The Biosynthesis of Phenols. Part XXIV.[1] Arugosin C, a Metabolite of a Mutant Strain of *Aspergillus Rugulosus*

By **James A. Ballantine, Victor Ferrito, Cedric H. Hassall,*† and Malcolm L. Jenkins,** Department of Chemistry, University College of Swansea, Singleton Park, Swansea SA2 8PP

It has been established that arugosin C, $C_{25}H_{28}O_6$, an optically active metabolite of *A. rugulosus*, has the dibenz-[*b.e*]oxepin structure (IV) and is, therefore, closely related to arugosin A.[2] This structure was established by mass spectrometry and by a comparison of the ^1H and ^{13}C n.m.r. spectra with those of arugosin A.

OUR investigations of the use of mutants to elucidate biosynthetic relationships of phenolic metabolites of fungi have utilised strains of *Aspergillus rugulosus*.[1-6] The wild-type produced two major metabolites, arugosins A and B,[2] with structures (Ia) and (Ib), respectively. Mutant strains gave various other novel phenolic compounds, including several related to asperugin (II).[3] Stages in the biosynthesis of asperugin have been deduced from those results.[1]

Preliminary studies of a mutant of *A. rugulosus* (designated A.R.M. 325) indicated that this strain produced traces of a compound related to arugosins A and B; this new compound, named arugosin C, has now been investigated further.

Arugosin C was isolated in very low yield from the mycelium and purified by counter-current distribution, followed by preparative layer chromatography and gel filtration. The optically active oil, $C_{25}H_{28}O_6$, had hydroxy-absorptions at 3600 and 3440 cm^{-1} (broad) in its i.r. spectrum and formed a crystalline mono-4-nitrobenzoate, mono- and di-acetates, and mono- and dimethyl ethers. The i.r. spectrum of the diacetate retained a broad band at 3400 cm^{-1}, indicating that arugosin C contained at least one hydroxy-function which resisted acylation.

Some of the features of the ^1H n.m.r. spectrum of arugosin C were also in the spectrum of arugosins A and B (Ia and b). Signals due to the aromatic protons, to one methyl and one isopentenyl group, each attached to an aromatic nucleus, and to the two chelated hydroxy-functions were in both spectra (Table 1). However, the signals associated with the isopentenyloxy side-chain in arugosins A and B were absent, as were the signals

† *Present address:* Roche Products Ltd., Welwyn Garden City, Herts.

[1] Part XXIII, J. A. Ballantine, V. Ferrito, and C. H. Hassall, *Phytochemistry*, 1971, **10**, 1309.

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

[3] J. A. Ballantine, C. H. Hassall, and G. Jones, *J. Chem. Soc.*, 1965, 4672.

[4] J. A. Ballantine, C. H. Hassall, B. D. Jones, and G. Jones, *Phytochemistry*, 1967, **6**, 1157.

[5] J. A. Ballantine, C. H. Hassall, and B. D. Jones, *Phytochemistry*, 1968, **7**, 1529.

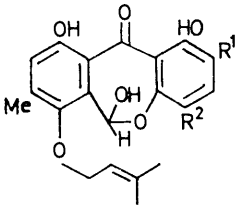
[6] J. A. Ballantine, V. Ferrito, C. H. Hassall, and V. I. P. Jones, *J. Chem. Soc.* (*C*), 1969, 56.

associated with the hemiacetal function. Instead, three new resonances were observed at τ 4·96, 5·76, and 7·67. Double-resonance experiments established that these signals were due to the presence of the partial structure (III). In addition, the signals due to the methyl groups of one of the isopentenyl functions in arugosins A and B were found at much higher field (τ 8·70 and 8·75); this was interpreted as due to deshielding of those methyl groups by a β oxygen atom in a saturated environment.

The close similarity of the spectra suggested that arugosin C was a close analogue of arugosins A and B, with some modification to the isopentenyloxy side-chain, and to the hemiacetal function.

If we assume the same basic skeleton and orientation for arugosins A, B, and C, two structures, (IV) and (V), can be proposed for arugosin C; both are in agreement with the ^{1}H n.m.r. data.

