Absolute Stereochemistry of (+)-Gigantecin from *Annona coriacea* (Annonaceae)

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Summary: The absolute stereochemistry of the cytotoxic polyketide gigantecin (1) has been established by single-crystal X-ray studies and analysis of Mosher ester derivatives by ^1H and ^{19}F NMR.

Bioactivity-directed fractionation of higher plants has resulted in the discovery of many novel cytotoxic compounds with potential as antineoplastic agents.[1,2] Recent research on the Annonaceae family has resulted in the discovery of a series of biologically active polyketides (known as the Annonaceous acetogenins).[3,4] These compounds fall into three major structural groups: the C_{35} or C_{38} polyketides[5,6] with a monotetrahydrofuran (THF) ring, the C_{37} polyketides with adjacent bis-THF rings,[7] and the C_{37} polyketides with nonadjacent bis-THF rings.[8] It is clear that the determination of the stereochemistry of this class of compounds will be crucial to the understanding of their biological activities, antitumor potential, and mechanism of action.[9] There have been chemical-shift based NMR approaches to the assignment of the relative[10] and absolute[11] stereochemistry of the adjacent bis-THF system as well as the relative[12] stereochemistry of the mono-THF system. X-ray analysis has been applied to two polyketide derivatives.[13] This is the first report of an X-ray analysis of an underivatized polyketide.

The seed of the Brazilian plant *Annona coriacea* Mart was fractionated using cytotoxicity in human tumor cell lines as a guide to give gigantecin (1). This paper reports the elucidation of the absolute stereochemistry of 1, which is a C_{37} polyketide with nonadjacent bis-THF rings. Its occurrence in *Goniothalamus giganteus* and its two-dimensional structure with partial relative stereochemistry were first reported by Alkofahi *et al.*[14] Compound 1 showed significant cytotoxicity against A-549 (lung carcinoma), HT-29 (colon adenocarcinoma), MCF-7 (breast adenocarcinoma), and U251MG (glioblastoma multiforme) human tumor cell lines at ED_{50} levels of 0.4, 0.001, 4.3, and 0.003 μg/mL, respectively.

The 95% ethanol extract of the defatted seed, after partitioning with dichloromethane and water and then with 90% methanol and hexane, gave rise to an active methanol fraction. Subsequent chromatography on silica with chloroform and methanol resulted in 1 in 0.1% overall yield as white crystalline needles, mp 108–9 °C, $[\alpha]^{25}_D$ +15.5° (*c* 0.22, $CHCl_3$). The IR absorptions at 3460 and 1764 cm^{-1} and the UV (acetonitrile) λ_{max} at 219.5 nm (log ε 3.62) confirmed the presence of hydroxyl and α,β-unsaturated lactone moieties. High-resolution FABMS, obsd 639.4809, calcd for $(MH)^+$ 639.4836, established the molecular formula as $C_{37}H_{66}O_8$.

The 2D-COSY and 2D-HETCOR NMR spectra (^1H at 500 MHz and ^{13}C at 125 MHz) showed the characteristic pattern of a C_{37} polyketide. The lactone moiety showed signals corresponding to the carbonyl (δ 174.59), olefinic (δ 7.19, 131.09, 151.82), and γ-methine carbon (δ 5.05, 77.94), positions consistent with the proposed structure. The acetate derivative 2 displayed a total of four acetoxyl groups (2.02 s, 3H; 2.06 s, 6H; 2.08 s, 3H) and, therefore, indicated the presence of four hydroxyl groups in 1. Three of the four methine protons geminal to the hydroxyl groups were coupled to three of the four ring junction methine protons. By virtue of the chemical shifts, these signals

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(6) The only C_{38} example is annonastatin (Lieb, F.; Nonfon, M.; Wachendorff-Neumann, U.; Wendisch, D. *Planta Med.* **1990**, *56*, 317).
(7) Examples are (a) uvaricin (Jolad, S. D.; Hoffmann, J. J.; Schram, K. H.; Cole, J. R.; Tempesta, M. S.; Kriek, G. R.; Bates, R. B. *J. Org. Chem.* **1982**, *47*, 3151), (b) rolliniastatin-1 (Pettit, G. R.; Gragg, G. M.; Polonsky, J.; Herald, D. L.; Goswami, A.; Smith, C. R.; Meretti, C.; Schmidt, J. M.; Weisleder, D. *Can. J. Chem.* **1987**, *65*, 1433), (c) asimicin (Rupprecht, J. K.; Chang, C.-j.; Cassady, J. M.; McLaughlin, J. L.; Mikolajczak, K. L.; Weisleder, D. *Heterocycles* **1986**, *24*, 1197), and (d) bullatacin (Hui, Y.-H.; Rupprecht, J. K.; Liu, Y. M.; Anderson, J. E.; Smith, D. L.; Chang, C.-j.; McLaughlin, J. L. *J. Nat. Prod.* **1989**, *52*, 463).
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(13) The two cases of indirect X-ray studies are (a) the absolute configuration of the 15-*O*-(*p*-bromophenyl)urethane of rolliniastatin 1 (ref 7a) and (b) the relative configuration of the dihydro-γ-hydroxy carboxylate salt of annonin I (Born, L.; Lieb, F.; Lorentzen, J. P.; Moeschter, H.; Nonfon, M.; Söllner, R.; Wendisch, D. *Planta Med.* **1990**, *56*, 312).

