship. Unlike that of shamixanthone (I), the mass spectrum of epi-isoshamixanthone showed an ion due to loss of 56 mass units. Since this is indicative of an *ortho*-hydroxyprenyl function,[4] it seemed likely that compound (X) contained the *C*-prenyl residue at C-2. This was confirmed by comparison of the ^{1}H n.m.r. spectra of the derived methyl ether (XI) in C_6D_6 and $CDCl_3$, which showed an upfield shift of the methoxy-signal [$\tau(C_6D_6) - \tau(CDCl_3)$ 0·20 p.p.m.] smaller than is required for a compound containing a free position *ortho* to the methoxy-group,[7] *cf.* 0·36—0·50 p.p.m. for *O*-methyltajixanthone [2] (XIX) and (XXIV). Hence epi-isoshamixanthone has structure (X), in which the absolute configuration has not been determined owing to lack of material.

The pattern of occurrence and relative quantities of the biogenetically related dibenzoxepins and xanthones from the various strains of *A. variecolor* are summarised in Table 2, which also lists the major metabolite, 4,7-dimethoxy-5-methylcoumarin (XXVIII) from strain IMI 53749. This biogenetically unrelated compound is discussed separately in the following paper.

We have suggested [3] that shamixanthone (I), tajixanthone (II), and arugosins A—C, (XVI), (XVII), and (XX), respectively, are derived biogenetically from chryosphanol anthrone by oxidative ring fission and introduction of *O*- and *C*-prenyl units from mevalonate, to give the *O*-formylbenzophenone from which arugosins A and B are derived by hemiacetal formation, and shamixanthone by further transformations. The co-occurrence of these compounds in several strains of *A. variecolor* (Table 2) tends to support this suggestion. Furthermore, equivalent oxidative fission has been shown to occur in the biosynthesis of the benzophenone sulochrin from the anthraquinone 5-*O*-methylemodin.[7,8] Although we have not been able to detect either anthrone or anthraquinone precursors in strains of *A. variecolor*, it is possible that the biological oxidation of such a precursor to arugosins A and B is so efficient as to prevent its accumulation.

The biosynthesis of shamixanthone (I) from arugosins A and B, (XVI) and (XVII), requires (*a*) cyclodehydration to give the xanthone ring and (*b*) cyclisation of the *o*-prenyloxy-aldehyde unit to the substituted dihydropyran ring, but it is not clear which of these reactions occurs first. Isolation of the variecoxanthones (XIII)—(XV) [1] tended to suggest that prior formation of the xanthone ring occurred and that these compounds were then formed by subsequent reduction of the benzaldehyde groups to the corresponding benzyl alcohols. However, although the aldehyde corresponding to variecoxanthone A readily cyclises *in vitro* to give a compound containing the requisite dihydropyran ring, this stereospecific isomerisation gives only the product in which the isopropenyl and hydroxy groups are *cis*-related [1] whereas in shamixanthone they are *trans*-related.[2] If the equivalent *in vivo* cyclisation follows the same stereochemical course it seems unlikely that shamixanthone arises in this way, although the minor metabolite epi-isoshamixanthone (X) may well do so. We have suggested that the *trans*-relationship in shamixanthone could arise if the dihydropyran ring is formed before the xanthone ring.[1] However, none of the metabolites isolated contains the dihydropyranobenzophenone structure which would support this hypothesis. On the other hand, it is probable that arugosin C (XX) arises from such an intermediate by the alternative cyclodehydration between C(25)–OH and C(10)–OH to the dibenzoxepin system.

The metabolites tajixanthone (II), the hydrate (III) and methanolate (IV), and the hydroxy- and methoxy-

TABLE 2

Distribution of metabolites in strains of *A. variecolor* (in mg l^{-1})

	Mycelium (g l^{-1})	(I)	(II)	(XIII)	(XIV)	(XVI) and (XVII)	(XVIII)	Others
IMI 112543	15·0	*ca.* 30	*ca.* 70		10·0	15·0	1·0	(III), 10; (IV), 15; (V) 5
CBS 134·55	5·5	6·0	9·6	8·9		11·0		
CBS 135·55 [1]	6·0	1·45		11·5	0·7	2·05		(XV), 0·8; sterigmatocystin,* 5·0
IMI 53749	7·6	2·0				2·3	0·6	(X), 0·45; (XX), 36; (XXI), 0·03; (XXVIII), 100;† sterigmatocystin,* 0·9
IMI 77894	7·0			45				
IMI 136·778	4·9	8·0	16·0			8·6		(V) 4·2; (VI), 15·0
IMI 146·289	6·9	7·3	11·0		6·1	11·0		

A. variecolor IMI 60316 and 75129 produced mycelium (4·9 and 6·1 g l^{-1}) but no metabolites could be detected.

* E. Bullock, J. C. Roberts, and J. G. Underwood, *J. Chem. Soc.*, 1962, 4179. † Isolation and structure elucidation in the following paper.

