Tetrahedron Letters,Vol.24,No.47,pp 5193-5196,1983 0040-4039/83 $3.00 + .00
Printed in Great Britain

THE STRUCTURE OF THE BAFILOMYCINS, A NEW GROUP OF MACROLIDE ANTIBIOTICS

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Summary: The structure of bafilomycins A_1, A_2, B_1, B_2, C_1 and C_2, produced by Streptomyces griseus ssp. sulphurus has been elucidated mainly by spectroscopic studies. The bafilomycins are macrolide antibiotics with a 16-membered lactone ring.

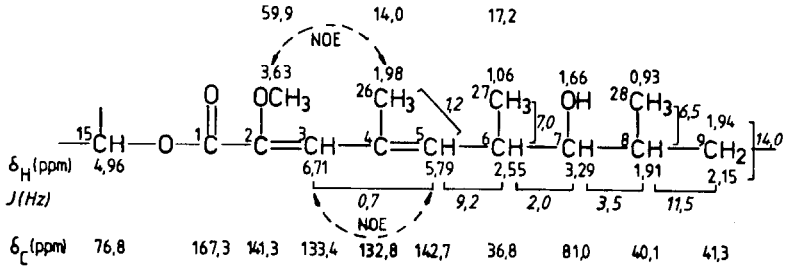
Isolation: The culture medium of Streptomyces griseus ssp. sulphurus[1] was extracted with ethyl acetate (pH 10). After removal of the solvent in vacuo three compounds with antifungal activities, bafilomycines A_1, B_1, and C_1 were isolated by column chromatography on silica gel (eluent: chloroform/methanol 9:1). Each component was purified by multiple column chromatography on silica gel (bafilomycin A_1: 1. column: chloroform/methanol 95:5; 2. column: ethylmethylketone/chloroform 1:1; B_1: 1. column: chloroform/methanol 9:1; 2. column: chloroform/ethyl acetate 1:1; C_1: linear gradient of chloroform/methanol 1:1 to methanol with 2 % ammonia, followed by preparative high pressure liquid chromatography (LiChrosorb RP18, 10 µm (MERCK), 250mm x 16mm); eluent: methanol/water 85:15).

Physico-chemical properties of bafilomycin A_1: colorless amorphous powder; m. p. 98-106°C (decomp.); $C_{35}H_{58}O_9$; FD-MS: M^+ m/z 622; λ_{max}^{MeOH} 245 nm (ε 25000) and 280 nm (ε 12100); ν_{max}^{KBr} 3400, 2900, 1700 (sh), 1680, 1250 cm^{-1}.

The ^{13}C NMR spectrum of bafilomycin A_1 (100.62 MHz in $^{12}CDCl_3$) revealed the following groups: CH_3 x 9, CH_2 x 2, CH x 6, CH_3O x 2, CHO x 6, O-C-O x 1, CH= x 5, C= x 3 and -COO- x 1. This accounted for 54 protons. All bafilomycins are unstable at pH < 6 and >11 and decompose at the melting point.

Structure elucidation of bafilomycin A_1[2]: With SFORD it was possible to correlate almost all proton resonances with the carbon resonances[3]. Analysis of the 400 MHz ^1H NMR spectrum of bafilomycin A_1 and its acetyl derivatives in $CDCl_3$ was accomplished with the aid of conventional proton spin decoupling, INDOR spectra and NOE experiments to give the following partial structures.[4,5]

Partial structure I:

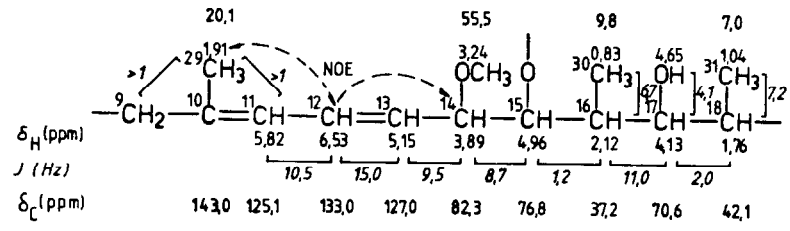


(measured in $^{12}CDCl_3$)

The strong IR bands at 1680 cm^{-1} and 1250 cm^{-1} and the intense UV absorption at 280 nm, attributed to a $n \rightarrow \pi^*$ transition are in agreement with the assumption of an α,γ-unsaturated ester. The substituents of the diene structure were determined by NMR spectroscopy. Only irradiation at H-15 (δ=4.96 ppm) and H-3 (δ=6.71 ppm) in the SFORD spectrum with ^{13}C-^{1}H-decoupling leads, besides a decoupling of the corresponding ^{13}C-resonances, to a sharp ^{13}C NMR-resonance of C1, indicating the substitution by an ester group.

