Structure Elucidation of the Macrocyclic Antibiotic Lipiarmycin

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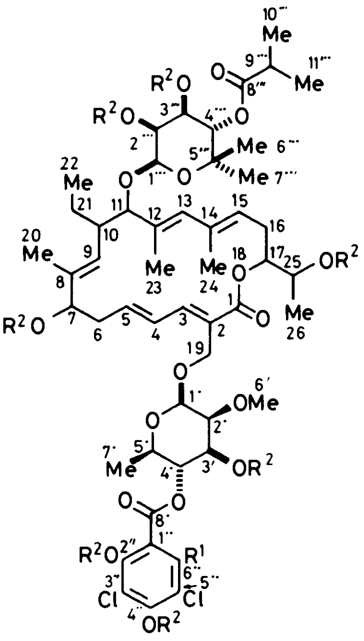
By a combination of chemical degradations and ^{1}H and ^{13}C n.m.r. studies, the structure of the antibiotic lipiarmycin, produced by *Actinoplanes deccanensis*, has been elucidated. The molecule contains two glycosyl moieties, namely 2-*O*-methyl-4-*O*-homodichloro-orsellinate-β-D-rhamnose and 4-*O*-isobutyrate-5-methyl-β-rhamnose, attached to a 18-membered unsaturated lactone ring.

Lipiarmycin, an antibiotic produced by fermentation of a strain of *Actinoplanes*,[1] is active mainly against gram-positive bacteria and particularly against strains of cariogenic *Streptococcus mutans*.[2] It inhibits the growth of susceptible bacteria by interfering with RNA synthesis.[3] Preliminary investigation led to the identification of six aliphatic moieties, one 5-methyl-β-rhamnose unit, and one 2-*O*-methyl-4-*O*-homodichloro-orsellinate-β-rhamnose unit.[4] In this paper we describe the complete structure elucidation of lipiarmycin on the basis of new chemical evidence and ^{1}H and ^{13}C n.m.r. experiments.

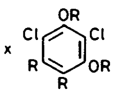
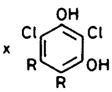
Reverse-phase thin-layer chromatography (t.l.c.) and high-performance liquid chromatography (h.p.l.c.) show that lipiarmycin is a mixture of two main components, designated A3 and A4 and present in a 3:1 ratio, which were separated by flash chromatography. Examination of the ^{1}H n.m.r. spectra of the two compounds indicated that factor A3 (**1a**) only differs from factor A4 (**2**) in that the ethyl group on the phenyl moiety is replaced by a methyl group (see Figure 1 and Table 2). In addition, by mild KOH–MeOH hydrolysis of lipiarmycin A4



(1a) R^1 = CH_2—Me ; R^2 = H
(1b) R^1 = CH_2—Me ; R^2 = Ac
(1c) R^1 = CH_2—Me ; R^2 = PhCO
(2) R^1 = Me ; R^2 = H

Figure 1.

Table 1. Carbon and proton counts for lipiarmycin A3 (**1a**) from ^{13}C and ^{1}H n.m.r. data

^{13}C N.m.r.	^{1}H N.m.r.	Number of carbons	Number of protons	Carbon assignment
11 × CH_3	1 × $(CH_3)_2CH$	11	6	10‴ and 11‴
	1 × $(CH_3)_2C$		6	6‴ and 7‴
	2 × CH_3CH_2		6	22 and 8″
	2 × CH_3CH		6	26 and 7′
	3 × CH_3C=		9	20, 23, and 24
4 × CH_2	4 × CH_2	4	8	6, 16, 21, and 7″
2 × CH	2 × CH	2	2	10 and 9‴
6 × CH=	2 × CH=	6	6	3, 4, 5, 9, 13, and 15
4 × C=		4		2, 8, 12, and 14
1 × 	1 × 	6	2	1″, 2″, 3″, 4″, 5″, and 6″
1 × CH_3OR	1 × CH_3OR	1	3	6′
1 × CH_2OR	1 × CH_2OR	1	2	19
11 × $CHOR$	5 × $CHOH$	11	10	7, 25, 3′, 2‴, and 3‴
	3 × $CHOR$		3	11, 2′, and 5′
	3 × $CHOCOR$		3	17, 4′, and 4‴
1 × COR_2		1		5‴
2 × $CH(OR)_2$	2 × $CH(OR)_2$	2	2	1′ and 1‴
3 × CO_2R		3		1, 8′, and 8‴
Total		54	74	

(**2**), methyl 2,4-dihydroxy-3,5-dichloro-6-methylbenzoate was obtained, confirming the substitution on the aromatic ring (see Experimental). We therefore concentrated our studies on lipiarmycin A3 (**1a**).

Fast atom bombardment mass spectrometry (f.a.b.-m.s.) of (**1a**) did not produce significant fragment ions but exhibited, in the positive ion spectrum, the $[M\text{Na}]^+$ ion at m/z 1 079 with a less abundant quasi molecular ion $[MH]^+$ at m/z 1 057. Thus, the molecular formula previously[4] assigned to lipiarmycin was revised and a molecular mass of 1 056 corresponding to the molecular formula $C_{52}H_{74}Cl_2O_{18}$ assigned to compound (**1a**).

Analysis of the ^{1}H and ^{13}C n.m.r. spectra of compound (**1a**) was fully consistent with the proton and carbon counts, as illustrated in Table 1.

In fact, the fully decoupled ^{13}C n.m.r. spectrum of compound (**1a**) showed the presence of 52 carbon atoms while the ^{1}H n.m.r spectrum indicated 74 hydrogen atoms, of which seven belong to hydroxy groups as demonstrated by formation of the hepta-acetate (**1b**) upon acetylation with Py-Ac_2O. The characteristic downfield shift experienced by 7-H, 25-H, 3′-H, 2‴-H, and 3‴-H (δ 0.95—1.45 p.p.m.) (Table 2) permitted us to assign five hydroxy groups at the corresponding carbon atoms, whereas

Table 2. ^{1}H N.m.r. chemical shifts (δ_H/p.p.m.) for compounds (**1a**), (**1b**), (**2**), (**3b**), (**3c**), (**14**), (**15**), and (**16**) in [$^2H_6$]acetone

