STRUCTURAL STUDIES ON LIPIARMYCIN

I. CHARACTERIZATION BY ^1H AND ^{13}C NMR SPECTROSCOPY AND ISOLATION OF METHYL 2-*O*-METHYL-4-*O*-HOMODICHLOROORSELLINATE-β-RHAMNOSIDE

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(Received for publication April 5, 1983)

^1H and ^{13}C NMR spectral studies of lipiarmycin in $CDCl_3$ and in pyridine-d_5 provided evidence for the six partial structures **I**~**VI** and the two sugar units **1** and **2**. Acid methanolysis led to the isolation of methyl 2-*O*-methyl-4-*O*-homodichloroorsellinate-β-rhamnoside, whose structure was determined by spectroscopic methods.

Lipiarmycin[1,2] is a chlorine containing antibiotic produced by *Actinoplanes deccanensis* ATCC 21983, active mainly against Gram-positive bacteria. While the weak *in vivo* activity delayed further developments, its mechanism of action has been the subject of many studies[3~8].

In a previous paper[2] the preliminary characterization of lipiarmycin was reported, showing the presence of a sugar moiety and of many branched aliphatic chains. Furthermore, homodichloroorsellinic acid was isolated after acid hydrolysis. In this paper we report the structure elucidation of the sugar components and of the two compounds obtained by hydrolysis. In addition, the partial structures of some aliphatic moieties are deduced from 270 MHz ^1H and 67.88 MHz ^{13}C NMR spectroscopy.

From elemental analysis and osmometric data, supported by ^1H and ^{13}C NMR spectroscopy, lipiarmycin has the molecular formula $C_{52}H_{72}Cl_2O_{18\sim19}$.

Spectroscopic Studies

Fig. 1 shows the IR spectrum of lipiarmycin in $CDCl_3$. Absorption bands at 3600, 3560, 3540 and 3500 cm^{-1} are assigned by deuteration to OH functions, the last three of them being intramolecularly H-bonded. The spectrum shows three carbonyl absorptions at 1730 and 1690 cm^{-1}, unchanged after deuteration, and at 1660 cm^{-1} which shifts to 1640 cm^{-1} after deuteration. The band at 1690 cm^{-1} is then assigned to a conjugated C=O and that at 1660 cm^{-1} to a C=O ester conjugated and H-bonded; the band at 1730 cm^{-1} is due to an aliphatic ester. In the double bond region a band at 1590 cm^{-1} (1580 cm^{-1} after D_2O addition) is associated with an aromatic moiety carrying one or more phenolic groups. These latter functions are confirmed by the presence of δ_{C-O} bands at 1410 and 1220 cm^{-1} shifted by deuteration. Finally, the strong band at 1070 cm^{-1} (δ_{C-O}) can be assigned to the anomeric group of a sugar.

The ^1H NMR spectra of lipiarmycin at 270 MHz in $CDCl_3$ and in pyridine-d_5, after addition of D_2O, are reported in Figs. 2 and 3, respectively. The relationships among the protons of lipiarmycin, as represented by their ^1H NMR chemical shifts and coupling constants obtained by ^1H homo-decoupling experiments, are reported in Table 1, where the various H-bearing groups are labelled with alphabetical letters. Examination of the ^1H NMR data in $CDCl_3$ indicates that the total number of protons is 72 of which 5 are exchangeable. Furthermore, the exchangeable H at δ 11.5 is attributed to a phenolic OH

Fig. 1. IR spectrum of lipiarmycin in $CDCl_3$ solution (------ plus D_2O).

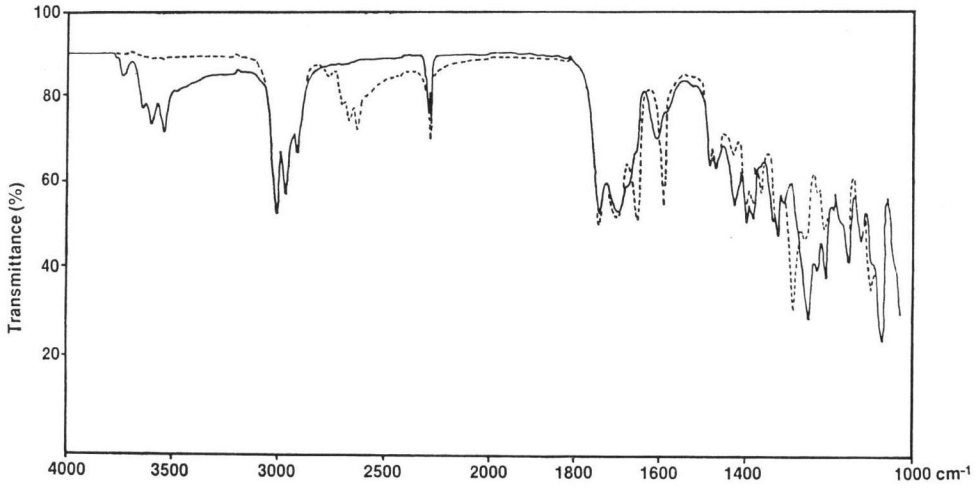
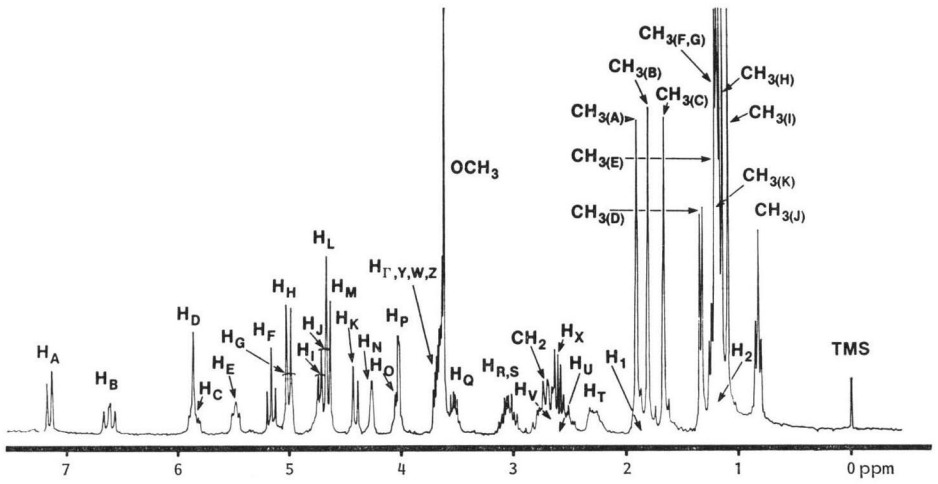


