Stereochemical Assignment of Roflamycoin by ^{13}C Acetonide Analysis

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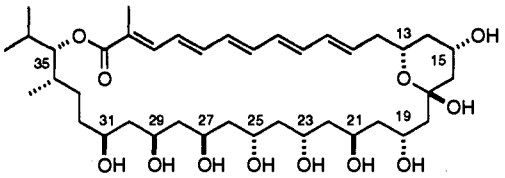
Complex polyol segments make the stereochemical elucidation of polyene macrolide antibiotics a serious challenge.[2] Polyene macrolides exhibit poor crystalline properties, and only two of the more than 200 known examples have had their structures determined by X-ray analysis.[3] The complete stereochemical structures of four other polyene macrolides have been determined by spectroscopic analysis, chemical degradation, and partial synthesis.[4] Several methods have been suggested for determining the stereochemistry of alternating polyol chains,[4h,5] but we favor the ^{13}C NMR acetonide analysis in which syn 1,3-diol acetonides (^{13}C NMR of methyls = 30, 19 ppm) are easily distinguished from anti 1,3-diol acetonides (^{13}C NMR of methyls = 25, 25 ppm).[6] We have applied the ^{13}C acetonide analysis, in conjunction with other spectroscopic data, to rapidly and efficiently determine the complete stereochemical structure of roflamycoin.

The antifungal agent roflamycoin (= flavomycoin) was isolated over 20 years ago.[7] The gross skeletal structure was determined by NMR and mass spectral analysis as well as careful degradative studies,[8] but the stereochemistry remained a mystery. Two polyene macrolides, roxaticin and mycoticin, have a common stereochemical pattern, and it was proposed that roflamycoin shared the same pattern at 9 of its 11 stereogenic centers.[3b] Following this assumption, we prepared the spiroacetal of such a roflamycoin by total synthesis.[9] We obtained an authentic sample of roflamycoin after completion of that synthesis and found that the spiroacetal **2**[10] derived from natural roflamycoin **1** (Dowex 50W-X1 (H^+), MeOH) did not match our synthetic material.[9] The stereochemical assignment of roflamycoin is described below.



Natural Roflamycoin (1)

Acylation of **2** gave peracetate **3** (Scheme 1). Several protons on the spiroacetal portion of **3** were identified by ^1H NMR and COSY analysis. The axial protons at C14 (1.18 ppm, q, J = 11.6 Hz), C18 (1.11 ppm, t, J = 12.2 Hz), and C20 (1.41 ppm, q, J = 1.17 Hz) are only consistent with the protons at C13, C15, C19, and C21 all being axial. Thus the relative (acyclic) configurations at 13/15 and 19/21 must be anti.

Spiroacetal **2** was subjected to acetonide-forming conditions followed by acetylation to give a mixture of diacetonides; careful separation by reverse-phase HPLC gave a single pure compound, **4**, for which nearly every proton could be assigned by ^1H NMR and COSY analysis. The chemical shifts for the protons at C13 and C21 are 3.40 and 4.32 ppm, respectively. If the relative configuration at 15/19 were anti, the spiroacetal portion of **4** would be approximately C_2 symmetric, and the protons at C13 and C21 would be in nearly equivalent environments. The large-chemical shift difference suggests that this is not the case and that the relative configuration at 15/19 is syn. This was confirmed by cross peaks in the NOESY spectra of **4** between the equatorial C18 proton and the axial protons at C13 and C15 (Figure 1).

The ^{13}C NMR spectrum of **4** (prepared with 99% [1,3-$^{13}C_2$]-acetone)[11] showed methyl doublets at 30.65, 30.48, 19.54, and 19.47 ppm. Thus, the relative configurations at 23/25 and 29/31 must be syn.

Treatment of roflamycoin with $NaBH_4$ reduced the C17 hemiacetal to give a 5:3 mixture of epimeric alcohols. Separation of the epimers by HPLC gave pure polyols that were treated separately with acetone to give pentaacetonides **7a** and **7b**. The ^{13}C DEPT NMR spectrum of **7a** showed two syn and three anti acetonides, while the same analysis of **7b** showed one syn and four anti acetonides. This is only consistent with the C17, C19 acetonide being syn in **7a** and anti in **7b**. We already know that the relative configuration at 29/31 is syn, so the remaining three relationships (13/15, 21/23, and 25/27) must be anti.

