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Glycosylative Inactivation of Chalcomycin and Tylosin by a Clinically Isolated *Nocardia asteroides* Strain

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Studies on the susceptibility of pathogenic *Nocardia* to macrolide antibiotics, chalcomycin and tylosin, showed that most of the *Nocardia* species examined were highly resistant to both antibiotics, although *N. nova* was moderately susceptible. *N. asteroides* IFM 0339 converted these macrolides into inactive metabolites by glycosylation at 2′-OH or glycosylation and reduction of the 20-formyl group. The structures of the metabolites were determined from NMR and MS data to be 2′-[*O*-(β-D-glucopyranosyl)]chalcomycin (**2**), 2′-[*O*-(β-D-glucopyranosyl)]tylosin (**5**) and 20-dihydro-2′-[*O*-(β-D-glucopyranosyl)]tylosin (**4**).

Our studies on the susceptibility of 75 clinical isolates of *Nocardia* species to macrolide antibiotics (rokitamycin, midecamycin and erythromycin) revealed species-specific drug resistance patterns, most of which involved metabolic inactivation of the drugs.[1] Depending on the *Nocardia* species (*N. asteroides*, *N. farcinica*, *N. brasiliensis*, *N. otitidiscaviarum* and *N. nova*) used, rokitamycin and midecamycin were reduced at the 18-formyl group (18-dihydrorokitamycin and 18-dihydromidecamycin), phosphorylated and reduced (18-dihydro-2′-*O*-phosphorylrokitamycin and 18-dihydro-2′-*O*-phosphorylmidecamycin), or deacylated (3″-de-*n*-propionyl-4″-de-*n*-butyrylrokitamycin, 4″-de-*n*-butyrylrokitamycin, or 4″-de-*n*-propionylmidecamycin). Erythromycin was phosphorylated (2′-*O*-phosphorylerythromycin) or glycosylated (2′-[*O*-(β-D-glucopyranosyl)]erythromycin).[2] Glycosylation of 2′-OH of desosamine in erythromycin was found only in the case of the inactivation by *N. asteroides* IFM 0339 strain.

These results prompted us to study if other macrolide antibiotics, with different sugar components on the lactone ring, were inactivated by *Nocardia* species. Chalcomycin (**1**)[3,4] which contains D-mycinose and a neutral sugar, D-chalcose, instead of a basic sugar, D-desosamine, and tylosin (**3**)[5,6] which contains D-mycinose, D-mycaminose and L-mycarose, were selected for this purpose. Here, we report the drug-susceptibility patterns of five *Nocardia* species to both antibiotics, and the elucidation of a resistance mechanism through the structural determination of the inactivated macrolides.

Experimental

General

^1H and ^{13}C NMR spectra were measured in $CDCl_3$ at 25°C on a JEOL ALPHA-500 NMR spectrometer at 500

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MHz and 125 MHz, respectively. Chemical shifts in ^{1}H and ^{13}C NMR spectra were recorded in δ units relative to chloroform (δ_H=7.24 ppm and δ_C=77.0 ppm, respectively). ^{1}H and ^{13}C NMR signal assignments and correlations between the signals were performed on the basis of COSY, pulse-field gradient heteronuclear single quantum coherence (PFG-HSQC), pulse-field gradient heteronuclear multiple-bond correlation (PFG-HMBC), total correlation spectroscopy (TOCSY)[7,8], HSQC-TOCSY[9,10], and NOESY experiments. FAB-MS and HRFAB-MS were measured on a JEOL JMS-HX110 double-focusing mass spectrometer with a JMS-DA7000 data system. The ion acceleration voltage was 10 kV, and the fast-atom xenon gas was accelerated at a voltage of 6 kV. *m*-Nitrobenzyl alcohol was used as the matrix.

Chemicals

Chalcomycin and tylosin were obtained from Sigma Chemical Co.