Fig. 2. ^1H NMR spectrum at 270 MHz of 2×10^{-2} M lipiarmycin in $CDCl_3$ solution (D=100%) after D_2O exchange.



intramolecularly H-bonded. Another exchangeable H at δ 6.9 is assigned to a not H-bonded phenolic OH. Moreover, three alcoholic groups are present, as deduced by the difference in integration of the spectrum around δ 2.5 before and after deuteration.

In pyridine-d_5 the signals of the protons resonating in the regions δ 3.5 ~ 5 and δ 1 ~ 1.5 are better separated and the J values can be better calculated. The observed values of chemical shifts and coupling constants yield the following structural information: 1) the protons designated H_Q, H_F, H_Z, H_Y and H_L are CH groups bonded to oxygen and form a network of coupled spins; the J_{vic} values are such that these protons very likely belong to a pyranose ring and the configuration of the anomeric center is β[9]; 2) in the same way, protons H_H, H_W, H_P and H_M are correlated and belong to another monosaccharide unit; 3) the protons H_F and H_H show, in comparison with the previous protons possessing two J_{vic}, a downfield shift of 1 ~ 1.5 ppm suggesting that they should be bonded to

Table 1. ^{1}H NMR data for lipiarmycin at 270 MHz in $CDCl_3+D_2O$ and pyridine-d_5+D_2O, respectively (δ in ppm, J_{vic} in Hz).

		$CDCl_3$			Pyridine-d_5		
$CH_{3(J)}$		0.82 (t)	$J_{CH_{3(J)},CH_{2(1,2)}}$	=7.5	0.78 (t)	$J_{CH_{3(J)},CH_{2(1,2)}}$	=7.5
$CH_{3(G)}$		1.18 (d)	$J_{CH_{3(G)},X}$	=7	1.18 (d)	$J_{CH_{3(G)},X}$	=7
$CH_{3(F)}$		1.18 (d)	$J_{CH_{3(F)},X}$	=7	1.18 (d)	$J_{CH_{3(F)},X}$	=7
$CH_{3(I)}$		1.09 (s)			1.37 (s)		
$CH_{3(K)}$		1.19 (t)	$J_{CH_{3(K)},CH_{2(R,S)}}$	=7.5	1.39 (t)	$J_{CH_{3(K)},CH_{2(R,S)}}$	=7
$CH_{3(H)}$		1.14 (s)			1.42 (s)		
$CH_{3(E)}$		1.20 (d)	$J_{CH_{3(E)},O}$	=6	1.51 (d)	$J_{CH_{3(E)},O}$	=6.5
$CH_{3(C)}$		1.67 (s)			1.64 (s)		
$CH_{3(D)}$		1.33 (d)	$J_{CH_{3(D)},Q}$	=6	1.68 (d)	$J_{CH_{3(D)},Q}$	=6
$CH_{3(B)}$		1.81 (s)			1.93 (s)		
$CH_{3(A)}$		1.91 (s)			2.09 (s)		
CH_2	H_2	1.18 (m)	$J_{gem\ 2,1}$	=ND		$J_{gem\ 2,1}$	=ND
			$J_{2,U}$	=ND	1.4 (m)	$J_{2,U}$	=ND
			$J_{2,CH_{3(J)}}$	=7		$J_{2,CH_{3(J)}}$	=7
〃	H_1	1.91 (m)	$J_{gem\ 1,2}$	=ND		$J_{gem\ 1,2}$	=ND
			$J_{1,U}$	=ND	2.1 (m)	$J_{1,U}$	=ND
			$J_{1,CH_{3(J)}}$	=7.5		$J_{1,CH_{3(J)}}$	=7.5
H_X		2.59 (qq)	$J_{X,CH_{3(F)}}$	=7	2.65 (qq)	$J_{X,CH_{3(F)}}$	=7
			$J_{X,CH_{3(G)}}$	=7		$J_{X,CH_{3(G)}}$	=7
CH_2		2.64 (dd)	$J_{CH_2,C}$	=7	2.75 (dd)	$J_{CH_2,C}$	=7
			$J_{CH_2,N}$	=ND		$J_{CH_2,N}$	=ND
H_U		2.5 (m)	$J_{U,CH_{2(1,2)}}$	=ND		$J_{U,CH_{2(1,2)}}$	=ND
			$J_{U,G}$	=11.5	2.75 (m)	$J_{U,G}$	=11.5
			$J_{U,I}$	=10		$J_{U,I}$	=10
CH_2	H_T	2.28 (m)	$J_{gem\ V,T}$	=ND		$J_{gem\ V,T}$	=ND
			$J_{T,I}$	= 5	2.8 (m)	$J_{T,I}$	= 5
			$J_{T,E}$	=10		$J_{T,E}$	=9.5
〃	H_V	2.7 (m)	$J_{gem\ V,T}$	=ND		$J_{gem\ V,T}$	=ND
			$J_{V,I}$	= 5	3.28 (m)	$J_{V,I}$	= 5
			$J_{V,E}$	=10		$J_{V,E}$	= 9.5
CH_2	H_S	3.01 (dq)	$J_{gem\ R,S}$	=16	3.13 (dd)	$J_{gem\ R,S}$	=ND
			$J_{S,CH_{3(K)}}$	= 7.5		$J_{S,CH_{3(K)}}$	=7
〃	H_R	3.16 (dq)	$J_{gem\ R,S}$	=15	3.13 (dq)	$J_{gem\ R,S}$	=ND
			$J_{R,CH_{3(K)}}$	= 7.5		$J_{R,CH_{3(K)}}$	= 7

