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Relative and Absolute Configurations of Ganefromycin α

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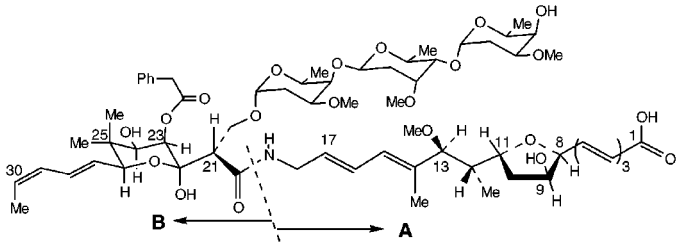
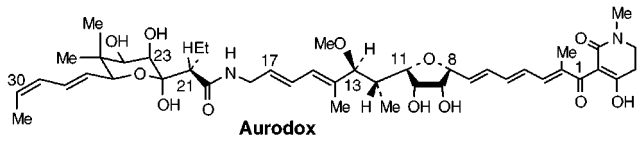
ABSTRACT



Ganefromycin α (1)

The full structure of ganefromycin α has been determined. The relative configurations were determined from $^3J_{H,H}$ coupling constants and NOE data, while the absolute configurations in moieties A and B were determined separately by difference CD of their acylate derivatives, which showed typical exciton couplets. The configurations of the stereogenic centers in ganefromycin α are 8*S*, 9*S*, 11*R*, 12*S*, 13*S*, 21*S*, 22*R*, 23*R*, 24*R*, and 26*S*.

Ganefromycin α (**1**) produced by *Streptomyces lydicus* ssp. *tanzanius* is an antibiotic with commercial potential as a performance enhancement agent in livestock. The discovery, isolation, and biosynthesis of ganefromycin have been reported,[1] and its "planar" structure has also been published.[2] However, the relative and absolute configurations remain to be determined. The absolute configuration of a related antibiotic, aurodox, has been determined by X-ray crystallography of degradation products, see Figure 1.[3]



Aurodox

Figure 1. Full structure of aurodox determined by X-ray.

Ganefromycin α can be divided into three sections: the trisaccharide and moieties **A** and **B**. The full structure of the trisaccharide was determined by X-ray crystallography and optical rotation, while the relative configuration of moiety **B** has been determined by NMR.[2] In the following, the relative configurations in moiety **A** have been determined by NMR and the absolute configurations of moieties **A** and **B** by exciton-coupled circular dichroic spectroscopy (CD).[4] To determine the configurations, ganefromycin was degraded to **2** and **3**,[5] according to previously described methods (Scheme 1).[2]

With respect to the tetrahydrofuran moiety in **A** (C-8 to C-13) the relative configurations were determined by evaluating *J* constants and NOEs of **2** and/or **3**.[5] For the acyclic

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(1) Carter, G. T.; Phillipson, D. W.; Goodman, J. J.; Dunne, T. S.; Borders, D. B. *J. Antibiot.* **1988**, *41*, 1511−1514. Carter, G. T.; Tischler, M.; West, R. R.; Bailey, A. E. *J. Antibiot.* **1995**, *48*, 1312−1319. McDonald, L. A.; Lotvin, J. A.; Bailey, A. E.; Carter, G. T. *J. Nat. Prod.* **1998**, *61*, 217−226.

(2) Carter, G. T.; Phillipson, D. W.; West, R. R.; Borders, D. B. *J. Org. Chem.* **1993**, *58*, 6588−6595.

(3) Maehr, H.; Leach, M.; Williams, T. H.; Blount, J. F. *Can. J. Chem.* **1980**, *58*, 501−526.

(4) (a) Harada, N.; Nakanishi, K. *Circular Dichroic Spectroscopy-Exciton Coupling in Organic Stereochemistry*; University Science Books: Mill Valley, CA; 1983. (b) Nakanishi, K.; Berova, N. The exciton chirality method. In *Circular Dichroism, Principals and Application*; Nakanishi, K., Berova, N., Woody, R., Eds.; New York: VHC Publishers: 1994; pp 361−398.