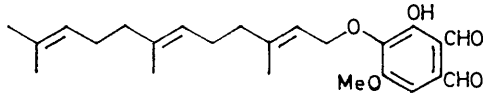
The OH signals at τ −3·85 and −0·69 indicate that the position of the isopentenyl side-chain in arugosin C is the same as for the major metabolite, arugosin A (Ia) (τ −3·37 and −0·80), but differs from that in arugosin B (Ib) (τ −2·7 and −1·09). The methylene protons (τ 5·76) were non-equivalent (AB octet); this is in accord with structures (IV) and (V) in which the heterocyclic



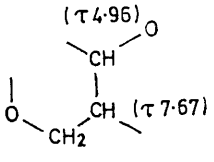
(I)

a; R^1 = CH_2·CH:CMe_2, R^2 = H

b; R^1 = H, R^2 = CH_2·CH:CMe_2



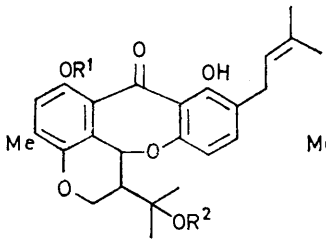
(II)

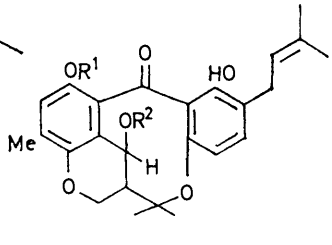


(III)

rings are relatively rigid. Arugosin C was stable to mild acid and alkali, in contrast to A and B which were degraded rapidly through modification of the hemiacetal function.

The structure (IV) accounts for the mass spectrum of arugosin C. In the case of arugosins A and B, the first major fragmentation involved loss of the *O*-linked isopentenyl side-chain and the formation of the ion $M - 68$. Instead, for arugosin C, there was loss of C_3H_6O from the molecular ion to give the ion *m/e* 366. The subsequent fragmentations of the ion *m/e* 366 (Scheme) are consistent with those of substituted benzophenones.[7] The structures assigned to the ions *m/e* 162





(IV) R^1 = R^2 = H (V) R^1 = R^2 = H

(VI) R^1 = Me, R^2 = H (VII) R^1 = Me, R^2 = H

(VIII) R^1 = R^2 = Ac (IX) R^1 = R^2 = Ac

and 188, formed from one segment of the benzophenone, and the ions *m/e* 205 and 149, formed from the other segment, lead to the structure (X) for the benzophenone ion *m/e* 366.

The ready six-centre rearrangement envisaged for the release of acetone in the Scheme presumably requires the six atoms to lie in one plane in the transition intermediate. The consideration of models established that this was possible for the structure (IV) but not for (V). Moreover the only reasonable mechanism for loss of acetone through electron bombardnment of (V) involves both the unfavourable cleavage of a Ph–O bond and the unlikely transference of an OH group.

Further support for the structure (IV) for arugosin C came from consideration of the ^{1}H n.m.r. spectrum of the diacetate (VIII) (Table 1). Acetylation occurs at one of the two phenolic hydroxy-groups (ArOAc at τ 7·81) and at the alcoholic hydroxy-group (ROAc at τ 8·17) but the signal (τ 4·96) for the methine proton in the alcohol was unchanged in the diacetate, although there is normally a large shift for such protons (*ca.* 1·1 p.p.m.) in the acetates of secondary alcohols. Also, the two methyl groups were found to be deshielded (0·22 p.p.m.) upon acetylation; this is consistent with the tertiary alcohol structure (IV).