ND: Not determined.

O–CO–R groups instead to OR groups; 4) the signals H_L and H_M (δ 4.66 and 4.62 in $CDCl_3$) having only one J_{vic} of about 1 Hz can be assigned to the anomeric protons of the two postulated sugars[10] (see also carbon signals later on). By combining the above information with that described in Table 1 for the other H atoms, the six moieties (**I**~**VI**) reported in Fig. 4 can be hypothesized to be present in the lipiarmycin molecule.

The ^{13}C NMR spectrum at 67.88 MHz in pyridine-d_5 is reported in Fig. 5. The ^{13}C NMR data in $CDCl_3$ and pyridine-d_5 obtained by the appropriate ^{13}C {^{1}H} selective decoupling experiments are listed in Table 2. The carbon atoms are designated with the same capital letters assigned to the corresponding hydrogens. The total number of carbons is calculated to be 52. The study of chemical shifts allows one to derive the following structural information in the various spectral regions: 1) δ180~165 ppm:

Table 1. (continued).

		$CDCl_3$			Pyridine-d_5		
$-O-CH_3$		3.62 (s)			3.68 (s)		
H_Γ		3.7 (d)	$J_{\Gamma,U}$	=10	3.87 (d)	$J_{\Gamma,U}$	=10
H_Q		3.53 (dq)	$J_{Q,F}$	= 9.5	3.87 (dq)	$J_{Q,F}$	=10
			$J_{Q,CH_{3(D)}}$	= 6		$J_{Q,CH_{3(D)}}$	= 6
H_Y		3.7 (dd)	$J_{Y,Z}$	= 3	3.94 (dd)	$J_{Y,Z}$	= 3
			$J_{Y,L}$	= 1		$J_{Y,L}$	= 1
H_Z		3.7 (dd)	$J_{Z,Y}$	= 3	4.16 (dd)	$J_{Z,Y}$	= 3
			$J_{Z,F}$	= 9.5		$J_{Z,F}$	=10
H_W		3.7 (dd)	$J_{W,P}$	= 3	4.23 (dd)	$J_{W,P}$	= 3
			$J_{W,H}$	=10		$J_{W,H}$	=10
H_P		4.02 (dd)	$J_{P,W}$	= 2.5	4.49 (dd)	$J_{P,W}$	= 3
			$J_{P,M}$	= 1		$J_{P,M}$	= 1
H_N		4.25 (m)	J_{N,CH_2}	=ND	4.52 (m)	J_{N,CH_2}	=ND
H_O		4.02 (dq)	$J_{O,CH_{3(E)}}$	= 6	4.58 (dq)	$J_{O,CH_{3(E)}}$	= 6
			$J_{O,I}$	= 6.5		$J_{O,I}$	= 6.5
CH_2	H_K	4.4 (d)	$J_{gem\ K,J}$	=11.5	4.79 (d)	$J_{gem\ K,J}$	=11
〃	H_J	4.74 (d)	$J_{gem\ J,K}$	=11.5	4.97 (d)	$J_{gem\ J,K}$	=11
H_M		4.62 (d)	$J_{M,P}$	= 1	4.9 (d)	$J_{M,P}$	= 1
H_L		4.66 (d)	$J_{L,Y}$	= 1	5.02 (d)	$J_{L,Y}$	= 1
H_I		4.74 (dt)	$J_{I,O}$	= 6.5	5.34 (dt)	$J_{I,O}$	= 6.5
			$J_{I,CH_{2(V,T)}}$	= 5		$J_{I,CH_{2(V,T)}}$	= 5
H_G		4.98 (d*)	$J_{G,U}$	=10	5.67 (d*)	$J_{G,U}$	=11.5
			$J_{*G,CH_{3(C)}}$	=ND		$J_{*G,CH_{3(C)}}$	=ND
H_H		4.99 (dd)	$J_{H,W}$	=10	5.77 (dd)	$J_{H,W}$	=10
H_F		5.14 (dd)	$J_{F,Q}$	= 9.5	5.8 (dd)	$J_{F,Q}$	=10
			$J_{F,Z}$	= 9.5		$J_{F,Z}$	=10
H_D		5.84 (s*)	$J_{*D,CH_{3(B)}}$	=ND	6.01 (s*)	$J_{*D,CH_{3(B)}}$	=ND
H_E		5.46 (t*)	$J_{E,CH_{2(V,T)}}$	=10	6.02 (s*)	$J_{E,CH_{2(V,T)}}$	= 9.5
			$J_{*E,CH_{3(A)}}$	=ND		$J_{*E,CH_{3(A)}}$	=ND
H_C		5.84 (dt)	$J_{C,B}$	=16	6.24 (dt)	$J_{C,B}$	=15
			J_{C,CH_2}	= 7		J_{C,CH_2}	= 7
H_B		6.58 (dd)	$J_{B,C}$	=16	6.88 (dd)	$J_{B,C}$	=15
			$J_{B,A}$	=11		$J_{B,A}$	=11
H_A		7.12 (d)	$J_{A,B}$	=11	7.81 (d)	$J_{A,B}$	=11

