THE COMPLETE ASSIGNMENT OF THE ^{13}C NMR SPECTRA OF LASALOCID AND THE SODIUM SALT-COMPLEX OF THE ANTIBIOTIC

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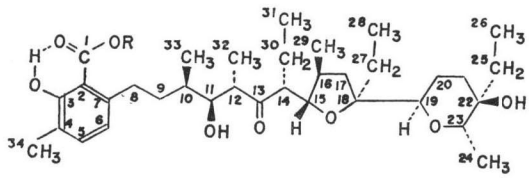
All thirty-four signals observed in the ^{13}C nmr of both the free acid form (**Ia**) and sodium salt (**Ib**) of the polyether antibiotic lasalocid have been assigned. This was achieved using model compounds such as 3-methylsalicylic acid, the retroaldol ketones from both lasalocid and lysocellin and a γ-lactone from a third polyether, salinomycin. The last assignments to be made were accomplished using biosynthetically enriched samples of the antibiotic.

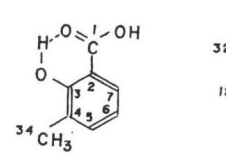
One of the earliest biosynthetic applications of ^{13}C nmr spectroscopy in the field of natural products was a study[1] that led to the first *bona fide* demonstration of butyric acid incorporation, by *Streptomyces lasaliensis* in the formation of the three C-ethyl groups in lasalocid (**Ia**). This discovery was later reported for another polyether antibiotic, monensin A[2]. A more recent utility for the ^{13}C nmr technique has been found in the study of the solution conformation of complex organic compounds such as salinomycin[3] and lysocellin[4], which also belong to the polyether class of antibiotics.

In this report, the partial assignments of lasalocid's ^{13}C nmr spectrum made from earlier biosynthetic studies[1,5] are completed. The results are presented for both the free acid, ethanolate (**Ia**) and the sodium salt-complex (**Ib**) and provide preliminary data for the eventual conformational analysis of lasalocid and its salts in solution by ^{13}C nmr. Conformational analyses of **Ia** have been

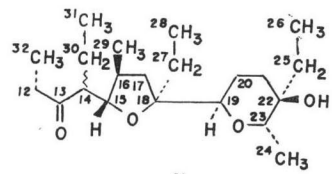
Fig. 1. Numbering system for compounds **I**, **II**, **III**, **IV** and **V** based on the lasalocid nomenclature (ref. 10).



Ia: R= H, C_2H_5OH; Lasalocid A, ethanolate.
Ib: R= Na; Lasalocid A, sodium salt-complex.



III



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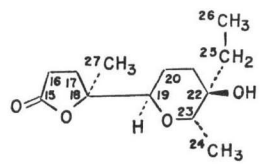
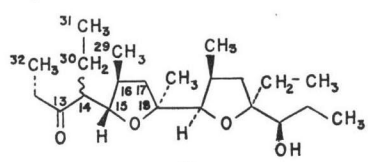


IV



V

reported using proton nmr spectroscopy[6,7] and our results should provide information to complement these analyses. For instance, a cursory examination of the results presented in Table 1 reveals that three of the seven quaternary carbons, C-1, 2 and 13 (see Fig. 1) are the three which undergo the largest downfield shift on sodium complexation.

Assignment of the ^{13}C NMR of the Lasalocid Retroaldol Ketone, II

Pyrolysis[8] or base treatment[9] of **Ia** (or **Ib**) produces *inter alia* the ketone **II**, a $C_{21}H_{34}O_4$ molecule in which the C-14 center[10] is epimerized but the other six asymmetric centers are unaffected. This result was expected on chemical grounds and was confirmed by ^{13}C nmr which gives double peaks for ten of the twelve carbons adjacent to C-14 whereas the eight carbons associated with the terminal tetrahydro-pyran all give single peaks. The paired signals are assigned by partially relaxed FOURIER transform (PRFT) spectra[11] assuming the relaxation times of similar carbons in both epimers are roughly equal (Table 1).