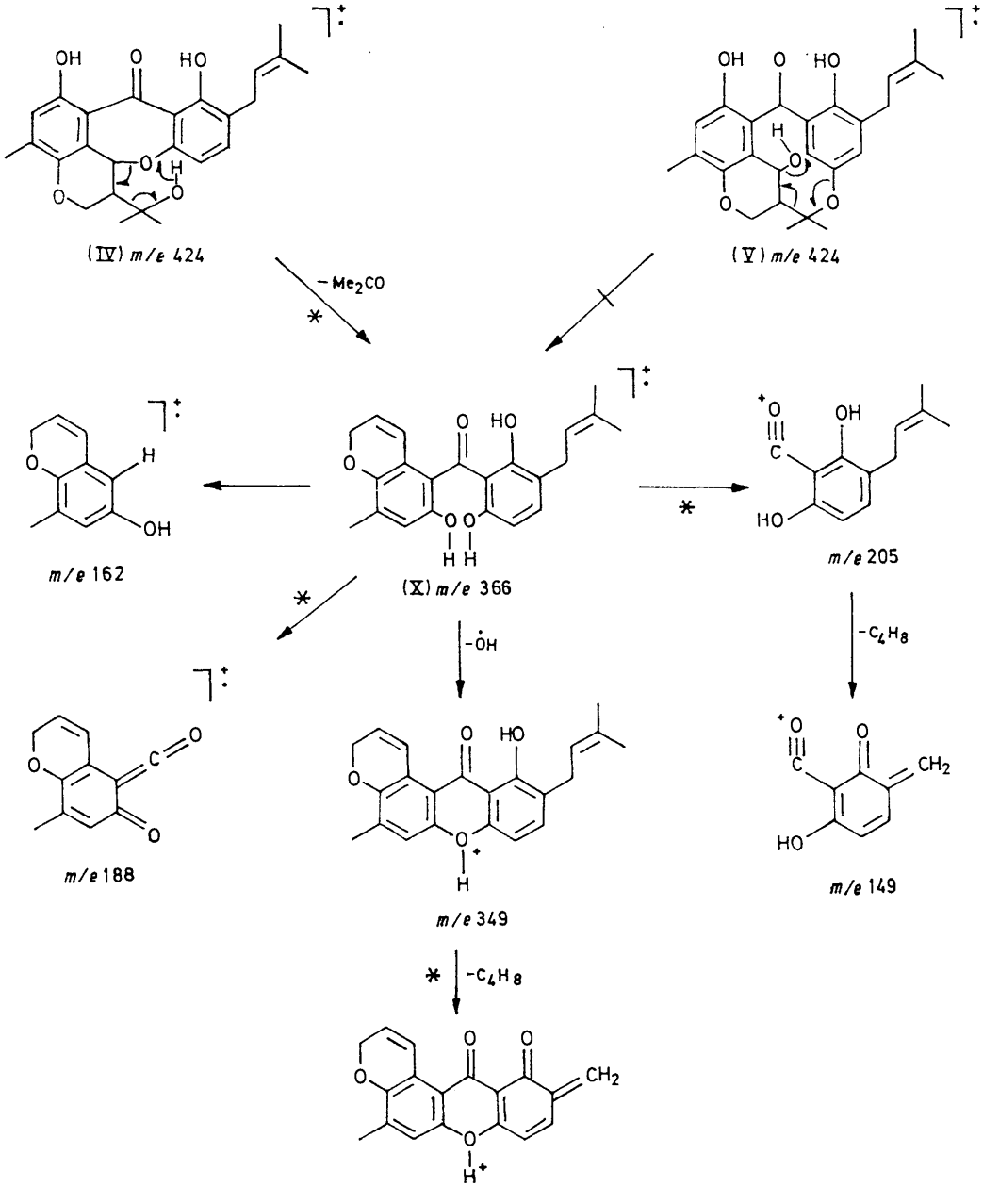
This conclusion was supported by the fact that mono-*O*-methylarugosin C [(VI) or (VII)] was unchanged after prolonged treatment with active manganese dioxide, a reagent which oxidises benzylic secondary alcohols.[8] Overall the chemical and physical evidence is clearly in favour of structure (IV) for arugosin C.

The small quantity of material available, combined with the chemical stability of arugosin C, prevented the confirmation of the orientation of the substituents by conventional degradative techniques, but the assignment of structure was supported by the comparison of the ^{13}C

[7] J. A. Ballantine and C. T. Pillinger, *Org. Mass Spectrometry*, 1968, **1**, 425.

[8] L. F. Fieser and M. Fieser, ' Reagents for Organic Synthesis,' Vol. 1, Wiley, New York, 1967, p. 640.

n.m.r. spectra of arugosins A and B with that of arugosin C. As ^{13}C n.m.r. spectroscopy is very sensitive to slight changes in the electronic environment of carbon atoms, any changes in the orientation of substituents should give rise to distinct differences in the proton-decoupled



SCHEME

^{13}C spectra of the compounds. The proton-decoupled spectrum of arugosin C contained the expected 25 resonances, whereas that of arugosins A and B contained 42 discernible peaks for the two position isomers.

