Austocystins. Six Novel Dihydrofuro[3',2':4,5]furo[3,2-*b*]xanthenones from *Aspergillus ustus*

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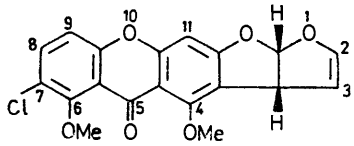
Extraction of maize meal cultures of *Aspergillus ustus* gave six novel metabolites, designated austocystins, to which were assigned the substituted 3a,12a-dihydrofuro[3',2':4,5]furo[3,2-*b*]xanthen-5-one structures (1), (20), (21), (23), (26), and (29). The substitution pattern of the austocystins was confirmed by the results of chemical degradation as well as by use of n.m.r. techniques such as acetylation shifts, benzene-induced solvent shifts, and nuclear Overhauser effect experiments.

MAIZE meal cultures of *Aspergillus ustus* (Bainier) Thom. and Church have been found to cause acute toxicoses in day-old ducklings. Recently we reported the constitution and absolute configuration of austdiol,[1] the main toxic metabolite from *A. ustus* (C.S.I.R. 1128), as well as the structures of five biogenetically related dioxopiperazines.[2] Fractionation of the toxic extract resulted in the isolation of the two known metabolites versicolorin C [3] and averufin [4] and six interrelated minor metabolites for which the collective trivial name austocystin is proposed. The six austocystins were assigned structures (1), (20), (21), (23), (26), and (29) on the basis of spectroscopic and chemical evidence.

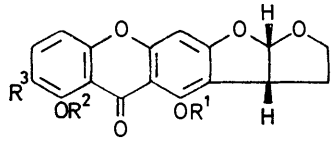
Austocystin A (1), $C_{19}H_{13}ClO_6$, $\nu_{max.}$ 1660 and 1630 (γ-pyrone) cm^{-1}, showed an n.m.r. spectrum (Table 1) compatible with the proposed structure. The chemical shifts and multiplicities of the signals assigned to the protons of the bisdihydrofuran ring system correspond closely with those reported for similar ring systems in, for example, sterigmatocystin,[5] aflatoxins B_1 and G_1,[6] parasiticol,[7] and versicolorin A.[3] The presence of a vinyl ether group was confirmed by catalytic hydrogenation to give dihydroaustocystin A (2).

Demethylation of dihydroaustocystin A (2) yielded mainly the dihydroxy-derivative (3). The chelated nature of this phenol was evident from the low n.m.r. chemical shifts of the two phenolic protons (τ −2·46 and −1·95) (Table 1). Two minor products, both $C_{18}H_{13}ClO_6$, were assigned structures (4) and (5); the location of the methoxy-groups was inferred from the n.m.r. spectra. The formation of these three chelated phenolic compounds located the two methoxy-groups in dihydroaustocystin A (2) at the positions *peri* to the carbonyl group.

Although the spectroscopic characteristics of austocystin A are compatible with the proposed structure (1),



(1)



(2) $R^1=R^2=Me, R^3=Cl$

(3) $R^1=R^2=H, R^3=Cl$

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

(5) $R^1=Me, R^2=H, R^3=Cl$

(6) $R^1=R^2=Ac, R^3=Cl$

(7) $R^1=R^2=Me, R^3=H$

the alternative structures (8)—(10) cannot be excluded solely on the basis of n.m.r. chemical shifts. Structures (8) and (9) are derivatives of sterigmatocystin (12). The 5-proton in sterigmatocystin and its derivatives [5] and in the aflatoxins [6,8] resonates as a singlet at τ 3·60—3·70

[1] R. Vleggaar, P. S. Steyn, and D. W. Nagel, *J.C.S. Perkin I*, 1974, 45.

[2] P. S. Steyn, *Tetrahedron*, 1973, **29**, 107.

[3] T. Hamasaki, Y. Hatsuda, N. Terashima, and M. Renbutsu, *Agric. and Biol. Chem. (Japan)*, 1967, **31**, 11.

[4] D. F. G. Pusey and J. C. Roberts, *J. Chem. Soc.*, 1963, 3542; J. S. E. Holker, S. A. Kagal, L. J. Mulheirn, and P. M. White, *Chem. Comm.*, 1966, 911; P. Roffey and M. V. Sargent, *ibid.*, p. 913.

[5] (*a*) E. Bullock, J. C. Roberts, and J. G. Underwood, *J. Chem. Soc.*, 1962, 4179; (*b*) E. Bullock, D. Kirkaldy, J. C. Roberts, and J. G. Underwood, *ibid.*, 1963, 829; H. J. Burkhardt and J. Forgacs, *Tetrahedron*, 1968, **24**, 717.

[6] T. Asao, G. Büchi, M. M. Abdel-Kader, S. B. Chang, E. L. Wick, and G. N. Wogan, *J. Amer. Chem. Soc.*, 1965, **87**, 882.

[7] R. D. Stubblefield, O. L. Shotwell, G. M. Shannon, D. Weisleder, and W. K. Rohwedder, *J. Agric. Food Chem.*, 1970, **18**, 391.

[8] M. F. Dutton and J. G. Heathcote, *Chem. and Ind.*, 1968, 418.

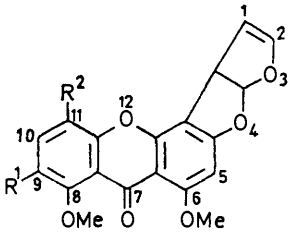
TABLE 1

Chemical shifts (τ) and multiplicities (J in Hz) in the n.m.r. spectra of the austocystins and derivatives (in $CDCl_3$)