ND: Not determined.
* Broad band.

three carbonyl groups can be observed, two of which (δ 168.5 and 167.9) are α,β unsaturated carbonyls and one (δ 176.5) is a saturated ester; 2) δ 160~110 ppm: six sp^2 carbons (C_A, C_B, C_C, C_D, C_E, C_G), among them C_A is presumably bonded to an oxygen. Ten quaternary sp^2 carbons among which six suggest the presence of one aromatic ring carrying both electron-donating and electron-withdrawing substituents; 3) δ 115~60 ppm: there are thirteen –CH–O– groups, among them carbons C_L and C_M (δ 101.7 and 97.3 in pyridine-d_5) are assigned to the anomeric carbons of the two sugar units previously hypothesized. Furthermore, the two ^1J values (152 and 153 Hz) are an additional indication of a β-configuration for both sugars. In addition, one $-\overset{|}{\underset{|}{C}}-O-$, one OCH_3 and the $-\overset{H_J}{\overset{|}{\underset{H_K}{\underset{|}{C}}}}-O-$ are present;

Fig. 3. ^{1}H NMR spectrum at 270 MHz of 2×10^{-2} M lipiarmycin in pyridine-d_5 solution (D=99%) after D_2O exchange.

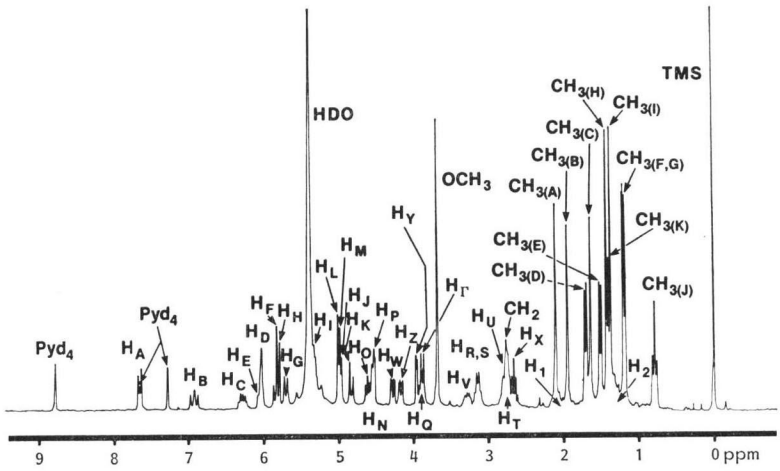


Fig. 4.

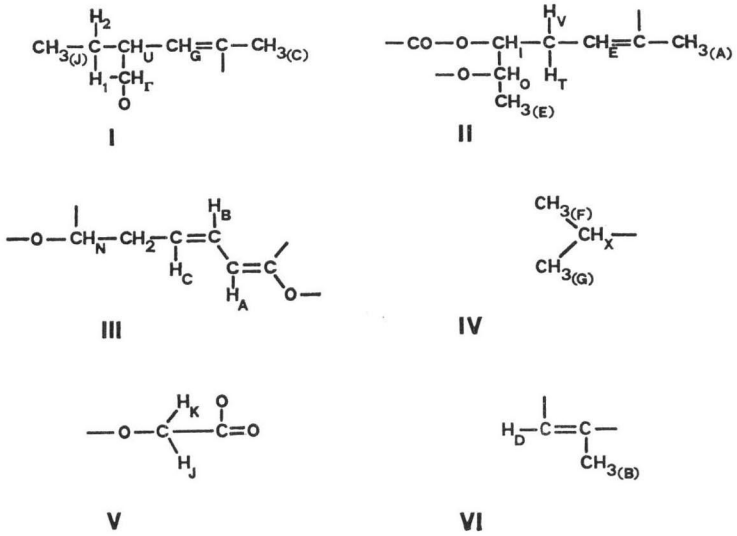


Fig. 5. ^{13}C NMR spectrum at 67.88 MHz of 0.46 M lipiarmycin in pyridine-d_5 solution (D=99%) (noise decoupled).