The assignment of a peak to a particular carbon atom was made possible by comparison with literature reports for model compounds and was greatly assisted by off-centre double-resonance techniques. These restore enough coupling for the multiplet structure of each resonance to be discerned and the number of protons attached to each carbon atom determined. The use of this procedure enabled the majority of the peaks to be assigned to particular carbon atoms and the rest of the peaks to groups of like atoms. The assignments are compared in Table 2 using the numbering system indicated for the carbon atoms in arugosin C (IV) and arugosin A (Ia).

TABLE 1

^{1}H N.m.r. assignments of arugosin C and related compounds (τ values; solvent $CDCl_3$)

Assignments	No. of protons	Multiplicity (J/Hz)	Arugosin C (IV)	Arugosins A and B (Ia and b)	Mono-*O*-methyl arugosin C (VI)	Di-*O*-acetyl arugosin C (VIII)
ArOH	1 *	s	−3·85	(−3·37, −2·78)	−3·09	−3·10
ArOH	1 *	s	−0·69	(−1·09, −0·87)		
ArH	1	d (8)	2·76	2·76	2·81	2·85
ArH	1	s	3·23	3·19	3·23	3·21
ArCH(O*H*)–O	1 *	d (5)		3·46		
ArH	1	d (8)	3·64	3·56	3·67	3·75
=C*H*–CH_2–O–	1	m		4·48		
ArCH_2–C*H*=	1	m	4·72	4·73	4·70	4·80
ArCH–O	1	d (4)	4·96		4·85	4·97
ArC*H*(OH)–O–	1	d (5)		5·0		
O–CH_2–CH	2	m (4, 9)	5·76	5·66 δ	(5·60, 6·97) ‡	(5·63, 6·06) ‡
ArO·CH_3	3	s			6·20	
ArCH_2–	2	d (8)	6·72	6·74	6·70	6·78
O–CH_2–C*H*–CH	1	m (4, 9)	7·67		7·63	7·33
ArCH_3	3	s	7·78	7·68	7·73	7·81
ArO·CO·CH_3	3	s				7·81
C–O–CO·CH_3	3	s				8·17
$(CH_3)_2$C=	6	d (7)	8·28	8·25 †	8·25	8·34
C–OH	1 *	s	8·44		8·43	
$(CH_3)_2$C–O–	3	s	8·70		8·70	8·50
$(CH_3)_2$C–O–	3	s	8·75		8·70	8·50

* Exchangeable. † 12H. ‡ Two 1H quartets, J 4 and 9 Hz. δ 2H doublet, J 7 Hz.

TABLE 2

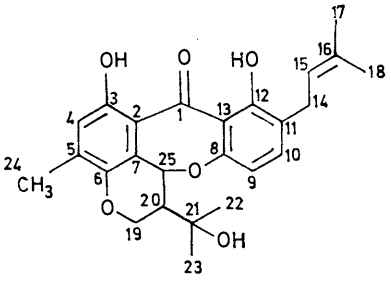
Comparison of ^{13}C n.m.r. spectra

Arugosin C (IV)		Arugosins A and B (Ia and b)		Ref. compounds	Ref.
Shift † δ_C (p.p.m.)	Assignment of C atom	shift † δ_C (p.p.m.)	Assignment of C atom	δ_C (p.p.m.) of analogous C atoms	
194·0	1	194·4	1	ArCO, 190—200	*a*
160·8, 156·6, 153·3, 143·0 *	3, 6, 8, 12	160·1, 159·4, 155·4, 154·8, 150·6, 149·9, 143·6, 143·5 *	3, 6, 8, 12	1,3-Dimethoxybenzene 162 Sterigmatocystin 154—164	*b* *c*
	*	139·0, 138·8	21		
	*	137·4 (d)	20		
135·3	15	135·8, 135·2 (d)	15	Me_2C=CH·$CHMe_2$, 130	*d*
134·2	7		*		
130·7	16	130·8, 130·6	16		
	*	128·5, 128·3	7		
122·1	5	121·9, 121·0	5	*o*-Cresol, 123·3	*b*
120·1	10	120·7, 120·1 (d)	10	1,3-Dimethoxybenzene, 129·2	*b*
118·7	2 or 13	118·9	2 or 13	Sterigmatocystin, 104—130	*c*
118·2 (d)	4	118·8 (d)	4	*m*-Cresol, 123·6	*b*
117·8	2 or 13	117·3, 116·3	2 or 13		
110·8	11	112·5, 116 (d)	9, 11	1,3-Dimethoxybenzene, 105·5	*b*
107·2 (d)	9	110·0, 108·4 (d)			
	*	92·4, 91·6 (d)	25	Lycorenine, 90·3	*e*
73·1 (d)	25	—	*	X–537A, 71·3	*f*
	*	70·8, 70·1 (t)	19		
69·8	21	—	*		
64·2 (t)	19	—	*		
48·7 (d)	20	—	•		
28·3, 27·7, 27·3, 25·3, 17·4, 16·1 (m)	14, 17, 18 22, 23, 24	30·2, 28·2, 27·3, 25·4, 25·0, 17·3, 17·4, 16·5 (m)	14, 17, 18 22, 23, 24	*o*-Cresol, 16—30 Radicirin, 16—30	*b* *g*

