Notes

NOVEL MACROLIDES FROM *MICROMONOSPORA ROSARIA*

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Rosaramicin (**3**), a sixteen-membered macrolide antibiotic, produced by *Micromonospora rosaria*, has been the subject of several investigations[1~7]. The antibiotic is highly active against Gram-positive and Gram-negative bacteria. During the course of large scale fermentation besides **3**, several related macrolides were isolated and characterized. Some of these have been previously reported[8~12]. However, the carboxylic acid macrolide, which was expected from a natural sequence of events (eq 1)

$$RCH_2CH_3 \xrightarrow[O]{} RCH_2CH_2OH \xrightarrow[O]{} RCH_2CHO \xrightarrow[O]{} RCH_2COOH \xrightarrow[-CO_2]{} RCH_3 \quad \text{(eq 1)}$$

has never been reported. We report here the isolation of this acid and other minor co-fermentation products. In addition, we also report the ^{13}C NMR data (Table 1) of all the macrolides (Fig. 1).

Results and Discussion

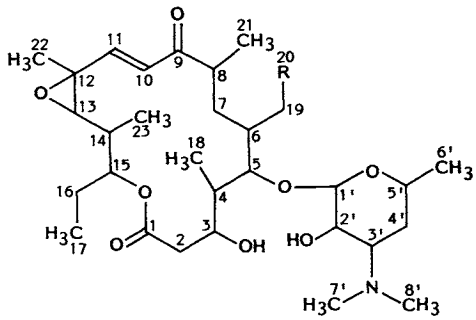
Physical constants are presented in Table 2. Compound **4** ($C_{31}H_{51}NO_{10}$, MW *m/z* 597), UV λ_{max}^{EtOH} at 238 nm (ε 13,120), $[\alpha]_D^{26}$ −24.9° (*c* 0.23 EtOH) was isolated from the fermentation broth using XAD-4 resin and subsequently purified *via* silica gel column chromatography. ^{13}C NMR ($CDCl_3$, ppm) indicated the presence of the methyl carbons at δ 8.6, 9.0, 14.5, 14.8 and 17.6, the methylene carbons at δ 24.5, 32.0, 35.9 and 40.1,

Table 1. ^{13}C NMR data of rosaramicins in $CDCl_3$ (δ, ppm).

Carbon	1	1a	1b	2	3[a]	3a	3b	4	5	6	6a	7	7a	8	9	10	11
1	174.0	174.6	174.0	173.5	173.5	174.1	173.6	172.7	174.1	175.5	175.3	173.6	169.8	174.1	165.5	165.5	174.5
2	38.6	39.8	38.7	39.8	39.7	39.6	39.6	40.1	39.7	41.1	41.0	39.7	38.2	39.6	120.7	120.4	40.2
3	67.1	67.7	67.2	67.1	66.8	67.2	66.7	66.9	67.2	67.4	67.2	65.7	68.4	67.0	151.0	150.9	67.3
4	41.0	40.5	41.0	41.1	41.2	41.0	41.1	42.8	41.8	42.1	42.2	41.1	40.6	41.1	41.8	41.5	42.2
5	80.2	80.2	81.0	81.2	81.3	81.2	81.8	83.8	86.7	80.3	80.3	80.6	80.5	81.7	82.5	82.0	88.6
6	39.9	38.7	39.9	33.1	31.4	31.4	31.4	33.6	31.4	35.4	35.4	32.8	33.7	36.2	31.1	33.6	69.4
7	33.2	33.9	33.4	31.7	31.8	32.5	31.9	32.0	34.2	38.1	38.2	32.1	32.2	32.8	31.1	30.7	36.4
8	45.2	44.9	45.3	45.3	45.1	45.1	45.1	45.3	45.3	49.6	49.3	45.0	44.7	45.2	45.1	45.6	45.6
9	201.3	204.2	201.4	201.5	200.3	203.3	200.6	201.5	201.2	160.0	159.9	201.1	200.4	200.9	200.7	200.3	203.5
10	123.2	118.9	123.2	123.2	122.8	118.3	122.9	123.0	123.3	111.8	111.8	123.1	123.4	122.9	122.9	122.8	125.1
11	150.2	145.2	150.4	150.6	150.9	146.0	151.0	150.7	150.0	161.9	161.9	150.6	150.5	150.7	150.9	151.5	151.4
12	59.8	133.6	59.8	59.7	59.7	133.4	59.8	59.8	59.8	62.5	62.5	59.7	59.9	59.7	60.1	60.1	61.0
13	67.9	147.5	68.2	68.1	68.0	148.2	68.0	68.6	67.8	72.1	72.2	68.0	68.4	68.0	66.9	66.8	69.3
14	37.8	38.7	37.9	37.7	37.8	38.9	37.8	37.8	37.8	36.8	36.7	37.7	36.7	37.8	37.2	37.2	38.7
15	76.7	78.7	76.8	76.7	76.8	78.6	76.9	76.3	76.9	79.2	79.2	76.7	77.3	76.9	76.3	76.2	78.2
16	24.7	24.7	24.8	24.7	24.7	24.8	24.7	24.5	24.8	25.9	25.9	24.7	24.4	24.7	24.9	25.0	25.5
17	9.0	9.0	9.1	9.0	9.0	8.8	8.9	8.6	9.0	8.5	8.5	9.0	8.5	9.0	8.9	8.9	9.4
18	9.2	9.6	9.6	9.1	9.0	9.7	9.1	9.0	9.3	9.4	9.5	9.2	10.5	9.1	19.1	18.8	9.5
19	21.1	21.0	21.3	33.1	43.9	43.7	43.9	35.9	17.5	138.4	138.3	35.7	34.3	32.3	39.5	44.6	
20	12.0	12.0	12.0	60.7	202.9	203.5	203.2	180.3	—	144.6	144.6	67.2	67.6	149.0	148.0	201.8	
21	17.5	17.6	17.5	17.4	17.4	17.6	17.4	17.6	17.4	18.3	18.2	17.4	17.6	17.4	17.4	17.4	17.7
22	15.0	16.2	15.0	14.9	15.0	16.2	15.0	14.8	15.0	16.1	16.1	14.9	15.5	14.9	15.8	15.9	15.3
23	14.5	12.9	14.5	14.4	14.5	12.9	14.5	14.5	14.5	14.8	14.9	14.5	14.6	14.5	14.6	14.6	14.7
24												50.7	48.3	132.3	132.9		
25												209.2	206.3	199.0	198.8		
26												31.1	30.8	26.6	27.1		