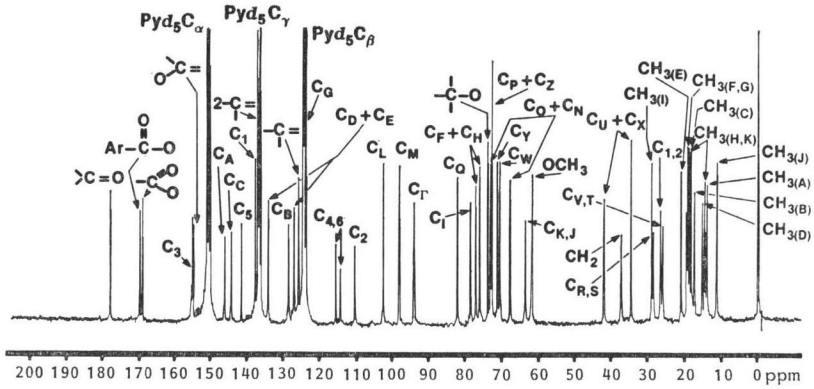


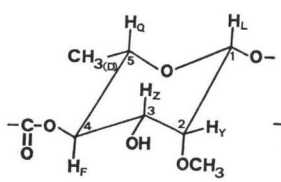
Table 2. ^{13}C NMR data for lipiarmycin at 67.88 MHz in $CDCl_3$ and in pyridine-d_5 (δ in ppm, $^1J_{C-H}$ in Hz).

Carbon		$CDCl_3$ δ	Pyridine-d_5 δ	Pyridine-d_5 $^1J_{C-H}$	Carbon		$CDCl_3$ δ	Pyridine-d_5 δ	Pyridine-d_5 $^1J_{C-H}$
C_A	(d)	144.1	145.1	152	$CH_{3(A)}$	(q)		13.7	116
C_B	(d)	128.9	127.8	157	$CH_{3(B)}$	(q)		17.3	124
C_C	(d)	140.7	143.2	154	$CH_{3(C)}$	(q)		18.2	116
C_D	(d)	134.1	133.3	147	$CH_{3(D)}$	(q)		15.1	124
C_E	(d)	127.5	126.3	142	$CH_{3(E)}$	(q)		20.9	126
C_F	(d)		76.8	152	$CH_{3(F)}$	(q)		19.3	131
C_G	(d)	123.4	123.7	151	$CH_{3(G)}$	(q)		19.0	118
C_H	(d)		75.7	152	$CH_{3(H)}$	(q)		18.6	129
C_I	(d)		78.2	142	$CH_{3(I)}$	(q)		28.6	129
$C_{K,J}$	(t)	63.5	63.3	145	$CH_{3(K)}$	(q)		14.4	124
C_L	(d)	101.7	101.7	152	$CH_{3(J)}$	(q)	10.9	11.1	121
C_M	(d)	94.8	97.3	153	O–CH_3	(q)	62.1	61.4	142
C_N	(d)		67.3	142	–C(–)–O	(s)		73.5	—
C_O	(d)		72.8	147					
C_P	(d)		72.4	147	>C=O	(s)	177.2	176.5	—
C_Q	(d)		81.8	147	Ar–CO–O	(s)	170.0	168.5	—
$C_{R,S}$	(t)	28.6	28.2	126	–CO–O		168.6	167.9	—
C_U	(d)	34.2	41.8	121	O>C=	(s)	157.4	153.8	—
$C_{T,V}$	(t)	26	25.8	142	>C=	(s)	136.7	135.85	—
C_Γ	(d)	92.4	93.3	142	>C=	(s)	134.6	135.85	—
C_W	(d)		70.2	142	>C=	(s)	125.2	125.1	—
C_X	(d)	41.7	34.4	139	C_1	(s)	136.1	136.9	—
C_Z	(d)		72.4	147	C_2	(s)	107.6	109.6	—
C_Y	(d)		70.9	134	C_3	(s)	152.6	154.0	—
CH_2	(t)	35.7	37.1	121	C_4	(s)	113.7	114.9	—
$C_{1,2}$	(t)	25.9	26.3	123	C_5	(s)	143.0	110.6	—
					C_6	(s)	103.0	113.6	—

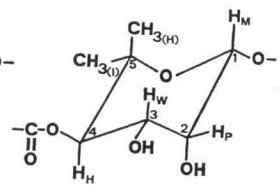
4) δ 45~10 ppm: there are two –C(–)H, four >CH_2 and eleven –CH_3 groups.

The selective decoupling $^{13}C\{^1H\}$ experiments allowed also the interpretation of some aspects of the proton spectrum. In fact, the ^1H NMR spectrum appears very complex in the region around δ 2.5 ppm, due to the superimposition of a large number of signals attributable to –CH and >CH_2 groups.

Fig. 6.



"Sugar 1"
2-*O*-Methyl-β-rhamnose
(shown L)moiety



"Sugar 2"
5-Methyl-β-rhamnose
(shown L)moiety

Homonuclear decoupling in this zone was not conclusive. The comparison between the ^{13}C off-resonance and the corresponding coupled spectra established that there are in this zone five CH_2 groups: since four of them were identified in a different zone, it can be derived that part of the signals around δ 2.5 ppm are due to a CH_2 and the remainder to a CH of allylic type.

The singlet at δ 73.5 ppm in the ^{13}C NMR spectrum assigned to a quaternary carbon bonded to an oxygen and the two methyl groups ($CH_{3(I)}$ and $CH_{3(H)}$) that in the ^1H NMR spectrum in pyridine-d_5

resonate as singlets at δ 1.37 and 1.42 ppm, respectively, suggest the presence of the moiety $\begin{matrix}CH_3\\CH_3\end{matrix}\!\!>\!C\!<\!^{O-}$ which should belong to one of the two postulated sugar units. The two sugar units have been designated as "sugar **1**" and "sugar **2**" (Fig. 6).