Microorganisms and Culture Conditions

Twenty-eight strains of five pathogenic species (*N. asteroides*, *N. brasiliensis*, *N. farcinica*, *N. nova* and *N. otitidiscaviarum*), including the type strains, were used. Matured seed cultures were prepared by the method previously described[1] and used as seed inocula for the inactivation or MIC experiments. MICs were determined by an agar dilution method using brain heart infusion agar (BHI medium, Difco Laboratories, Detroit, Mich.)[11]. Inactivation of macrolide antibiotics was monitored by bioassay, using *Bacillus subtilis* PCI 219 as the indicating organism.

Preparation of Inactivated Compounds

A seed culture of *N. asteroides* IFM 0339 was used for preparation of inactivated compounds. Two milliliters of the seed culture were inoculated into 500 ml shake flasks containing 200 ml of BHI medium (2% glucose). After 24 hour of incubation by a rotary shaker (250 rpm, 2.5 cm) at 32°C, 20 mg of each antibiotic was added, and the mixture was incubated for 5 days. After separation from the mycelia by centrifugation at 6,000 rpm (5,800×*g*), the supernatant was extracted with 200 ml of EtOAc, and the organic solution was washed with an equal volume of 5% $NaHCO_3$ solution three times. The washed extracts were combined and concentrated *in vacuo*. The dry residues were purified by silica gel column chromatography (EtOAc and MeOH, 4:1). The inactivated compounds [CHIP-0339 (**2**) from chalcomycin (**1**), and TIP-0339-1 (**4**) and TIP-0339-2 (**5**) from tylosin (**3**)] were further purified by preparative TLC with BuOH - AcOH - H_2O (3 : 3 : 1), followed by Sephadex LH-20 column chromatography eluted with a solvent mixture of chloroform and MeOH (1 : 1). An anisaldehyde-sulfuric acid spray reagent was used to detect sugars on TLC plates. From 20 mg of chalcomycin (**1**) and tylosin (**3**), 9.2 mg of CHIP-0339 (**2**), and 4.8 mg of TIP-0339-1 (**4**) and 5.0 mg of TIP-0339-2 (**5**) were obtained, respectively. The incubation, extraction and purification were repeated, and 56 mg of **2**, 9 mg of **4**, and 17 mg of **5** were obtained. The Rf values of **1** and **2** on a silica gel TLC plate developed with EtOAc - Me_2CO (3 : 1) were 0.79 and 0.16, and those of **3**, **4** and **5** developed with EtOAc - MeOH (3 : 1) were 0.63, 0.20 and 0.26, respectively.

Results

Susceptibility to Chalcomycin and Tylosin and Inactivation Profiles of Five *Nocardia* species

The susceptibility of pathogenic *Nocardia* to chalcomycin (**1**) and tylosin (**3**) was examined using erythromycin as a reference drug (Table 1). Among five *Nocardia* species tested, *N. asteroides*, *N. brasiliensis*, *N. farcinica* and *N. otitidiscaviarum* were highly resistant to **1** and **3** as well as to the reference drug erythromycin; except for a few strains, MIC values were greater than 100 μg/ml. On the other hand, *N. nova* showed a different susceptibility pattern to macrolide antibiotics. Although the MIC values of *N. nova* against chalcomycin were more than 100 μg/ml, the species was moderately susceptible to tylosin, with MIC values of 3.1 to 25 μg/ml. All strains of *N. nova* were also susceptible to the reference erythromycin. These results confirmed our previous finding that pathogenic *Nocardia* show species-specific drug susceptibility patterns, and *N. nova* is the most susceptible species to macrolides tested hitherto.[1]

Inactivation of Chalcomycin and Tylosin by *N. asteroides* IFM 0339

Among the susceptible strains to chalcomycin (**1**) and tylosin (**3**), only *N. asteroides* IFM 0339 converted **1** to **2**, and **3** to **4** and **5**. Antimicrobial activities of the products were measured by the size of the inhibition zone on *Bacillus subtilis* PCI 219 using paper disc[12] (Table 2). These data indicate that the products **2**, **4** and **5** lack antimicrobial activity.