[a] Assignments based upon ^{13}C-^{13}C INADEQUATE data.

Fig. 1. Rosaramicin macrolides.



1 R=CH_3; **1a** 12,13-desepoxy; **1b** *N*-oxide
2 R=CH_2OH
3 R=CHO; **3a** 12,13-desepoxy; **3b** *N*-oxide
4 R=COOH
5 R=H
6 "2N" component; **6a** *N*-oxide
7 R=$-CH(OH)\overset{24}{C}H_2\overset{25}{C}O\overset{26}{C}H_3$; **7a** 3,20,2′-triacetate
8 R=$-CH=\overset{24}{C}H\overset{25}{C}O\overset{26}{C}H_3$
9 R=$-CH=CH-CO-CH_3$, Δ^2
10 R=CHO, Δ^2
11 C-6=OH; **11a** 3,6,2′-triacetate

the methine carbons at δ 33.6, 37.8, 42.8, 45.3, 66.9, 68.6, 76.3 and 83.8 and the olefinic and singlet carbons at δ 59.8, 123.0, 150.7, 172.7, 180.3 and 201.5. The comparison of the data with that of **3** indicated that C-20 has been oxidized from $-CHO\rightarrow$ COOH (δ 202.9→180.3) with concurrent perturbations in the chemical shifts of other carbons, *e.g.*, C-19 (δ 43.9→35.9). ^1H NMR ($(CD_3)_2CO$, ppm) showed the presence of diagnostic resonances at δ 0.90 (t, CH_3), 1.10 (d, $2\times CH_3$), 1.12 (d, CH_3), 1.20 (d, CH_3), 1.24 (d, CH_3), 1.50 (s, CH_3), 3.69 (m, $-CHO-$), 4.86 (m, $-CHOCO$), 6.43 (d, =CH) and 6.88 (d, =CH). The spectrum lacked the presence of a $-CHO$ function (δ 9.76 in **3**).†

HPLC of the bulk material led to the detection of a new co-fermentation product, purified by silica gel column chromatography (CH_2Cl_2-EtOH-H_2O-HOAc, 24:8:1:1). ^{13}C NMR (CD_3OD-$CDCl_3$, ppm) of the purified product (**11**, MW *m/z* 555) indicated the presence of the methyl carbons at δ 9.4, 9.5, 14.7, 15.3 and 17.7, the methylene carbons at δ 25.5, 36.4 and 40.2, the methine carbons at δ 38.7, 42.2, 45.5, 67.3, 69.3, 69.4, 78.2 and 88.2 and the olefinic and singlet carbons at δ 61.0, 125.1, 151.4, 174.5 and 203.5. ^1H NMR ($(CD_3)_2CO$, ppm) indicated all the diagnostic resonances at δ 0.91 (t, CH_3), 1.15 (d, $2\times CH_3$), 1.22 (d, CH_3), 1.24 (d, CH_3) 1.50 (s, CH_3), 2.84 (d, $-CHO$), 4.92 (m, $CHOCO$), 6.40 (d, =CH) and 6.90 (d, =CH).