Proton[a]	(**1a**)	(**1b**)	(**2**)	(**3b**)[b]	(**3c**)	(**14**)	(**15**)	(**16**)
3	7.24	7.17	7.24	7.34	7.34	7.39	7.39	7.39
4	6.63	6.66	6.63	6.63	6.74	6.71	6.71	6.69
5	5.96	5.97	5.96	6.14	6.16	6.27	6.28	6.19
6a	2.68	*ca.* 2.7	2.70	2.44	2.59	2.50	2.50	2.73
6b	2.52	*ca.* 2.6	2.52	2.42	2.57	2.46	2.46	2.50
7	4.28	5.23	4.28	4.06	5.16	4.12	4.12	4.34
9	5.22	4.94	5.22	4.95	5.03	5.22	5.22	5.35
10	2.63	*ca.* 2.7	2.63	2.54	*ca.* 2.5	3.15	3.15	3.21
11	3.73	3.77	3.73	3.61	3.71	5.09	5.07	5.28
13	5.84	5.98	5.84	5.71	5.79	5.91	5.90	6.13
15	5.63	5.57	5.63	5.35	5.27	4.44	4.37	5.00
16a	2.75	*ca.* 2.7	2.76	2.27	*ca.* 2.5	1.91	2.34	2.14
16b	2.43	*ca.* 2.4	2.45	2.25	*ca.* 2.5	1.87	1.75	1.71
17	4.74	4.91	4.74	3.65	5.05	3.89	3.89	5.10
19a	4.61	4.59	4.60	4.67	4.66	4.65	4.65	4.60
19b	4.43	4.46	4.42	4.49	4.49	4.52	4.52	4.46
20	1.66	1.73	1.66	1.63	1.67	1.69	1.69	1.70
21a	1.93	1.75	*ca.* 1.9	1.83	*ca.* 1.8	1.35	1.35	*ca.* 1.4
21b	1.25	*ca.* 1.2	*ca.* 1.2	*ca.* 1.2	*ca.* 1.2	1.35	1.35	*ca.* 1.4
22	0.82	0.75	0.83	0.78	0.76	0.85	0.85	0.87
23	1.82	1.83	1.82	1.68	1.64	1.75	1.75	1.91
24	1.74	1.86	1.74	1.74	1.74	1.72	1.75	1.75
25	4.05	5.29	4.05	3.83	5.00	3.75	3.75	4.07
26	1.19	1.25	1.19	1.18	1.23	1.19	1.19	1.22
1′	4.69	4.75	4.68	4.53	4.79	4.69	4.69	4.69
2′	3.61	3.79	3.61	*ca.* 3.6	3.81	3.62	3.62	3.61
3′	3.81	4.95	3.83	3.58	4.94	3.78	3.78	3.81
4′	5.11	5.31	5.10	5.06	5.32	5.13	5.12	5.11
5′	3.60	3.69	3.63	3.39	3.69	3.59	3.59	3.60
6′	3.53	3.46	3.52	3.64	3.48	3.55	3.55	3.53
7′	1.32	1.38	1.30	1.40	1.40	1.33	1.33	1.29
7″a	3.01	2.81	2.56	2.80	2.82	3.01	3.02	3.06
7″b	3.01	2.77		2.78	2.78	3.01	3.02	2.99
8″	1.22	1.18		1.19	1.19	1.22	1.22	1.19
1‴	4.78	5.06	4.78	4.62	5.01			
2‴	3.97	5.42	3.97	4.00	5.44			
3‴	3.74	5.16	3.74	*ca.* 3.6	5.14			
4‴	5.00	5.11	5.00	*ca.* 3.6	5.11			
6‴	1.10	1.25[c]	1.10	1.28[c]	1.27[c]			
7‴	1.16	1.17[c]	1.16	1.10[c]	1.17[c]			
9‴	2.57	2.55	2.57					
10‴	1.16[c]	1.12[d]	1.16[c]					
11‴	1.14[c]	1.10[d]	1.14[c]					

[a] Compounds (**1a**) and (**2**) exhibited OH resonances at δ_H 10.66, 9.30, 4.35, 4.05, *ca.* 3.6, *ca.* 3.6, and 3.35, and at 10.87, 9.38, 4.35, 4.05, *ca.* 3.6, *ca.* 3.6, and 3.35, respectively; compounds (**1b**) and (**3c**) exhibited OAc resonances at δ_H 2.40, 2.28, 2.19, 2.11, 2.05, 2.01, and 1.96, and at 2.44, 2.34, 2.11, 2.10, 2.02, 2.01, 1.98, 1.98, and 1.92, respectively, and (**3c**) a CO_2H resonance at δ_H 10.50; compound (**3b**) exhibited OMe resonances at δ_H 3.91, 3.88, and 3.78. [b] $CDCl_3$. [c,d] Assignments within each column may be interchanged.

Table 3. ^{1}H-{^{1}H} coupling constants (*J*/Hz) for compounds (**1a**), (**1b**), (**2**), (**3b**), (**3c**), (**14**), (**15**), and (**16**)

J	(**1a**)	(**1b**)	(**2**)	(**3b**)	(**3c**)	(**14**)	(**15**)	(**16**)
3,4	11.3	11.5	11.3	11.5	11.4	11.3	11.3	11.3
3,5	0.8	*ca.* 1	0.8	0.8	0.8	*ca.* 1	*ca.* 1	0.8
3,19a	*ca.* 0.5	*ca.* 0.5	*ca.* 0.5	*a*	*ca.* 0.5	*a*	*a*	*ca.* 0.5
4,5	14.8	15.0	14.8	15.1	15.1	15.0	15.0	15.0
4,6a	1.8	*ca.* 2	1.8	*a*	*ca.* 1.4	*ca.* 1	*ca.* 1	1.8
4,6b	1.0	*ca.* 1	1.0	*a*	*ca.* 1.4	*ca.* 1	*ca.* 1	1.1
5,6a	4.6	4.8	4.6	*ca.* 7	*ca.* 7.5	7.0	7.0	5.0
5,6b	9.4	10.0	9.4	*ca.* 7	*ca.* 7.5	7.6	7.6	9.3
6a,6b	*a*	*a*	*a*	*ca.* 14.8	*a*	14.0	14.0	15.3
6a,7	*ca.* 4	*ca.* 4	*ca.* 4	*ca.* 7.5	*ca.* 6.5	6.8	6.8	3.2
6b,7	*ca.* 4	*ca.* 4	*ca.* 4	*ca.* 6	*ca.* 6.5	6.0	6.0	3.6
7,9	1.6	1.4	1.6	*a*	1.2	*ca.* 1	*ca.* 1	1.7
7,20	0.6	*a*	0.6	*a*	*a*	*a*	*a*	0.6
9,10	10.4	10.7	10.4	10.6	10.5	9.3	9.3	9.8
9,20	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.6
10,11	9.6	10.0	9.6	9.3	9.2	9.3	9.3	7.8
10,21a	*a*	*a*	*a*	3.2	*a*	7.2	7.2	7.4
10,21b	*a*	*a*	*a*	9.3	*a*	7.2	7.2	7.4
11,13	0.5	*a*	0.5	*a*	0.8	1.3	1.3	1.6
11,23	*a*	*a*	*a*	*a*	*ca.* 0.5	1.6	1.6	1.4
13,15	1.0	1.0	1.0	*a*	1.3	1.2	1.2	*a*
13,23	1.3	1.3	1.3	1.4	1.4	*a*	*a*	*ca.* 0.5
13,24	0.5	*a*	0.5	*a*	*a*	1.5	1.5	1.6
15,16a	7.2	*ca.* 8	7.2	*ca.* 7	*ca.* 7.5	8.8	6.6	11.5
15,16b	9.0	*ca.* 8	9.0	*ca.* 7	*ca.* 7.5	6.7	9.4	3.8
15,24	1.3	1.4	1.3	1.4	1.4	*a*	*a*	*a*
16a,16b	14.2	*a*	14.2	*a*	*a*	13.0	12.3	13.0
16a,17	5.1	7.0	5.1	*ca.* 6.5	*ca.* 9.5	5.7	6.7	4.0
16b,17	4.4	4.6	4.4	*ca.* 6.5	*ca.* 5.5	3.4	*a*	*ca.* 1
17,25	6.2	5.4	6.2	4.0	4.0	3.8	3.8	*ca.* 1
19a,19b	11.3	11.8	11.3	11.8	11.5	11.5	11.5	11.5
21a,22	7.3	7.5	7.3	7.3	7.2	7.4	7.4	7.3
21b,22	7.3	7.5	7.3	7.3	7.2	7.4	7.4	7.3
25,26	6.3	6.5	6.3	6.4	6.4	6.3	6.3	6.5
1′,2′	0.8	1.0	0.8	1.0	0.9	0.8	0.8	0.8
2′,3′	3.3	3.3	3.3	*a*	3.2	3.3	3.3	3.3
3′,4′	9.7	10.3	9.7	9.3	10.3	9.8	9.8	9.8
4′,5′	9.7	9.6	9.7	9.4	9.6	9.6	9.6	9.5
5′,7′	6.1	6.2	6.1	6.2	6.2	6.2	6.2	6.1
7″a,7″b	*a*	14.0		13.5	13.5	*a*	*a*	12.6
7″a,8″	7.3	7.5		7.5	7.5	7.4	7.4	7.3
7″b,8″	7.3	7.5		7.5	7.5	7.4	7.4	7.3
1‴,2‴	1.1	1.4	1.1	1.5	1.4			
2‴,3‴	3.3	2.9	3.3	3.1	2.7			
3‴,4‴	10.0	10.7	10.0	*a*	10.7			
9‴,10‴	6.9	7.0	6.9					
9‴,11‴	6.9	7.0	6.9					