It is interesting that the higher MIC values of *Nocardia* species against **1** and **3** (Table 1) did not always coincide with the presence of inactivation activity. This may be due

Table 1. Comparison of susceptibility of pathogenic *Nocardia* to chalcomycin, tylosin and erythromycin.

Nocardia species and strain number		MIC values (μg/ml) (Inactivation ability)[a]		
		chalcomycin	Tylosin	erythromycin
N. asteroides	IFM 0258	>100 (-)[a]	50 (-)	12.5 (-)
	IFM 0262	>100 (-)	>100 (-)	>100 (+)
	IFM 0280	>100 (-)	>100 (-)	>100 (+)
	IFM 0299	>100 (+)	>100 (+)	>100 (+)
	IFM 0319^T	>100 (-)	>100 (-)	>100 (-)
	IFM 0339	>100 (+)	>100 (+)	>100 (+)
	IFM 0347	>100 (-)	>100 (+)	>100 (+)
N. brasiliensis	IFM 0236^T	>100 (+)	>100 (+)	>100 (+)
	IFM 0246	>100 (+)	>100 (+)	>100 (+)
	IFM 0249	>100 (-)	>100 (+)	>100 (+)
	IFM 0256	>100 (+)	>100 (+)	>100 (+)
	IFM 0279	>100 (-)	25 (+)	25 (+)
N. farcinica	IFM 0221	>100 (-)	>100 (-)	>100 (-)
	IFM 0228	>100 (-)	50 (-)	>100 (-)
	IFM 0275	100 (-)	50 (-)	>100 (-)
	IFM 0284^T	>100 (-)	>100 (-)	>100 (-)
	IFM 0294	>100 (-)	>100 (-)	>100 (-)
	IFM 0320	>100 (-)	>100 (-)	>100 (-)
N. nova	IFM 0274	>100 (-)	25 (-)	0.39 (-)
	IFM 0290^T	>100 (-)	3.13 (-)	0.39 (-)
	IFM 0297	>100 (-)	25 (-)	0.39 (-)
	IFM 0356	>100 (-)	12.5 (-)	0.2 (-)
N. otitidiscaviarum	IFM 0192	>100 (-)	>100 (+)	>100 (-)
	IFM 0205	>100 (-)	>100 (+)	>100 (+)
	IFM 0239^T	>100 (-)	>100 (+)	>100 (+)
	IFM 0300	>100 (-)	>100 (+)	>100 (+)
	IFM 0303	>100 (-)	>100 (+)	>100 (+)
	IFM 0337	>100 (-)	>100 (+)	>100 (+)

[a] Inactivation ability was monitored by bioassay using *Bacillus subtilis* PC1 219

Table 2. Comparison of antimicrobial activities of chalcomycin (**1**), tylosin (**3**) and their converted products, CHIP-0339 (**2**), TIP-0339-1 (**4**) and TIP-0339-2 (**5**) against *Bacillus subtilis* PCI 219 using paper disc.

Compound	Inhibition zone around paper disc	
	5 μg/disc[a)]	50 μg/disc[a)]
1	13.9	19.4
2	0	0
3	20.0	23.0
4	0	0
5	0	0

a) each paper disc contains respective compound

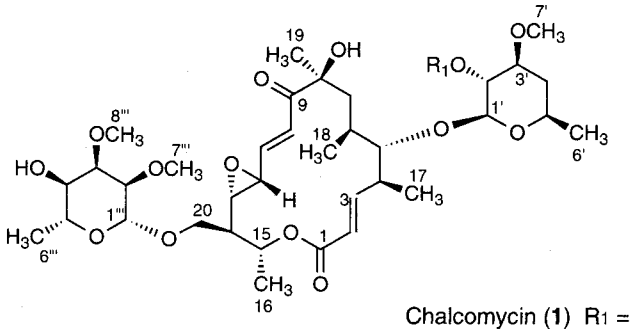
to the presence of other resistance mechanisms.