Comparison of the data of **5**, **3** and **11** (Table 3) showed that in **5** and **11** C-5 and C-7 are shifted downfield compared to **3** where they are influenced by the γ-effect of C-20. The data are consistent with structure **11** (6-hydroxy) for this isolate. Treatment with acetic anhydride in pyridine resulted in the formation of 3, 6, 2′-triacetate (*m/z* 681.4) supported by ^{13}C and ^1H data.

Spectroscopic Data

^{13}C NMR spectrum of **5** (R=H) indicated the presence of a lactone group at δ 174.1, α,β-unsaturated keto moiety at δ 201.2, 123.3 and 150.0, an epoxide ring at δ 59.8 and 67.8, three oxygen bearing methine carbons at δ 67.2 (HCOH), 76.9 (HCOCO) and 86.7 (HC−O−sugar), the latter having desosamine β-glycosidically linked. Desosamine ring does not undergo severe conformational changes and is discussed only in the case of

Table 2. Physical constants.

	1a		**1b**		**3b**		**4**		**6a**	
Formula	$C_{31}H_{53}NO_7$		$C_{31}H_{53}NO_9$		$C_{31}H_{51}NO_{10}$		$C_{31}H_{51}NO_{10}$		$C_{31}H_{48}N_2O_8$	
MW	552 (M+1)		583		597		597		576	
Elemental	Calcd	Found	Calcd	Found	Calcd	Found	Calcd	Found	Calcd	Found
Anal										
C	67.48	66.62	63.78	62.37	62.29	57.73	62.29	59.60	64.56	60.07
H	9.68	9.27	9.15	8.59	8.60	8.05	8.60	7.82	8.39	8.35
N	2.54	2.64	2.40	2.45	2.34	2.21	2.34	2.56	4.86	4.23
UV λ_{max}^{EtOH} nm	282		238, 283		238		238		238, 278, 287	
ε	20,400		4,750, 1,540		12,130		13,120		7,100, 2,050, 1,780	
$[\alpha]_D^{26}$ (*c* 0.2∼0.5 EtOH)	−11.8°		−22.3°		−39.7°		−24.9°		—	

† The ^{13}C and ^1H NMR data for desosamine sugar were not affected and are not discussed unless otherwise specified.

Table 3[a]. ^{13}C NMR chemical shifts of **5**, **3** and **11**.

Carbon	Compounds		
	5	**3**	**11**
4	41.8	41.3	42.2
5	86.7	80.9	88.6
6	31.4	31.7	69.4
7	34.2	31.7	36.4
8	45.3	45.5	45.6
9	201.2	201.7	203.7

Chemical shifts given in ppm.
[a] Solvent: $CDCl_3$ (**5**); CD_3OD-$CDCl_3$ (**3** and **11**).

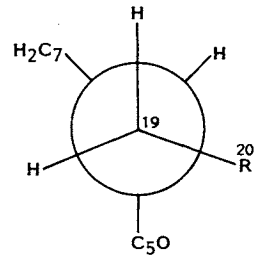
N-oxidation. The resonances at δ 31.4 and 17.5 are attributed to carbons 6 and 19, respectively.

For RCH_3, three compounds **1** (R=CH_3), **1a** (12,13-desepoxy) and **1b** (desosamine *N*-oxide) were isolated. The introduction of a methyl group (δ 12.0) at C-19 shifted C-6 to 39.9 ($\Delta\delta$=+8.5 ppm) and C-19 to 21.1 ($\Delta\delta$=+3.6 ppm). C-5 suffered an upfield shift ($\Delta\delta$=−6.5 ppm), a fact attributed to a combination of γ gauche effect and steric compression.

Desepoxidation in **1a** resulted in an additional double bond leading to a five carbon conjugation and significant planarity in one-half of the aglycone. Methyl-23 was shifted upfield ($\Delta\delta$=−1.6 ppm) as expected. Compound **1b** was more polar than **1** and the ^{13}C NMR analysis indicated *N*-oxidation (discussed separately). The presence of a polar group (R=CH_2OH) in **2**, shifted C-19 to 33.1 vs. 21.1 in **1** and the CH_2OH carbon appeared at δ 60.7. C-6 experienced an upfield shift to 33.1 from 39.9 in **1**.

Further oxidation resulted in rosaramicin **3** (R=CHO), the major and the most important isolate of the fermentation. C-19 shifted further downfield from 21.2→43.9 with concurrent generation of a CHO function at δ 202.9 ppm. Desepoxy rosaramicin (**3a**) has aglycone carbon shifts similar to **1a** and the *N*-oxide (**3b**) has chemical shifts of desosamine identical to that of the same in **1b**. C-6 appeared upfield at δ 31.4 in **3**, **3a** and **3b** compared to δ 39.9 ppm in **1**, **1a** and **1b**. Compound **4** (R=COOH) has never been mentioned before because of the difficulty in its isolation. The acid was isolated from the aqueous mother liquor by precipitation. C-19 and C-20 appeared at δ 35.9 and 180.3, respectively.