[a] Not determined.

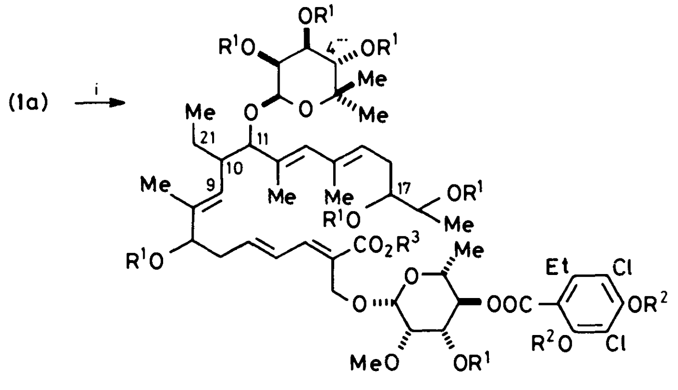
the two remaining hydroxy groups were assigned to the homodichloro-orsellinate moiety. The assignment of the resonances in the ^{1}H and ^{13}C n.m.r. spectra of compound (**1a**) (Tables 2—4) followed from chemical-shift criteria and from ^{1}H-{^{1}H} and ^{13}C-{^{1}H} low-power specific decoupling experiments, whereas multiplicities and (C,H) coupling constants were obtained from analysis of fully ^{1}H coupled ^{13}C n.m.r. spectra.

Some reactions were performed on compound (**1a**) in order to obtain additional structural evidence. Treatment of compound (**1a**) with KOH gave compound (**3a**) *via* opening of a lactone function and removal of an isobutyryl moiety, as depicted in Scheme 1.

F.a.b.-m.s. of compound (**3a**) indicated a molecular mass of 1 004.02 m.u., in agreement with the molecular formula $C_{48}H_{70}Cl_2O_{18}$. Moreover, compound (**3a**) on treatment with CH_2N_2 afforded the trimethyl derivative (**3b**), and with Py–Ac_2O the nona-acetate (**3c**). The three OMe resonances in compound (**3b**) have to be ascribed to the methylation of the CO_2H group formed by hydrolysis of the lactone bond, and of the two phenolic hydroxy groups of the homodichloro-orsellinate unit. On the other hand, the two additional acetate groups in (**3c**) relative to the hepta-acetate (**1b**) must arise from the esterification of the new formed 17- and 4‴-OH's. The downfield shift of the resonances of the corresponding protons (δ *ca.* 1.5 p.p.m.) with respect to (**3b**) confirm their assignment. The ^{1}H n.m.r. data of compounds (**3b**) and (**3c**), which are fully consistent with the proposed structures, are given in Tables 2 and 3.

On the basis of the above-described and of the following evidence five fragments (**4**)—(**8**), shown in Figure 2, could be identified.

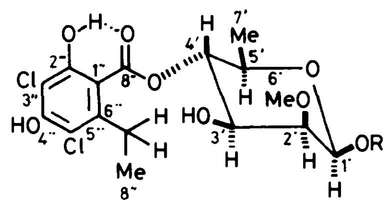
Fragment (**4**). This fragment was characterized by the number of protons which are located on oxygen-bearing carbon atoms.



(3)

a; $R^1 = R^2 = R^3 = H$
b; $R^1 = H$, $R^2 = R^3 = Me$
c; $R^1 = R^2 = Ac$, $R^3 = H$

Scheme 1. *Reagents*: i, 0.5M KOH; N_2



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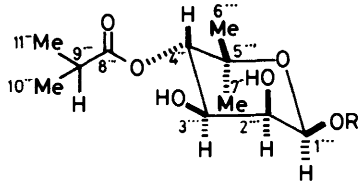

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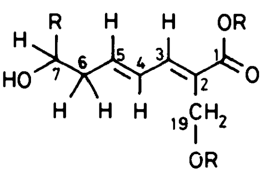

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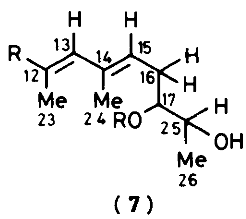

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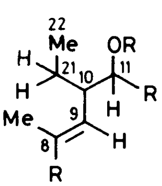


(8)

Figure 2.

The doublet of quartets at δ_H 3.60 ($^3J_{4',5'}$ 9.7 and $^3J_{5',7'}$ 6.1 Hz), assigned to 5'-H, served as the starting point in the analysis of this spin system. Its irradiation caused collapse of the 3 H doublet at δ_H 1.32 (7'-H_3) to a singlet and simplified the signal at δ_H 5.11 (4'-H) to a doublet, the remaining *trans*-diaxial coupling of 9.7 Hz being due to 3'-H (δ_H 3.81). The small couplings of 0.8 and 3.3 Hz observed for the equatorially-disposed 2'-H must arise from interactions with its neighbouring 1'- and 3'-H. The location of the OMe group at C-2' followed from the three-bond (C,H) coupling constant exhibited by the oxygen-bearing C-6' [δ_C 61.67; ^3J(CH) 5.5 Hz] with 2'-H. Furthermore, irradiation of 1'-H in a n.O.e. experiment enhanced, in addition to 2'-H (7%), 3'-H and 5'-H (4 and 7%) indicating that these protons are 1,3 *syn*-diaxially disposed.

