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DETERMINATION OF THE ABSOLUTE STEREOCHEMISTRY AT THE C16 ORTHOESTER OF EVERNINOMICIN ANTIBIOTICS; A NOVEL ACID-CATALYZED ISOMERIZATION OF ORTHOESTERS

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Abstract: The absolute stereochemistry at C16 orthoester center of Ziracin (**1**) was unequivocally established *via* a novel acid-catalyzed orthoester isomerization. Structures of the resulting isomers **2** and **3** were determined by chemical degradation and extensive spectroscopic analyses. This novel isomerization was successfully extended to other related everninomicins (**11-13**), thus completing the entire structural assignment of everninomicin antibiotics. © 1997 Elsevier Science Ltd.

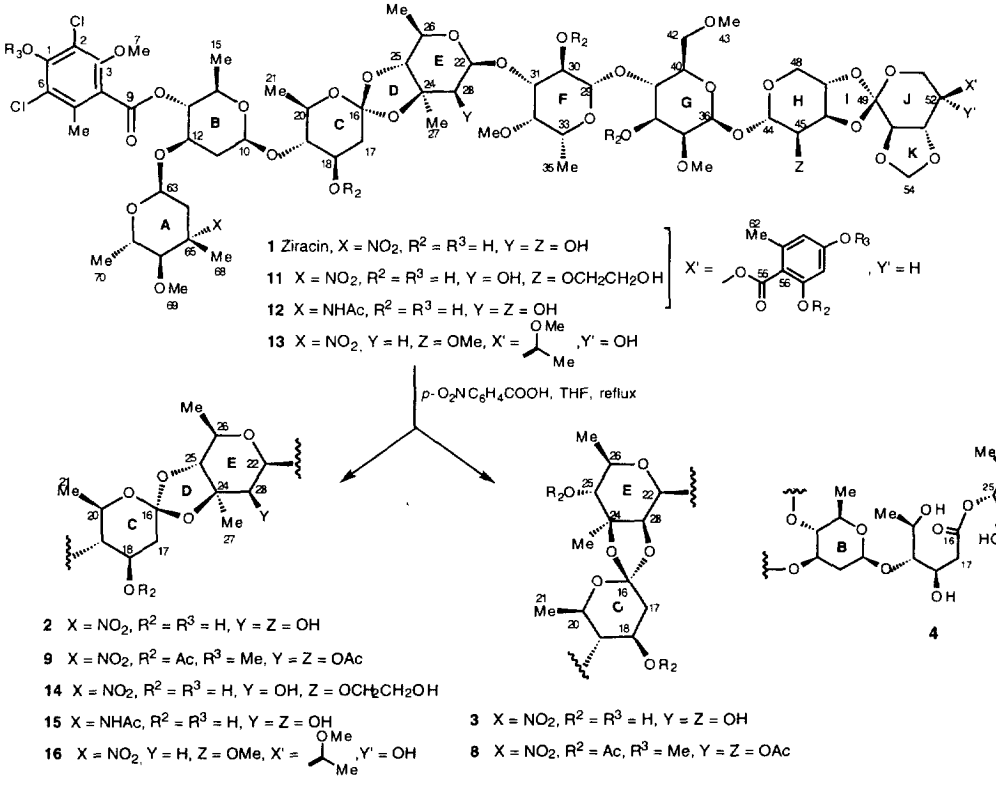
Everninomicins, a group of complex oligosaccharides produced by *Micromonospora carbonaceae*, are highly active against Gram positive bacteria including methicillin resistant *Staphylococci* and vancomycin resistant *Enterococci*. Ziracin (Sch 27899; **1**), a member of this group of antibiotics, is undergoing extensive clinical trial to determine its activity against both sensitive and resistant strains and if successful it will become one of the major new antibiotics to be used in human medicine in the last couple of decades.