Structural Elucidation of CHIP-0339 (**2**), TIP-0339-1 (**4**) and TIP-0339-2 (**5**)

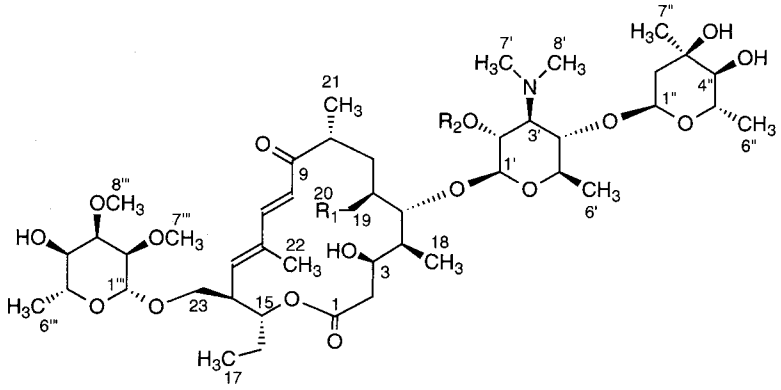
The structures of **2**, **4** and **5** were determined by comparison of the MS and NMR data with those of chalcomycin (**1**) and tylosin (**3**) (Fig. 1). The ^{13}C NMR signals of **1**[13], and the ^1H and ^{13}C NMR signals of **3** have been assigned,[13~17] although some ambiguities remain. In this work, we present our assignment of the ^1H and ^{13}C NMR signals of **1** and **3** in $CDCl_3$ at 25°C (Tables 4 and 5).

FAB-MS data of **2** indicated the molecular weight to be 862, and the molecular formula was determined by HRFAB-MS to be $C_{41}H_{66}O_{19}$. This product was assumed to be glycosylated chalcomycin [$(M_1+C_6H_{10}O_5)$, where M_1 is the MW of chalcomycin] (Table 3). Similarly, the molecular

Fig. 1



Chalcomycin (**1**) R_1 = H
CHIP-0339 (**2**) R_1 = β-D-glucopyranosyl



Tylosin (**3**) R_1 = CHO, R_2 = H
TIP-0339-1 (**4**) R_1 = CH_2OH, R_2 = β-D-glucopyranosyl
TIP-0339-2 (**5**) R_1 = CHO, R_2 = β-D-glucopyranosyl

Table 3. HRFAB-MS data for CHIP-0339 (**2**), TIP-0339-1 (**4**) and TIP-0339-2 (**5**).

Compound	Molecular formula	Calculated	Found
chalcomycin (**1**)	$C_{35}H_{56}O_{14}$ (M_1) (MW 700)		
CHIP-0339 (**2**)	$C_{41}H_{66}O_{19}$ (M_2) (MW 862) (M_1+$C_6H_{10}O_5$)	863.4277 (M_2+H)	863.4309
tylosin (**3**)	$C_{46}H_{77}NO_{17}$ (M_3) (MW 915)		
TIP-0339-1 (**4**)	$C_{52}H_{89}NO_{22}$ (M_4) (MW 1079) (M_3+2H+$C_6H_{10}O_5$)	1080.595 (M_4+H)	1080.602
TIP-0339-2 (**5**)	$C_{52}H_{87}NO_{22}$ (M_5) (MW 1077) (M_3+$C_6H_{10}O_5$)	1078.580 (M_5+H)	1078.585

Table 4. ^{1}H NMR chemical shifts (δ_H, ppm) , multiplicities and coupling constants (J, Hz) of chalcomycin (**1**), CHIP-0339 (**2**), tylosin (**3**) and TIP-0339-2 (**5**) in $CDCl_3$ (Reference value: δ_H 7.24 ppm of chloroform).