Table 1. Assignment of the ^{13}C nmr spectra of lasalocid free acid **Ia** and sodium salt complex **Ib** together with the retroaldol ketone **II**

Carbon number[a]	Functional group	^{13}C Shift in ppm[b]		
		Ia (in CD_2Cl_2)	**Ib** (in CD_2Cl_2)	**II** (in $CDCl_3$)
1	$-CO_2H$	173.6	176.4	
2	$=C-$	111.2	118.2	
3	$=C-OH$	161.6	161.3	
4	$=C-CH_3$	124.1	123.0	
5	$=CH$	135.1	131.5	
6	$=CH$	121.6	119.6	
7	$=C-CH_2$	144.4	143.5	
8	$-CH_2$	34.8	33.4	
9	$-CH_2$	37.0	38.1	
10	$-CH(CH_3)$	34.9	34.5	
11	$-CH(OH)$	72.8	71.0	
12	$-CH(CH_3)$	48.9	49.0	37.1 (36.4)
13	$C=O$	214.1	219.9	212.6
14	$-CH(C_2H_5)$	55.4	55.9	58.9 (57.2)
15	$-CH-O$	84.2	83.1	86.5 (85.7)
16	$-CH(CH_3)$	34.8	34.1	38.3 (36.9)
17	$-CH_2$	38.7	37.9	41.0 (40.7)
18	$-C(O)$	86.7	87.6	84.4 (84.3)
19	$-CH(O)$	70.9	68.6	72.9
20	$-CH_2$	20.0	19.5	21.3
21	$-CH_2$	30.2	29.2	29.5
22	$-C(O)$	72.4	71.5	70.5
23	$-CH(O)$	76.6	77.2	76.6
24	$-CH_3$	14.0	13.6	14.2
25	$-CH_2$	30.7	31.1	30.6
26	$-CH_3$	6.6	6.7	6.4
27	$-CH_2$	30.6	29.8	28.7 (28.4)
28	$-CH_3$	9.2	9.5	8.1
29	$-CH_3$	15.9	16.0	17.6 (16.7)
30	$-CH_2$	16.7	16.2	21.3 (21.0)
31	$-CH_3$	12.9	12.4	12.5 (12.2)
32	$-CH_3$	13.2	12.6	7.4
33	$-CH_3$	13.4	13.3	
34	$-CH_3$	15.7	15.3	

[a] For the numbering system, see Fig. 1.
[b] Downfield from internal Me_4Si.

The resonance of 212.6 ppm is characteristic of a ketone carbon (C-13) and comparison of **II** with the γ-lactone, **IV** (Table 2) obtained from salinomycin[3] facilitates the assignment of the eight carbons, C-19 to C-26 in **II** as shown in Table 1. By elimination, the remaining oxymethine (C-15) and quaternary carbon (C-18) are assigned to the peaks at 86.7 (85.7)* and 84.6 (84.3) ppm respectively. The carbons, C-12 and C-14, adjacent to the carbonyl can be assigned using deuterium exchange, and this leaves one methine peak at 38.3 (36.9) ppm, due to C-16. Carbons C-17, 29, 30, 31 and 32 of the lasalocid retroaldol ketone **II** can be conveniently assigned by comparison with the retroaldol ketone **V** (Table 2) from lysocellin solved earlier[12]. The remaining methylene carbon at δ28.7 (28.4) ppm and the methyl at 8.1 ppm arise from the ethyl C-27 and C-28 respectively. Due to the proximity of C-29 and C-31 to the epimerized center at C-14, both peaks due to these methyls are doubled, whereas the four other methyls in **II**; C-24, 26, 28 and 32 give single peaks as indicated in Table 1.

Assignment of ^{13}C Spectrum of Lasalocid A, Ethanolate

Crystallization of lasalocid A free acid from ethanol yields a monoethanolate, **Ia** ($C_{34}H_{54}O_8 \cdot$

Table 2. Assignment of the ^{13}C nmr spectra of model compounds related to lasalocid

Carbon number[a]	Functional group	δ-^{13}C Shift in ppm[b] of **III**, **IV** & **V** in $CDCl_3$		
		III	**IV**	**V**
1	$-CO_2H$	173.4		
2	$=C-$	112.6		
3	$=C-OH$	160.8		
4	$=C-CH_3$	126.7		
5	$=CH$	136.8		
6	$=CH$	118.9		
7	$=C-CH_2$	[128.6][c]		
12	$-CH(CH_3)$			37.3 (37.1)
13	$C=O$			213.6
14	$-CH(C_2H_5)$			59.3 (57.2)
15	$-CH(O)$		[177.3][c]	87.3 (86.7)
16	$-CH(CH_3)$		29.6	36.9 (36.4)
17	$-CH_2$		29.1	42.5
18	$-C(O)$		87.3	
19	$-CH(O)$		73.0	
20	$-CH_2$		21.2	
21	$-CH_2$		29.0	
22	$-C(O)$		70.8	
23	$-CH(O)$		76.9	
24	$-CH_3$		14.3	
25	$-CH_2$		30.7	
26	$-CH_3$		6.3	
27	$-CH_2$		[22.9][c]	
28	$-CH_3$			
29	$-CH_3$			16.8 (16.2)
30	$-CH_2$			21.5 (21.0)
31	$-CH_3$			12.6 (12.2)
32	$-CH_3$			7.3
34	$-CH_3$	15.5		

[a] For the carbon numbering system, see Fig. 1.
[b] Downfield from internal Me_4Si.
[c] Functionalities of these carbons are different from those of the corresponding carbons in **Ia**, **Ib** and **V**.