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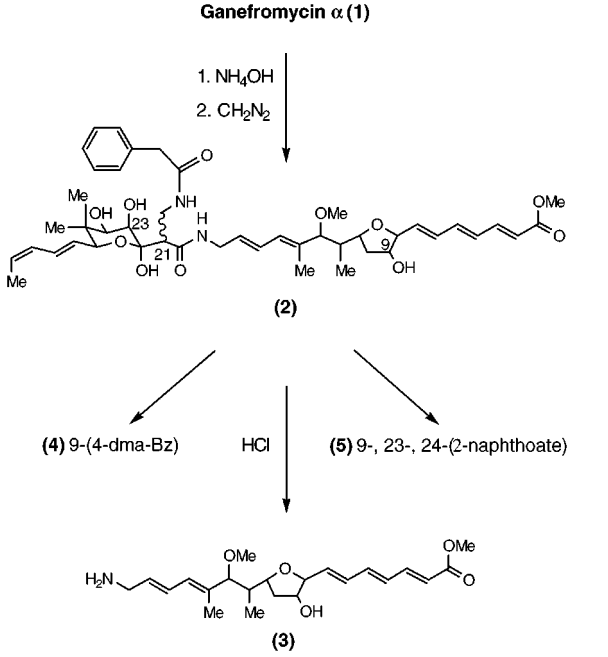
Scheme 1



C-12/C-13 moiety, the NOE intensities were compared with distances based on molecular mechanics calculations.[6] All NMR experiments were performed in deuterated methanol to avoid intramolecular hydrogen bonding. The absolute configuration of the cyclic hemiacetal moiety in **B** was determined using aglycon **2** that is formed upon ammonolysis and esterification of **1**. As this 23-benzyloxo to a 21-methyleneamino group transformation is postulated to occur via an sp^2 hybridized C-21 intermediate,[2] the C-21 configuration in **2** is epimerized; however, this does not affect the conclusion regarding the absolute configuration.

Moiety A (Figures 2 and 3). The three stereogenic centers at C-8, -9, and -11 on the tetrahydrofuran ring give rise to four possible isomers with different relative stereochemistries. Of the four possibilities, the relative configurations at these centers and conformation of the ring were determined as represented by **I** on the basis of *J* values and NOE data of **2** and/or **3**.

The intense NOEs observed (Figure 2) between 8-H/10-Ha and 9-H/10-Ha, the medium NOE between 9-H/10-Hb,

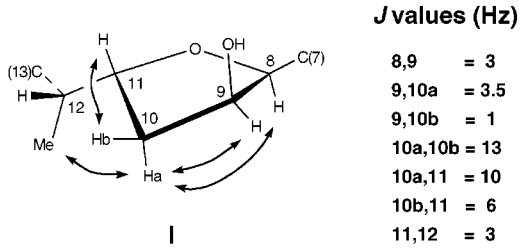


Figure 2. Configuration and conformation of the THF moiety derived from *J* and NOE data (arrows) of **2** and/or **3** and molecular mechanics calculation.

in connection with the values $J_{8,9} = 3$ Hz, $J_{9,10a} = 3.5$ Hz, and $J_{9,10b} = 1$ Hz define the stereochemistry as follows: C-7 triene chain pseudoequatorial, 8-H pseudoaxial, 9-H pseudo-eq, 10-Ha pseudo-ax, and 10-Hb pseudo-eq; since 9-H is pseudo-eq the 9-OH is pseudo-ax (or pointing up). The NOE between 8-H and 9-H could not be estimated due to the small shift difference between the two protons. The strong 9-H/10-Ha NOE and medium 9-H/10-Hb NOE show that 9-H bisects the 10-Hs but is closer to 10-Ha. Finally, the *J* values of 10a/11-H and 10b/11-H, together with NOE data (structure **I**), define the C-11 configuration, where the C-12 side chain adopts a pseudo-eq orientation. The lowest energy conformation of the THF moiety based on molecular mechanics calculations performed with the 9-methyl ether (to avoid H-bonding effects) yielded a conformer that was in full accord with the NMR data.

The relative configurations at C-11/C-12 (Figures 2 and 3, **II**) were determined from the gauche coupling between 11-H and 12-H (3 Hz) and the NOE data: 12-Me/10-Ha (strong), 12-Me/10-Hb (medium), 12-H/10-Ha (medium), and 12-H/10-Hb (medium). The relative configurations at C-12/C-13 (Figure 3, **III**) were determined from the anti coupling between 12-H and 13-H (10 Hz) and the intense NOE between 12-Me/14-Me and 13-H/15-H. Projection structure **III** is the only one that satisfies both the *J* values and NOE results.