The structure of "2N" impurity **6** has been reported earlier[8]. However, further improvement in the methodology led to the isolation of *N*-oxide (**6a**).



1 R=CH_3
2 R=CH_2OH

Compound **7** isolated as an acetone adduct, in which 20-CHO function underwent aldol reaction:

$$CH_2CHO+(CH_3)_2CO \longrightarrow -CH_2-\underset{\underset{OH}{|}}{CH}-CH_2\overset{\overset{O}{\|}}{C}CH_3$$

In **7**, new carbons appeared at δ 67.2 (CHOH), 209.2 (C=O), 50.7 (CH_2) and 31.1 (CH_3). Further characterization was achieved by converting **7** to its 3,20,2′-triacetate (**7a**) with expected chemical shift changes except for an upfield shift of C=O (209.2→206.3) and ester (COO) (173.6→169.8) carbons, indicative of the loss of intramolecular hydrogen bonding.

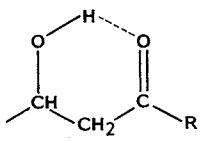
^{13}C T_1 relaxation data for **7** was obtained for comparison with that of **3**[4]. The average NT_1 values for the aglycone and desosamine moieties were 0.50±0.21 and 0.51±0.11 seconds, respectively. The same values for **3** were 0.40±0.05 seconds and 0.41±0.03 seconds, respectively. Compounds **7** and **3** undergo isotropic globular motion in solution. However, the side chain at C-6 displayed increased mobility as shown by the following T_1 values.

$$\overset{\overset{|}{H}}{\underset{|}{C}}-CH_2CH(OH)CH_2COCH_3$$

0.62 0.39 1.12 seconds

High T_1 value (1.15 seconds) for methyl-22 suggested free rotation even though methyl groups at quarternary carbons in 14-membered macrolides were restricted in their mobility.

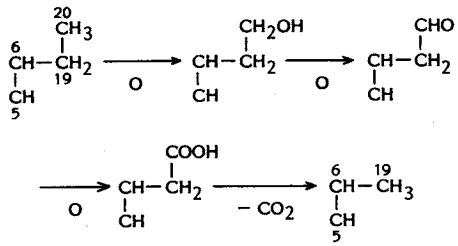
Dehydration of **7** led to the isolation of another UV absorbing component which was assigned structure, **8**, based upon the presence of two new olefinic carbons at δ 149.0 (C-20) and 132.3 (C-24) and shifting $COCH_3$ carbons to 199.0 and 26.6 ppm,



R=CH_3 or OCHR'

respectively. Attempts to prepare **8** from **7** *via* triacetate, **7a**, followed by dehydration led to the isolation of **9**. Compound **9** had three α,β-unsaturated keto moieties encompassing C-1, C-2, C-3, C-9, C-10, C-11 and C-20, C-24, and C-25. The double bond at 2,3-position (Δ^2) resulted in the appearance of olefinic carbons at δ 120.7 and 151.0 with concurrent upfield shift of the ester carbonyl (δ 174.4→165.5; loss of intramolecular hydrogen bond and unsaturation effect). Except for the presence of C-20, C-24 double bond and its proximity effects, the data of **9** were comparable to that of Δ^2-rosaramicin (**10**).

Incorporation (^{13}C- and ^{14}C-labeled precursors) studies indicated that carbons 20, 19, 6 and 5 of the aglycone moiety of **3** originated from a butyrate molecule[6] and the isolation of all components in the sequence,



suggests successive oxidation during fermentation. Similar transformations have been observed for rifamycins antibiotics[13]. Attempts to establish the kinetics of this oxidation in **3** utilizing 4-^{13}C-labeled butyrate were not successful.

Sugar Moiety

^{13}C Chemical shifts of desosamine are identical to the reported values and are consistent with all *trans* stereochemistry. However, *N*-oxidation imparts significant changes as a result of N→O bond orientation. The chemical shifts (δ, ppm) of the sugar carbons are shifted from, *e.g.*, 1' (104.5→103.2), 2' (70.4→72.2), 3' (65.8→76.1), 4' (28.4→34.9), 5' (69.7→67.5), 6' (21.1→21.0) and 7', 8' (40.2→52.2, 59.1). The influence of N–O bond for carbons 2', 3', 4', 7' and 8' suggests its usefulness as a diagnostic probe.

The *in vitro* antibacterial activity of **4** was 2~64, and >64 μg/mg (MIC) for Gram-positive and Gram-negative organisms, respectively.

Acknowledgments

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