We have previously reported the structure of several everninomicins,[1] including everninomicin B,[2] C,[3] D[4] and antibiotic 13-384[5] components 1 and 5 (Sch 27899 and Sch 27900, respectively). A unique structural feature shared among these compounds is the presence of two acid sensitive orthoester linkages[6] at C16 and C49, the former being relatively more acid labile. The absolute configuration of C49 center was determined by X-ray crystallography.[7] In a model study, the stereochemistry at C16 was postulated by Beau and coworkers[8] based on different acid hydrolysis products of the two isomers of the synthetic disaccharide fragments (ring C-E). We wish to report here the preparation of the more stable stereoisomers of everninomicins *via* a novel acid-catalyzed orthoester isomerization, and consequently, the unequivocal assignment of stereochemistry at C16.

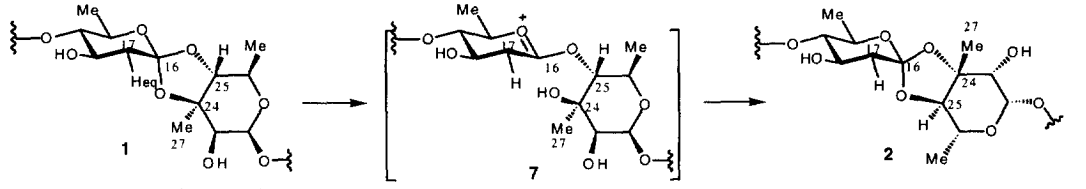
During our studies towards the chemical modifications of **1**, we have discovered two unexpected isomerization products **2** and **3** involving the C16 orthoester. Experimental conditions were optimized to give either isomer as the major product. When a solution of **1** in anhydrous THF was treated with *p*-nitrobenzoic acid under refluxing conditions, a mixture of **2** and **3** along with some **1** was obtained (Scheme 1). The mixture was separated by column chromatography on silica gel to yield **2** as major product (**2**:**3** = 5:1). Prolonged heating shifted the product ratio in favor of **3** (**2**: **3** = 1:3).

Compound **2**, $[\alpha]_D$ = -49.4 ($CHCl_3$, *c* 1.035), is a colorless, amorphous solid which has the same molecular composition and similar fragmentation pattern as **1**, $C_{70}H_{97}Cl_2NO_{38}$ (M+Na, HRFAB found 1652.4983, calc. 1652.4963). All proton and carbon resonances were assigned using ^{1}H-NMR, ^{13}C-NMR, HMQC, HMBC and HMQC-TOCSY spectra. Examination of the NMR data of compound **2** against those of **1**[9] revealed that the significant changes in chemical shifts involved only rings **C**, **D** and **E**. Specifically, major shifts occurred at H17, H25, H27 and C16, C17, C24, C25 (Table 1). However, the coupling constants among protons in rings **C-E** remained unchanged. More importantly, the two

Scheme 1 Acid-catalyzed isomerization



Scheme 2 Observed NOE differences in **1**-**2**

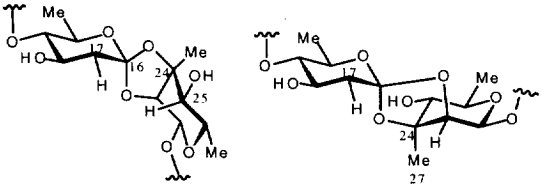


characteristic ^{13}CNMR signals at δ 119.20 and 119.24 ppm confirmed the presence of two orthoester carbons. The NOE experiments provided key information on the configuration of the C16 center. The NOE difference observed in NOESY of **2** between H17eq in **C** ring and H27 (methyl) in ring **E** indicated that these protons are *cis* with respect to the dioxolane ring **D**; such NOE was absent in **1**. While NOE between H17eq and H25 (or H18) was observed in **1**, it was inconclusive due to

Table 1. NMR Chemical Shifts in Compounds **1-3** (in ppm)

position	Sch 27899 (**1**)		**2**		**3**	
	^{13}CNMR	^{1}HNMR	^{13}CNMR	^{1}HNMR	^{13}CNMR	^{1}HNMR
16	120.40		119.24		118.71	
17	39.79	1.81, 2.41	40.43	1.99, 2.24	40.00	1.96, 2.29
18	68.16	3.93	68.38	3.88	68.53	3.83
19	88.18	3.07	88.10	3.06	88.20	3.03
20	70.05	3.84	69.43	3.75	68.90	3.76
21	17.77	1.25	17.84	1.28	17.86	1.25
22	101.02	5.03	101.49	5.01	98.49	5.03
23	72.95	4.08	72.75	4.08	79.35	4.11
24	80.50		81.93		84.06	
25	78.45	3.93	76.11	4.12	76.48	3.44
26	68.80	3.82	68.53	3.81	71.44	3.29
27	18.80	1.35	18.96	1.22	16.59	1.38