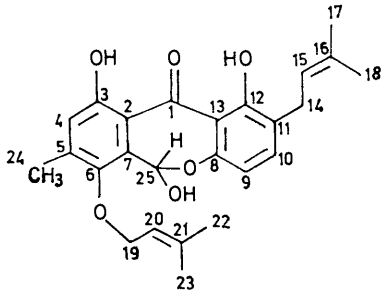
* Significant deviations in chemical shift values. † The multiplicity indicated is the result of off-resonance experiments.

[a] J. B. Stothers and P. C. Lauterbur, *Canad. J. Chem.*, 1964, **42**, 1563. [b] P. C. Lauterbur, *J. Amer. Chem. Soc.*, 1961, **83**, 1846. [c] M. Tanabe, T. Hamasaki, and H. Seto, *Chem. Comm.*, 1970, 1539. [d] R. A. Friedel and H. L. Retcofsky, *J. Amer. Chem. Soc.*, 1963, **85**, 1300. [e] W. O. Crain, jun., W. C. Wildman, and J. D. Roberts, *J. Amer. Chem. Soc.*, 1971, **93**, 990. [f] J. W. Westley, D. L. Pruess, and R. G. Pitcher, *J.C.S. Chem. Comm.*, 1972, 161.

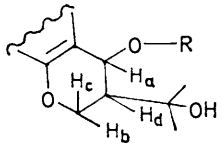
When the chemical shifts found from the arugosin C spectrum were compared with those of the major isomer in arugosins A and B it became evident that signals for 19 of the 25 carbon atoms were almost identical (within 1·5 p.p.m.), but for the other 6 carbon atoms there were much greater differences.



(IV)



(Ia)



(IVa)

The signal corresponding to the hemiacetal carbon atom (C-25) had moved upfield from 9·24 p.p.m. in arugosins A and B to 73·1 p.p.m. in arugosin C. This was attributed to the transfer of one of the oxygen atoms. The signal for the oxygen-linked atom (C-19) had also moved upfield from 70·8 to 64·2 p.p.m., as expected on saturation of the adjacent double bond.

In addition, the signals due to the alkene carbon atoms (C-20 and C-21) at 139 and 137·4 p.p.m. were replaced by resonances at 48·7 and 69·8 p.p.m., respectively, for two saturated carbon atoms. Finally, the resonances assigned to the ring junction carbon atoms (C-7 and C-8) had moved downfield by 6 and 3 p.p.m., respectively, presumably owing to the constraint imposed upon them by the formation of the tetracyclic system.

In this way, the six differences found in the ^{13}C n.m.r. spectrum of arugosin C, when compared with data for arugosins A and B, can be completely rationalised in terms of the suggested modification of the isopentenyloxy side-chain. As the spectra are virtually identical in other respects the main carbon skeleton must be identical.

It was not possible to define the stereochemistry at the asymmetric centres (C-20 and C-25) in the benzopyran ring system in arugosin C (IVa), for although the ^1H n.m.r. coupling constants (Table 1) indicated the relationships, H_a/H_d-*gauche*, H_b/H_d-*gauche*, and H_c/H_d-*trans*, an examination of models of the tetracycline ring system established that there were a number of conformations where these relationships were valid.