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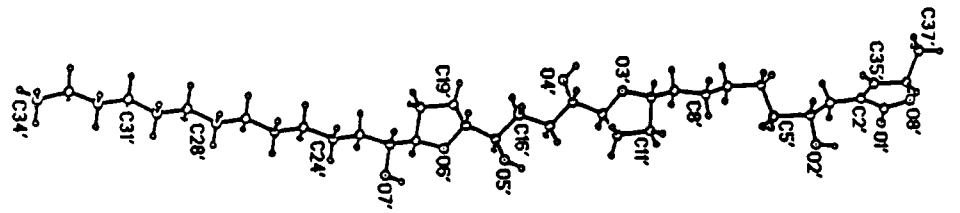
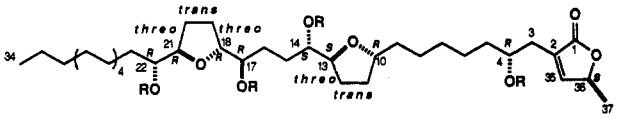


Figure 1. A computer-generated perspective drawing of the final X-ray model of gigantecin (1).

implied a *threo* relationship between the hydroxyl groups and the THF rings.

The assignment of the positions of the hydroxyl groups and the THF rings was based on diagnostic mass spectral fragment ions. High-resolution EIMS of 1 showed a major ion at 281.1761 (calcd for $C_{16}H_{25}O_4$ 281.1753) as a result of the fragmentation at C-13/C-14. Another major ion at 281.2444 (calcd for $C_{18}H_{33}O_2$ 281.4625) derived from the splitting along the C16–C17 bond and the loss of one molecule of water. Furthermore, an ion at 351.2166 (calcd for $C_{20}H_{31}O_5$ 351.2171) came from the C-17/C-18 breakage and the loss of one molecule of water. The fragment ions at 211.1312, 421.2600, and 199.2047 established the positions of hydroxyl groups at C-4, C-14, C-17, and C-22 and those of the tetrahydrofuranyl rings at C-10/C-13 and C-18/C-21. The relative stereochemistry of the two mono-THF rings was determined by converting 1 to its tetramesitoate and applying the ^{1}H-NMR method.[12,15] Accordingly, the assignment of C-10 through C-14 is *threo-trans* and C-17 through C-22 is *threo-trans-threo*.

The overall relative stereochemistry of 1 was achieved by single-crystal X-ray diffraction on a sample recrystallized from butanone as clear colorless flat plates, representing the first example of direct X-ray study of natural polyketides from Annonaceae. The crystals belonged to the monoclinic space group $P2_1$ with a = 5.0980(10) Å, b = 76.452(4) Å, c = 10.115(2) Å, and β = 103.700(2)° and contained two independent molecules in the asymmetric unit (Z = 4). All unique diffraction maxima with $2\theta \leq 95°$ were collected on a computer-controlled four-circle diffractometer using graphite-monochromated Cu Kα radiation. After corrections for Lorentz, polarization, and background effects, 1692 (56%) were judged observed ($|F_o| > 4\sigma|F_o|$). Finding a phasing model was difficult, but repeated attempts with the Rantan methodology finally yielded a small starting fragment that was extended to the complete structure using the tangent formula expansion method.[16] The model was refined using full-matrix least-squares techniques with isotropic heavy atoms and fixed riding hydrogen atoms to a final crystallographic residual of 10.07%. See the supplementary material for additional details. A computer-generated drawing of 1 is given in Figure 1.[17]



Gigantecin (1) R = H
Gigantecin Tetraacetate (2) R = Ac

Figure 2. Numbered structure of gigantecin (1).

The absolute configuration of 1 was determined by ^{1}H- and ^{19}F-NMR of (*R*)- and (*S*)-Mosher ester derivatives of 1.[11] The analysis of the carbinol at C(4) was performed with certainty, since all the signals involved could be unambiguously assigned.[18] By use of the chemical shift equations $\Delta\delta_H = \delta_S - \delta_R$ and $\Delta\delta_F = \delta_S - \delta_R$, the signs of the $\Delta\delta$ values of Mosher ester derivatives are as follows: H-5 positive, H-3 negative, H-35 negative, H-36 negative, H-37 negative, and CF_3-4 positive. Combining this information with that of the X-ray, the stereochemistry at all chiral centers is as follows: 4*R*, 10*R*, 13*S*, 14*S*, 17*R*, 18*R*, 21*R*, 22*R*, and 36*S*. Therefore, (+)-gigantecin (1) is 3-[7-[5-[1(*S*),4(*R*)-dihydroxy-4-[tetrahydro-5-(1(*R*)-hydroxytridecyl)-2(*R*),5(*R*)-furanyl]butyl]tetrahydro-2(*R*),5(*S*)-furanyl]-2(*R*)-hydroxyheptyl]-5(*S*)-methyl-2(5*H*)-furanone (Figure 2).

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Supplementary Material Available: Tables of ^{1}H, ^{13}C, and ^{19}F NMR data of gigantecin and derivatives (5 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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(15) The ring junction methine and the carbinol methine signals of the model compounds in C_6D_6 are δ 3.97 and 5.29 for the *threo-cis*, 3.93 and 5.45 for the *erythro-cis*, 4.09 and 5.35 for the *threo-trans*, and 4.05 and 5.48 for the *erythro-trans*. The observed signals for the tetramesitoate of 1 are δ 4.05 (H-13) and 5.32 (H-14), 4.11 (H-18) and 5.30 (H-17), and 4.09 (H-21) and 5.30 (H-22). **1983**, *A39*, 566.

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(17) The author has deposited atomic coordinates for gigantecin with the Cambridge Crystallographic Data Centre. The coordinates can be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, CB2 1EZ, UK.

(18) It should be noted that the analysis of the carbinols at C(14), (17), and (22) was very difficult because of the overlapping nature of the signals.