Proton	1	2	3	5
2a	5.79 d,15.5	5.79 d,15.5	1.89 d, 16.0	1.91 d, 16.5
2b	---	---	2.44*	2.46 dd, 16.5, 10.5
3	6.62 dd,15.5,10.5	6.63 m	3.77 d, 10.0	3.78*
4	2.67 m	2.61 m	1.59*	1.62*
5	3.18*	3.14*	3.65 d, 9.0	3.71*
6	1.23*	1.26*	2.20 brm	2.05*
7a	1.85 dd,15.0, 2.5	1.80 m	1.42 m	1.41 brm
7b	1.92 dd,15.0, 12.0	1.80 m	1.57*	1.61*
8	---	---	2.54 brm	2.53*
10	6.54 d, 15.5	6.57 d, 15.5	6.22 d, 15.0	6.28 d, 15.5
11	6.55 dd, 15.5, 7.5	6.54 dd, 15.5, 8.0	7.26 d, 15.0	7.31 d, 15.5
12	3.29*	3.29 dd, 8.0, 2.0	---	---
13	3.12 dd, 9.0, 2.0	3.10 dd, 9.0, 2.0	5.86 d, 10.0	5.89 d, 10.5
14	1.35*	1.36*	2.90*	2.93*
15	5.30 dq,10.5, 6.5	5.29 dq,11.0, 6.0	4.93 ddd, 10.0, 10.0, 2.0	4.95 ddd, 9.5, 9.5, 2.0
16a	1.32 d, 6.5	1.30 d, 6.0	1.54*	1.59*
16b	---	---	1.83 m	1.84*
17	1.18 d, 7.0	1.16 d, 6.5	0.88 t, 7.0	0.91 t, 7.0
18	0.98 d, 6.5	0.92 d, 6.5	0.95 d, 6.5	1.04 d, 6.5
19a	1.35 s	1.35 s	2.35*	2.41*
19b	---	---	2.83 dd, 18.0, 10.0	2.73*
20a	3.63 dd,10.0, 3.0	3.61 dd,10.0, 2.5	9.62 s	9.64 s
20b	4.15 dd,10.0, 3.0	4.13 dd,10.0, 2.5	---	---
21	---	---	1.16 d, 7.0	1.21 d, 7.0
22	---	---	1.74 s	1.78 s
23a	---	---	3.49*	3.53*
23b	---	---	3.94 dd, 9.0, 4.0	3.97 dd, 9.0, 3.5
1'	4.18 d, 7.5	4.25 d, 7.0	4.16 d, 7.5	4.36, brd, ca.6.0
2'	3.29*	3.40*	3.48*	3.40*
3'	3.19*	3.33*	2.42*	2.84*
4'a	1.21*	1.22*	3.21*	3.54*
4'b	2.01 ddd, 12.0, 5.0, 1.5	2.08 m	---	---
5'	3.44 m	3.42*	3.21*	3.33*
6'	1.19 d, 6.0	1.18 d, 6.0	1.19* d	1.25*
7'	3.38 s	3.38 s	2.43 s	2.65 brs
8'	---	---	2.43 s	2.65 brs
1"	---	---	5.01 d, 3.5	5.08 brd, ca.2.0
2"a	---	---	1.70 dd, 14.5, 3.5	1.81*
2"b	---	---	1.97 d, 14.5	2.04 d, 14.0
4"	---	---	2.88*	3.00*
5"	---	---	4.01 dq, 10.0, 6.0	3.83*

Table 4. Continued

Proton	1	2	3	5
6"	---	---	1.24 d, 6.0	1.28, d, 6.0
7"	---	---	1.18 s	1.24, s
1"'	4.55 d, 7.5	4.53 d, 7.5	4.51 d, 7.5	4.54 d, 7.5
2"'	3.05 dd, 7.5, 2.5	3.03 dd, 7.5, 2.5	2.97 dd, 7.5, 2.5	3.00*
3"'	3.74 dd, 3.5, 2.5	3.73 dd, 3.5, 2.5	3.70 dd, 3.0, 2.5	3.72 dd, ca.3.0,3.0
4"'	3.18*	3.16*	3.13 brd, ca.8.0	3.16 m
5"'	3.50 dq, 9.5, 6.0	3.51*	3.46*	3.48*
6"'	1.24 d, 6.0	1.22 d, 6.0	1.20*	1.23, d, 6.0
7"'	3.53 s	3.51 s	3.43 s	3.46, s
8"'	3.59 s	3.57 s	3.56 s	3.59 s
Glu-1	---	4.47 d, 7.5	---	4.40, br
Glu-2	---	3.34*	---	3.33*
Glu-3	---	3.57*	---	3.53*
Glu-4	---	3.51*	---	3.52*
Glu-5	---	3.38*	---	3.35*
Glu-6a	---	3.78 m	---	3.79*
Glu-6b	---	3.89*	---	3.86*