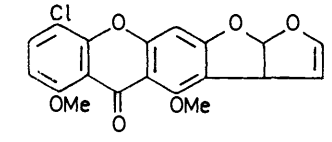
Compound	2-H	3-H	3a-R	4-OR	6-OR	7-H	8-H	9-H	11-H	12a-H	1'-H	2'-H	3'-Me$_2$	3'-OR
Austocystin A (1)	3·56t	4·68t	5·28dt	6·00	6·02		2·43d	2·95d	3·44	3·27d				
	J 2·5	*J* 2·5	*J* 7·0, 2·5 (H)	(Me)	(Me)		*J* 9·0	*J* 9·0		*J* 7·0				
Dihydroaustocystin (A)	5·88m	7·72m	6·36m	5·96	5·98		2·41d	2·90d	3·47	3·59d				
(2)			(H)	(Me)	(Me)		*J* 9·0	*J* 9·0		*J* 5·5				
(3)	5·90m	7·70m	6·34m	−1·95	−2·46		2·42d	3·20d	3·69	3·56d				
			(H)	(H)	(H)		*J* 9·0	*J* 9·0		*J* 5·5				
(4)	5·90m	7·68m	6·34m	−3·30	6·00		2·34d	2·85d	3·69	3·57d				
			(H)	(H)	(Me)		*J* 9·0	*J* 9·0		*J* 5·5				
(5)	5·88m	7·72m	6·32m	5·96	−3·79		2·44d	3·21d	3·42	3·57d				
			(H)	(Me)	(H)		*J* 9·0	*J* 9·0		*J* 5·5				
(6)	5·92m	7·84m	6·36m	7·52	7·52		2·34d	2·73d	3·31	3·54d				
			(H)	(Ac)	(Ac)		*J* 9·0	*J* 9·0		*J* 5·5				
(7)	5·86m	7·72m	6·35m	5·95	6·01	3·25dd	2·50dd	3·06dd	3·46	3·60d				
			(H)	(Me)	(Me)	*J* 8·5, 1·0	*J* 8·5, 8·5	*J* 8·5, 1·0		*J* 5·5				
Austocystin B (20)	3·56t	4·68t	5·30dt	−1·74	−2·16	3·30d	2·59d		3·57	3·24d	7·16m	8·23m	8·66	
	J 2·5	*J* 2·5	*J* 7·0, 2·5 (H)	(H)	(H)	*J* 8·5	*J* 8·5			*J* 7·0				
Austocystin C (21)	3·54t	4·68t	5·26dt	5·96	−2·93	3·32d	2·64d		3·36	3·26d	7·14m	8·24m	8·67	
	J 2·5	*J* 2·5	*J* 7·0, 2·5 (H)	(Me)	(H)	*J* 8·5	*J* 8·5			*J* 7·0				
(22)	3·55t	4·65t	5·26dt	5·94	6·04	3·29d	2·62d		3·35	3·27d	7·11m	8·21m	8·67	8·37
	J 2·5	*J* 2·5	*J* 7·0, 2·5 (H)	(Me)	(Me)	*J* 8·5	*J* 8·5			*J* 7·0				(H)
Austocystin D (23) *	3·36d	4·32d	4·60	−1·52	−2·20	3·37d	2·50d		3·52	3·64	7·19m	8·30m	8·73	6·60
	J 3·0	*J* 3·0	(OH)	(H)	(H)	*J* 8·5	*J* 8·5							(H)
(24)	3·43d	4·41d	6·64	5·90	6·04	3·28d	2·64d		3·35	3·57	7·12m	8·22m	8·66	8·02
	J 3·0	*J* 3·0	(OMe)	(Me)	(Me)	*J* 8·5	*J* 8·5							(H)
(25)	3·48d	4·45d	7·3	5·92	6·02	3·27d	2·59d		3·57	3·77	7·13m	8·23m	8·66	7·3
	J 3·0	*J* 3·0	(OH)	(Me)	(Me)	*J* 8·5	*J* 8·5							(H)
Austocystin E (26)	3·46d	4·43d	6·7	5·95	−2·40	3·37d	2·66d		3·61	3·64	7·26m	8·24m	8·67	6·7
	J 3·0	*J* 3·0	(OH)	(Me)	(H)	*J* 8·5	*J* 8·5							(H)
Dihydroaustocystin D	5·80m	*ca.* 7·5m	4·81	−1·57	−2·35	3·34d	2·48d		3·57	4·06	7·18m	8·26m	8·73	6·64
(27) *	6·30m		(OH)	(H)	(H)	*J* 8·5	*J* 8·5							(H)
(28) *	5·78m	*ca.* 7·4m	8·02	7·68	7·59	3·08d	2·37d		3·10	3·56	7·04m	7·82m	8·45	8·02
	6·30m		(OAc)	(Ac)	(Ac)	*J* 8·5	*J* 8·5							(Ac)
Austocystin F (29) *	3·32d	4·31d	4·60	−1·70	−2·20	3·22dd	2·31dd	3·06dd	3·50	3·61				
	J 3·0	*J* 3·0	(OH	(H)	(H)	*J* 8·5, 1·0	*J* 8·5, 8·5	*J* 8·5, 1·0						
(30)	3·46d	4·44d		5·91	6·00	3·21dd	2·56dd	3·05dd	3·59	3·62				
	J 3·0	*J* 3·0		(Me)	(Me)	*J* 8·5, 1·0	*J* 8·5, 8·5	*J* 8·5, 1·0						

* In $(CD_3)_2CO$.

whereas the singlet due to the aromatic proton in austocystin A (1) resonates at τ 3·44. This result mitigates against an angular structure such as (8) or (9).



(8) R^1=Cl, R^2=H
(9) R^1=H, R^2=Cl



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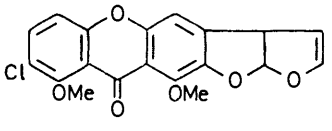


(11)

Conclusive evidence in favour of a linear structure is now presented. It has been shown [9] that the n.m.r. signal of the proton located *para* to a phenolic hydroxy-group shows a downfield shift (0·35—0·50 p.p.m.) upon acetylation of the hydroxy-group, whereas an *ortho*-proton signal is shifted downfield by 0·20—0·25 p.p.m. A *meta*-proton suffers no marked downfield shift (0·03—0·10 p.p.m.). Acetylation of the hydroxy-groups in dihydrosterigmatocystin (14) and 6-*O*-demethyldihydrosterigmatocystin (16) resulted in comparable downfield shifts of the signals of the various types of aromatic protons (see Table 2). Acetylation of compound (3)

TABLE 2

Shift of the aromatic proton signals on acetylation of the phenolic hydroxy-groups * (in p.p.m.)

		5-H (*ortho*)	9-H (*ortho*)	10-H (*meta*)	11-H (*para*)
Dihydrosterigmato-cystin	(14)		−0·21	−0·11	−0·49
	(16)	−0·24	−0·19	−0·07	−0·43
		7-H (*ortho*)	8-H (*meta*)	9-H (*para*)	11-H (*para*)
	(3)		−0·08	−0·47	−0·38
	(27)	−0·26	−0·11		−0·47

* Negative values denote a downfield shift.

caused downfield shifts of the 11- and 9-proton signals (0·38 and 0·47 p.p.m., respectively) characteristic of protons *para* to a hydroxy-group. The chlorine atom must therefore be located at C-7 and structure (1) is thus verified for austocystin A. These findings are in agreement with the results obtained for austocystin A by use of n.m.r. techniques such as benzene-induced solvent shifts and the nuclear Overhauser effect (n.O.e.) (see below).

