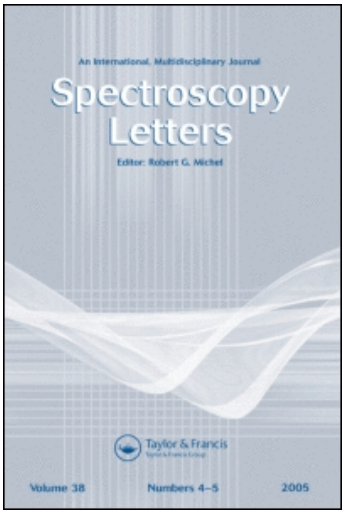

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László Szilágyi[a]; János Samu[b]; Ilona Harsányi[c]
[a] Chair of Organic Chemistry, L. Kossuth University, Debrecen [b] L. Eötvös University, [c] Reanal Co., Budapest, epes, Hungary

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STRUCTURE ELUCIDATION OF TWO ACETYLATED DERIVATIVES OF OLIGOMYCIN A

Keywords: 2D NMR, ^{1}H, ^{13}C, assignments, macrolide, biological testing.

László Szilágyi[1]*, János Samu[2] and Ilona Harsányi[3]

Chair of Organic Chemistry, L. Kossuth University[1], H-4010. Debrecen, P.O.B.20.; L. Eötvös University[2], H-1518 Budapest 112 P.O.B.32; Reanal Co.[3], H-1147 Budapest, Telepes u. 53, Hungary

ABSTRACT

Partial acetylation of oligomycin A (**1**) resulted in the formation of 5,9,33-tri-O-acetyl (**2**) and 5,9,13,33-tetraacetyl (**3**) derivatives whose structures have been established through complete assignments of their ^{1}H and ^{13}C NMR spectra. These derivatives were devoid of inhibitory activity when tested on *Aspergillus niger* spores.

* Author for correspondence.

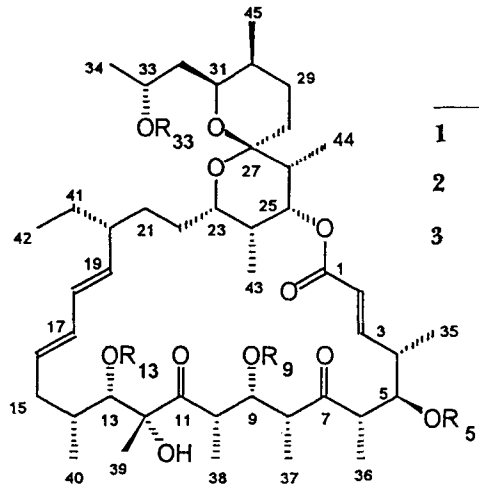
INTRODUCTION

The oligomycin/rutamycin/cytovaricin family of macrolide antibiotics has been of considerable value in studies of oxidative phosphorylation as these compounds are specific inhibitors of mitochondrial ATPase [1,2]. The structures of oligomycin A, -C and -B have been determined by chemical degradation[3] and X-ray crystallography[4], respectively. Chemical modification of substrates or inhibitors is one of the most powerful approaches to map enzyme binding sites and to get insight into structural requirements for substrate or inhibitor binding. Acetylated derivatives of macrolide antibiotics like oleandomycin and erythromycin were found to show *in vivo* biological activity; this was partly attributed to increased hydrophobicity favoring induction and formation of cytochrome P450 metabolic complexes[5]. We have chosen to synthesize acetylated derivatives of oligomycin A **(1)** for biological testing.