[a] Multiplicities, s: singlet, d: doublet, t: triplet, q: quartet, m: multiplet, br: broad

* Overlapping with other signals.

formula of **5** [MW: 1077 (M_3+$C_6H_{10}O_5$), where M_3 is the MW of tylosin] was consistent with that of glycosylated tylosin. Compound **4** [MW: 1079 (M_3+2H+$C_6H_{10}O_5$)] was suggested to be a dihydrogenated product of **5**.

The structure of **2** was determined to be 2′-[*O*-(*β*-D-glucopyranosyl)]chalcomycin based on the NMR data (Tables 4 and 5). The presence of the *β*-D-glucopyranose moiety was indicated by signals at δ_C 105.2 (Glu-1), 75.5 (Glu-2), 75.9 (Glu-3), 70.4 (Glu-4), 76.2 (Glu-5) and 62.6 (Glu-6) ppm, and at δ_H 4.47 (Glu-1), 3.34 (Glu-2), 3.57 (Glu-3), 3.51 (Glu-4), 3.38 (Glu-5), 3.78 and 3.89 (Glu-6) ppm.[18~20] The presence of the *β*-D-glucopyranose moiety was confirmed by a TOCSY experiment. As expected, correlations of H-Glu1 to H-Glu2~H-Glu6 were observed.[7,8] The site of glycosylation was determined by PFG-HMBC correlation of H-2′ with C-Glu1, and H-Glu1 with C-2′. The downfield shift of the C-2′ signal ($\Delta\delta_C$: 7.9 ppm) relative to that of **1** is also in accord with glycosylation at 2′-OH.[2] No other significant differences were observed in the NMR spectra of **2** and chalcomycin **1**, indicating that the remaining structures of these two compounds are the same.

The structure of **5** was determined to be 2′-[*O*-(*β*-D-glucopyranosyl)]tylosin from the NMR data (Tables 4 and 5). The presence of the *β*-D-glucopyranose moiety was indicated by signals at δ_C 105.8 (Glu-1), 75.2 (Glu-2), 76.4 (Glu-3), 70.3 (Glu-4), 76.6 (Glu-5) and 62.5 (Glu-6) ppm, and at δ_H 4.40 (Glu-1), 3.33 (Glu-2), 3.53 (Glu-3), 3.52 (Glu-4), 3.35 (Glu-5), 3.79 and 3.86 (Glu-6) ppm. The ^{13}C-chemical shift values of Glu1~Glu6 of **5** were very similar to those of **2**. The glycosylation at 2′-OH was indicated by the shift of the C-2′ signal in **5** from that of tylosin (**3**) ($\Delta\delta_C$: 9.0 ppm). The glycosylation site was determined by a NOESY experiment: NOE was observed between H-Glu1 and H-2′. Attempts to correlate H-2′ with C-Glu1 and H-Glu1 with C-2′ by PFG-HMBC were unsuccessful. The signals due to the other protons and carbons in the spectra of **5** were similar to those of **3**, indicating that the remaining structure was unchanged.

The structure of **4** was determined to be 20-dihydro-2′-[*O*-(*β*-D-glucopyranosyl)]tylosin by comparing the ^{13}C NMR (Table 4) and ^1H NMR data with those of **5**.