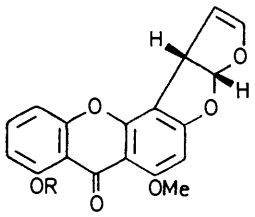
The methoxy-proton shifts obtained upon change of solvent from chloroform to benzene are useful in structure determination.[10] Pelter *et al.*[11] have shown that signals

[9] J. Massicot, J.-P. Marthe, and S. Heitz, *Bull. Soc. chim. France*, 1963, 2712; Dun-Mei Yang, N. Takeda, Y. Iitaka, U. Sankawa, and S. Shibata, *Tetrahedron*, 1973, **29**, 519.

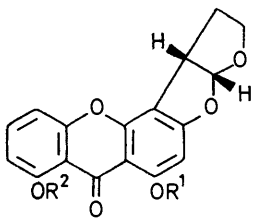
[10] H. M. Fales and K. S. Warren, *J. Org. Chem.*, 1967, **32**, 501; R. G. Wilson, J. H. Bowie, and D. H. Williams, *Tetrahedron*, 1968, **24**, 1407; J. H. Bowie, J. Ronayne, and D. H. Williams, *J. Chem. Soc.* (*B*), 1967, 535.

[11] A. Pelter and P. Amenechi, *J. Chem. Soc.* (*C*), 1969, 887.

of methoxy-groups *ortho* to an aromatic proton move upfield by more than 0·30 p.p.m. on change of solvent from chloroform to benzene, but signals of methoxy-groups lacking such a neighbouring proton move upfield by 0·00—0·20 p.p.m. The solvent shifts obtained for the methoxy-groups of sterigmatocystin (12) (0·65 p.p.m.), dihydrosterigmatocystin (14) (0·59 p.p.m.), as well as those for their *O*-methyl derivatives (13) and (18), respectively, are as expected (Table 3). The low shift values obtained for the 6- and 4-methoxy-group protons (0·17 and 0·04 p.p.m.) in austocystin A argue in favour of structure (1).



(12) R=H
(13) R=Me



(14) R^1=Me, R^2=H
(15) R^1=Me, R^2=Ac
(16) R^1=R^2=H
(17) R^1=R^2=Ac
(18) R^1=R^2=Me

The substitution pattern of the xanthone ring in austocystin A (1) was confirmed by n.O.e.[12] studies. Irradiation at the resonance frequency of either of the methoxy-groups in (1) gave no observable increase in the integrated intensity of any of the aromatic proton signals. In contrast a considerable n.O.e. was observed on irradiation at the frequencies of the methoxy-groups in sterigmatocystin (12) (33%) and 8-*O*-methyldihydrosterigmatocystin (18) (33%, 6-OMe; 20%, 8-OMe) (Table 4).

Chemical evidence for the linear structure (1) of austocystin A was obtained by reductive dechlorination[13] of dihydroaustocystin A (2) with Raney nickel to give compound (7), which differed from authentic 8-*O*-methyldihydrosterigmatocystin (18). The n.m.r. spectrum (Table 1) of the dechlorinated product is in good agreement with structure (7). The assignment of the signals to the aromatic protons and the methoxy-group protons was facilitated by n.O.e. experiments which indicated the presence of an aromatic proton *ortho* to the 6-methoxy-group. No n.O.e. was observed on irradiation at the frequency corresponding to the 4-methoxy-group (τ 5·95) of compound (7) but upon irradiation at the frequency of the 6-methoxy-group (τ 6·01) a 20% increase in the integrated intensity of the 7-proton signal (τ 3·25) was observed (see Table 4). The benzene-induced solvent shifts (Table 3) for the 6- and 4-methoxy-group signals (0·55 and 0·18 p.p.m., respectively) were compatible with structure (7).

TABLE 3

Benzene-induced solvent shifts of the methoxy-proton signals

Compound	$\Delta = \tau(C_6D_6) - \tau(CDCl_3)$ (in p.p.m.)		
	6-OMe	8-OMe	
Sterigmatocystin (12)	+0·65		
O-Methylsterigmatocystin (13)	+0·60	+0·49	
Dihydrosterigmatocystin (14)	+0·59		
O-Methyldihydrosterigmatocystin (18)	+0·56	+0·50	
	4-OMe	6-OMe	
Austocystin A (1)	+0·04	+0·17	
Dihydroaustocystin A (2)	+0·07	+0·21	
(4)		+0·17	
(5)	+0·06		
(7)	+0·18	+0·55	
Austocystin C (21)	+0·28		
(22)	+0·16	+0·46	
(24)	+0·01	+0·42	
(25)	+0·11	+0·52	
Austocystin E (26)	+0·18		
(30)	+0·15	+0·58	
	1-OMe	3-OMe	8-OMe
(19)	+0·03	+0·74	+0·05

TABLE 4

Nuclear Overhauser effects *

Compound	Protons irradiated	Protons observed	% N.O.e (± 2%) †
Sterigmatocystin (12)	6-OMe	5-H	33
(18)	6-OMe	5-H	33
	8-OMe	9-H	20
Austocystin A (1)	4-OMe	9-H; 11-H	Nil; nil
	6-OMe	9-H; 11-H	Nil; nil
(7)	4-OMe	7-H; 11-H	Nil; nil
	6-OMe	7-H	20
(19)	1-OMe	4-H; 5-H	Nil; nil
	3-OMe	4-H	21
	8-OMe	4-H; 5-H	Nil; nil
(24)	4-OMe	12a-H	23
(25)	4-OMe	7-H; 11-H	Nil; nil
	6-OMe	7-H	20
Austocystin E (26)	4-OMe	7-H; 11-H	Nil; nil

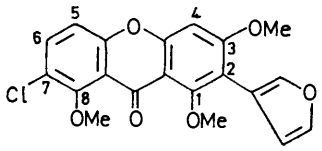
* All n.O.e. experiments were carried out on filtered and carefully degassed $CDCl_3$ solutions. Sample concentration *ca.* 10% w/v. † Given as % increase in integrated intensity on irradiation.