RESULTS AND DISCUSSION

Assignments

The structures of **2** and **3** have been determined by NMR spectroscopy. Unassigned ^{13}C chemical shifts were published[3] for the parent compound **1** in $CDCl_3$ while complete ^{1}H- and ^{13}C assignments were achieved[6] for a solution in methanol. In the present case $CDCl_3$ proved to be more useful as a solvent for comparative NMR studies mainly because of the lack of disturbing residual signals and a slightly better dispersion of the 11 methyl resonances in the ^{1}H NMR spectrum. Since both ^{1}H- and ^{13}C chemical shifts are solvent dependent it was necessary to reassign the NMR spectra in $CDCl_3$ solution. This was accomplished by concerted use of 2D methods such as DQ-filtered pure phase H,H-COSY[7], H,H-RELAY[8] and H,C-COSY[9]. These methods, supplemented with ^{13}C-HSQC[10] were also instrumental in obtaining essentially complete ^{1}H and



	R_5	R_9	R_{13}	R_{33}
1	H	H	H	H
2	Ac	Ac	H	Ac
3	Ac	Ac	Ac	Ac

^{13}C assignments for the acetyled derivatives **2** and **3** (Table 1). Regular (1D) ^{13}C NMR spectra indicated the presence of three and four acetyl carbonyl resonances for **2** and **3** respectively. The corresponding methyl signals are partially overlapped by other C-methyl resonances which could, however, be readily distinguished in the H,C-COSY maps. The positions of the acetyl groups could then be established on the basis of the characteristic downfield shifts of αH resonances upon acetylation of the respective OH-groups. Comparison of ^1H chemical shifts of **3** with those in the parent compound **1** reveals downfield shifts of 1.40, 1.60, 1.02 and 0.95 ppm for H-5, H-9, H-13 and H-33, respectively. Respective values for **2** are: 1.30, 1.48, -0.08 and 0.87 ppm (Table 1). It follows therefore that **2** is 5,9,33-tri-O-acetyl-oligomycin A whereas **3** has the single free OH-group in position 12 *i.e.*, it is 5,9,13,33-tetra-O-acetyl-oligomycin A. A "diacetate" of oligomycin A has been described[11] in 1958. Correct determination of the molecular masses of oligomycins later then established[12] this compound to be a tetraacetyl derivative. An analytical sample of oligomycin A

TABLE 1

^{1}H- and ^{13}C chemical shifts[a] of **1**,**2**, and **3**

	1		**2**		**3**	
Position	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	165.14	-	165.37	-	166.24	-
2	122.76	5.82	123.08	5.80	123.19	5.91
3	148.42	6.64	147.93	6.84	149.06	6.90
4	40.18	2.39	40.68	2.46	39.63	2.63
5	73.03	3.76	73.22	5.07	73.35	5.17
6	46.54	2.72	43.32	2.84	43.64	2.98
7	220.01	-	210.87	-	211.16	-
8	45.75	2.76	45.78	2.61	46.16	2.90
9	72.69	3.97	72.59	5.45	71.35	5.57
10	41.99	3.63	40.06	3.75	42.54	3.66
11	220.29	-	219.02	-	215.09	-
12	83.05	-	83.10	-	83.08	-
13	72.29	3.9	72.93	3.85	78.42	5.05
14	33.54	~1.9	33.56	1.86	33.55	2.03
15	38.47	~1.98;2.18	38.57	~1.90;2.17	39.07	2.00
16	129.40	5.45	129.27	5.43	129.63	5.52
17	132.45	6.02	132.51	5.98	133.39	6.10
18	130.32	5.92	130.34	5.89	130.85	5.97

Table 1. (Cont.)

	1		2		3	
Position	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
19	137.82	5.24	137.78	5.22	137.82	5.36
20	46.07	~1.85	45.18	1.80	45.34	1.84
21	31.50		31.60		30.62*	
22	31.01	1.04;1.61	30.46	~1.0;1.50	30.23*	
23	69.09	3.80	69.17	3.61	68.23	3.75
24	35.88	2.13	35.66	2.07	35.19	1.93
25	76.23	4.94	76.08	4.91	75.96	5.09
26	37.75	1.80	37.67	1.72	37.99	1.71
27	99.25	-	99.35	-	99.22	-
28	26.02	1.25;1.91	26.43*		26.39*	
29	26.54	1.40;2.12	28.23*		26.87*	
30	30.53	~1.57	30.05	1.51	29.68	1.55
31	67.29	3.99	69.17	3.70	68.12	3.75
32	42.62	1.31;1.61	39.77	1.55	39.42	1.53;1.62
33	64.73	4.02	68.08	4.89	68.91	4.97
34	24.81	1.23	21.08	1.20	20.86	1.2
35	17.97	1.18	17.31	0.94	16.70	0.97
36	9.29	1.07	10.18	0.98	9.91	0.99*
37	8.33	1.03	9.00	0.95	9.91	0.95*