The absolute configuration in moiety **A** was determined by using the trienoate chromophore, λ_{max} 290 nm, and acylating the allylic 9-OH with a chromophore that would couple with the trienoate. The amplitudes of exciton couplets

(5) NMR data for **2**: ^{1}H NMR (MeOD, 500 MHz) δ_H 0.70 (3H, d, $J_{12-Me,12} = 7$, 12-Me), 0.88 (3H, s, 25eq-Me), 0.89 (3H, s, 25ax-Me), 1.62 (3H, s, 14-Me), 1.66 (1H, ddd, $J_{12,11} = 3$, $J_{12,12-Me} = 7$, $J_{12,13} = 10$, H-12), 1.73 (3H, dd, $J_{30-Me,30} = 7$, $J_{30-Me,29} = 1$, 30-Me), 1.96 (1H, ddd, $J_{10b,9} = 1$, $J_{10b,10a} = 13$, $J_{10b,11} = 6.4$, H-10b), 2.03 (1H, ddd, $J_{10a,9} = 4$, $J_{10a,10b} = 13$, $J_{10a,11} = 10$, H-10a), 3.11 (H, m, H-21), 3.13 (3H, s, 13-OMe), 3.41 (1H, d, $J_{13,12} = 10$, H-13), 3.43 (H, dd, $J_{21-CH2a,21} = 4$, $J_{21-CH2a,21-CH2b} = 14$, 21-CH_2a), 3.48 (2H, s, CH_2Ph), 3.57 (H, d, $J_{24,23} = 4$, H-24), 3.59 (H, dd, $J_{21-CH2b,21} = 4$, $J_{21-CH2b,21-CH2a} = 14$, 21-CH_2b), 3.68 (H, d, $J_{24,23} = 4$, H-23), 3.72 (3H, s, -COOMe), 3.75 (H, dd, $J_{18a,17} = 7$, $J_{18a,18b} = 16$, H-18a), 3.87 (H, dd, $J_{18b,17} = 7$, $J_{18b,18a} = 16$, H-18b), 4.19 (1H, d, $J_{26,27} = 6$, H-26), 4.28 (1H, broad, H-9), 4.33 (1H, dd, $J_{8,7} = 7$, $J_{8,9} = 3$, H-8), 4.65 (1H, ddd, $J_{11,10a} = 10$, $J_{11,10b} = 6$, $J_{11,12} = 3$, H-11), 5.47 (1H, dq, $J_{30,30-Me} = 7$, $J_{30,29} = 11$, H-30), 5.59 (1H, dt, $J_{17,16} = 15$, $J_{17,18} = 7$, H-17), 5.59 (1H, dd, $J_{27,26} = 6$, $J_{27,28} = 15$, H-27), 5.89 (1H, d, $J_{2,3} = 15$, H-2), 6.05 (H, dd, $J_{7,8} = 7$, $J_{7,6} = 15$, H-7), 5.96 (1H, m, H-15), 5.96 (1H, m, H-29), 6.05 (1H, dd, $J_{7,6} = 15$, $J_{7,8} = 7$, H-7), 6.40 (H, dd, $J_{4,3} = 11$, $J_{4,5} = 15$, H-4), 6.43 (H, dd, $J_{6,5} = 11$, $J_{6,7} = 15$, H-6), 6.47 (1H, $J_{16,15} = 11$, $J_{16,17} = 15$, H-16), 6.51 (1H, dd, $J_{28,27} = 15$, $J_{28,29} = 11$, H-28) 6.67 (1H, dd, $J_{5,6} = 11$, $J_{5,4}$ 15, H-5), 7.26 (5H, m, Ph), 7.33 (H, m, H-3); MS (ESI-pos.) *m/z* 807.2 ($M + H^+$). NMR data for **3**: ^{1}H NMR (MeOD, 500 MHz) δ_H 0.71 (3H, d, $J_{12-Me,12} = 7$, 12-Me), 1.68 (3H, s, 14-Me), 1.68 (1H, m, H-12), 1.97 (1H, ddd, $J_{10eq,9} = 1$, $J_{10eq,10ax} = 13$, $J_{10eq,11} = 6.4$, H-10eq), 2.03 (1H, ddd, $J_{10ax,9} = 4$, $J_{10ax,10eq} = 13$, $J_{10ax,11} = 10$, H-10ax), 3.16 (3H, s, 13-OMe), 3.44 (1H, d, $J_{13,12} = 10$, H-13), 3.57 (2H, d, $J_{18,17} = 7$, H-18), 3.72 (3H, s,-COOMe), 4.28 (1H, broad, H-9), 4.33 (H, dd, $J_{8,7} = 7$, $J_{8,9} = 3$, H-8), 4.65 (1H, ddd, $J_{11,10ax} = 10$, $J_{11,10eq} = 6$, $J_{11,12} = 3.3$, H-11), 5.75 (1H, dt, $J_{17,16} = 15$, $J_{17,18} = 7$, H-17), 5.92 (1H, d, $J_{2,3} = 15$, H-2), 6.05 (H, dd, $J_{7,8} = 7$, $J_{7,6} = 15$, H-7), 6.06 (H, d, $J_{15,16} = 11$, H-15), 6.40 (H, dd, $J_{4,3} = 11$, $J_{4,5} = 15$, H-4), 6.43 (H, dd, $J_{6,5} = 11$, $J_{6,7} = 15$, H-6), 6.70 (H, dd, $J_{16,15} = 11$, $J_{16,17} = 15$, H-16), 7.33 (H, dd, $J_{3,2} = 15$, $J_{3,4} = 11$, H-3). The assignments of **2** and **3** were based on chemical shifts, *J* values, and NOE data.