Table 5. ^{13}C NMR chemical shifts (δ_C, ppm) of chalcomycin (**1**), CHIP-0339 (**2**), tylosin (**3**), TIP-0339-1 (**4**) and TIP-0339-2 (**5**) in $CDCl_3$ (Reference value: δ_C 77.0 ppm of chloroform).

Carbon	1	2	3	4	5
1	165.3	165.5	173.8	174.2	173.9
2	120.7	120.5	39.3	39.2	39.3
3	151.6	151.7	67.2	67.6	67.0
4	41.7	41.3	40.3	40.4	40.6
5	87.8	86.3	81.2	80.1	80.1
6	34.0	33.7	31.7	31.6	31.6
7	36.9	36.9	32.6	33.2	32.2
8	78.4	78.2	44.6	44.7	44.8
9	200.1	200.0	203.0	203.9	203.0
10	124.8	124.9	118.4	118.5	118.3
11	146.4	146.5	148.0	148.2	148.3
12	58.7	58.7	134.8	135.1	135.0
13	59.0	58.9	142.3	142.1	142.4
14	49.4	49.4	45.0	44.9	45.1
15	68.7	68.7	75.1	75.3	75.2
16	18.3	18.2	25.4	25.4	25.4
17	20.8	20.7	9.6	9.7	9.7
18	19.2	18.9	9.0	9.7	9.3
19	27.7	27.7	43.7	29.7	43.7
20	66.9	66.9	202.8	60.9	202.9
21	---	---	17.3	17.6	17.5
22	---	---	12.9	13.0	13.1
23	---	---	69.0	69.0	69.0
1'	103.1	102.1	103.6	102.1	102.0
2'	75.0	82.9	71.7	*	80.7
3'	80.4	79.9	68.7	69.0	68.8
4'	36.7	36.9	75.0	*	76.4
5'	67.7	67.2	73.1	72.0	72.1
6'	18.5	18.3	18.9	18.9	18.9
7'	56.7	56.5	41.9	41.4	41.3
8'	---	---	41.9	41.4	41.3
1"	---	---	96.4	97.6	97.6
2"	---	---	40.8	41.4	41.3
3"	---	---	69.4	69.8	69.8
4"	---	---	76.3	*	76.4
5"	---	---	66.0	66.9	67.0
6"	---	---	18.2	17.9	17.9
7"	---	---	25.3	25.8	25.8
1"'	100.9	100.8	101.0	101.1	101.1
2"'	81.9	81.8	81.8	81.9	81.9
3"'	79.6	79.6	79.8	79.8	79.8
4"'	72.6	72.6	72.6	72.7	72.7
5"'	70.7	70.6	70.5	70.6	70.6
6"'	17.7	17.7	17.7	17.8	17.8
7"'	59.6	59.6	59.6	59.7	59.7
8"'	61.7	61.6	61.7	61.8	61.8
Glu-1	---	105.2	---	105.5	105.8
Glu-2	---	75.5	---	75.0	75.2
Glu-3	---	75.9	---	*	76.4
Glu-4	---	70.4	---	70.2	70.3
Glu-5	---	76.2	---	*	76.6
Glu-6	---	62.6	---	62.4	62.5

* Overlapping with other signals

Reduction of the formyl group (C-20) to a hydroxymethylene group was indicated by the spectra of **4**, which lacked the signals of the formyl group (δ_H 9.64, δ_C 202.9 ppm) and C-19 (δ_C 43.7 ppm) and, instead, exhibited new C-19 (δ_C 29.7 ppm) and C-20 (δ_C 60.9 ppm) signals. The signals due to the other carbons in the spectrum of **4** were similar to those of **5**, indicating that the remaining structure was unchanged.