An alternative structure such as (11) for austocystin A is biogenetically improbable and could be excluded as a result of the following experiment. Treatment of austocystin A (1) with alkali followed by methylation gave the xanthone (19). The n.m.r. spectrum of (19) showed the 5- and 6-protons as an AB system (*J* 9·0 Hz) at τ 2·92 (d) and 2·44 (d) and the signal at τ 3·35 (s) was assigned to the 4-proton. The presence of a β-

[12] G. E. Bachers and T. Schafer, *Chem. Rev.*, 1971, **71**, 617 and references cited therein.

[13] H. Kämmerer, L. Horner, and H. Beck, *Chem. Ber.*, 1958, **91**, 1376.

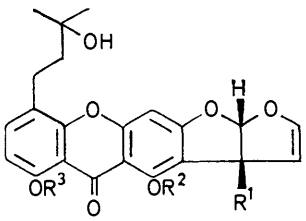
substituted furan ring was confirmed by the multiplets at τ 2·17 (2′-H), 2·54 (5′-H), and 3·11 (4′-H).[5a] The three-proton signal at τ 6·08 was assigned to the 3-methoxy-group because irradiation at the frequency of



(19)

this group resulted in a 21% increase in the integrated intensity of the 4-proton signal (τ 3·35). Irradiation of the signals at τ 6·28 (3H) and 5·98 (3H) (1- and 8-methoxy-groups) gave no observable n.O.e. Benzene-induced solvent shifts confirmed that only the 3-methoxy-group (upfield shift 0·74 p.p.m.) was *ortho* to an aromatic proton (Table 3).

Austocystin B (20), $C_{22}H_{20}O_7$, $\nu_{max.}$ 3600 (OH) and



(20) $R^1=R^2=R^3=H$

(21) $R^1=R^3=H, R^2=Me$

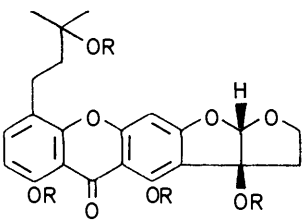
(22) $R^1=H, R^2=R^3=Me$

(23) $R^1=OH, R^2=R^3=H$

(24) $R^1=OMe, R^2=R^3=Me$

(25) $R^1=OH, R^2=R^3=Me$

(26) $R^1=OH, R^2=Me, R^3=H$



(27) R=H

(28) R=Ac

1665 and 1635 (γ-pyrone) cm^{-1}, showed n.m.r. singlets at τ −2·16 (1H) and −1·74 (1H) characteristic of two intramolecular hydrogen-bonded phenolic protons. The presence of a 3-hydroxy-3-methylbutyl side-chain was inferred from the singlet at τ 8·66 (6H, CMe_2) and the centrosymmetric multiplets at τ 7·16 (2H) and 8·23 (2H) (CH_2·CH_2).[14] The assignments of the other signals are summarized in Table 1. The molecular ion (M^+ 396) in the mass spectrum shows a loss of 73 mass units (C_4H_9O) due to fission of the benzylic C–C bond in the side-chain.

Austocystin C, $C_{23}H_{22}O_7$, was assigned structure (21) and is the 4-*O*-methyl derivative of austocystin B (20). The n.m.r. spectrum (Table 1) shows the methoxy-signal as a singlet at τ 5·96, characteristic of a 4-methoxy-group (see earlier). The benzene-induced solvent shift of the methoxy-proton signal of austocystin C was ambiguous (Table 3). The value (0·28 p.p.m.) is such that it is not possible to decide on the presence of a hydrogen atom *ortho* to the methoxy-group. It has been shown that the method may be misleading for compounds containing phenolic hydroxy-groups.[11,15] Accordingly austocystin C was methylated to give 6-*O*-methyl-austocystin C (22), identical with the di-*O*-methyl derivative obtained from austocystin B (20). The singlets at τ 5·94 (3H) and τ 6·04 (3H) in the n.m.r. spectrum (Table 1) were assigned to the 4- and 6-methoxy-groups, respectively. The benzene-induced solvent shifts of the 4- and 6-methoxy-proton signals (0·16 and 0·46 p.p.m., respectively) confirmed the substitution patterns of both austocystin B and C.

Austocystin D, $C_{22}H_{20}O_8$, $\nu_{max.}$ 3620 cm^{-1} (OH), showed an n.m.r. spectrum in accord with the assigned structure (23). The presence of hydroxy-groups was confirmed by the two singlets at τ 4·60 (1H) and 6·63 (1H), removed by treatment with D_2O. Two low-field D_2O-removable singlets at τ −2·20 (1H) and −1·52 (1H) were assigned to the two chelated phenolic protons. The chemical shifts of the olefinic protons [τ 4·32 and 3·34 (d, *J* 3·0 Hz)] correspond closely with values reported for similar systems, *e.g.* aflatoxin M_1.[16] The singlet at τ 3·64 (1H) is assigned to the 12a-proton (see later).

Catalytic hydrogenation of austocystin D (23) gave the dihydro-derivative (27). Chemical evidence for the presence of four hydroxy-groups in austocystin D was provided by acetylation of (27) to give the tetra-acetate (28) ($\nu_{max.}$ 1765 and 1725 cm^{-1}). The n.m.r. spectrum (Table 1) shows that the signal of the 11-proton [τ 3·10 (s)] is shifted strongly downfield (0·47 p.p.m.) in comparison with that in the free phenol (27) [τ 3·57 (s)], and this is characteristic of a proton *para* to a phenolic hydroxy-group.[9] The comparable downfield shift of the 7-proton is only 0·26 p.p.m., indicating that this proton is *ortho* to the phenolic hydroxy-group (Table 2).[9] The 3-hydroxy-3-methylbutyl side-chain is therefore at C-9.

Methylation of austocystin D (23) yielded the di-(25) and tri-*O*-methyl (24) derivatives. An n.O.e. experiment enabled the assignment of the singlet at τ 3·57 in the n.m.r. spectrum of (24) to the 12a-proton as well

[14] R. Vleggaar, T. M. Smalberger, and H. L. de Waal, *J.S. African Chem. Inst.*, 1973, **26**, 71.

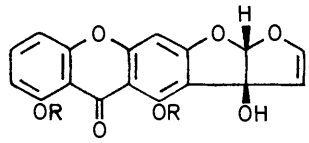
[15] F. Scheinmann, *Chem. Comm.*, 1967, 1015; G. H. Stout, M. M. Krahn, P. Yates, and H. B. Bhat, *ibid.*, 1968, 211.