The relative configurations of the hydrogens were deduced from the application of the Karplus equation to the J_{vic} values (see Table 1), while the absolute configuration is still undefined.

Hydrolysis of Lipiarmycin

Treatment of lipiarmycin with saturated methanolic HCl at reflux yielded degradation compound **I**, which by further treatment with 0.1 N NaOH at 100°C gave compound **II**. Their structures, as well as the correlation with lipiarmycin, were investigated by mass spectrometry, and by ^1H and ^{13}C NMR spectroscopy.

Compound II

The mass spectrum showed molecular ions at *m/z* 206/208 with a ratio indicating the presence of two chlorine atoms and corresponding to the formula $C_8H_8Cl_2O_2$. The fragmentation pattern is shown in Fig. 7. A loss of chlorine leading to a fragment at *m/z* 171/173 and of CH_3 leading to a fragment at *m/z* 191/193 $(C_7H_5Cl_2O_2)^+$ are present. The ^1H NMR spectrum ($CDCl_3$, 60 MHz) is consistent with the presence of a CH_3–CH_2–aryl group (δ 1.92, t, $CH_{3(K)}$; δ 2.65, q, $CH_{2(R,S)}$; $J_{CH_{3(K)},\,CH_{2(R,S)}}$=7 Hz),

Fig. 7.

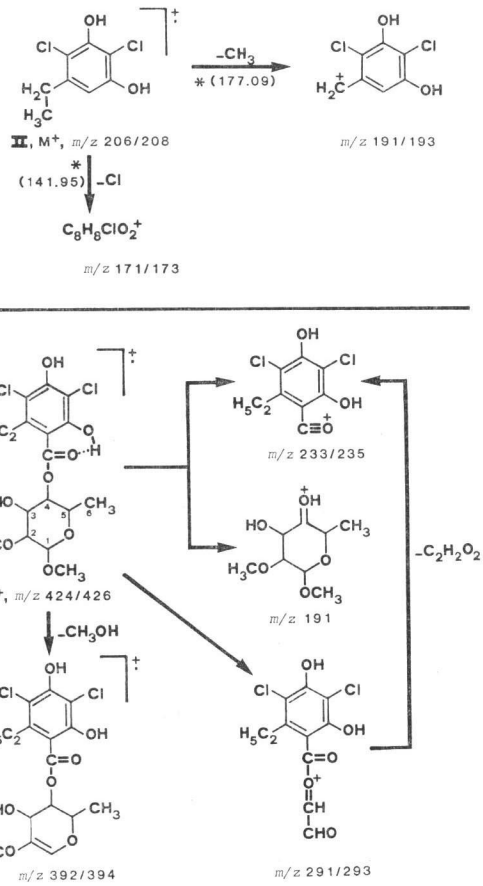
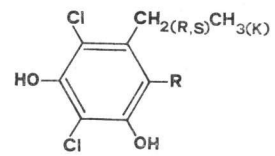


Table 3. ^{13}C NMR chemical shifts for compound **II** at 25.2 MHz in $CDCl_3$ (δ in ppm).

C_1	(s)	148
C_2	(s)	110.7
C_3	(s)	152
C_4	(s)	113
C_5	(s)	142.8
C_6	(d)	109.2
$C_{R,S}$	(t)	27.2
$CH_{3(K)}$	(q)	13.8

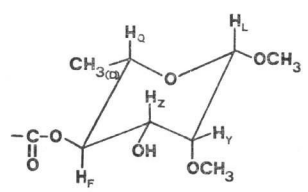
Fig. 8.



R= H 2,4-Dichloro-5-ethylresorcinol (Compound II)

R= COOH Homodichloroorsellinic acid

R= (Compound I)



an aromatic proton (δ 6.51, s, H_6) and two equivalent phenolic OH at δ 5.6 that should be in *meta* position because any attempt to obtain the corresponding 1,2- or 1,4-benzoquinone by mild oxidation failed. The ^{13}C NMR data of compound **II** are listed in Table 3. Signals attributable to six aromatic carbons, one CH_3 and one CH_2 groups are observed. All the above data fully support the structure of 2,4-dichloro-5-ethylresorcinol already reported in the literature[11], which is related to the homodichloroorsellinic acid previously isolated from lipiarmycin[2] (Fig. 8).

Compound I

The mass spectrum gives molecular ions at *m/z* 424/426 indicating the presence of two chlorine atoms and corresponding to the formula $C_{17}H_{22}Cl_2O_8$. The interpretation of the main peaks is shown in Fig. 7. The ion at *m/z* 233/235 corresponds to an acylium ion derived from the cleavage of an ester bond between the homodichloroorsellinic acid moiety and a sugar moiety (see the complementary ion at *m/z* 191/193). The peak at *m/z* 233/235 may also derive from the peak at 291/293 by loss of glyoxal. Since this latter fragment originates from the M^+ peak, the presence of the hydroxyl group in the ester linkage can be demonstrated (see also later). In the IR spectrum in $CDCl_3$ two bands at 3560 and 3510 (ν_{OH}) and a broad band at 3320 cm^{-1} (intramolecularly H-bonded OH) are present. Some other absorption bands can be assigned: 1590 and 1570 cm^{-1} ($\nu_{C=C}$); 2840 cm^{-1} (ν_{C-H}) of an aliphatic OCH_3 group; 1660 cm^{-1} ($\nu_{C=O}$, conjugated ester group intramolecularly H-bonded); 1410 and 1220 cm^{-1} (δ OH of phenolic hydroxyls); 1240 cm^{-1} (ν_{C-O-C} ester). Finally, a series of bands in the region 1180~1000 cm^{-1} correspond to the sugar moiety.

