Notes

3-*O*-DEMETHYLMONENSINS A AND B PRODUCED BY *STREPTOMYCES CINNAMONENSIS*

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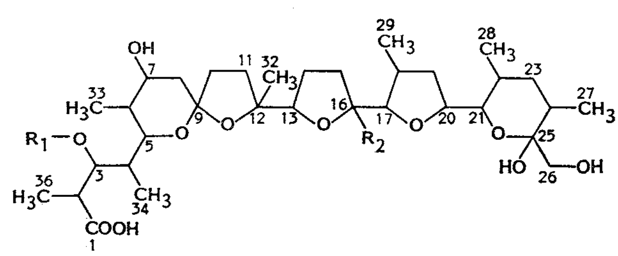
Monensin A derivatives lacking the C-3 methoxyl group have been described so far as the products of animal metabolism[1]. We report here their occurrence as co-metabolites of monensins A (**1**) and B (**2**) in *Streptomyces cinnamonensis*. They exhibit a different color reaction with vanillin reagent[2] (yellow-orange instead of red).

The strain *Streptomyces cinnamonensis* LO-63 (from our collection) was cultivated as described[3]. Monensins A and B were extracted from the methanolic extract of the mycelium into heptane. The methanolic layer was diluted with water (10%) and extracted with $CHCl_3$. Repeated preparative TLC on Silica gel G in the systems heptane - ethyl acetate - methanol (50:40:10 and 25:65:10), using UV detection after spraying with 0.1% ethanolic solution of morin, afforded besides residual **1**-Na and **2**-Na the pure compounds **3**-Na and **4**-Na. The compounds were eluted from the scraped silica gel with diethyl ether - methanol (97:3). **3**-Na: MP 236~239°C (diethyl ether); $[\alpha]_D^{20}$ +73.6° (*c* 0.0037, MeOH). **4**-Na: MP 224~227°C (diethyl ether); $[\alpha]_D^{20}$ +88.0° (*c* 0.0038, MeOH). Rf values in the system heptane - ethyl acetate - methanol (5:4:1) are: **1**-Na 0.44, **2**-Na 0.38, **3**-Na 0.25, **4**-Na 0.22.

Mass spectrometry (Table 1) suggests that **3**-Na is 3-*O*-demethylmonensin A and **4**-Na its lower homologue. The absence of methoxyl is also evident from ^1H and ^{13}C NMR spectra that confirm this deduction. Very close $J_{2,3}$ values (Table 2) indicate the same configuration at C-3 as in **1**-Na. Chemical shifts of carbons 7~36 are within 0.3 ppm identical with those of parent compounds[4]. The differences observed for C-2~C-6 are due to the removal of methoxyl. These compounds represent probably the last step in the biosynthesis of monensins. The amount of **3**-Na and **4**-Na increases upon addition of inhibitors of methylation. The activity against *Bacillus subtilis* decreases in order **1**-Na>**2**-Na≫**3**-Na>**4**-Na. This effect might be ascribed to greater polarity of **3**-Na and **4**-Na that can hamper their transport through membranes.



1 $R_1 = CH_3$ (35) $R_2 = CH_2CH_3$ (30, 31)
2 $R_1 = CH_3$ (35) $R_2 = CH_3$ (30)
3 $R_1 = H$ $R_2 = CH_2CH_3$ (30, 31)
4 $R_1 = H$ $R_2 = CH_3$ (30)

Table 1. Electron impact mass spectra[a].