[7] S. Gatenbeck and L. Malmström, *Acta Chem. Scand.*, 1969, **23**, 3493.

[8] R. F. Curtis, C. H. Hassall, and D. R. Parry, *Chem. Comm.*, 1970, 1512.

derivatives (V) and (VI), respectively, appear to be derived biogenetically from shamixanthone by oxidations of the *C*-prenyl residue. Presumably epoxidation to tajixanthone occurs first and this is the parent of the other compounds. Arugosin D (XXI) would be formed similarly from arugosin C (22), by rearrangement of a hypothetical epoxide.

EXPERIMENTAL

The general methods used for spectral measurements are identical to those outlined in Part V.[1] Spectral data indicated with an asterisk are listed in Supplementary Publication No. SUP 21245 (9 pp.).†

Isolation of Metabolites.—The cultures of the individual strains of *A. variecolor* were made and the isolations of the mycelial metabolites from the methanol-soluble fractions of the light petroleum extracts were carried out as reported for shamixanthone and tajixanthone in Part III. The individual metabolites were separated by preparative t.l.c. on silica gel GF (Merck) and known metabolites were characterised by i.r., u.v., and ^{1}H n.m.r. spectra (also by m.p. and mixed m.p. for crystalline compounds). In the case of arugosin C final purification was effected by chromatography on Sephadex LH-20 as previously described.[5] The amounts of the purified metabolites from each strain are summarised in Table 2, which also contains comparative data for strain CBS 135·5, previously reported in Part V.[1]

Tajixanthone Hydrate (III) (with K. Young).—Isolated from mycelium, this *compound* {(1R,2S)-8-[(2S)-2,3-*dihydroxy*-3-*methylbutyl*]-2,3-*dihydro*-1,11-*dihydroxy*-2-*isopropenyl*-5-*methylpyrano*[3,2-a]*xanthen*-12(1H)-*one*} formed yellow needles from acetone, m.p. and mixed m.p. 195—196° [with a sample prepared by acidic hydrolysis of tajixanthone (II) as previously described [3]].

Tajixanthone Methanolate (IV).—(*a*) Isolated from mycelium, this *compound* {(1R,2S)-2,3-*dihydro*-1,11-*dihydroxy*-8-[(2S)-2-*hydroxy*-3-*methoxy*-3-*methylbutyl*]-2-*isopropenyl*-5-*methylpyrano*[3,2-a]*xanthen*-12(1H)-*one*} formed yellow needles from acetone, m.p. 190—191°, $[\alpha]_D$ −65·8° (*c* 5·6 in $CHCl_3$), $\nu_{max.}$,* $\lambda_{max.}$,* *m/e* * (Found: C, 68·6; H, 6·8%; M^+, 454·196. $C_{26}H_{30}O_7$ requires C, 68·7; H, 6·7%; *M*, 454·198).

(*b*) Tajixanthone (100 mg) in boiling methanol was treated with 72% perchloric acid (2 ml) for 5 min. After cooling, the mixture was diluted with water (200 ml) and the product isolated in ethyl acetate (3 × 50 ml) and purified by t.l.c. The band at R_F 0·20 [benzene–ether (4 : 1 v/v)] gave tajixanthone methanolate as yellow needles (80 mg) from methanol, m.p. and mixed m.p. 189—191°, identical (spectroscopic properties) with the natural product.

14-*Hydroxytajixanthone* 25-*Acetate* (V).—Isolated from mycelium, this *compound* {1,2-trans-1-*acetoxy*-8-[2,3-*epoxy*-1-*hydroxy*-3-*methylbutyl*]-2,3-*dihydro*-11-*hydroxy*-2-*isopropenyl*-5-*methylpyrano*[3,2-a]*xanthen*-12(1H)-*one*} formed yellow needles from light petroleum (b.p. 60—80°), m.p. 224—227°, $[\alpha]_D$ +45·0° (*c* 0·5 in $CHCl_3$), $\nu_{max.}$,* $\lambda_{max.}$,* *m/e* * (Found: C, 67·7; H, 6·9%; M^+, 480·176. $C_{27}H_{28}O_8$ requires C, 67·5; H, 5·9%; *M*, 480·176).

14-*Methoxytajixanthone* 25-*Acetate* (VI).—Isolated from mycelium, this *compound* {1,2-trans-1-*acetoxy*-8-[2,3-*epoxy*-1-*methoxy*-3-*methylbutyl*]-2,3-*dihydro*-11-*hydroxy*-2-*isopropenyl*-5-*methylpyrano*[3,2-a]*xanthen*-12(1H)-*one*} formed yellow needles from acetone–light petroleum (b.p. 60—80°), m.p. 198—200°, $[\alpha]_D$ −19·6° (*c* 1·0 in $CHCl_3$), $\nu_{max.}$,* $\lambda_{max.}$,* *m/e* * (Found: C, 67·6; H, 6·3%; M^+, 494·195. $C_{28}H_{30}O_8$ requires C, 68·0; H, 6·1%; *M*, 494·193).