[16] C. W. Holzapfel and P. S. Steyn, *Tetrahedron Letters*, 1966, 2799; J. V. Rodricks, E. Lustig, A. D. Campbell, L. Stoloff, and K. R. Henery-Logan, *ibid.*, 1968, 2975; A. C. Waiss, M. Wiley, D. R. Black, and R. E. Lundin, *ibid.*, p. 3207; R. D. Hutchison and C. W. Holzapfel, *Tetrahedron*, 1971, **27**, 425.

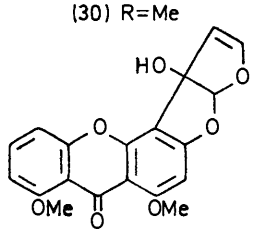
as determining the location of the third methoxy-group (at C-3a). Irradiation at the frequency of the methoxy-proton signal at τ 6·64 resulted in a 23% increase in the integrated intensity of the signal due to the 12a-proton (τ 3·57), and established the *cis*-configuration for the ring junction in the bisdihydrofuran unit.

N.O.e. studies of the di-*O*-methyl derivative (25) gave the following results: no n.O.e. was observed upon irradiation at the frequency of the 4-methoxy-group (τ 5·92) but irradiation at that of the 6-methoxy-group (τ 6·02) resulted in a 20% increase in intensity of the 7-proton signal [τ 3·27 (d)] (Table 4). The above results together with the benzene-induced solvent shifts of the methoxy-proton signals of (24) and (25) (Table 3) confirm the structure (23) for austocystin D.

Austocystin E (26), $C_{23}H_{22}O_8$, is the 4-*O*-methyl derivative of austocystin D. The chemical shift of the methoxy-protons (τ 5·95), the solvent-induced shift of



(29) R=H
(30) R=Me

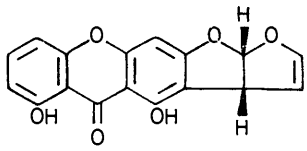


(31)

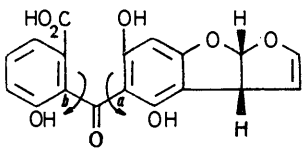
the methoxy-proton signal (0·18 p.p.m.), and the fact that no n.O.e. was observed on irradiation at the methoxy-group frequency, located this group at C-4. Methylation of austocystin E gave the di- and tri-*O*-methyl derivatives (25) and (24), thereby correlating it with austocystin D.

Austocystin F (29), $C_{17}H_{10}O_7$, was identified from its n.m.r. spectrum and that of its di-*O*-methyl derivative (30) (Table 1). The methoxy-proton shifts of (30) (0·15 and 0·58 p.p.m.) on change of solvent from chloroform to benzene, together with the compound's non-identity with aspertoxin (31) (t.l.c.), established the linear fusion of the xanthone ring with the bisdihydrofuran ring system.

Sterigmatin [17] (32), isolated from *Aspergillus versicolor*, was the first characterized metabolite with a linear fusion between the xanthone and bisdihydrofuran systems. The austocystins comprise an important addition to this new type of metabolite. The presence of the five-carbon side-chain (derived from γγ-dimethylallyl pyrophosphate) and the chlorine atom are novel features of these nonaketide-derived metabolites. The angular-fused compounds, *e.g.* sterigmatocystin (12) and derivatives, and the biogenetically related aflatoxins, are the more common metabolites.

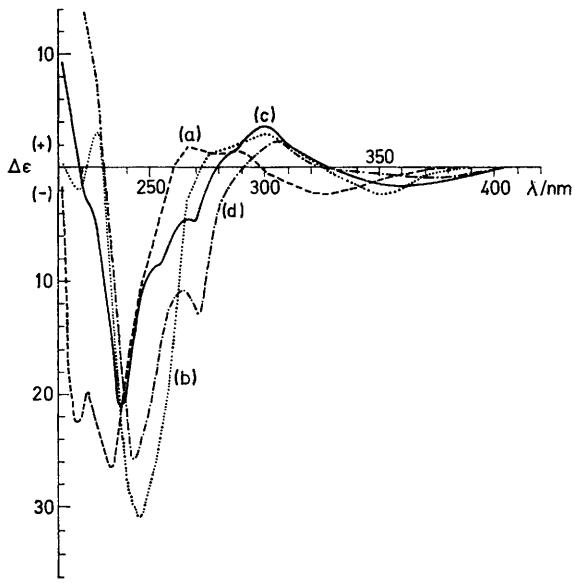


(32)



(33)

The biosynthesis of the austocystins can be readily explained in terms of the hypothetical pathway proposed by Büchi for the biosynthesis of the aflatoxins.[18] A key intermediate in this pathway is the acid (33).[18] Rotation around bond *a* in (33) leads to sterigmatocystin, whereas no rotation or rotation around *b* would lead to the austocystins. The presence of versicolorin C[3] in *A. ustus* and the co-occurrence of sterigmatocystin (12), versicolorin A and C, and sterigmatin (32) in *A. versicolor* [3,17] argue in favour of this pathway for the biosynthesis of the austocystins. Austocystin D (23) is almost certainly derived from austocystin B (20) rather than *vice versa* because the additional hydroxy-group present in this



C.d. spectra of (a) sterigmatocystin (12), (b) austocystin A (1), (c) austocystin C (21), and (d) austocystin (D) (23)

metabolite is attached to a carbon atom derived from an acetate *methyl* group, as in aflatoxin M_1.[18] This stereo-

[17] T. Hamasaki, K. Matsui, K. Isono, and Y. Hatsuda, *Agric. and Biol. Chem.* (*Japan*), 1973, **37**, 1759.

[18] M. Biollaz, G. Büchi, and G. Milne, *J. Amer. Chem. Soc.*, 1968, **90**, 5017; 1968, **90**, 5019; 1970, **92**, 1035.

specific replacement of a hydrogen atom occurs with retention of configuration.[19]

The above proposal of a common biosynthetic pathway for sterigmatocystin (12) and the austocystins suggests that the absolute configuration of the latter is the same as that of sterigmatocystin.[20] The *cis*-ring fusion of the bisdihydrofuran unit is well established [20] and is borne out for austocystins A—C by our n.m.r. results. Comparison of the c.d. spectrum of (3a-*R*,12c-*S*)-sterigmatocystin (12) with the spectra of autocystins A (1), C (21), and D (23) (Figure) confirms the suggested absolute configuration of the austocystins as indicated in the formulae.