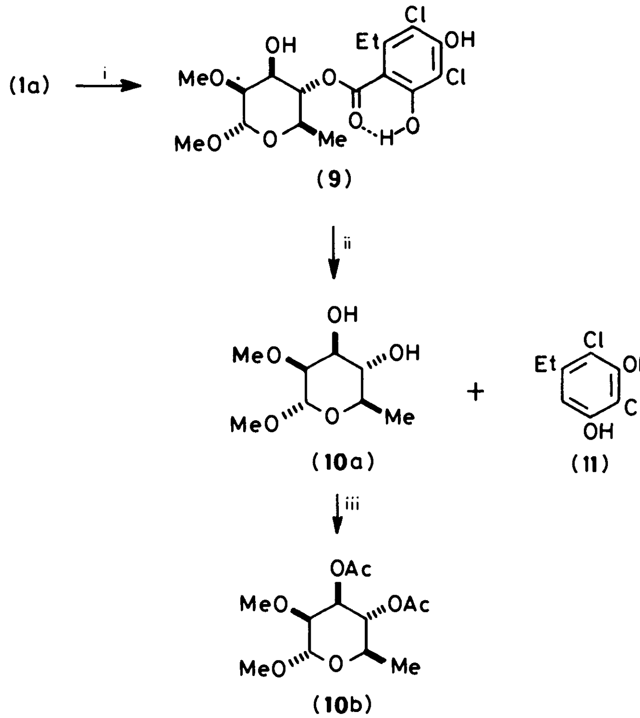
Table 4. ^{13}C N.m.r. data for lipiarmycin A3 (**1a**) in [$^2H_6$]acetone

Carbon	δ_C[a]/p.p.m.	^1J(CH)/Hz	Carbon	δ_C/p.p.m.	^1J(CH)/Hz
1	167.16 S		2'	81.56 D	146
2	125.39 S		3'	72.35 D	143
3	145.21 D	159	4'	77.60 D	149
4	128.22 D	155	5'	70.62 D	d
5	142.99 D	158	6'	61.67 Q	142
6	37.24 T	126	7'	18.16 Q	127.5
7	72.91 D	d	8'	169.54 S	
8	136.79[b] S		1''	110.40[c] S	
9	124.06 D	154	2''	156.11 S	
10	42.08 D	128	3''	108.27[c] S	
11	93.19 D	142	4''	153.82 S	
12	136.02[b] S		5''	114.60[c] S	
13	133.85 D	149	6''	142.72 S	
14	135.94[b] S		7''	26.12 T	130
15	126.46 D	152	8''	14.30 Q	128
16	28.47 T	127	1'''	96.59 D	155
17	78.29 D	148	2'''	72.65 D	d
19	63.46 T	148	3'''	70.28 D	142
20	15.19 Q	125	4'''	75.68 D	148
21	26.45 T	128	5'''	73.78 S	
22	11.10 Q	126	6'''	28.66 Q	127
23	13.78 Q	125	7'''	18.64 Q	127
24	17.44 Q	d	8'''	176.78 S	
25	67.96 D	143	9'''	34.73 D	130
26	20.47 Q	126.5	10'''	19.33 Q	128
1'	101.78 D	156	11'''	19.12 Q	128

[a] Capital letters refer to the pattern resulting from one-bond (C,H) coupling constants; S = singlet, D = doublet, T = triplet, and Q = quartet. [b,c] Assignments within each column may be interchanged. [d] Not detected.

These findings pointed to the presence of a 2-*O*-methylrhamnosyl unit adopting a chair conformation, and indicated that it is linked to the rest of the molecule as the β anomer. The one-bond (C,H) coupling possessed by the anomeric C-1' [δ_C 101.78; ^1J(CH) 156 Hz] supported the above conclusion.[5] Its absolute configuration was defined by comparing spectral and physicochemical properties of the methylglycoside diacetate (**10b**) ([α]$_D$ +64° in MeOH), obtained by acetylation of compound (**10a**) (Scheme 2), with those of the corresponding enantiomeric methylglycoside-L-α-rhamnose diacetate ([α]$_D$ −69° in MeOH).[6] In fact, they showed closely similar ^1H n.m.r. spectra (Table 5)* but opposite α_D values, thus permitting us to assign a D-α-configuration to (**10b**). These results, as corroborated by the aforementioned n.O.e. experiment, clearly indicated that this sugar is present in compound (**1a**) as a β-D-rhamnosyl unit. Finally, treatment of compound (**1a**) with saturated methanolic HCl gave compound (**9**) which by further treatment with MeONa in MeOH afforded, besides compound (**10a**), 2,4-dichloro-5-ethylresorcinol (**11**) (Scheme 2), whose structure has been previously determined.[4] The three-bond (C, H) coupling exhibited by the ester carbonyl C-8' [δ_C 169.54; ^3J(CH) 4 Hz] with 4'-H confirmed that the homodichloroorsellinic moiety is linked at C-4'.

* The numbering system for all derivatives is that used for lipiarmycin A3 (**1a**).



Scheme 2. *Reagents:* i, HCl-saturated MeOH; ii, MeONa–MeOH; iii, Py–Ac_2O

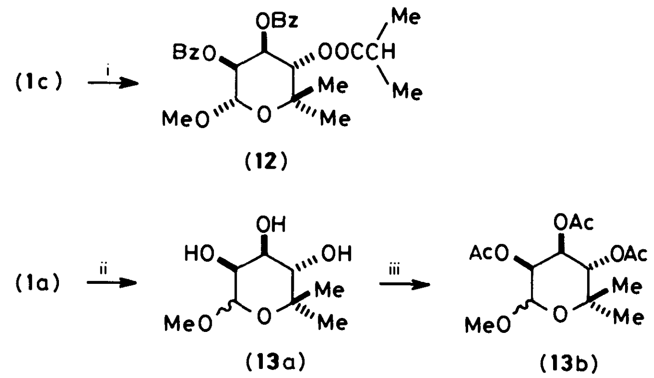
Fragment (**5**). The starting point of this spin system was formed by a doublet ($^{3}J_{1''',2'''}$ 1.1 Hz) at δ_H 7.8 which was assigned to 1'''-H because it was correlated with the anomeric C-1''' [δ_C 96.59; ^{1}J(CH) 155 Hz]. Its irradiation simplified to a doublet the resonance at δ_H 3.97 (2'''-H), the remaining splitting being the vicinal coupling to 3'''-H ($^{3}J_{2''',3'''}$ 3.3 Hz). The latter, in turn, was *trans*-diaxially coupled to 4'''-H (δ_H 5.00, $^{3}J_{3''',4'''}$ 10.0 Hz). Irradiation of the heptet at δ_H 2.57 (9'''-H) collapsed the two 3 H doublets at δ_H 1.16 and 1.14 (10'''- and 11'''-H_3) to a singlet, whilst irradiation of the two doublets caused the multiplet at δ 176.78 due to the C-8''' ester carbonyl to simplify to a doublet of doublets in the ^{13}C n.m.r. spectrum [^{3}J(CH) 7 and 4 Hz], these splittings being removed by irradiation of the protons 4'''- and 9'''-H. These experiments confirmed the presence of the isobutyryl moiety and proved that it is attached at C-4'''. The two singlet methyl groups resonating at δ_H 1.10 and 1.16 (6'''- and 7'''-H_3) were placed at the sole quaternary oxygen-bearing sp^2-hybridized carbon atom (C-5''') present in the ^{13}C n.m.r. spectrum of compound (**1a**). Irradiation of 1'''-H in an n.O.e. experiment led to enhancement, in addition to 2'''-H (6.5%), of 3'''-H and 7'''-H_3 (4.5 and 2%, respectively) indicating their 1,3-*syn* relationship, whereas irradiation of the equatorially-disposed 6'''-H_3 enhanced 4'''-H (14%). These results provided evidence for the presence of a 4-*O*-isobutyrate-5-methyl-β-rhamnosyl unit which adopts a chair confirmation and is β-linked to the rest of the molecule. Additional proof for the structure of this sugar, whose absolute configuration was not determined, was obtained from acidic methanolysis of per-benzoyl-lipiarmycin A3 (**1c**) which afforded the dibenzoyl sugar derivative (**12**) and from acidic methanolysis of compound (**1a**) which yielded 1-*O*-methyl-5-methylrhamnose (**13a**) which in turn gave the corresponding triacetate (**13b**) upon treatment with Py–Ac_2O (Scheme 3). The ^{1}H n.m.r. data of these three sugars are given in Table 5. In compound (**12**) irradiation of the methyl protons at δ_H 1.56 (7'''-H_3) in [$^{2}H_6$]acetone + D_2O enhanced 3'''-H (8%) and 1'''-OMe (4%), whereas irradiation of