Epi-isoshamixanthone (X).—Isolated from mycelium, this *compound* {1,2-cis-2,3-*dihydro*-1,11-*dihydroxy*-2-*isopropenyl*-5-*methyl*-10-[3-*methylbut*-2-*enyl*]*pyrano*[3,2-a]*xanthen*-12-(1H)-*one*} formed yellow needles from methanol, m.p. 114—115°, $[\alpha]_D$ −51·6° (*c* 0·8 in $CHCl_3$), $\nu_{max.}$,* $\lambda_{max.}$,* *m/e* * (Found: M^+, 406·178. $C_{25}H_{26}O_6$ requires *M*, 406·178).

Prepared from this compound (10 mg) with methyl iodide and potassium carbonate in acetone, the O-*methyl derivative* (XI) formed needles (6 mg) from benzene–hexane, m.p. 121—122°, $[\alpha]_D$ −42·4° (*c* 0·4 in $CHCl_3$), $\nu_{max.}$,* $\lambda_{max.}$,* τ * (Found: M^+, 420·194. $C_{26}H_{28}O_5$ requires *M*, 420·194).

25-O-*Methylarugosin A* (XVIII).—(*a*) Isolated from mycelium, this *compound* {1,10-*dihydroxy*-6-*methoxy*-8-*methyl*-2-(3-*methylbut*-2-*enyl*)-7-(3-*methylbut*-2-*enyloxy*)*dibenz*[b,e]*oxepin*-11(6H)-*one*} was a viscous orange oil, R_F 0·50 (benzene), $\nu_{max.}$,* $\lambda_{max.}$,* *m/e* * (Found: M^+, 438·207. $C_{26}H_{30}O_6$ requires *M*, 438·204).

(*b*) Arugosins A and B [(XVI) and (XVII)] (50 mg) were dissolved in methanol (20 ml) containing concentrated hydrochloric acid (0·5 ml). After 15 min at room temperature the solution was diluted with chloroform (100 ml), washed with 2M-sodium hydrogen carbonate (2 × 25 ml) and then water (2 × 25 ml), dried ($MgSO_4$), and evaporated; the residue was purified by preparative t.l.c. Development with benzene gave a band, R_F 0·50, which was eluted with ethyl acetate to give an orange oil having spectroscopic properties identical with those of compound (XVIII).

Prepared from the above compound (100 mg) with methyl iodide and potassium carbonate in acetone, and purified by t.l.c., the *di*-O-*methyl derivative* (XIX) was obtained as a viscous yellow oil (78 mg), R_F 0·60 [benzene–acetone (4 : 1)], $\nu_{max.}$,* $\lambda_{max.}$,* τ * (Found: M^+, 466·235. $C_{28}H_{34}O_6$ requires *M*, 466·236).

Arugosin D (XXI).—Isolated from mycelium, this *compound* {1,12a-*dihydro*-6,8-*dihydroxy*-1-(1-*hydroxy*-1-*methylethyl*)-4-*methyl*-9-(2-*hydroxy*-3-*methylbut*-3-*enyl*)[1]*benzopyrano*[4,5-bc][1]*benzoxepin*-7(2H)-*one*} was a viscous yellow oil, R_F 0·40 [ether–benzene (1 : 1)], $[\alpha]_D$ −31·2° (*c* 0·95 in $CHCl_3$), $\nu_{max.}$,* $\lambda_{max.}$,* *m/e* * (Found: M^+, 440·182. $C_{25}H_{28}O_7$ requires *M*, 440·182).

Dihydroarugosin C (XXIII).—Arugosin C (110 mg) in ethanol (25 ml) was shaken at room temperature with palladium–carbon (20 mg; 10%) for 24 h under hydrogen at 1 atm. After removal of the catalyst and solvent, the residue was purified by preparative t.l.c. [benzene–ether (95 : 5)]. Dihydroarugosin C (XXIII) {1,12a-*dihydro*-6,8-*dihydroxy*-1-(1-*hydroxy*-1-*methylethyl*)-4-*methyl*-9-(3-*methylbutyl*)[1]*benzopyrano*[4,5-bc][1]*benzoxepin*-7(2H)-*one*} was obtained as a yellow oil (80 mg), R_F 0·3 [benzene–ether (95 : 5)], $[\alpha]_D$ −5·2° (*c* 2·2 in $CHCl_3$), $\nu_{max.}$,* $\lambda_{max.}$,* τ * (Found: M^+, 426·204. $C_{25}H_{30}O_5$ requires *M*, 426·204). Similar hydrogenations with platinum oxide catalyst under various conditions of temperature and pressure gave essentially similar results.