EXPERIMENTAL

M.p.s were determined on a Kofler hot-stage apparatus. U.v. absorptions were measured (for solutions in ethanol) on a Unicam SP 800 spectrometer, i.r. spectra on a Perkin-Elmer 237 spectrometer, mass spectra on an A.E.I. MS 9 double-focusing spectrometer, and n.m.r. spectra (for solutions in $CDCl_3$) on a Varian HA-100 spectrometer (with tetramethylsilane as internal standard). C.d. spectra were measured for solutions in methanol (JASCO J-20 spectropolarimeter). T.l.c. was carried out on Merck pre-coated silica plates (thicknesses 0·25 and 2 mm for analytical and preparative purposes, respectively).

Isolation of the Austocystins.—*A. ustus* was grown in bulk on wet sterilized maize meal for 20 days. The dried, milled, mouldy maize (20·0 kg) was extracted with chloroform–methanol (1 : 1 v/v) for 2 days and the solvent was removed under reduced pressure to yield homogeneous crystalline material (austdiol [1]) (860 g) and soluble material (1520 g). The latter in chloroform (4 l) was twice extracted with water (1·5 l). Evaporation of the chloroform solution yielded material (840 g) which was partitioned between 90% methanol (3 l) and hexane, yielding toxic material (145 g) in the methanol layer. This toxic material was separated by chromatography on formamide-impregnated cellulose powder (2·0 kg). The cellulose column was developed consecutively with hexane, mixtures of hexane and benzene, and benzene; 2500 fractions (each 30 ml) were collected.

Fraction A (9·2 g) (tubes 201—400) contained austocystin A (1). Fraction B (6·6 g) (tubes 401—1000) contained austocystins B (20), C (21), and F (29) and averufin.[4] Fraction C (12·2 g) (tubes 1001—1300) contained austamide.[2] Fraction D (1·7 g) (tubes 1301—1400) contained austamide [2] and cyclo[prolyl-2-(1,1-dimethylallyl)tryptophyl].[2] Fraction E (9·4 g) (tubes 1401—1850) contained cyclo[2,3-didehydroprolyl-2-(1,1-dimethylallyl)tryptophyl] [2] and the corresponding azocine (derived by cyclization),[2] versicolorin C,[3] austocystin D (23), and austocystin E (26).

Purification of Austocystin A (7-*Chloro*-3a,12a-*dihydro*-4,6-*dimethoxyfuro*[3′,2′:4,5]*furo*[3,2-b]*xanthen*-5-*one*) (1).—Fraction A (9·2 g) was separated by column chromatography on alumina (activity III) with benzene as eluant to give *austocystin A* (1) (1·04 g), m.p. 204—205° (from benzene–n-hexane), $[\alpha]_D^{22}$ −301° (*c* 1·45 in $CHCl_3$); $\lambda_{max.}$ 229sh, 239sh, 247, 285sh, 303, and 335 nm (log ε 4·46, 4·52, 4·54, 4·03, 4·09, and 3·75); c.d. $\Delta\varepsilon_{390}$ 0, $\Delta\varepsilon_{350}$ −2·3, $\Delta\varepsilon_{325}$ 0, $\Delta\varepsilon_{300}$ +3·0, $\Delta\varepsilon_{271}$ 0, $\Delta\varepsilon_{245}$ −31·0, $\Delta\varepsilon_{229}$ 0, $\Delta\varepsilon_{226}$ +3·1, $\Delta\varepsilon_{221}$ 0, $\Delta\varepsilon_{218}$ −2·1, and $\Delta\varepsilon_{212}$ 0; $\nu_{max.}$ ($CHCl_3$) 1660 and 1630 (γ-pyrone) and 1610 cm^{-1} (Found: C, 61·3; H, 3·5; Cl, 9·75. $C_{19}H_{13}ClO_6$ requires C, 61·2; H, 3·5; Cl, 9·5%).

Purification of Austocystins B {3a,12a-*Dihydro*-4,6-*dihydroxy*-9-(3-*hydroxy*-3-*methylbutyl*)*furo*[3′,2′:4,5]*furo*[3,2-b]*xanthen*-5-*one*} (20), *C* (*its* 4-O-*Methyl Derivative*) (21), *and F* (*its* 9-*Unsubstituted* 3a-*Hydroxy*-*analogue*) (29).—Column chromatography of fraction B (6·6 g) on silica with chloroform–methanol (98 : 2 v/v) yielded a mixture (1·6 g) of the above austocystins and averufin.[4] Preparative t.l.c. with benzene–acetone (4 : 1 v/v) yielded austocystins B (20) (15 mg), C (21) (33 mg), and F (29) (77 mg), and averufin [4] (490 mg).

Austocystin B (20) had m.p. 172—173° (from benzene–n-hexane), $[\alpha]_D^{21}$ −278° (*c* 0·40 in $CHCl_3$); $\lambda_{max.}$ 227, 240sh, 254, 263, 274, 328, and 372 nm (log ε 4·39, 4·40, 4·52, 4·34, 4·37, 4·21, and 3·86); $\nu_{max.}$ ($CHCl_3$) 1670 and 1635 (γ-pyrone) and 1610 cm^{-1} (Found: C, 66·65; H, 5·0. $C_{22}H_{20}O_7$ requires C, 66·7; H, 5·1%).

Austocystin C (21) had m.p. 168—170° (from benzene–n-hexane), $[\alpha]_D^{22}$ −245° (*c* 0·88 in $CHCl_3$); $\lambda_{max.}$ 229sh, 238, 255, 277, 304, and 368 nm (log ε 4·31, 4·38, 4·32, 4·26, 4·10, and 3·66); c.d. $\Delta\varepsilon_{420}$ 0, $\Delta\varepsilon_{360}$ −1·6, $\Delta\varepsilon_{325}$ 0, $\Delta\varepsilon_{300}$ +3·7, $\Delta\varepsilon_{279}$ 0, $\Delta\varepsilon_{270}$ −4·8, $\Delta\varepsilon_{265}$ −4·5, $\Delta\varepsilon_{237}$ −20·9, and $\Delta\varepsilon_{219}$ 0; $\nu_{max.}$ ($CHCl_3$) 1645 and 1635 (γ-pyrone) and 1605 cm^{-1} (Found: C, 67·5; H, 5·6. $C_{23}H_{22}O_7$ requires C, 67·3; H, 5·4%).