* Where two peaks were observed for a single carbon, the one appearing at higher field is arbitrarily expressed in parentheses. Therefore, chemical shift values in parentheses do not necessarily correspond to one epimer.

C_2H_6O). The assignment of the ^{13}C nmr spectrum of **Ia** was accomplished by comparison with the retroaldol ketone, **II** (*vide supra*), and 2-hydroxy-3-methyl-benzoic acid (**III**), which was assigned by selective proton decoupling. Both of these models are assigned as shown in Table 2. Single frequency off-resonance decoupling experiments were employed in the assignment of all the carbons between C-13 to C-31 in **Ia**, except C-17 and C-19. Although the chemical shift of these two carbons in **II** suggested the peaks at δ 38.7 and 72.8 ppm as arising from C-17, 19 in **Ia**, biosynthetic enrichment[5] results (Table 3) showed that although the signal at δ 38.7 was due to C-17, the correct assignment of the C-19 peak is at δ 70.9 ppm. Of the remaining seven carbons; C-8 to C-12, C-32 and C-33, the peak at δ 48.9 ppm is assigned to C-12* due to the characteristic chemical shift for a methine adjacent to a ketone. The assignment of C-9 and C-11 (like C-19) was deduced from the biosynthetic enrichments summarized in Table 3. This implies that the remaining methine peak at δ 34.9 corresponds to C-10 and the remaining methylene peak at δ 34.8 ppm corresponds to C-8. The last of the methyl peaks at δ 13.2 and 13.4 ppm are tentatively assigned to C-32 and C-33 respectively, completing the assignment of **Ia**. From these results, together with our earlier proposals[5] for **Ib**, the complete assignment of the latter can be achieved. It is noteworthy that on going from **Ia** to **Ib**, the order of shifts is the same except for carbons C-9, 17 and C-11, 22 (Table 1).

Examining the change in chemical shifts (Table 1) for the free acid (**Ia**) on conversion to the sodium salt (**Ib**), the most obvious downfield shifts are observed for carbons C-2 (7.0 ppm), C-13 (5.8 ppm) and C-1 (2.8 ppm) whereas upfield shifts on sodium complexation are greatest for C-5 (3.6 ppm), C-19 (2.3 ppm) and C-6 (2.0 ppm). Results using other cation complexes are needed before any meaningful conclusions can be drawn regarding the solution conformations of **Ia** and **Ib** by ^{13}C nmr spectroscopy.

Table 3. Incorporation results from **Ia** used to assign the ^{13}C nmr peaks at δ 37.0, 38.7, 70.9 and 72.8 ppm

Carbon number in **Ia**	Incorporation of [1-^{13}C] acetate, [1-^{13}C] propionate and [1-^{13}C] butyrate into lasalocid as determined by ^{13}C nmr of the free acid			
	^{13}C Shift in ppm in CD_2Cl_2	% Abundance* of ^{13}C in lasalocid produced from		
		$CH_3{}^{13}CO_2Na$	$CH_3CH_2{}^{13}CO_2Na$	$CH_3CH_2CH_2{}^{13}CO_2Na$
9	37.0	1.0	3.5	1.0
17	38.7	1.0	1.0	4.0
19	70.9	2.0	1.0	1.0
11	72.8	1.0	3.5	1.0

* Corrected to nearest 0.5%.

Experimental

The ^{13}C nmr spectra of lasalocid A sodium salt[1] and γ-lactone[3] have been reported previously. The ^{13}C nmr spectrum of lasalocid A free acid ethanolate was run on a Varian XL-100 spectrometer (25.2 MHz) in CD_2Cl_2 and those of the retroaldol ketones (in $CDCl_3$) and 2-hydroxy-3-methyl-benzoic acid (in CD_3OD) on a JEOL FX-100 spectrometer (25.05 MHz).

Deuteration of **II** was carried out under the same conditions as for the retroaldol reaction of **I**[5] with H_2O being replaced by D_2O.

* In an earlier paper (ref. 5), this signal was assigned to C-8. Single frequency off-resonance decoupling experiments, however, showed the signal was due to a methine and therefore the assignment is revised as shown in Table 1.

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