The ^1H NMR data in $CDCl_3$ and in pyridine-d_5 are reported in Table 4. They show the presence of two phenolic OH, one of which is intramolecularly H-bonded, an alcoholic OH and two OCH_3 groups. The chemical shifts and the J_{vic} values of the protons designated H_F, H_Z, H_Y, H_L, H_Q and $CH_{3(D)}$ are the same in compound **I** and in lipiarmycin (see Table 1). The CH_3CH_2 group bonded to the aromatic ring is the one designated as $CH_{3(K)}CH_{2(R,S)}$ in lipiarmycin. The ^{13}C NMR data of compound **I** are reported in Table 5. They indicate the presence of a conjugated ester carbonyl (δ 169), six aromatic

Table 4. ^1H NMR data for compound **I** at 270 MHz in $CDCl_3$ and in pyridine-d_5, respectively (δ in ppm, J_{vic} in Hz).

$CDCl_3$				Pyridine-d_5			
$CH_{3(K)}$	1.18 (t)	$J_{CH_{3(K)},\,CH_{2(R,S)}}$	= 7	$CH_{3(K)}$	1.41 (t)	$J_{CH_{3(K)},\,CH_{2(R,S)}}$	= 7
$CH_{3(D)}$	1.24 (d)	$J_{CH_{3(D)},Q}$	= 6.5	$CH_{3(D)}$	1.63 (d)	$J_{CH_{3(D)},Q}$	= 6
$CH_{2(R,S)}$	3.04 (dq)	$J_{gem\ R,S}$	=13	$CH_{2(R,S)}$	3.14 (q)	$J_{R,CH_{3(K)}}$	= 7
		$J_{R,CH_{3(K)}}$	= 7.5				
Two OCH_3	3.50 (s)			OCH_3	3.38 (s)		
H_Y	3.51 (dd)	$J_{Y,L}$	= 1	OCH_3	3.48 (s)		
		$J_{Y,Z}$	= 3.5				
H_Q	3.80 (dq)	$J_{Q,CH_{3(D)}}$	= 6.5	H_Y	3.87 (dd)	$J_{Y,L}$	= 1
		$J_{Q,F}$	= 9.5			$J_{Y,Z}$	= 3.5
H_Z	3.96 (dd)	$J_{Z,Y}$	= 3.5	H_Q	4.13 (dq)	$J_{Q,CH_{3(D)}}$	= 6.5
		$J_{Z,F}$	= 9.5			$J_{Q,F}$	=10
H_L	4.86 (d)	$J_{L,Y}$	= 1	H_Z	4.51 (dd)	$J_{Z,Y}$	= 3.5
						$J_{Z,F}$	=10
H_F	5.18 (dd)	$J_{F,Q}$	= 9.5	H_L	5.07 (d)	$J_{L,Y}$	= 1
		$J_{F,Z}$	= 9.5				
R–OH	2.3			H_F	5.88 (dd)	$J_{F,Q}$	=10
ϕ–OH	6.7					$J_{F,Z}$	=10
ϕ–OH	11.6			Three OH	=7.5		

carbons ($C_1 \sim C_6$) and all the oxygen linked carbons (C_F, C_Z, C_Y, C_L, C_Q) assigned to "sugar **1**" in lipiarmycin.

Since compound **I** gives a negative TOLLEN's reaction it can be deduced that the anomeric hydroxyl is methylated. The other OCH_3 present should be in position 2 due to the chemical shift value of the signal of H_Y (bonded to C_2 of the sugar) in the ^1H NMR spectrum. This hypothesis is confirmed by the ion at *m/z* 291 in the mass spectrum deriving from the breaking of the bonds C_2/C_3 and C_4/C_5 of the sugar (Fig. 7). In fact, the mass spectrum recorded after deuteration shows in addition to the doublet of isotopic peaks at *m/z* 291/293 a doublet at *m/z* 294/296 corresponding to a fragment containing three exchangeable protons, *i.e.* two from the phenolic OH's and one from the OH in the β-position to the point of attachment of the carboxyl of the homodichloroorsellinic acid.

Table 5. ^{13}C NMR chemical shifts for compound **I** at 67.88 MHz in pyridine-d_5 (δ in ppm).

$CH_{3(K)}$	(q)	13.9
$CH_{3(D)}$	(q)	17.5
$CH_{2(R,S)}$	(t)	25.3
OCH_3	(s)	54.6
OCH_3	(s)	58.6
C_Q	(d)	66.4
C_Z	(d)	69.8
C_Y	(d)	77.0
C_F	(d)	81.2
C_L	(d)	99.9
C_2	(s)	100.1
C_4	(s)	110.8
C_6	(s)	114.0
C_1	(s)	116.6
C_5	(s)	141.8
C_3	(s)	154.4
$-\overset{\overset{O}{\|}}{C}=O$	(s)	169.0

Since it is obvious under the conditions of methanolysis of lipiarmycin that the anomeric hydroxyl becomes methylated, the only reasonable conclusion is that the OCH_3 group in position 2 of "sugar **1**" is the one that is originally present in lipiarmycin. In fact, the ^1H NMR chemical shift of H_Y in position 2 in compound **II** and in lipiarmycin is δ 3.87 *vs* 3.94.