Austocystin F (29) had m.p. 230—233° (decomp.) (from methanol), $[\alpha]_D^{22}$ −244° (*c* 0·91 in pyridine); $\lambda_{max.}$ 224, 252, 259sh, 268, 325, and 368 nm (log ε 4·44, 4·54, 4·48, 4·40, 4·17, and 3·63); c.d. $\Delta\varepsilon_{410}$ 0, $\Delta\varepsilon_{368}$ −1·3, $\Delta\varepsilon_{325}$ 0, $\Delta\varepsilon_{303}$ +2·7, $\Delta\varepsilon_{287}$ 0, $\Delta\varepsilon_{267}$ −23·6, $\Delta\varepsilon_{260}$ −23·2, $\Delta\varepsilon_{245}$ −32·4, $\Delta\varepsilon_{231}$ 0, $\Delta\varepsilon_{224}$ +19·5, and $\Delta\varepsilon_{220}$ −17·6; $\nu_{max.}$ (KBr) 1660 and 1635 (γ-pyrone) and 1610 cm^{-1} (Found: C, 62·3; H, 3·0. $C_{17}H_{10}O_7$ requires C, 62·6; H, 3·1%).

Purification of Austocystins D {3a,12a-*Dihydro*-3a,4,6-*trihydroxy*-9-(3-*hydroxy*-3-*methylbutyl*)*furo*[3′,2′:4,5]*furo*-[3,2-b]*xanthen*-5-*one*} (23) *and E* (*its* 4-O-*Methyl Derivative* (26).—Fraction E (9·4 g) in chloroform (200 ml) was extracted with saturated sodium carbonate solution to yield versicolorin C (1·4 g).[3] The solid residue from the chloroform solution was purified by preparative t.l.c. with benzene–acetone (4 : 1 v/v) to give austocystin D (23) (600 mg) and austocystin E (26) (90 mg).

Austocystin D (23) had m.p. 114—116° (from benzene), $[\alpha]_D^{21}$ −186° (*c* 1·14 in Me_2CO); $\lambda_{max.}$ 227, 254, 263sh, 273, 329, and 370 nm (log ε 4·29, 4·50, 4·36, 4·37, 4·11, and 3·64); c.d. $\Delta\varepsilon_{410}$ 0, $\Delta\varepsilon_{370}$ −1·0, $\Delta\varepsilon_{325}$ 0, $\Delta\varepsilon_{305}$ +2·3, $\Delta\varepsilon_{290}$ 0, $\Delta\varepsilon_{271}$ −13·2, $\Delta\varepsilon_{265}$ −10·8, $\Delta\varepsilon_{242}$ −25·9, $\Delta\varepsilon_{230}$ 0; $\nu_{max.}$ ($CHCl_3$) 3620 (OH), 1665 and 1635 (γ-pyrone), and 1615 cm^{-1} (Found: C, 64·2; H, 5·0. $C_{22}H_{20}O_8$ requires C, 64·1; H, 4·9%).

Austocystin E (26) is a yellow glass, $\lambda_{max.}$ 228, 236, 257, 262sh, 273, 304, and 368 nm (log ε 4·29, 4·37, 4·36, 4·35, 4·33, 4·01, and 3·64); $\nu_{max.}$ (KBr) 1635 and 1605 cm^{-1} (Found: M^+, 426·1319. $C_{23}H_{22}O_8$ requires *M*, 426·1314).

Hydrogenation of Austocystin A (1).—Austocystin A (410 mg) in acetic acid (40 ml) was hydrogenated over 10% palladium–charcoal (50 mg) for 1 h (uptake 1 mol. equiv.). The mixture was filtered, diluted with water (50 ml), and extracted with chloroform to give a colourless solid. Crystallization from benzene–n-hexane gave *dihydroaustocystin A* (2) (370 mg) as needles, m.p. 175—177°, $[\alpha]_D^{22}$ −146° (*c* 1·18 in $CHCl_3$); $\nu_{max.}$ ($CHCl_3$) 1660 and 1630 (γ-pyrone) and 1605 cm^{-1} (Found: C, 60·9; H, 4·1; Cl, 9·6. $C_{19}H_{15}ClO_6$ requires C, 60·9; H, 4·0; Cl, 9·5%).

[19] E. R. H. Jones, *Pure Appl. Chem.*, 1973, **33**, 39.
[20] J. S. E. Holker and L. J. Mulheirn, *Chem. Comm.*, 1968, 1576.

Demethylation of Dihydroaustocystin A (2).—A mixture of dihydroaustocystin (200 mg) and potassium iodide (1 g) in acetic acid (10 ml) was refluxed for 6 h, diluted with water (20 ml), and extracted with chloroform to give a yellow solid. Column chromatography on silica with chloroform–benzene (1 : 1 v/v) as eluant gave the diphenol (3) (86 mg) and a mixture of the monomethyl ethers (4) and (5) (78 mg). The latter was separated on preparative t.l.c. with benzene–acetone (4 : 1 v/v) to give the 6-methyl (4) (40 mg) and the 4-methyl ether (5) (9 mg).

The *diphenol* (3) had m.p. 238—240° (from benzene–n-hexane); $\nu_{max.}$ ($CHCl_3$) 1668, 1630, and 1610 cm^{-1} (Found: C, 59·05; H, 3·4; Cl, 10·2. $C_{17}H_{11}ClO_6$ requires C, 58·9; H, 3·2; Cl, 10·2%).

The 6-*methyl ether* (4) had m.p. 228—230° (from benzene–n-hexane); $\nu_{max.}$ ($CHCl_3$) 1660 and 1620 cm^{-1} (Found: C, 59·8; H, 3·7; Cl, 9·75. $C_{18}H_{13}ClO_6$ requires C, 59·9; H, 3·6; Cl, 9·8%).

The 4-*methyl ether* (5) had m.p. 187—189° (from benzene–n-hexane); $\nu_{max.}$ ($CHCl_3$) 1630 and 1600 cm^{-1} (Found: C, 59·8; H, 3·75; Cl, 9·8. $C_{18}H_{13}ClO_6$ requires C, 59·9; H, 3·7; Cl, 9·8%).

Acetylation of the Diphenol (3).—A solution of the diphenol (3) (50 mg) in acetic anhydride (2 ml) and pyridine (2 ml) was stirred at room temperature for 1 h. The *diacetate* (6) (45 mg) crystallized from benzene–n-hexane as needles, m.p. 203—205°; $\nu_{max.}$ ($CHCl_3$) 1775, 1640, and 1610 cm^{-1} (Found: C, 58·7; H, 3·5; Cl, 8·2. $C_{21}H_{15}ClO_8$ requires C, 58·55; H, 3·5; Cl, 8·2%).