(6) All molecular mechanics calculations were obtained using the MMFF force field on Spartan V5.0, Wavefunction, Inc.

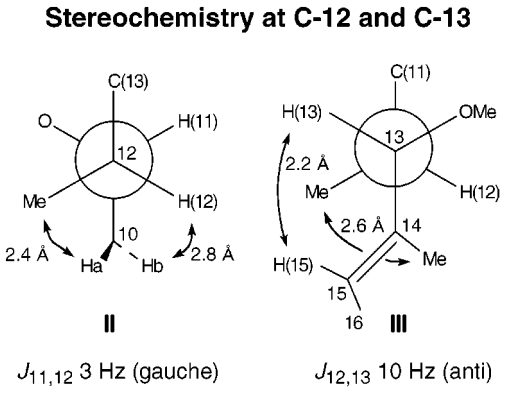


Figure 3. **II**. Projection from C-12 to C-11. **III**. Projection from C-13 to C-12. The conformations are derived from *J* and NOE data (arrows) and distances (Å) from molecular mechanics calculation.

originating from the interaction between two different chromophores are larger the closer the λ_{max} of the two chromophores. This led to the employment of *p*-dimethylaminobenzoate with λ_{max} 307 nm (Figure 4). A further

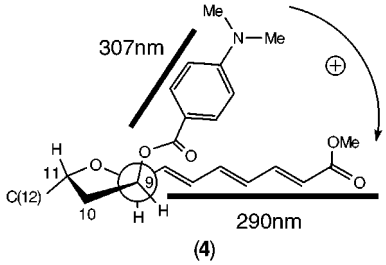


Figure 4. Conformation of **4** in moiety **A**. The dihedral angle between the two chromophores is estimated to be ca. 60°.

advantage in choosing this chromophore is that the 242 nm negative Cotton effect of **2** (Figure 5) does not interfere with the exciton coupling. The angle between the triene and the 9-OH was estimated from NMR and molecular mechanics to be ca. 60° in **I**, which is close to the optimal 70° angle for exciton coupling (Figure 4).[4]

Thus, the 9-OH in **2** was converted into its *p*-dimethylaminobenzoate (dma-Bz) **4** by reaction of **2** with dimethylaminobenzoic acid in the presence of EDC and DMAP. The UV and CD of **4** are shown in Figure 5. Since **2** shows a positive Cotton effect around 300 nm due to the trienoate, the difference CD between **4** and **2** with A = +71 represents the true positive exciton coupling contribution between the 2-dmaBz and trienoate chromophores in **4**. This establishes the configuration as 8*S*,9*S*, i.e., the configurations of this moiety are 8*S*, 9*S*, 11*R*, 12*S*, and 13*S*.

Moiety B (Figure 6). The relative configuration of the hemiacetal portion in the **B** half of ganefromycin α (**1**) determined earlier[2] was confirmed in the present work

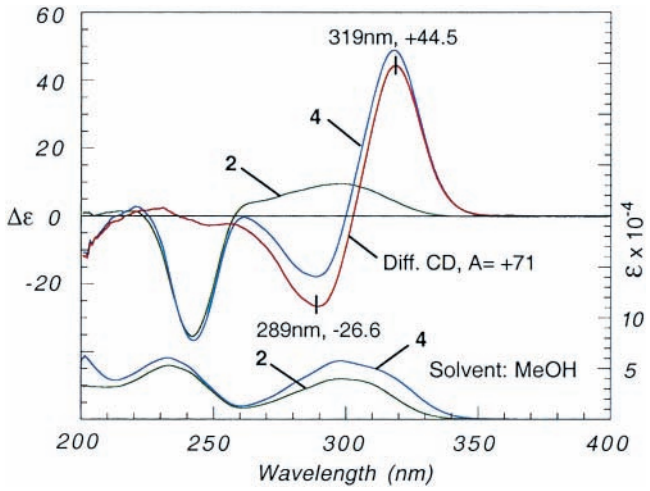


Figure 5. CD and UV spectra of **2** (green) and **4** (blue) and the difference CD (red) between **4** and **2**.

