Communications to the editor

PREPARATION AND CHARACTERIZATION OF DEDESOSAMINYL 5-*O*-MYCAMINOSYL-10,11-DIHYDROMYCINAMICIN IV

Sir:

During the course of search for new antibiotics produced by *Micromonospora* sp., in addition to the formation of AR-5 (mycinamicin) antibiotics, we isolated three neutral components (**1**~**3**, Fig. 1) which were characterized from their spectroscopic (PMR, CMR and MS) data[1]. These compounds were named[2] as mycinolide IV (**1**), dedesosaminylmycinamicin IV (**2**) and dedesosaminylmycinamicin V (**3**). It was envisioned that a non-antibiotic producing blocked-mutant may be utilized to produce an antibiotic by glycosidation at C-5 of the neutral isolates. We now disclose the formation of dedesosaminyl-5-*O*-mycaminosyl-10,11-dihydromycinamicin IV (**4**) from **2**.

Non-antibiotic producing mutant XJ-174 was isolated from a population of tylosin-producing *Streptomyces fradiae* (ATCC 19609)[3] after treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG). The blocked mutant itself does not produce any detectable amounts of antibiotic but produces tylosin when incubated with 20-deoxy-20-dihydro-12,13-desepoxy-12,13-dehydrorosaranolide[4]. Incubation of mutant XJ-174 with **2** yielded compound (**4**). Similar experiment with **3** produced a more polar antibiotic than **4** with characteristics anticipated, based upon differences between **2** and **3**, for **5**. However, small quantities of **5** prevented rigorous spectroscopic characterization.

For a preparative fermentation the mutant was incubated in the presence of 1 g of **2** at 30°C for 120 hours in a 14-liter fermentor containing 10 liters medium consisting of 25 g dextrose, 25 g soy flour, 5 g $CaCO_3$, 2 g NaCl, 5 ml corn steep liquor and 30 ml sunflower oil in tap water. Fermentation broth was harvested and extracted twice (10 liters each) with ethyl acetate, and the solvent was concentrated to obtain dark brownish oily residue. The residue was precipitated with acetone and ethyl ether - hexane (6: 4) and further purified by preparative silica-gel thin-layer chromatography using the lower phase of the solvent system consisting of chloroform - methanol - 17% ammonia (40: 12: 20) to obtain 8 mg of **4**.

Compound **4** ($C_{37}H_{63}O_{12}N$, *m/z* 713.4) showed UV λ_{max}^{MeOH} at 211 (ε 14,400) and 275 nm (ε 2,750). Proton NMR spectrum in $(CD_3)_2CO$ indicated the presence of resonances at δ 0.88 (t, 7.0 Hz, CH_3), 1.00~1.20 (d, 6.5 Hz, 5×CH_3), 2.50 (s, N(CH_3)$_2$), 3.50 (s, OCH_3), 3.54 (s, OCH_3), 4.40 (d, 6.0 Hz, H-1'), 4.50 (d, 8.0 Hz, H-1''), 4.98 (m, H-15), 5.40 (bm, H-12,13) and a *trans* coupled α,β unsaturated lactone moiety at δ 5.84 (d, J=16.0 Hz) and 6.78 (dd, J=16.0 and 9.6 Hz) assigned to the H-2 and H-3, respectively. ^{13}C NMR data of **1**, **2** and **4**, presented in Table 1, suggest that the aglycone portion of the antibiotic consisted of four methyl carbons at δ 9.7, 17.2, 17.8 and 18.7, four olefinic carbons at δ 121.6, 129.0, 132.6 and 150.7, two carbonyl carbons at δ 166.0 (C-1) and 213.6 (C-9), six methine carbons at δ 34.6, 40.9, 44.9, 48.3, 74.5 and 86.5 and the remaining five methylene carbons appeared at δ 25.5, 26.2, 32.8, 38.6 and 70.1 (OCH_2 at C-23).

The chemical shifts of carbons of mycinose and mycaminose are comparable to the literature

Fig. 1

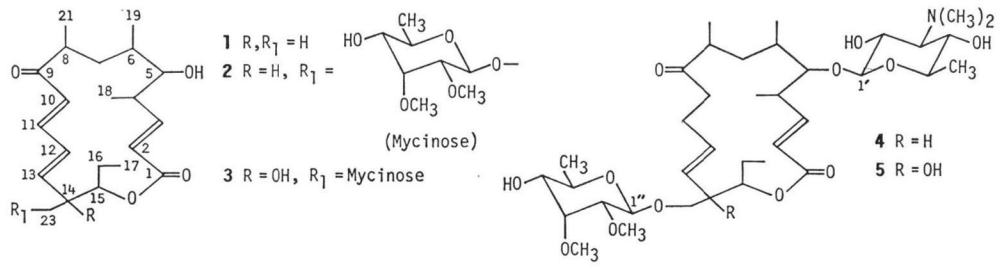

Table 1. ^{13}C NMR data in $CDCl_3$[a].

Carbon	1	2	4
1	166.3	166.2	166.0
2	121.3	121.1	121.6
3	151.3	151.6	150.4
4	40.5	40.6	40.9
5	80.1	79.9	86.5
6	33.9	34.0	34.6
7	31.7	31.7	32.8
8	44.8	44.8	44.9
9	203.9	203.4	213.6
10	123.9	123.2	38.6
11	142.2	141.6	26.2
12	131.7	133.0	129.0
13	145.2	142.0	132.6
14	43.4	49.3	48.3
15	77.1	73.8	74.5
16	24.7	25.3	25.5
17	9.6	9.7	9.7
18	19.4	19.4	18.7
19	17.7	17.8	17.8
21	17.4	17.4	17.2
23	15.7	68.6	70.1
	(CH_3)	(CH_2O-myc)	(CH_2O-myc)
Mycinose			
1″		101.2	101.1
2″		81.9	82.0
3″		80.0	80.0
4″		73.0	72.8
5″		70.5	70.6*
6″		17.5	17.9
2″-OCH_3		59.7	59.8
3″-OCH_3		61.7	61.7
Mycaminose			
1′			104.5
2′			71.2
3′			70.8*
4′			70.3*
5′			73.4
6′			18.8
3′-$N(CH_3)_2$			41.8

[a] Both fully decoupled and SFOR experiments were performed to distinguish between CH, CH_2 and CH_3 carbons.

* Interchangeable.

values[5]. The major differences in the ^{13}C NMR data are the substitution of mycinose at C-23 (**1**→**2**), the saturation of a double bond at C-10 and C-11 and the glycosidation at C-5 (**2**→**4**). Low resolution mass spectrum indicated molecular ion at *m/z* 713.4. Major fragments were at *m/z* 698 (M^+-CH_3), 682 (M^+-OCH_3), 538 (*O*-aglycone-*O*-mycaminose, loss of 175 for mycinose), 522 (aglycone-*O*-mycaminose, loss of 191 for *O*-mycinose), 348 (aglycone-O), 333 (aglycone-H), 332 (aglycone), 191 (mycinose-O), 190 (mycaminose-O), 175 (mycinose), 174 (mycaminose) and 88 ($CH_3O-CH=CH-OCH_3$). Base peak at *m/z* 88 is typical of mycinose. Based upon the above spectroscopic data structure **4** was assigned.

In summary, the isolation of **4** indicates that the functions of mutant XJ-174 were the 5-*O*-glycosidation with mycaminose and the saturation of a double bond at C-10 and C-11. Compound **4** has tylosin type biological activity.

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