Reductive Dechlorination of Dihydroaustocystin A (2).—Dihydroaustocystin (75 mg) in methanol (10 ml) containing potassium hydroxide (12 mg) was hydrogenated over Raney nickel (150 mg). After 2 h (uptake 1 mol. equiv.) the mixture was filtered, diluted with water (20 ml), and extracted with chloroform to give a solid. Crystallization from methanol gave 7-*dechlorodihydroaustocystin A* (7) (60 mg) as needles, m.p. 203—204°; $\nu_{max.}$ ($CHCl_3$) 1650, 1625, and 1610 cm^{-1} (Found: C, 64·65; H, 5·1. $C_{19}H_{16}O_6,CH_3OH$ requires C, 64·5; H, 5·4%).

Alkaline Degradation of Austocystin A (1).—A solution of austocystin A (100 mg) in methanolic 25% potassium hydroxide (10 ml) was refluxed under nitrogen for 2 h, diluted with water (10 ml), acidified (6N-HCl), and extracted with chloroform. The product (95 mg) was methylated with methyl iodide (1 ml) and anhydrous potassium carbonate (1 g) in acetone (10 ml). The crude product was purified by preparative t.l.c. with chloroform to give 7-*chloro*-2-(3-*furyl*)-1,3,8-*trimethoxyxanthone* (19) (69 mg), m.p. 155—157° (from benzene–n-hexane); $\nu_{max.}$ ($CHCl_3$) 1660 and 1610 cm^{-1} (Found: C, 62·0; H, 3·9; Cl, 9·1. $C_{20}H_{15}ClO_6$ requires C, 62·1; H, 3·9; Cl, 9·2%).

Methylation of Austocystin C (21).—A mixture of austocystin C (8 mg), anhydrous potassium carbonate (100 mg), and methyl iodide (0·5 ml) in acetone (5 ml) was refluxed for 6 h to give 6-O-*methylaustocystin C* (22) (8 mg) as a glass (Found: M^+, 424·1525. $C_{24}H_{24}O_7$ requires M, 424·1521).

Methylation of Austocystin B (20).—Austocystin B (2 mg) was methylated with methyl iodide as described above to give the dimethyl ether (22) (2 mg).

Hydrogenation of Austocystin D (23).—Austocystin D (210 mg) in methanol (20 ml) was hydrogenated over 10% palladium–charcoal (25 mg) for 30 min (uptake 1 mol. equiv.) to give *dihydroaustocystin D* (27) (208 mg), m.p. 166—167°, $[\alpha]_D^{22}$ −73° (*c* 1·26 in pyridine); $\lambda_{max.}$ 227, 238sh, 253, 262sh, 272, 329, and 370 nm (log ε 4·34, 4·37, 4·53, 4·34, 4·31, 4·19, and 3·65); $\nu_{max.}$ ($CHCl_3$) 3600, 1670, 1635, and 1610 cm^{-1} (Found: C, 63·7; H, 5·4. $C_{22}H_{22}O_8$ requires C, 63·8; H, 5·35%).

Acetylation of Dihydroaustocystin D (27).—A solution of dihydroaustocystin D (30 mg) in acetic anhydride (3 ml) and pyridine (3 ml) was refluxed for 2·5 h to give the *tetra-acetate* (28) (38 mg) as a glass; $\nu_{max.}$ ($CHCl_3$) 1765, 1725, 1660, 1640, and 1615 cm^{-1} (Found: C, 61·9; H, 5·5. $C_{30}H_{30}O_{12}$ requires C, 61·85; H, 5·2%).

Methylation of Austocystin D (23).—A mixture of austocystin D (60 mg), anhydrous potassium carbonate (600 mg) and methyl iodide (1 ml) in acetone (10 ml) was refluxed for 12 h to give a mixture of two products. Separation by preparative t.l.c. on alumina with chloroform–methanol (96 : 4 v/v) gave the *trimethyl ether* (24) (39 mg) as a glass (Found: M^+, 454·1619. $C_{25}H_{26}O_8$ requires M, 454·1626), and the *dimethyl ether* (25) (23 mg) as a glass (Found: M^+, 440·1468. $C_{24}H_{24}O_8$ requires M, 440·1470).

Methylation of Austocystin E (26).—Methylation of austocystin E (35 mg) with methyl iodide as described above gave the trimethyl (24) (11 mg) and dimethyl (25) (18 mg) ethers.

Methylation of Austocystin F (29).—Austocystin F (10 mg) was methylated with methyl iodide as described above to give 4,6-*di*-O-*methylaustocystin F* (30) (8 mg) as a glass (Found: M^+, 354·0733. $C_{19}H_{14}O_7$ requires M, 354·0737).

Sterigmatocystin Derivatives.—8-*O*-Methylsterigmatocystin (13), m.p. 265—267° (lit.,[21] 265—267°), dihydrosterigmatocystin (14), m.p. 229—230° (lit.,[21] 229—230°) and its acetate (15), m.p. 214—216° (lit.,[21] 215—216°), and 8-*O*-methyldihydrosterigmatocystin (18), m.p. 282—283° (lit.,[21] 282—283°) were prepared from sterigmatocystin (12) and characterized by standard procedures. Demethylation of dihydrosterigmatocystin (14), by the method described for dihydroaustocystin A (2), gave 6-*O*-demethyldihydrosterigmatocystin (16), m.p. 202—204° (lit.,[22] 202—204°). Acetylation of compound (16) gave the *diacetate* (17), m.p. 202—205° (from benzene–n-hexane); τ 7·7 (2H, m, 1-H), 7·68 (6H, s, 6- and 8-OAc), 6·30 (1H, m, 12c-H), 5·80 (2H, m, 2-H), 3·52 (1H, s, 5-H), 3·50 (1H, d, $J_{3a.12c}$ 6·0 Hz, 3a-H), 3·05 (1H, dd, J 8·0 and 1·0 Hz, 9-H), 2·69 (1H, dd, J 8·0 and 1·0 Hz, 11-H), and 2·38 (1H, dd, J 8·0 and 8·0 Hz, 10-H) (Found: C, 63·7; H, 4·2. $C_{21}H_{16}O_8$ requires C, 63·6; H, 4·1%).

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