(continued)

Table 1. (Cont.)

	1		2		3	
Position	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
38	14.13	1.10	14.24	0.92	12.57	0.89
39	21.04	1.13	21.47	1.08	22.50	1.27
40	14.52	0.99	14.52	0.93	15.72	0.88
41	28.56	1.30;1.37	29.68	1.22;1.33	28.39	1.18;1.34
42	12.11	0.81	12.11	0.77	11.76	0.74
43	6.11	0.83	5.81	0.79	5.86	0.81
44	11.81	0.97	11.84	0.90	11.76	0.84
45	11.26	0.90	10.99	0.85	10.87	0.84
C=O(Ac)	-		170.72		170.73	
			170.31 (2x)		170.65	
					170.43	
					169.60	
CH_3(Ac)			21.08		21.39	
			20.93		20.79	
			20.74		20.61	

[a] Measured for solutions in $CDCl_3$ at 50 (**1**) and 100 MHz (**2** and **3**) for ^{13}C and 400 MHz for ^1H. δ-values are referenced to the solvent signal at 77.00 ppm for ^{13}C and to tetramethylsilane for ^1H. * Interchangeable assignments.

"diacetate" was prepared by the method of Masamune et al.[11]. The ^{1}H- and ^{13}C NMR spectra of this product were identical with those of **3**.

EXPERIMENTAL

1 (33 mg, 4.2 mmol) was acetylated in a mixture of pyridine and acetic anhydride (1 ml, 1:1) at 40 °C. After 5 hr, t.l.c. monitoring (Kieselgel 60, cyclohexane-ethyl acetate 1:1 v/v) of the reaction indicated formation of two new products **2** and **3**, in approximately equal amounts. Prolonging the reaction time to 8 hr resulted in the formation of **3** exclusively. The reaction was quenched after 5 hr by pouring the mixture into a large excess of ice-water and extracted with $CHCl_3$ twice. The organic phase was washed with water, dried over $MgSO_4$ and evaporated. The crude product was purified by column chromatography on silica gel (Kieselgel 60, 0.9x50 cm), using cyclohexane - ethyl acetate 2:1 (v/v) as eluent, to yield **2** (16 mg, 44%), and **3** (13 mg, 33%). **2** was a syrup with $[\alpha]_D^{25}$ -58.4 (c=0.8, ethanol) while **3** had m.p. 98-99 °C (crystallized from cyclohexane-pentane), and $[\alpha]_D^{25}$ -79.0 (c=0.7, ethanol), lit.[11] m.p. 112-113 °C, lit.[11] $[\alpha]_D^{24}$ -86.1 (c=1.74, ethanol).

For biological testing 0.5 ml of a spore suspension of *Aspergillus niger* (10^9 spores/ml) was added at 45 °C to 100 ml of a sterile culture medium containing yeast extract (0.1 g) beef meat extract (0.1 g), tripton (0.2 g), $Fe^{II}SO_4$ (0.3 mg), glucose (1 g) and agar (1.5 g). The test substances **1**, **2** and **3** were developed on a chromatographic plate (Kieselgel 60), dried, poured upon with the medium above and incubated at 37 °C for 24 hr. **2** and **3**, in contrast to **1**, did not show any inhibition zone on treatment with p-iodonitrotetrazolium violet (10 mg in 20 ml ethanol-water 1:1 v/v).

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