Table 5. ^{1}H n.m.r. data for compounds (**10b**), (**12**), (**13a**), and (**13b**)

Protons	(**10b**)[a] δ_H/p.p.m.	Proton	(**12**)[b] δ_H/p.p.m.	(**13a**)[a] δ_H/p.p.m.	(**13b**)[a] δ_H/p.p.m.
1'	4.72	1'''	4.95	4.69	4.69
2'	3.62	2'''	5.64	3.97	5.47
3'	5.19	3'''	5.77	3.95	5.11
4'	5.11	4'''	5.69	3.70	5.23
5'	3.79	6'''	1.34	1.32[c]	1.32
6'	1.22	7'''	1.56	1.34[c]	1.32
1'-OMe	3.47[c]	9'''	2.51		
2'-OMe	3.39[c]	10'''	1.04[c]		
3'-OAc	2.07[d]	11'''	0.95[c]		
4'-OAc	2.04[d]	1'''-OMe	3.53	3.41	3.48
		2'''-OR	7.3—8.2	*ca.* 2.0	2.19[c]
		3'''-OR	7.3—8.2	*ca.* 2.0	2.06[c]
		4'''-OR		*ca.* 2.0	1.99[c]

J(HH)/Hz	(**10b**)	J(HH)/Hz	(**12**)	(**13a**)	(**13b**)
1',2'	1.9	1''',2'''	1.9	1.2	1.4
1',5'	0.7	2''',3'''	3.0	3.1	3.3
2',3'	3.1	3''',4'''	10.5	9.0	10.6
3',4'	10.2	9''',10'''	6.8		
4',5'	9.4	9''',11'''	6.8		
5',6'	6.3				

[a] In $CDCl_3$. [b] In [$^{2}H_6$]acetone. [c,d] Assignments within each column may be interchanged.



Scheme 3. *Reagents:* i, HCl-saturated MeOH; ii, MeOH 0.5M H_2SO_4, N_2; iii, Py–Ac_2O

the methyl protons at δ_H 1.34 (6'''-H_3) led to enhancement of 4'''-H (8.5%), the n.O.e. experiments proving that the sugar is in this case an α-anomer.

Fragment (**6**). This fragment showed the presence of three vinylic protons at δ_H 7.24, 6.63, and 5.96, assigned to 3-, 4-, and 5-H respectively, which were part of an α-methyleneoxy dienoic moiety. In fact, decoupling experiments demonstrated that 3-H was allylically coupled to 5-H and to the oxygen-bearing C-19 proton at δ_H 4.61, and vicinally coupled to 4-H (^{4}J 0.8, ^{4}J 0.5, and ^{3}J 11.3 Hz, respectively). In addition irradiation of 3-H and 19-H_2 simplified the resonance at δ_C 167.16 (C-1), whose chemical shift well agreed for a conjugate lactone carbonyl carbon atom. The presence of coupling constants between 4- and 5-H and 6-methylene protons, which in turn were both coupled to the signal at δ_H 4.28 (7-H), were accounted for by the proposed structure of this fragment.

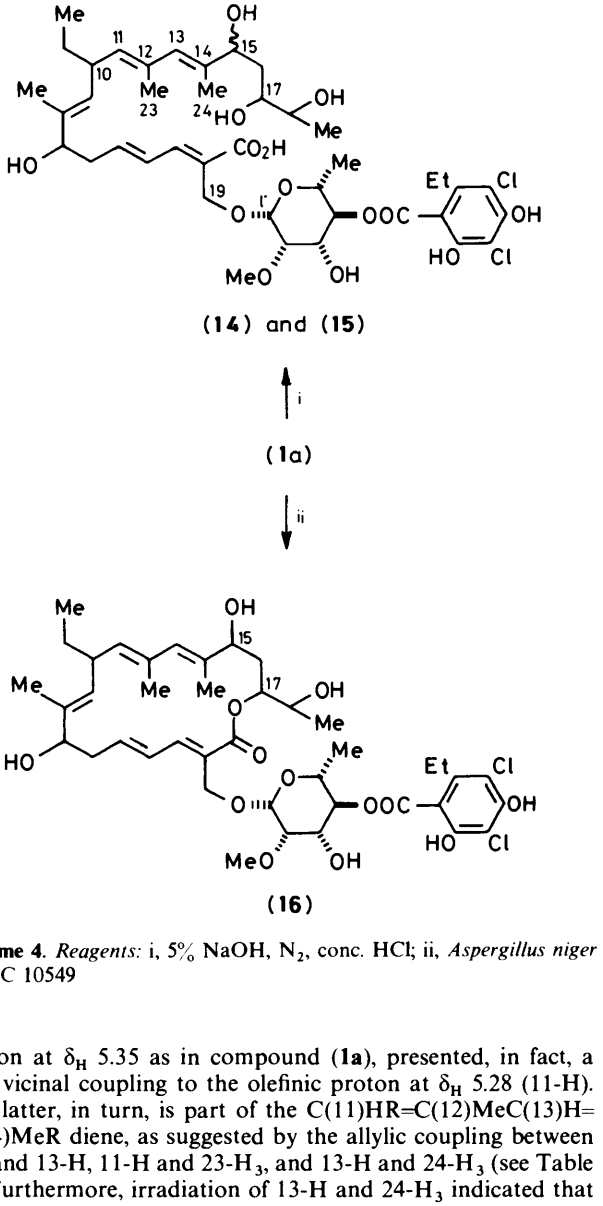
Fragment (**7**). The chemical shift values and the magnitudes of the geminal and vicinal coupling constants presented by 16-H_2, 17-H, 25-H, and 26-H_3 (see Tables 2 and 3) pointed to the presence of a C(16)H_2C(17)H(OR)C(25)H(OH)Me grouping which was allocated to C-15 because both 16-methylene protons (δ_H 2.75 and 2.43) were vicinally coupled to the 15-olefinic proton at δ_H 5.63. Moreover, the coupling constants between 13- and 15-H, 13-H and 23-H_3, and 15-H and 24-H_3 [^4J(HH) 1.0, ^4J(HH) 1.3, and ^4J(HH) 1.3 Hz, respectively] and the couplings between C(24) and each of 13- and 15-H [^3J(CH) 4 and ^3J(CH) 8.5 Hz, respectively] indicated the presence of the C(12)–C(15) diene moiety.

Fragment (**8**). Besides the five protons already assigned to 6-H_2, 16-H_2, and 9‴-H, the region between 2.4 and 2.8 p.p.m. contained another proton, assigned to 10-H, which was shown by n.O.e. experiments carried out on 20- and 23-H_3 to resonate at δ_H 2.63. Selective irradiation of 10-H resulted in decoupling of the resonances at δ_H 5.22 and 3.73, assigned to the 9-vinylic and 11-ethereal protons, and affected the multiplet at δ_H 1.93 (21a-H), the other 21b-methylene proton at δ_H 1.25 being obscured by methyl signals. Moreover, the multiplicity exhibited by the 3 H triplet at δ_H 0.82 (22-H_3), which was vicinally coupled to both 21-H_2, was indicative of the presence of an ethyl moiety, whereas the allylic coupling between 9-H and the 3 H signal at δ_H 1.66 (20-H_3) placed 20-H_3 at C-8. A clear analysis of 10-H followed from the ^{1}H n.m.r. spectrum of compound (**3b**) in which 10-H presented four vicinal couplings of 10.6, 9.3, 3.2, and 9.3 Hz to 9-H, 11-H, and 21-H_2, respectively, the latter two couplings confirming that the ethyl group is located at C-10.