Reduction of Arugosin C Dimethyl Ether (XXII).—Prepared as previously described,[5] this compound (200 mg) in ethanol (5 ml) and liquid ammonia (80 ml) at −80° was treated with sodium (55 mg). After 10 min the solution was treated with ammonium chloride (400 mg) and allowed to evaporate at room temperature, and the residue was diluted with 2M-hydrochloric acid (2 ml) in water (25 ml). After

† For details of Supplementary Publications see Notice to Authors No. 7 in *J.C.S. Perkin I*, 1973, Index issue.

isolation in chloroform the product was purified by preparative t.l.c. [benzene–acetone (4 : 1)]. The band at R_F 0·7 gave the *benzophenone* (XXV) as a yellow oil (16 mg), $[\alpha]_D$ −94·0° (*c* 0·65 in $CHCl_3$), $\nu_{max.}$,* $\lambda_{max.}$,* *m/e*,* τ * (Found: M^+, 454·235. $C_{27}H_{34}O_6$ requires *M*, 454·236).

Isomerisation of the αβ-Unsaturated Ketone (XXVI).—Prepared as previously described,[2] this compound (34 mg) was heated under reflux for 15 min in ethyl acetate (10 ml) containing acetic acid (0·2 ml). The product was separated by preparative t.l.c. [benzene–ether (95 : 5)]. Eluted with ethyl acetate, 2-*acetyl*-11-*hydroxy*-8-[(2S)-2-*hydroxy*-3-*methylbut*-3-*enyl*]-5-*methylpyrano*[3,2-a]*xanthen*-12(3H)-*one* (XXVII), separated from acetone–light petroleum (b.p. 60—80°) in yellow needles (21 mg), m.p. 200—202°, $[\alpha]_D$ −33·3° (*c* 0·27 in $CHCl_3$), $\nu_{max.}$,* $\lambda_{max.}$,* τ,* *m/e* * (Found: C, 70·5; H, 5·8. $C_{24}H_{22}O_6$ requires C, 70·9; H, 5·5%).

Hydrolysis of 14-*Methoxytajixanthone* 25-*Acetate* (VI).—This compound (70 mg) in 50% aqueous tetrahydrofuran (30 ml) was heated to boiling and treated with 72% perchloric acid (0·5 ml). After 5 min the mixture was cooled and poured into water and the product isolated in chloroform (3 × 50 ml). After washing with 2M-sodium hydrogen carbonate (2 × 25 ml) and water (2 × 25 ml) and drying (Na_2SO_4), the solvent was evaporated off and the residue purified by preparative t.l.c. [ether–benzene (1 : 4)]. The band at R_F 0·23 was eluted and the product crystallised from aqueous ethanol, giving yellow needles (40 mg) of 1,2-trans-1-*acetoxy*-8-(2,3-*dihydroxy*-1-*methoxy*-3-*methylbutyl*)-2,3-*dihydro*-11-*hydroxy*-2-*isopropenyl*-5-*methylpyrano*[3,2-a]-*xanthen*-12(1H)-*one* (VIII), m.p. 180—182°, $[\alpha]_D$ −36·0° (*c* 1·4 in $CHCl_3$), $\nu_{max.}$,* $\lambda_{max.}$,* τ * (Found: C, 65·2; H, 6·4%; M^+, 512·205. $C_{28}H_{32}O_9$ requires C, 65·6; H, 6·3%; *M*, 512·204).

Lead Tetra-acetate Cleavage of the Diol (VIII).—Lead tetra-acetate (60 mg) was added to a stirred solution of the diol (VIII) (45 mg) in acetic acid (2 ml) at room temperature. After 1·5 h the mixture was diluted with water (50 ml) and extracted with chloroform (2 × 25 ml). After thorough washing with water, the extract was dried ($MgSO_4$) and evaporated. The yellow oily residue (40 mg) was purified by preparative t.l.c. [ether–benzene (3 : 7)]. The band at R_F 0·40 was extracted with acetone to give 1,2-trans-1-*acetoxy*-8-[*formyl*(*methoxy*)*methyl*]-2,3-*dihydro*-11-*hydroxy*-2-*isopropenyl*-5-*methylpyrano*[3,2-a]*xanthen*-12(1H)-*one* (IX) as pale yellow needles (14 mg) from acetone–light petroleum (b.p. 60—80°), m.p. 145—146°, $\nu_{max.}$,* $\lambda_{max.}$,* τ * (Found: M^+, 452·151. $C_{25}H_{24}O_8$ requires *M*, 452·146).

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