Ion type[b]	3-Na			4-Na		
	m/z	Rel. (%)	Composition	m/z	Rel. (%)	Composition
M	678	7	$C_{35}H_{59}O_{11}Na$	664	8	$C_{34}H_{57}O_{11}Na$
a	647	72	$C_{34}H_{56}O_{10}Na$	633	48	$C_{33}H_{54}O_{10}Na$
b	617	33	$C_{34}H_{58}O_8Na$	603	33	$C_{33}H_{56}O_8Na$
c	561	15	$C_{30}H_{50}O_8Na$	547	19	$C_{29}H_{48}O_8Na$
d	547	14	$C_{29}H_{48}O_8Na$	533	19	$C_{28}H_{46}O_8Na$
e	477	35	$C_{25}H_{42}O_7Na$	463	41	$C_{24}H_{40}O_7Na$
f	463	74	$C_{24}H_{40}O_7Na$	449	88	$C_{23}H_{38}O_7Na$
g	405	66	$C_{22}H_{38}O_5Na$	391	80	$C_{21}H_{36}O_5Na$
h	365	14	$C_{18}H_{30}O_6Na$	365	19	$C_{18}H_{30}O_6Na$
k	321	100	$C_{17}H_{30}O_4Na$	307	100	$C_{16}H_{28}O_4Na$
l	435	14	$C_{22}H_{36}O_7Na$	421	19	$C_{21}H_{34}O_7Na$
m	337	14	$C_{16}H_{26}O_6Na$	337	19	$C_{16}H_{26}O_6Na$
x[c]	575	38	$C_{31}H_{52}O_8Na$	561	36	$C_{30}H_{50}O_8Na$

[a] Varian MAT-311 (70 eV, direct inlet at 200°C).
[b] Nomenclature of ions see ref 1.
[c] Ions not reported in ref 1 but analogous ion present in the mass spectrum of sodium monensin A[5].
Rel.: Relative intensity.

Table 2. Signals of distinct protons in the ^1H NMR spectra of compounds 1-Na, 3-Na and 4-Na.

Proton	1-Na[a]			3-Na[b]			4-Na[b]		
	δ	Mult.	Couplings (Hz)	δ	Mult.	Couplings (Hz)	δ	Mult.	Couplings (Hz)
2	2.530	dq	10.3, 6.7	2.502	dq	10.0, 7.0	2.503	dq	10.0, 7.0
3	3.819	dd	10.3, 1.6	3.701	dd	10.0, 1.6	3.700	dd	9.5, 1.7
5	4.035	dd	11.5, 1.9	3.810	mt		3.803	mt	
7	3.890	ddd	2.3, 3.6, 2	3.881	mt		3.883	mt	
13	3.543	mt	ΣJ=15.3	3.531	mt	ΣJ=16.3	3.563	mt	ΣJ=15.5
17	3.974	d	3.4	3.940	d	3.5	3.930	d	3.3
20	4.397	ddd	4.1, 7, 9.8	4.393	ddd	4, 7, 9.7	4.402	ddd	4, 7.3, 9.0
21	3.828	dd	4.1, 9.5	3.810	mt		3.803	mt	
26A	3.980	d	11.9	3.979	d	11.8	4.002	d	12.0
26B	3.297	d	11.9	3.297	d	11.8	3.304	d	12.0
27	0.805	d	5.6	0.805	d	5.9	0.812	d	5.8
28	0.850	d	5.9	0.852	d	6.2	0.856	d	6.0
29	0.937	d	7.1	0.892	d	6.6	0.888	d	7.0
30	0.937	t	7.6	0.938	t	7.3	1.174	s	
32	1.509	s		1.506	s		1.488	s	
33	0.896	d	6.9	0.884	d	7.1	0.879	d	7.0
34	1.179	d	6.9	1.136	d	6.5	1.191	d	7.0
36	1.239	d	6.7	1.183	d	7.0	1.243	d	6.6

[a] ref 6.
[b] Varian XL-200, 200 MHz, $CDCl_3$, internal TMS, 25°C.
Mult.: Multiplicity.

Table 3. Comparison of selected ^{13}C chemical shifts of compounds **1**-Na, **2**-Na, **3**-Na and **4**-Na.

Carbon	**1**-Na[a]	**3**-Na	**2**-Na[a]	**4**-Na	Δ_1[b]	Δ_2[c]
1	181.4	181.0	181.3	181.1	−0.4	−0.2
2	45.1	45.5	45.0	45.5	+0.4	+0.5
3	83.2	73.8	83.0	73.6	−9.4	−9.4
4	37.6	42.1	37.5	42.2	+4.5	+4.7
5	68.5	67.8	68.3	67.8	−0.7	−0.5
6	34.9	35.7	34.9	35.7	+0.8	+0.8

Our data: Jeol FX-60, 15.036 MHz, $CDCl_3$, internal TMS standard, 25°C.

[a] ref 4.

[b] $\Delta_1=\delta$(**3**-Na)$-\delta$(**1**-Na).

[c] $\Delta_2=\delta$(**4**-Na)$-\delta$(**2**-Na).

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