The final unassigned stereochemical relationship in the polyol portion is 27/29. Roflamycoin spiroacetal (**2**) was silaylated and then subjected to ozonolysis followed by treatment with $NaBH_4$. The resulting diol was silylated again and reduced with LAH to remove the lactate ester and leave only the C35 hydroxyl unprotected. Mesylation followed by deprotection with Bu_4NF resulted in cyclization of the C31 alcohol by displacement of the C35 mesylate with inversion. Acetonide formation followed by benzoylation gave the diacetonide **6**. The ^{13}C NMR spectrum of **6** (prepared with 33% [1,3-$^{13}C_2$]acetone)[11] showed signals at 30.74(2), 20.22, and 20.04 ppm that are consistent only with syn relative configuration at 23/25 and 27/29.

The relative configuration of C31 with respect to C34 and C35 was determined by analysis of the peracylated tetrahydropyan **5**. The ^1H NMR and COSY spectra of **5** showed that the proton at C35 was a doublet of doublets at 3.18 ppm with J = 3.9 and

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(2) For a review of polyene macrolide antibiotics, see: Omura, S.; Tanaka, H. In *Macrolide Antiobiotics: Chemistry, Biology, and Practice*; Omura, S. Ed.; Academic Press: New York, 1984; pp 351–404.

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(10) in addition to other methods, compounds were characterized by ^1H and ^{13}C NMR spectroscopy and HRMS.

(11) The natural abundance acetonide was treated with Dowex 50W-X1 (H^+) and MeOH followed by enriched [1,3-$^{13}C_2$]acetone and CSA.

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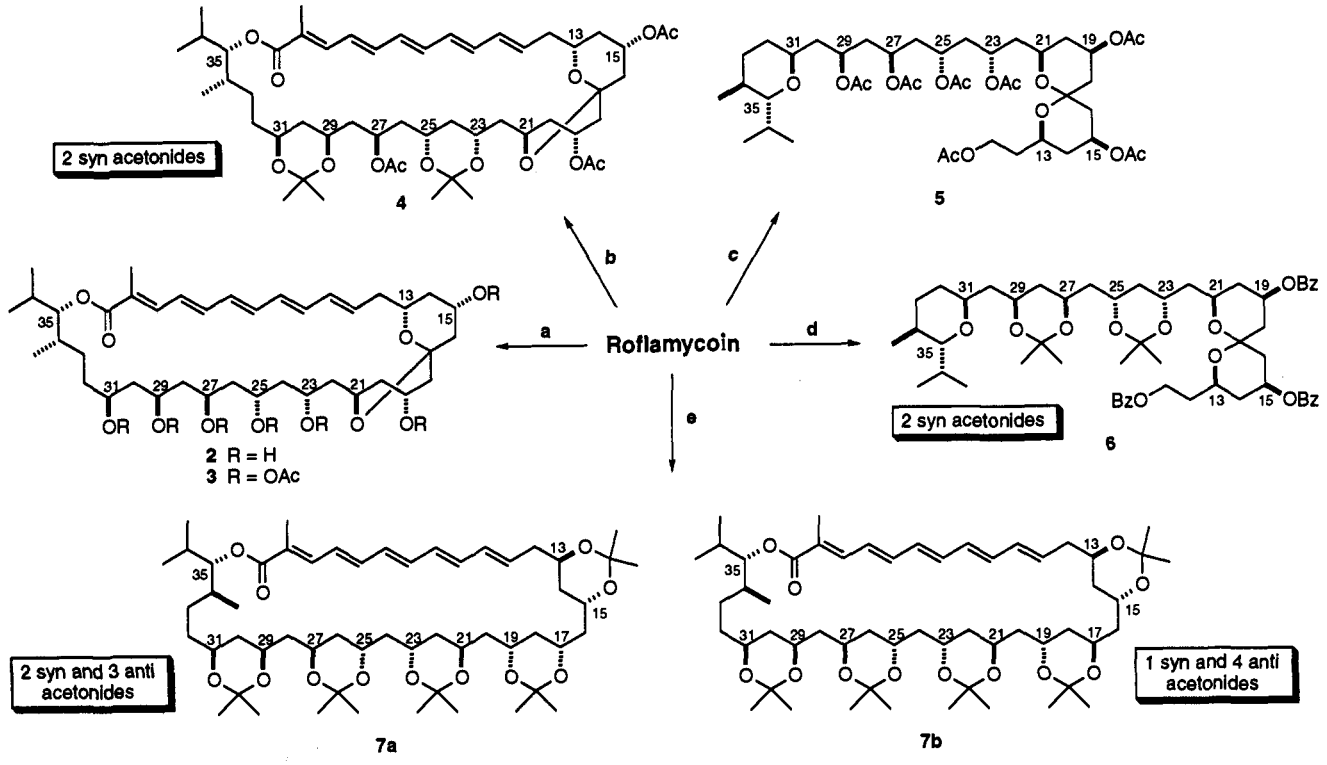
Scheme 1[a]