Discussion

We found that the susceptibility of pathogenic *Nocardia* to chalcomycin (**1**) and tylosin (**3**) was species-specific, and that *N. asteroides* IFM 0339 inactivated chalcomycin (**1**) and tylosin (**3**) by glycosylation. Both antibiotics were glycosylated at 2′-OH with glucose to form 2′-[*O*-(β-D-glucopyranosyl)]chalcomycin and 2′-[*O*-(β-D-glucopyranosyl)]tylosin. The formyl group of the latter compound was further reduced to a hydroxymethylene group. We have reported that erythromycin was also glycosylated by *N. asteroides* IFM 0339 to form 2′-[*O*-(β-D-glucopyranosyl)]erythromycin, but the 16-membered macrolides rokitamycin and midecamycin were only deacylated by the organism.[1,2]

Inactivation of macrolide antibiotics by glycosylation was first observed for oleandomycin. CELMER *et al.* found that D-glucosyloleandomycin, most likely 2′-*O*-D-glucosyloleandomycin, was formed by *Streptomyces antibioticus* ATCC 11891, an oleandomycin producer, and that it was converted to oleandomycin and D-glucose by a soluble enzyme of the organism.[21] VILCHES *et al.* carried

out these reactions with ^{3}H-labeled UDP-glucose and cell extracts, and with extracellular enzyme of *S. antibioticus*. They found that some other macrolide antibiotics were also inactivated, but tylosin (**3**) was unchanged.[22] The glycosylation was suggested to afford protection from the antibacterial activity of oleandomycin during biosynthesis. A gene *mgt* from *Streptomyces lividans* TK21, which encodes a glycosyl transferase that inactivates macrolides using UDP-glucose as a cofactor, was cloned and sequenced.[23] The substrates of the *mgt* product MGT were investigated, and both chalcomycin (**1**) and tylosin (**3**) were shown to be substrates of the enzyme.[24,25] Angolamycin, which contains angolasamine (2-deoxymycaminose), was not modified by MGT, and the glycosylation site was suggested to be 2′-OH of mycaminose.

A glycosylated inactivation product of macrolide was first isolated from fermentation of erythromycin by *Streptomyces vendargensis* ATCC 25507, and the structure was determined to be 2′-[*O*-(*β*-D-glucopyranosyl)]erythromycin.[26] Recently, SASAKI *et al.* obtained inactivated erythromycin derivatives with cell extracts of *Streptomyces hygroscopicus* ATCC 31080 in the presence of UDP-glucose or UDP-galactose. The inactivated derivatives had been formed by glycosylation at 2′-OH of the desosamine moiety. They also reported that cell extract of *S. antibioticus* ATCC 11891, a producer organism of oleandomycin, glycosylated tylosin (**3**) with both UDP-glucose and UDP-galactose. The glycosylation of tylosin was shown by a specific spot on a TLC plate, but the product was not isolated.[27]

The glycosylation site of 16-membered macrolide antibiotics has not been determined, but our data clearly showed that 2′-OH of mycaminose in tylosin (**3**) and that of chalcose in chalcomycin (**1**) were glycosylated. Glycosylation of **1** and **3** with galactose was excluded by a TOCSY experiment on CHIP-0339 (**2**) as described above; correlation of H-Glu1 with only H-Glu2~H-Glu-4 would have been observed for the *β*-D-galactopyranosyl derivative.[28]

We have reported that some strains of *N. brasiliensis* and *N. pseudobrasiliensis* produce novel antibiotics such as brasilicardin A, brasilidine A, brasilinolides, and nocardicyclins.[29~32] In the course of our continuing search for antibiotics from pathogenic *Nocardia* isolated from clinical sources, we found that *N. brasiliensis* IFM 0466 strain produces macrolide antibiotics such as 32-membered brasilinolides.[31] In addition, our recent study showed that this *N. brasiliensis* IFM 0466 strain is also a producer of erythromycins.[33] We confirmed that the strain has glycosylation activity for the 2′-OH group of erythromycins (data not shown). Considering that antibiotic-producing strains should have inactivation enzymes to protect themselves from their own produced antibiotics, it would be interesting to know whether the antibiotic-inactivation enzyme of *N. brasiliensis* IFM 0466 is also responsible for the biosynthesis of erythromycin.

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