Connection Between the Various Fragments.—At this stage of the structural analysis it was necessary to assemble the lipiarmycin A3 (**1a**) structure by interconnecting the fragments (**4**)—(**8**). Evidence for the linkage between C(7) and C(8) followed from the allylic coupling arising between 7- and 9-H, and from the three-bond coupling between C-20 and 7-H [^4J(HH) 1.6 and ^3J(CH) 2 Hz], whereas the linkage between C(11) and C(12) derived from the allylic coupling between 11- and 13-H, and from the three-bond coupling between C(23) and 11-H [^4J(HH) 0.5 and ^3J(CH) 4 Hz]; the final lactone ring structure was provided by the aforementioned hydrolysis of compound (**1a**) affording (**3a**).

We can now turn our attention to the interconnections between the two sugar units (**4**) and (**5**) and the C(11) and C(19) carbon atoms of the aglycone. Alkaline hydrolysis of compound (**1a**) followed by strong acidification gave a mixture of two compounds (**14**) and (**15**), diastereoisomeric at C-15 (Scheme 4), which could not be separated. Their formation could be explained by lactone ring opening followed by nucleophilic OH attack at C-15, which caused, *via* a shift of the C(12) and C(14) double bonds to C(11) and C(13) positions, the expulsion of the 5-Me-rhamnose moiety (**5**), which must therefore be originally linked at C-11. The n.O.e. experienced by 11-H (5%) in compound (**1a**) by irradiation of the 1‴- anomeric proton in [$^2H_6$]acetone + D_2O gave further support to the above evidence. The remaining 2-*O*-methylrhamnosyl moiety (**4**) must then be linked at C-19. The n.O.e. experienced by the 1′-anomeric proton (2%) by irradiation of 19b-H in [$^2H_6$]acetone + D_2O confirmed the close proximity of these two protons. A similar diene shift with expulsion of the 5-methylrhamnose unit (**5**) (Scheme 4) was also observed by treatment of compound (1a) with a culture of *Aspergillus niger* ATCC 10549, which stereoselectively afforded the 15-OH compound (**16**) without lactone ring opening; the same compound, as an epimeric mixture at C-15, was obtained together with (**13a**) during the treatment of (**1a**) with methanolic 0.25M H_2SO_4.

Accordingly, comparison of the ^{1}H n.m.r. spectrum of compound (**16**) and the spectrum of the mixture of compounds (**14**) and (**15**), with that of compound (**1a**) revealed the lack of the resonances ascribed to the 5-methylrhamnose and the downfield shift exhibited by 10-H (δ 0.58 p.p.m.), reflecting its diallylic nature. This proton, which is vicinally coupled to the 9-olefinic



Scheme 4. *Reagents:* i, 5% NaOH, N_2, conc. HCl; ii, *Aspergillus niger* ATCC 10549

proton at δ_H 5.35 as in compound (**1a**), presented, in fact, a new vicinal coupling to the olefinic proton at δ_H 5.28 (11-H). The latter, in turn, is part of the C(11)HR=C(12)MeC(13)H=C(14)MeR diene, as suggested by the allylic coupling between 11- and 13-H, 11-H and 23-H_3, and 13-H and 24-H_3 (see Table 3). Furthermore, irradiation of 13-H and 24-H_3 indicated that these protons interact *via* a four-bond coupling with the resonance at δ_H 5.00 which was then assigned to 15-H. Its chemical shift was in agreement with that of an allylic carbynol proton, whereas the upfield shift exhibited by the 16-H_2 protons when compared with their chemical shift values in compound (**1a**) (δ −0.61 and −0.72 p.p.m.) indicated that they were no longer adjacent to a double bond.

The lactone ring opening for compounds (**14**) and (**15**) emerged from the upfield shift experienced by 17-H relative to compound (**16**) (δ −1.21 p.p.m.). Finally, the diastereoisomeric relationship between compounds (**14**) and (**15**) was deduced from the close similarity of their ^{1}H n.m.r. spectra except for the signals due to the 16-H_2 which are in proximity to the epimeric hydroxy-bearing C-15.

Configurations of the Double Bonds.—The magnitude of the ^{1}H-^{1}Hcoupling constant between 4- and 5-H [^3J(HH) 14.8 Hz] in (**1a**) and its relevant derivatives (Table 3) established the *trans* configuration for the C(4)=C(5) double bond. For the tri-substituted double bonds, namely those between C(2) and C(3), C(8) and C(9), C(12), and C(13), and C(14) and C(15), there is no coupling constant information and the geometries were established by using n.O.e. effects. Thus, the n.O.e.s between 3-

and 5-H (7.5%) and 4-H and 19-methylene protons (3.5 and 2%) indicated the *E* configuration for the C(2)=C(3) double bond, the n.O.e.s between 20-H_3 and 10-H (4.5%), and 7- and 9-H (10%) the *E* configuration of the C(8)=C(9) double bond, and the n.O.e.s between 11- and 13-H (3%), and 24-H_3 and 16-H_2 (1.5 and 2.5%) the *E,E* configurations of the C(12)=C(15) diene. Moreover, in the fully ^{1}H-coupled ^{13}C n.m.r. spectrum of compound (**1a**), the quartets centred at δ_C 15.19, 13.78 and 17.44, due to 20-, 23-, and 24-H_3, respectively, showed additional fine structure in that each leg of each quartet presented a three-bond coupling [^3J(CH) 8.5 Hz] with 9-, 13-, and 15-H, respectively. These values[7] are in agreement for a *trans* relationship between each H, Me pair. Furthermore, the value[7] of the coupling between 3-H and C(1) [^3J(CH) 7 Hz] is indicative of a *cis* geometry between C(1)OOR and 3-H.

In conclusion, the structures depicted in Figure 1 are assigned to lipiarmycin, the two components A3 (**1a**) and A4 (**2**) being differentiated by a methylene group on the phenyl ring. The 18-membered lactone ring contains two independent conjugate diene systems, and a number of hydroxy and methyl functions. Two neutral sugars are linked through hemi-acetalic bonds to the core aglycone. One is 2-*O*-methyl-D-rhamnose, linked as the β-anomer, which is esterified by homodichloro-orsellinic acid in (**1a**) and dichloro-orsellinic acid in (**2**). This sugar has been found as the L-form in the macrolide antibiotics aranciamycin,[6] scopamycin A,[8] and leucanicidin,[9] and in the anthracycline antibiotic steffimycin,[10] whereas the D-form is present in specific mycosides produced by certain strains of *Mycobacteria* and *Myxobacteria*,[6] and by *Bacterium faecalis alcaligenes*.[11] The other sugar is the hitherto undescribed 5-methylrhamnose, also linked as the β-anomer, which is esterified with isobutyric acid.

The biosynthesis of lipiarmycin has not been investigated but the structure assigned to the aglycone part may be derived from the incorporation of 3 acetate, 5 propionate and 1 butyrate units.

Therefore, lipiarmycin appears to be a new macrocyclic antibiotic somewhat different from the classical macrolides.