(Figure 6). After determination of relative configurations in this moiety from NMR data of ganefromycin α (**1**) itself, the absolute configuration was determined from the exciton coupling between the 23- and 24-biacylates of aglycon **2**.

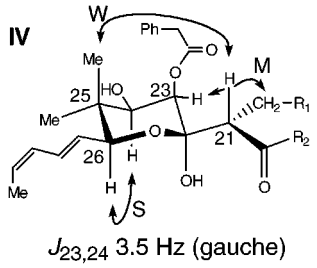


Figure 6. Relative configurations in moiety **B**. The NOEs are denoted S (strong), M (medium), and W (weak).

A strong NOE is found between 24-H/26-H (both hydrogens ax) and $J_{23,24}$ = 3.5 Hz (23-H is eq). For C-21 in **1**, molecular mechanics calculation shows that 21-H and 22-OH are anti periplanar, a reasonable conformation since the two large 21-C substituents are directed away from the ring; the weak NOE between 21-H and the axial 25-Me confirms this conformation. A medium intensity NOE between 21-CH_2/23-H places the 21-CH_2 on the side closest to 23-H as shown in **IV**.

The absolute configuration of the hemiacetal sugar moiety in **2** was determined by naphthoylation of 23- and 24-hydroxyls in **2** with 2-naphthoic acid in the presence of EDC and DMAP.[7] Since the 9-OH is the most reactive in **2**, a chromophore that does not couple strongly with the 8-trienoate

(7) Dong, J. G.; Akritopoulou-Zanze, I.; Guo, J.; Berova, N.; Nakanishi, K.; Harada, N. *Enantiomer* **1997**, *2*, 397−409.

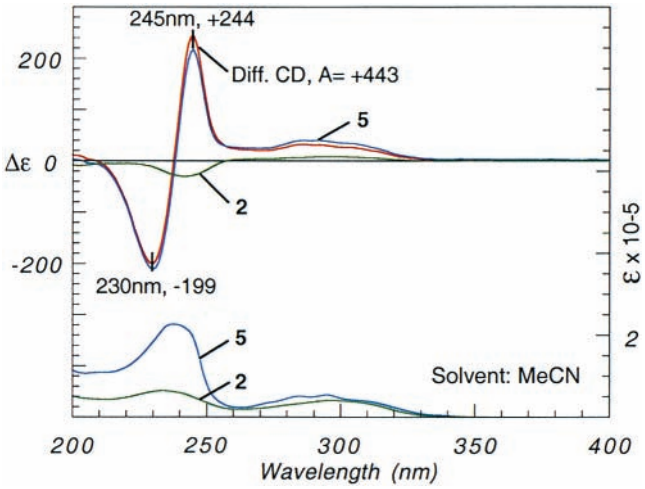


Figure 7. CD and UV spectra of **2** (green) and **5** (blue) and the difference CD (red) between **5** and **2**.

absorbing at 290 nm should be selected. 2-Naphthoates absorb at 235 nm and therefore would not interact strongly with the triene system. Aglycon **2** was thus converted into its 9,23,24-trisnaphthoate **5**; for CD see Figure 7. The intense positive couplet, A = +443, observed in the difference CD between **5** and **2** establishes the configuration as 23*R*,24*R*; note that there is no observable coupling between the 290 nm trienoate and naphthalenoid chromophores. The configurations in moiety **B** are therefore 21*S*, 22*R*, 23*R*, 24*R*, and 26*S* (as in Figure 6, **IV**). This leads to the full structure of ganefromycin α depicted in structure **1**.

Acknowledgment. We thank NIH for Grant GM 34509 (to K.N. and N.B.) and the Jacob and Alice Wallenbergs Foundation (to T.A.) for financial support.

Supporting Information Available: (1) NOE data for **2**; (2) CD and UV spectra of compounds **1**, **2**, and **3**; (3) experimental procedures with spectroscopic data for **4** and **5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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