Finally, the fact that the ^1H NMR chemical shift of the anomeric proton H_L is almost identical in compound **II** and in lipiarmycin (δ 5.07 *vs* 5.02, respectively) indicates that in lipiarmycin the anomeric carbon is bonded with the rest of the molecule. Thus, the sugar bonded to homodichloroorsellinic acid in lipiarmycin is 2-*O*-methyl-β-rhamnose as shown by the structure assigned to compound **I** (Fig. 8). The absolute configuration of the sugar has not yet been established.

Conclusions

The data presented indicate that by acid methanolysis of lipiarmycin the methyl glycoside of 2-*O*-methyl-4-*O*-homodichloroorsellinate-β-rhamnose was obtained. The sugar 2-*O*-methyl-β-rhamnose ("sugar **1**") has also been found in the antibiotic aranciamycin[12], while the α-isomer has been isolated from scopamycin A[9].

The other sugar unit evidentiated in lipiarmycin ("sugar **2**") differs from "sugar **1**" for the substitution at carbon 5, and for the fact that the OH in position 2 is free because of the downfield shift of 0.6 ppm of the proton H_P in respect to the corresponding H_Y in "sugar **1**". Then, "sugar **2**" is 5-methyl-β-rhamnose. The anomeric protons H_M and H_L possess about the same values of chemical shift and $J_{1a,2e}$ (about 1 Hz), then it can be concluded that also in lipiarmycin the anomeric OH of "sugar **2**" is involved in a β-glycosidic linkage[10,18]. Studies on the interconnections of the structural units (**I**~**VI**) outlined in this paper (see Fig. 4) are under way.

Experimental

Osmometric determinations of molecular weight of lipiarmycin were made with a Knauer apparatus in CH_3OH at 37°C and in pyridine at 90°C.

TLC was run on Silica gel F_{254} plates to a distance of 10 cm with the eluent mixture $CH_3COOC_2H_5$ - benzene (1: 1). The spots were detected by visual examination at 254 nm.

HPLC was run with a Varian model 5000 LC equipped with a 20 μl loop injector Rheodyne model 7125 and a fixed wavelength detector at 254 nm. Column:Zorbax ODS 250×4 mm, eluent: 0.025 M NaH_2PO_4 - CH_3CN (55: 45) buffered at pH 6.0. Flow rate: 2 ml/minute. Internal reference: deoxycorticosterone, t_R=9.4 minutes.

IR spectra were recorded on a Perkin Elmer model 580 spectrometer.

Mass spectra were registered on a Hitachi RMU-6L spectrometer at 70 eV.

^1H and ^{13}C NMR spectra and data were obtained on a Bruker WH 270 spectrometer equipped with disk unit using tetramethylsilane (TMS) as internal reference. NMR spectra of compound **II** were registered with a Varian A-60 D (^1H) and a Varian XL-100 (^{13}C) spectrometer.

UV spectra were run on a Beckman DK-2 spectrophotometer.

Potentiometric titrations were made in methylcellosolve - H_2O (4: 1) with 0.01 N NaOH.

Lipiarmycin

A small sample of highly pure product (Rf 0.09) was used which melted at 173°C (differential scanning calorimetry). Its t_R relative to the internal reference by HPLC is 0.7.

From microanalytical data and NMR spectra the molecular formula $C_{52}H_{72}Cl_2O_{18\sim19}$ was calculated corresponding to a molecular weight of 1,056~1,072 which was in agreement with both the values determined by osmometry (1,070 in CH_3OH and 1,080 in pyridine) and with the value calculated from potentiometric titrations (1,020).

Lipiarmycin did not give a significant mass spectrum under electron impact.

Preparation of Compound I

Lipiarmycin (180 mg) was dissolved in 10 ml of anhydrous CH_3OH, then 2 ml of CH_3OH saturated with gaseous HCl was added. After refluxing for 2 hours the solvent was removed under vacuum, CH_3OH was added and the solution was evaporated again. The residue was dissolved in water and extracted three times with 10 ml of $CHCl_3$. The extracts were pooled, concentrated to a small volume and chromatographed over 20 g of Silica gel Merck 0.06~0.2 mm slurried in a 95: 5, $CHCl_3$ - CH_3OH mixture.

The column was eluted with the same solvent mixture collecting a number of 5 ml fractions that were checked by TLC. Fractions 40~47 containing a product with Rf 0.67 were combined and evaporated to dryness yielding 30 mg of a solid insoluble in *n*-hexane, soluble in ether which gave a brown-purple color with alcoholic $FeCl_3$. The t_R relative to the internal reference (HPLC) is 0.3. The UV spectrum (CH_3OH) showed the following maxima(nm, log ε): 217 (4.26), 252 (3.92), 315 (4.05).

Preparation of Compound II

A solution of 115 mg of compound **I** in 15 ml of 0.1 N NaOH was refluxed for 2 hours. The reaction mixture was cooled, neutralized with dil. HCl solution then extracted with $CHCl_3$. The extracts were collected and evaporated to dryness yielding a gummy residue with Rf 0.8.

Acknowledgments

We thank Dr. P. FERRARI for IR and UV spectra. We are indebted to Dr. C. CORONELLI and to Prof. G. C. LANCINI for criticism and helpful discussions.

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