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An Unusually Facile Preparation of 21-Alkoxyderivatives of Bafilomycin A_1

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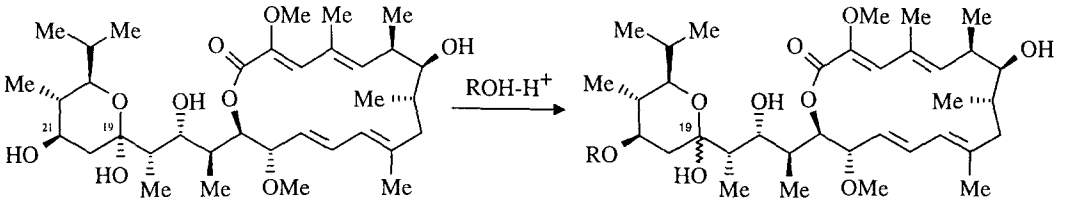
Abstract: 21-alkoxy derivatives of bafilomycin A_1 can be obtained easily by treatment of bafilomycin A_1 with linear, primary alcohols in the presence of an aqueous solution of oxalic acid. Reaction proceeds with complete retention of configuration. Copyright © 1996 Elsevier Science Ltd

Bafilomycin A_1 (**1**), a macrolide antibiotic produced by *Streptomyces griseus*,[1] is a specific inhibitor of vacuolar H^+-ATPase,[2] a proton pump which acidifies intracellular organelles and affords the protonmotive force required for many biological processes in all eukariotic cells. This macrolide is endowed with several interesting biological activities, including antitumor,[3] antiviral[4] and immunosuppressant[5] properties. Recently, bafilomycin A_1 has also been found to inhibit *in vitro* the release of β-amyloid[6] and the mitogen-induced DNA synthesis.[7]

During a research effort aimed at studying structure-activity relationships of bafilomycin derivatives, a few *O*-alkylated compounds had to be prepared. The methods of etherification reported in the literature entail the reaction of **1** with alcohols in the presence of acetic or phosphoric acid under forcing conditions.[8] In our hands, these methods afforded a very complex mixtures from which 19-*O*-alkyl, 21-*O*-alkyl and 19,21-*O*-dialkyl derivatives could be isolated, albeit in very low yields, by means of preparative HPLC. The synthesis of 7-*O*-methylbafilomycin was reported by treatment of **1** with methyl iodide and sodium hydride;[8] in this case, however, we were not able to resolve the complex reaction mixture and no useful alkylated product could be isolated, even by preparative HPLC. The reaction was unsuccessful also when sodium hydride was replaced by silver oxide or potassium carbonate.

We have found that the treatment of an alcoholic solution (methanol, ethanol or propanol) of **1** with 10% aqueous oxalic acid (ROH: acid 4:1 w/w) at room temperature afforded, in high yield, the corresponding 21-alkoxy derivatives **2a-c** with complete retention of configuration at C-21. The reaction with linear primary alcohols was complete after 3-5 hours at room temperature and isolated yields were

beetween 60-90%. On the ôther hand, branched or secondary alcohols, such as 2-propanol, 2-butanol or isobutanol failed to give any reaction at room temperature, whilst at 50-60°C only extensive decomposition of **1** was observed.



1, Bafilomycin A_1

2a R = Methyl (19R/19S 4:1)
b R = Ethyl (19R/19S 4:1)
c R = Propyl (19R/19S 5:1)

Ethers **2a-c** were always accompanied by a minor amount (about 10-20% by HPLC) of another compound. Only in the case of the methyl ether, this compound could be isolated by column chromatography and identified as the (19S)-epimer of **2a**. It proved to be very labile and inter-converted into the more stable (19R)-epimer even at low temperature (2°C) in the solid state (moist amorphous solid).

The structure of 19R-**2a** was assigned on the basis of its mass spectrum (negative FAB, diethanolamine: *m/z* 635) and of ^{1}H and ^{13}C NMR spectra. A clear evidence of the presence of an OMe group at position 21 appeared from the difference in chemical shifts of H-21 ($\Delta\delta$ = –0.5 ppm) and of C-21 ($\Delta\delta$ = +9.7 ppm) in comparison with the parent compound **1.** Further confirmation was achieved by detection of n.O.e. contacts to H-21, H-20_{ax}, H-20_{eq} and 22-Me on irradiation of 21-OMe.