[a] (a) (i) Dowex 50W-X1 (H^+), MeOH; (ii) Ac_2O, DMAP, THF. (b) (i) Dowex 50W-X1 (H^+), MeOH; (ii) acetone, 2,2-dimethoxypropane, CSA; (iii) Ac_2O, DMAP, THF. (c) (i) Dowex 50W-X1 (H^+), MeOH; (ii) TBSOTf, 2,6-lutidine, CH_2Cl_2; (iii) O_3, MeOH/$CHCl_3$ (2:1), then $NaBH_4$; (iv) TBSOTf, 2,6-lutidine, CH_2Cl_2; (v) $LiAlH_4$, THF; (vi) MsCl, DMAP, Et_3N, CH_2Cl_2; (vii) TBAF, THF; (viii) Ac_2O, DMAP; THF. (d) (i) Repeat sequence c (i–vii); acetone, 2,2-dimethoxypropane, PPTS; (iii) Bz_2O, DMAP, THF. (e) (i) $NaBH_4$, 50% aqueous EtOH; (ii) HPLC separation; (iii) acetone, 2,2-dimethoxypropane, CSA.

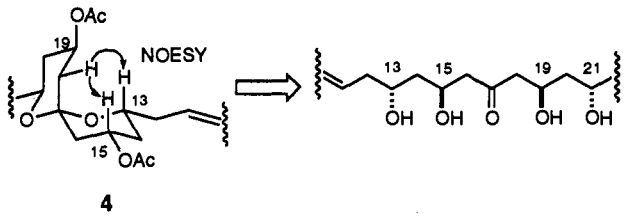


Figure 1. Selected Nosey data for the spiroacetal portion of compound **4**.

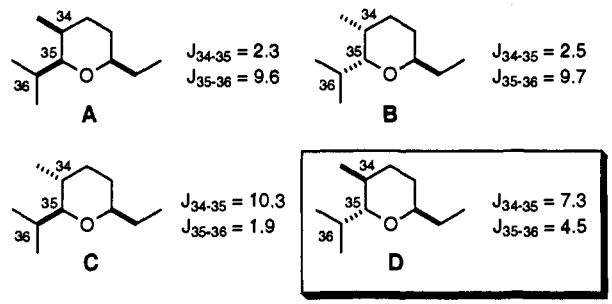


Figure 2. Calculated coupling constants for the tetrahydropyran diastereomers A–D.

8.0 Hz, and decoupling identified the $J_{34,35}$ = 8.0 Hz and the $J_{35,36}$ = 3.9 Hz. A complete conformational search[12] was carried out on each of the four possible tetrahydropyran diastereomers **A–D**, and the predicted coupling constants[13] for each conformer within 10 kJ/mol of the minimum were weighted according to a Boltzmann distribution at 25 °C. The predicted coupling constants for **A–D** are shown in Figure 2. Only stereoisomer **D** is consistent with the observed coupling constants in **5**, so the relative configurations at 31/34 and 34/35 in roflamycoin are syn.

The absolute configuration at C35 was determined by the advanced Mosher's method.[14] Sequential treatment of **7b** with O_3, $NaBH_4$, LAH, and Mosher's acid chlorides gave the C11, C35 bis-Mosher's ester derivatives **8**. The $\Delta\delta$ ($= \delta S - \delta R$) values are consistent with an *S* configuration at C35.[15]

The data presented here are consistent only with the roflamycoin structure illustrated. The structure differs at three (21, 25, 27) of the nine stereocenters proposed by Maehr.[4b] We have identified the stereochemical structure of natural roflamycoin from among the 2048 possible stereoisomers in 2 months using ca. 150 mg of natural material. This work demonstrates the power of the ^{13}C acetonide analysis for the structure determination of polyene macrolide antibiotics.

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Supplementary Material Available: Spectral data for compounds **1–7** (18 pages). This material is contained in many 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.

(12) A Monte Carlo search with 500 initial structures using the MM2 force field was carried out using Macromodel 3.5 (Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440–467).

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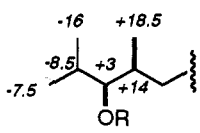
(15) $\Delta\delta$ (=δS Mosher's ester – δR Mosher's ester) values for compound **8** in hertz at 500 MHz.



8 (R = *R* and *S* Mosher's Ester)