Experimental

U.v. spectra were measured in 95% EtOH on a Beckmann DK-2 spectrophotometer and i.r. spectra with a Perkin Elmer 137 instrument. Mass spectra were taken at 70 eV on a VG-ZAB2 instrument equipped with a f.a.b. source. ^{1}H (300.13 MHz) and ^{13}C (75.47 MHz) N.m.r. spectra were recorded on a Bruker CXP-300 spectrometer. Chemical shifts are in p.p.m. (δ) from $SiMe_4$ as internal standard. N.O.e. difference spectra were obtained by subtracting alternatively right-off resonance-free induction decays (f.i.d.s) from right-on resonance-induced f.i.d.s. N.O.e. values reported in the test have only qualitative significance.

Column chromatography was performed with Merck silica gel (0.04—0.063 mm) at medium pressure. Unless otherwise indicated, the purity of products was checked by t.l.c., n.m.r. and mass spectra, and was deemed sufficient for the purpose of structural elucidation. M.p.s were measured on a Kofler apparatus and are uncorrected.

H.p.l.c. was run with a Constametric III pump (LDC Milton Roy) equipped with a 20 μl loop injector Reodyne 7125 and a Perkin-Elmer LC-15 detector at 254 nm. Column: Spheri-5 Brownlee Labs RP-8 (250 × 4.6 mm). Eluant: MeCN–H_2O (3:1). Injection: 20 μl. Flow rate 1 ml/min.

Separation of Lipiarmycin A3 (**1a**) *and A4* (**2**).—Lipiarmycin[2] (500 mg) was adsorbed on the top of a chromatographic column filled with silica gel and eluted under N_2 pressure with EtOAc–hexane (3:1) to give the pure metabolite (**1a**) (250 mg). Residual material was eluted with EtOAc to yield compounds (**1a**) and (**2**) as a 1:1 mixture (200 mg); this was subjected to flash chromatography on RP-C18 silica gel using acetone–water (2:1, 0.1% Na_2SO_4) as eluant to give pure compounds. *Lipiarmycin A3* (**1a**) crystallized from EtOAc–hexane as white crystals, m.p. 161—165 °C; $[\alpha]_D$ −6.2° (*c* 2.0 in MeOH); t.l.c. [silanized silica gel plates 60 HF254 Merck; acetone–water (1:1, 0.1% Na_2SO_4)] R_F 0.45; h.p.l.c. R_T 2.33 min. Some physical constants of compound (**1a**) have already been reported.[2,4] The molecular formula was determined by ^{13}C, ^{1}H n.m.r. data and fast atom bombardment (f.a.b.) mass spectral analysis, which showed a molecular species at *m/z* 1 079 (M^+ + Na) (Found: C, 58.9; H, 6.9; Cl, 6.5. $C_{52}H_{74}Cl_2O_{18}$ requires C, 59.03; H, 7.05; Cl, 6.70%); ^{1}H and ^{13}C N.m.r. data are reported in Tables 1—3. *Lipiarmycin A4* (**2**) was isolated as a white solid, m.p. 138—140 °C (EtOAc–hexane); $[\alpha]_D$ −9.4° (*c* 0.15 in MeOH); t.l.c. [as for compound (**1a**)] R_F 0.58; h.p.l.c. R_T 2.03 min; f.a.b.-m.s. *m/z* 1 065 (M^+ + Na) (Found: C, 58.5; H, 6.8; Cl, 6.7. $C_{51}H_{72}Cl_2O_{18}$ requires C, 58.67; H, 6.97; Cl, 6.79%). ^{1}H N.m.r. data are reported in Tables 2 and 3.

Acetylation of Lipiarmycin A3 (**1a**).—Compound (**1a**) (200 mg), dissolved in pyridine (1 ml) and Ac_2O (2 ml) was left for 12 h at room temperature. The reaction mixture was dissolved in chloroform, and the solution was successively stirred with saturated aqueous $NaHCO_3$, water, saturated aqueous $KHSO_4$, and water and finally dried (Na_2SO_4). Preparative t.l.c. (p.l.c.) [silica gel; EtOAc–hexane (1:1)] yielded the *peracetate* (**1b**) as a colourless amorphous solid, m.p. 115—118 °C; $[\alpha]_D$ −28.9° (*c* 0.5 in $CHCl_3$) (Found: C, 58.4; H, 6.4. $C_{66}H_{88}Cl_2O_{25}$ requires C, 58.62; H, 6.56%). ^{1}H N.m.r. data are reported in Tables 2 and 3.

Perbenzoylation of Lipiarmycin A3 (**1a**).—Compound (**1a**) (300 mg) was treated with pyridine (5 ml) and benzoyl chloride (0.5 ml). After 30 min the precipitate was filtered off, washed with Et_2O, and crystallized from EtOAc–hexane. Compound (**1c**) was obtained as a white solid, m.p. 118—122 °C; $[\alpha]_D$ −76.2° (*c* 0.2 in $CHCl_3$); $\lambda_{max.}$ 232, 270, and 280sh nm (ε 157 000, 32 700, and 26 300); $\nu_{max.}$ (KBr) 1 760 (lactone CO), and 1 725 (aryl ester) cm^{-1} (Found: C, 67.6; H, 5.5. $C_{101}H_{102}Cl_2O_{25}$ requires C, 67.89; H, 5.75%).

Methyl 3,5-*Dichloro*-2,4-*dihydroxy*-6-*methylbenzoate from Lipiarmycin A4* (**2**).—Compound (**2**) (100 mg) was treated with 0.5M KOH in MeOH for 2 days at room temperature. Evaporation of the solvent, neutralization and extraction of the solution with Et_2O gave after p.l.c. with EtOAc–hexane (1:4) a few mgs of the title compound, m.p. 106—109 °C, *m/z* 250/252 (M^+), (Found: M^+, 249.9828 ± 0.007. $C_9H_8Cl_2{}^{35}O_4$ requires *M*, 249.9798); δ_H (90 MHz; $CDCl_3$) 2.60 (3 H, s, 6″-Me), 4.00 (3 H, s, CO_2Me), 6.45 (1 H, br s, 4″-OH), 10.05 (1 H, s, 2″-OH).

Alkyaline Hydrolysis of Lipiarmycin A3 (**1a**).—Compound (**1a**) (300 mg) was treated with 0.5M KOH for 20 h at room temperature under an N_2 stream. The reaction mixture was acidified with diluted HCl and extracted with EtOAc. Work-up and t.l.c. using $CHCl_3$–MeOH (5:1) as eluant reveaied a more polar compound (**3a**) which was purified from EtOAc–hexane. Compound (**3a**) was a glassy solid, m.p. 108—110 °C, $[\alpha]_D$ −49.5° (*c* 0.1 in MeOH); $\lambda_{max.}$ 228, 263, and 316 nm (ε 33 600, 29 000, and 5 200); f.a.b.-m.s. *m/z* 1 027 (M^+ + Na) (Found: C, 56.8; H, 7.1; $C_{48}H_{70}Cl_2O_{18}$ requires C, 57.31; H, 7.01%).

Methylation of Compound (**3a**).—Compound (**3a**) (50 mg) dissolved in dry CH_2Cl_2–MeOH, was treated with an excess of CH_2N_2 in CH_2Cl_2 and the mixture was allowed to remain at −50 °C for 15 min. The excess CH_2N_2 was blown off with an N_2 stream and the solution evaporated to dryness. The residue was submitted to p.l.c. using CH_2Cl_2–MeOH (15:1) as eluant to give the *trimethyl derivative* (**3b**), m.p. 100—105 °C (CH_2Cl_2–hexane). ^{1}H N.m.r. data are reported in Tables 2 and 3.