EXPERIMENTAL

M.p.s were determined with a Kofler hot-stage microscope. U.v. spectra were measured with a Unicam SP 800 spectrophotometer. O.r.d.–c.d. measurements were performed at Westfield College and we are grateful to Professor W. Klyne for these measurements. I.r. spectra were determined with a Perkin-Elmer 257 spectrophotometer. ^1H N.m.r. spectra were obtained with a Varian HA-100 instrument. ^{13}C N.m.r. spectra were obtained with a Varian XL-100 instrument at Zug, Switzerland, and we are grateful to Varian A.G. for these measurements. Mass spectra were measured at 70 eV with an A.E.I. MS-9 spectrometer. All molecular formulae quoted for ions were established by accurate mass measurement. Each of the samples analysed by mass spectrometry was shown to be homogeneous by t.l.c. in the three solvent systems: (a) benzene–methanol–acetic acid (10 : 2 : 1), (b) light petroleum (b.p. 60—80°)–ethyl acetate (3 : 1), (c) chloroform. The ^1H n.m.r. spectra which are recorded confirm, through integration studies and through the absence of stray signals, that material of a high standard of purity was examined. R_F Values refer to thin-layer chromatograms [light petroleum (b.p. 60—80°)–ethyl acetate (72 : 28 v/v) as eluting solvent].

Extraction and Purification of Arugosin C.—A mutant strain, A.R.M. 325, of *Aspergillus rugulosus* was grown from a spore suspension for 14 days at 25° on a low-nitrogen medium.[9] The stationary cultures were in batches of 800 flat-sided bottles (*ca.* 1 l capacity) each containing 200 ml of medium. The mycelium, after separation from the culture fluid with a wire mesh strainer, was macerated in a blender with distilled water (100 ml for 5 bottles) and the macerate was extracted with ether (250 ml for 5 bottles). Concentration of the ether solution from 800 bottles *in vacuo* yielded a dark brown viscous residue (22 g) which was shown by t.l.c. to contain a complex mixture of metabolites. Portions of the residue (*ca.* 7 g) were distributed equally between the first six tubes of a 200-tube automatic countercurrent distribution instrument (H.O. Post Scientific Instrument Co.) and subjected to 200 transfers with 10 ml phases of light petroleum–methanol–water (10 : 10 : 1 v/v). Tubes 50—80 yielded impure arugosin C as a yellow syrup (807 mg), which was subjected to preparative layer chromatography on Kieselgel G plates (200 mg on each 30 × 20 × 0·1 cm plate). The light yellow band of R_F 0·55 was collected and the metabolite was eluted with ethyl acetate. The product (248 mg) was contaminated with farnesol, which was removed by gel filtration through Sephadex LH20 in methanol to yield *arugosin C* (IV) {1,12a-*dihydro*-6,8-*dihydroxy*-1-(1-*hydroxy*-1-*methylethyl*)-4-*methyl*-9-(3-*methylbut*-2-*enyl*)[1]*benzopyrano*[4,5-bc][1]*benzoxepin*-7(2H)-*one*} as a golden yellow oil (107 mg) (Found: M^+, 424·1886 ± 0·002.

[9] C. H. Hassall and K. Lawrence, *J. Gen. Microbiol.*, 1964, **35**, 483.

$C_{25}H_{28}O_6$ requires M, 424·1886), $\nu_{max.}$ ($CHCl_3$) 3600 (OH), 3440br (OH) and 1620 (CO) cm^{-1}, $\lambda_{max.}$ (EtOH) 227, 271, 293, 309, and 407 nm (log ε 4·34, 3·94, 3·97, 4·01, and 3·95), o.r.d. (MeOH, *c* 8·4) λ 446, 371, 333, 295, 274, and 236 nm (mol. rotation −4950, +16,800, +25,500, −9100, +10,900, and +23,600°), c.d. (MeOH, *c* 8·4) λ 409, 307, 262, and 223 nm (mol. ellipticity −12,640, +12,060, +10,200, and +44,220), *m/e* 424 (31%), 366 (10), 349 (7), 293 (11), 205 (14), 203 (17), 189 (54), 188 (100), 163 (12), 162 (58), 161 (21), 149 (25), and 59 (26).

Mono-O-4-bromobenzoylarugosin C.—Treatment of arugosin C (50 mg) with 4-bromobenzoyl chloride in pyridine at room temperature yielded the *mono-4-bromobenzoate* as yellow prisms (from light petroleum), m.p. 76° (44 mg) (Found: M^+, 608·1234 ± 0·003. $C_{32}H_{31}{}^{81}BrO_7$ requires M, 608·1234), $\nu_{max.}$ ($CHCl_3$) 3610 (OH) and 1730 (ester) cm^{-1}.