Scheme 3 Expected NOE differences in the isomeric orthoesters **3** and **10**



3 NOE H17eq/H25 **10** NOE H17eq/H23

the overlap resonances of H25 and H18 (since NOE is expected between H17/H18); no NOE was found between H17 and H25 in **2** (Scheme 2). Our findings of NOE differences were consistent with what was observed with the synthetic **C-E** disaccharide fragments.[8]

Upon mild two-phase acidic hydrolysis[5] (0.1N HCl aq/EtOAc 1:4 v/v), **2** was converted to the same ester **4**, obtained earlier from **1**.[5] Although the same hydrolysis product was obtained from both **1** and **2**, the rate of hydrolysis for **2** (29 h, 50% conversion) was much slower than that for **1** (7 h, 100%). Further treatment of **4** with diazomethane afforded lactone **5** and olgose-like compound **6**.[5] These degradation products (**4** - **6**) were fully characterized and found to be identical with the degradation products obtained from **1**. Putting together all the evidence presented above, we propose that compound **2** is the C16 stereoisomer of **1**. In addition, NOE evidence cited above for both **1** and **2** establishes the absolute stereochemistry at C16 for both **1** and **2**, as shown in Scheme 2.

This novel isomerization is presumably achieved *via* an acid catalyzed ring opening of **D**-ring to form the oxonium ion intermediate **7** and subsequent ring closure from either face of the **C** ring to give both **1** and **2**. Isomer **2** is thermodynamically more stable, and therefore, it accumulates in the reaction mixture (Scheme 2).

Compound **3**, $[\alpha]_D$ = -54.3 ($CHCl_3$, *c* 1.00), a colorless, amorphous solid, has the same molecular formula $C_{70}H_{97}Cl_2NO_{38}$ (M+Na, HRFAB found 1652.4967, calc. 1652.4963) and mass spectral fragmentation patterns as the parent antibiotic. It is slightly more polar than **2**. Assignments of proton and carbon resonances by extensive NMR experiments unfolded a similar phenomenon found in **2**, *i.e.* major changes in chemical shifts are within **C - E** rings, and to a less extent at C/H30, C/H31. The changes were more substantial than those in **2**. The largest shifts in NMR occurred at C23 ($\Delta\delta$ = 6.4

ppm) and C24 ($\Delta\delta$ = 3.6 ppm), H25 ($\Delta\delta$ = -0.49 ppm) and H26 ($\Delta\delta$ = -0.53 ppm) suggesting change at C23 position in the formation of **3** from **1**. ^{13}C-NMR resonances at δ 118.71, 119.20 ppm again indicate the presence of two orthoester carbons.

Acid hydrolysis of **3** under the same biphasic conditions[5] was very slow and the C49 orthoester hydrolyzed first. Both **2** and **3** were derivatized *via* methylation with diazomethane followed by acetylation with excess acetic anhydride. The products were again characterized by extensive NMR experiments. Based on chemical shift changes of the relevant methine protons upon acylation, it was evident that compound **8**, the peracetylated derivative of compound **3**, possessed an acetoxy function at C25 but not at C23, an indication of the fact that in compound **3** C25-hydroxyl was free and C23-hydroxyl group was tied up in the orthoester linkage. Consistent with the assignment of structure **2**, compound **9**, the peracetate of compound **2**, showed acetylation at 23-OH but not at 25-OH. Mechanistically, it is conceivable that 23-OH being in close proximity could participate in the ring closure of the intermediate **7** to give two new orthoesters **3** and its isomer **10** (Scheme 3). In 2D NOE spectrum of **3**, the presence of crosspeak between H17eq and H25 is consistent only with the stereochemistry depicted in structure **3**.