Acetylation of Compound (**3a**).—Compound (**3a**) (50 mg) was acetylated with Py–Ac_2O at 0 °C overnight and standard work-up gave the *peracetate* (**3c**), m.p. 108—112 °C (CH_2Cl_2–hexane); $[\alpha]_D$ −35° (*c* 2 in MeOH). ^{1}H N.m.r. data are reported in Tables 2 and 3.

Hydrolysis of Compound (**9**) *to Compounds* (**10a**) *and* (**11**).—Compound (**9**) (200 mg), obtained by treating compound (**1a**) with saturated methanolic HCl as previously described,[4] was dissolved in MeOH (10 ml) and treated with MeONa (100 mg) at 60 °C for 2 h (t.l.c. control). Evaporation of the solvent, dilution, acidification and extraction with EtOAc gave a mixture which by p.l.c. using CH_2Cl_2–MeOH (9:1) as eluant afforded compounds (**10a**) and (**11**). The more polar compound was 1,2-O-*dimethyl*-D-α-*rhamnose* (**10a**), isolated as an oil, $[\alpha]_D$ +22.4° (*c* 0.2 in $CHCl_3$); *m/z* 192 (M^+), 160 (M^+ − 32), and 130; δ_H ($CDCl_3$; 90 MHz), 1.30 (3 H, d, 6.2 Hz, 7'-H_3), *ca.* 2.2 (2 H br, 3'- and 4'-OH), 3.3—3.8 (4 H, m, 2'-, 3'-, 4'-, and 5'-H), 3.38 and 3.49 (6 H, s, 2 × OMe), and 4.75 (1 H, br s, 1'-H). Compound (**11**) was identified as 2,4-*dichloro*-5-*ethylresorcinol* by direct comparison with an authentic sample previously isolated.[4]

Acetylation of Sugar (**10a**).—Compound (**10a**) (10 mg) was acetylated with Py–Ac_2O and standard work-up yielded compound (**10b**) (8 mg) as crystals, m.p. 55 °C (from Et_2O–hexane); $[\alpha]_D$ +64.4° (*c* 0.7 in MeOH); *m/z* 276 (M^+) (Found C, 52.05; H, 7.2. $C_{12}H_{20}O_7$ requires C, 52.16; H, 7.30%). ^{1}H N.m.r. data are reported in Table 5.

Acidic Degradation of Compound (**1c**).—A solution of compound (**1c**) (300 mg) in dry MeOH (10 ml) was treated with HCl-saturated MeOH (1 ml) and the mixture was refluxed for 10 min. Solvent was removed under reduced pressure and the residue was chromatographed on p.l.c. with EtOAc–hexane (1:2) as eluant to give 1-O-*methyl*-2,3-O-*dibenzoyl*-4-O-*isobutyryl*-5-*methyl*-α-*rhamnose* (**12**) as white crystals, m.p. 115—118 °C; $[\alpha]_D$ −83.7° (*c* 0.2 in MeOH), $\lambda_{max.}$ 197, 225, and 266 nm (ε 20 100, 26 250, and 4 400); $\nu_{max.}$(Nujol) 1 735 (aliphatic ester) and 1 700 cm^{-1} (aryl esters); *m/z* 470 (M^+), 456 (M^+ − 14), 440, 412, 352, and 247 (Found: C, 66.2; H, 6.3. $C_{26}H_{30}O_8$ requires C, 66.37; H, 6.43%). ^{1}H N.m.r. data are reported in Table 5.

Acidic Hydrolysis of Compound (**1a**).—Compound (**1a**) (300 mg) was treated with 0.5M H_2SO_4 in MeOH (1:2) (15 ml) for 5 h at 60 °C under N_2; the solvent was removed and the residue was added to water (10 ml), extracted first with Et_2O and then with BuOH. Work-up and p.l.c. of the ethereal extracts using EtOAc–hexane (1:1) as eluant gave compound (**16**) (100 mg) as an inseparable mixture of C-15 epimers. P.l.c. of the BuOH extract with CH_2Cl_2–MeOH (7:1) as eluant afforded 1-O-*methyl*-5-*methyl*-α-*rhamnose* (**13a**) (5 mg) as a glassy solid, m.p. 90—95 °C; $[\alpha]_D$ +46.6° (*c* 0.15 in MeOH); ^{1}H n.m.r. data are reported in Table 5. Compound (**16**) had m.p. 130—135 °C; $[\alpha]_D$ −72.7° (*c* 1 in MeOH); $\lambda_{max.}$ 230, 269, and 320 nm (ε 34 200, 23 600, and 4 700); $\nu_{max.}$(KBr) 1 740 (lactone CO), and 1 690 cm^{-1} (aryl ester) (Found: C, 59.2; H, 6.7. $C_{41}H_{56}Cl_2O_{13}$ requires C, 59.49; H, 6.82%).

Acetylation of Sugar (**13a**).—Compound (**13a**) (5 mg) was acetylated with Py–Ac_2O to yield after p.l.c. with EtOAc–hexane (1:2) as eluant the *triacetyl derivative* (**13b**) as crystals, m.p. 95—97 °C (CH_2Cl_2–hexane); *m/z* 318 (M^+), 303 (M^+ − 15), 286 (M^+ − 32), and 258 (M^+ − 60) (Found: C, 52.5; H, 6.4. $C_{14}H_{22}O_8$ requires C, 52.82; H, 6.97%). ^{1}H N.m.r. data are reported in Table 5.

Treatment of Lipiarmycin A3 (**1a**) *with* Aspergillus niger.—A pre-inoculum of a strain of *A. niger* ATCC 10549 was incubated in 5 Erlenmayer flasks (containing each 50 ml of medium) for 3 days at 24 °C in a liquid culture containing malt extract–yeast–glucose (10, 10, 30 g l^{-1}) and the pH was corrected to 7. After this time, compound (**1a**) (10 mg in 0.2 ml EtOH for flask) was added. After 24 h of incubation the liquid culture was extracted twice with EtOAc. P.l.c. using EtOAc–hexane (1:1) as eluant gave a single compound (**16**) (10 mg), identical on t.l.c. with the compounds obtained by acidic hydrolysis as described above. ^{1}H N.m.r. data are reported in Table 3.

Compounds (**14**) *and* (**15**) *as Racemic Mixture at C-15*.—Compound (**1a**) (300 mg) was submitted to alkaline hydrolysis to give compound (**3a**) which was successively refluxed for 3 h in a mixture of concentrated HCl (1 ml) and MeOH (2 ml). Evaporation of the solvent, dilution and extraction with EtOAc gave by p.l.c. using CH_2Cl_2–MeOH (9:1) as eluant, compounds (**14**) and (**15**) a mixture of C-15 epimers, as a glassy solid, m.p. 90—95 °C (CH_2Cl_2–hexane); $[\alpha]_D$ −21.3° (*c* 1 in MeOH); $\lambda_{max.}$ 200, 215, 253, and 312 nm (ε 33 600, 32 400, 32 800 and 8 200); (Found: C, 57.8; H, 6.4; $C_{41}H_{58}Cl_2O_{14}$ requires C, 58.22; H, 6.91%). ^{1}H N.m.r. data are reported in Table 3.

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