The $^{3}J_{21,20eq}$ (4.5 Hz), $^{3}J_{21,20ax}$ (12 Hz) and the large value of the $^{4}J_{19\text{-}OH,20ax}$ (2.4 Hz), was in agreement with the antiperiplanar orientation of the 19-OH proton with respect to H-20_{ax} already observed in the parent compound. These values can be taken as a demonstration of the retention of conformation and stereochemistry of the tetrahydropyran ring. Further support was obtained by n.O.e. experiments on irradiation of H-23 (Figure 1) giving a result similar to that observed in bafilomycin A_1.[9] Similar spectroscopic results provided structural confirmation of the ethoxy and propoxy derivatives **2b,c**.

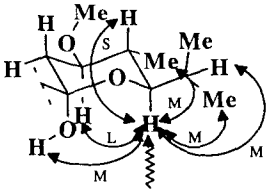
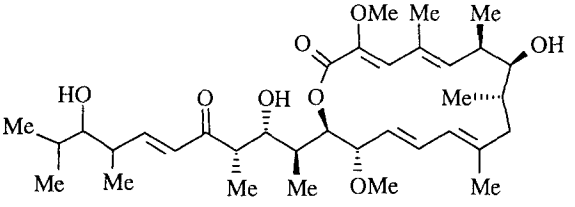


Figure 1. n.O.e. contacts after irradiation of 23-H of 19R-**2a**

Structural characterization of the 19S-epimer of **2a** was based on the negative FAB mass spectrum showing that only one methylation occurred in the molecule; moreover, the presence of the fragment at *m/z* 419 indicated that methylation did not occur at 7-OH and 17-OH. The ^{1}H-NMR spectrum displayed the methoxy signal resonance at 3.35 ppm, significantly different from the chemical shift of 19-OMe (δ= 3.02 ppm),[10] ruling out a possible alkylation at 19-OH. The signal of 19-OH was still present in the spectrum, but shifted up field ($\Delta\delta$= -0.7 ppm) compared with the 19R-epimer, suggesting that the group was no longer involved in the hydrogen bonding with 17-OH[11] (confirmed by $\Delta\delta$ = -0.7 ppm of 17-H). The 7-H signal at δ= 3.3 ppm as in bafilomycin A_1, clearly indicated that 7-OH was not methylated. Further investigation was not possible due to interconversion of this product into the more stable natural epimer.

The presence of a catalytic amount of water was necessary to obtain the desired 21-alkoxy bafilomycins. The same reaction carried out in anhydrous methanol afforded different products, *i.e.* the 19-*O*-methyl derivative was formed in the presence of a catalytic amount of acetic acid, while a mixture of dehydrated products was obtained in the presence of 3% anhydrous oxalic acid. Also, oxalic acid could be replaced by other acids of sufficient strength *e.g.* p-toluenesulphonic acid. An acidic pH, between 2 and 4, was necessary since no reaction occurred at pH 7, contrary to what reported for a similar *O*-methylation of concanamycin[12] obtained during deglycosylation in neutrally buffered medium.

Few insight into the reaction has been obtained. Although the opening of the tetrahydropyran ring is suggested by the formation of 19S-epimers, the involvement of an α,β-unsaturated ketone derivative is excluded since bafilomycin D[13] is recovered unchanged when submitted to the same reaction conditions (MeOH, aqueous oxalic acid, r.t., 5 h).



Bafilomycin D

On the other hand, the intermediate formation of a carbocation seems unlikely in view of the stereochemical outcome of the reaction which occurs with complete retention of stereochemistry at C-21, albeit the influence of the conformational bias of pyran and/or macrolide rings cannot be excluded. A plausible mechanism entails an intramolecular displacement of 21-OH by the hemiketalic α-oriented OH at C-19 with formation of an intermediate oxetane. Nucleophilic opening of the oxetane by the alcohol occurs from the β face of the molecule thus generating stereoselectively a 21β-alkoxide.[14]

In conclusion, a new simple procedure for selective and high-yielding preparation of 21-ethers of bafilomycin A_1 has been discovered which proceeds with complete retention of configuration and whose reaction mechanism deserves further exploration.

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