Methylation of Arugosin C.—Arugosin C (30 mg) was refluxed with dimethyl sulphate and anhydrous potassium carbonate in anhydrous acetone for 18 h. The resultant methyl derivatives were separated by preparative layer chromatography on Kieselgel G plates (chloroform as eluant). This afforded mono- and di-*O*-methyl ethers (10·4 and 4·3 mg, respectively) as pure yellow viscous oils.

Mono-O-methylarugosin C (VI) (Found: M^+, 438·2042 ± 0·0010. $C_{26}H_{30}O_6$ requires M, 438·2042) showed $\nu_{max.}$ ($CHCl_3$) 3610 (OH) and 3440br (OH) cm^{-1}, $\lambda_{max.}$ (EtOH) 212, 264, 285, and 372 nm (log ε 4·38, 3·96, 3·95, and 3·92), *m/e* 438 (23%), 380 (7), 350 (22), 349 (89), 294 (20), 293 (100), 203 (27), 176 (83), and 175 (22).

Di-O-methylarugosin C (Found: M^+, 452·2199 ± 0·0010. $C_{27}H_{32}O_6$ requires M, 452·2199) showed $\nu_{max.}$ ($CHCl_3$) 3640 (OH) cm^{-1}, τ ($CDCl_3$) 2·85 (1H, d), 3·30 (1H, s), 3·40 (1H, d), 4·80 (1H, t), 4·77 (1H, d), 5·70 (2H, m), 6·17 (3H, s), 6·24 (3H, s), 6·70 (2H, d), 7·70 (1H, m), 7·80 (3H, s), 8·28 (6H, s), 8·47 (1H, s, exchangeable with D_2O, OH), and 8·72 (6H, s), $\lambda_{max.}$ (EtOH) 233, 278, and 342 nm (log ε 4·17, 4·08, and 3·62).

Attempted Oxidation of Mono-O-methylarugosin C.—Activated manganese dioxide [10] (250 mg) was added to mono-*O*-methylarugosin C (21 mg) in chloroform (10 ml) and the suspension was stirred for 24 h at room temperature. Mono-*O*-methylarugosin C was obtained unchanged on work-up.

Mono-O-acetylarugosin C.—Arugosin C (60 mg) was shaken with acetic anhydride (1·5 ml) and anhydrous sodium acetate (0·5 ml) for 18 h at room temperature before pouring into an excess of potassium hydrogen carbonate solution. Extraction, followed by preparative layer chromatography, yielded *mono-O-acetylarugosin C* as a clear yellow viscous oil (37 mg) (Found: M^+, 466·1992 ± 0·0010. $C_{27}H_{30}O_7$ requires M, 466·1992), $\nu_{max.}$ ($CHCl_3$) 3600 (OH), 3440br (OH), and 1755 (ester) cm^{-1}, τ ($CDCl_3$) −3·20 (1H, s, exchangeable with D_2O, ArOH), 2·76 (1H, d), 3·18 (1H, s), 3·70 (1H, d), 4·74 (1H, t), 4·88 (1H, d), 5·58 (1H, q), 6·0 (1H, q), 6·74 (2H, d), 7·62 (1H, m), 7·79 (6H, s, $ArCH_3$ and OAc), 8·28 (6H, d), 8·42 (1H, s, OH), and 8·73 (6H, d), $\lambda_{max.}$ (EtOH) 221, 258, 295, and 347 nm (log ε 4·25, 3·99, 3·99, and 3·77).

Di-O-acetylarugosin C.—Arugosin C (54 mg) was refluxed with acetic anhydride (10 ml) and anhydrous sodium acetate (3 g) for 1 h then poured into an excess of potassium hydrogen carbonate solution. Extraction followed by preparative layer chromatography separated the mono-*O*-acetyl derivative (R_F 0·35) from *di-O-acetylarugosin C* (R_F 0·70), which was obtained as a yellow viscous oil (12 mg) (Found: M^+, 508·2097 ± 0·0010. $C_{29}H_{32}O_8$ requires M, 508·2097), $\nu_{max.}$ ($CHCl_3$) 3449br (OH), 1755 (ester), and 1730 (ester) cm^{-1}, $\lambda_{max.}$ (EtOH) 223, 256, 297, and 347 nm (log ε 4·07, 3·76, 3·76, and 3·62).

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