To test the generality of this orthoester isomerization, several related everninomicins **11-13** were found to undergo similar rearrangement to afford **14 -16** respectively. As an added proof, when everninomicin D (**13**), which lacks 23-OH, was subjected to the same isomerization conditions, only one product, compound **16**, was obtained.

The everninomicins and their isomers were tested in *in vitro* antibacterial assays against both methicillin-susceptible and methicillin-resistant *Staphylococci,* and vancomycin-susceptible and vancomycin-resistant *Enterococci.* Interestingly, while compound **3** showed virtually no activity, isomer **2** type compounds retained high antibiotic activities, even though they are slightly less potent than the parent antibiotic Ziracin (**1**).

In conclusion, we prepared[10] and characterized two isomers of everninomicin antibiotics *via* a novel acid-catalyzed orthoester isomerization. The absolute stereochemistry at C16 orthoester center was elucidated, thus completing the entire structural assignment of everninomicin antibiotics. We expect similar transformation applicable to other orthosomycins[6] and the stereochemistry of their orthoester centers be clarified in the same fashion.

References and Notes

1. Girijavallabhan, V. M.; Ganguly, A. K. Kirk-Othmer Encyclopedia of Chemical Technology 4th Ed., John Wiley & Son, 1992; Vol. No. 3, pp 259-266.
2. Ganguly, A. K.; Saksena, A. K. *J. Antibiotics* **1975**, *28*, 707.
3. Ganguly, A. K.; Szmulewicz, S. *J. Antibiotics* **1975**, *28*, 710.
4. Ganguly, A. K.; Sarre, O. Z.; Greeves, D.; Morton, J. *J. Am. Chem. Soc.* **1975**, *97*, 1982.
5. Ganguly, A. K.; Pramanik, B.; Chan, T. M.; Sarre, O. Z.; Liu, Y.-T.; Morton, J.; Girijavallabhan, V. *Heterocycles* **1989**, *28*, 83.
6. Everninomicins are members of a family of oligosaccharide antibiotics, also referred to as orthosomycins. For a review, see Wright, D. E. *Tetrahedron* **1979**, *35*, 1207.
7. Ganguly, A. K.; Sarre, O. Z.; McPhail, A. T.; Miller, R. *J. Chem. Soc. Chem. Com.* **1979**, 22.
8. Beau, J.-M.; Jaurand, G.; Esnault, J.; Sinay, P. *Tetrahedron Lett.* **1987**, 1105.
9. Chan, T.-M; Osterman R. M.; Morton, J. B.; Ganguly, A. K. *Magn. Res. Chem.* **1997**, *35* (8), 529.
10. Representative experimental procedure: a solution containing **1** (976 mg, 0.599 mmol) and *p*-nitrobenzoic acid (300 mg, 1.796 mmol, 3.0 eq) in dry THF (20 mL) was kept at reflux for 16 h (70°C, oil bath). Upon cooling, the solution was concentrated to a small volume and diluted with EtOAc (100 mL). The solution was washed successively with aqueous sodium bicarbonate, water and brine, dried over Na_2SO_4, and concentrated to afford the crude product. Chromatography on silica gel (5% MeOH in CH_2Cl_2) gave **2** (212 mg, white solid): Rf 0.38 (7% MeOH-CH_2Cl_2) and **3** (43 mg, white solid): Rf 0.31 (7% MeOH-CH_2Cl_2).

    A solution containing **1** (1000 mg, 0.613 mmol) and *p*-nitrobenzoic acid (304 mg, 1.820 mmol, 3.0 eq) in dry THF (20 mL) was kept at reflux for 44 h (70°C, oil bath). Upon cooling, solvent was evaporated and the residue was dissolved in EtOAc (100 mL). The solution was washed successively with aqueous sodium bicarbonate, water and brine, dried over Na_2SO_2, and concentrated to afford the crude product. Chromatography on silica gel (5% MeOH in CH_2Cl_2) gave **2** (67 mg, white solid) and **3** (224 mg, white solid).

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