The proton H-5 shows allylic coupling with Me-26 and H-3. By irradiation at H-5 (δ=5.79 ppm) the long range coupling of Me-26 and H-3 disappears. By irradiation at H-3 in a NOE experiment only the resonance of H-5 is seen in the difference spectrum. In the NOE difference spectra with saturation at MeO-C2 (δ=3.63 ppm) and Me-27 (δ=1.06 ppm) only the resonance signal of the corresponding group appears. This is in accordance with an E, E configuration of diene structure C2-C3-C4-C5, methoxy substitution at C2 and methyl substitution at C4.
The structural features of C5 through C9 could be deduced with the aid of ^{1}H spin decoupling and INDOR experiments. Coupling of H-8 and H-9 was recognized only in INDOR difference spectra. Between δ=1.8 ppm and δ=2.2 ppm the resonances in ^{1}H-^{1}H decoupling experiments could not be interpreted due to the signal density.

Partial structure II:



(measured in $^{12}CDCl_3$)

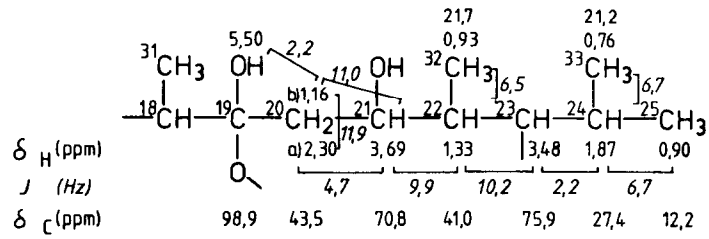
The connections of C11 to C15 and C16 to C18 can be recognized by ^{1}H-^{1}H spin decoupling. Connection of C9 to C10 can be shown by INDOR difference spectra, were the long range coupling of H-9a (δ=2.15 ppm) and Me-29 (δ=1.91 ppm) respectively disappears. The connection of C15 and C16 was difficult to recognize, since the resonance of H-16 (δ=2.12 ppm) is superimposed by the resonance of H-9a (δ=2.15 ppm). Decoupling experiments show, however, that the coupling resonance of H-15 (δ=4.96 ppm) lies at δ_H=2.1 ppm. Graphical analysis of the signal at 2.1 ppm as well as the decoupling spectra allow the identification of H-16 as the coupling partner of H-15.

From NOE difference spectra (irradiation at H-12 and Me-29), topless with the coupling constants typical for trans oriented protons ($^{3}J_{H-11/H-12}$=10.5 Hz and $^{3}J_{H-12/H-13}$=15.0 Hz)

results E, E configuration of the diene C10-C11-C12-C13.

The connection of the partial structure I and II followed from an INDOR experiment. Irradiation at Me-29 (δ=1.91 ppm) shows in the difference spectrum the coupling proton H-9a (δ=2.15 ppm), H-11 (δ=5.82 ppm) and H-12 (δ=6.53 ppm).

Partial structure III:



(measured in $^{12}CDCl_3$)

^1H spin decoupling experiments yield the connections C19 to C25 of the partial structure III. The connection C19-C20 is deduced from the allylic coupling of C19-OH with H-20b (δ=1.16 ppm, ^4J=2.2 Hz). Ring closure of C19 with C23 to a tetrahydropyran ring is in agreement with the ^1H-^1H coupling constants, which on the other hand allowed the conclusion, that the orientation of all substituents in the tetrahydropyran ring is equatorial with the exception of the axial C19-OH group of the hemiketal[6]. Only an axial C19-O<u>H</u> can couple with an axial proton (H-20b) in a zig zag "W" geometry.

Two carbon bonds remain open. Since no further coupling can be seen for H-18 and C19-OH, C18 is connected with C19. This leads to the total structure of bafilomycin **A_1**.

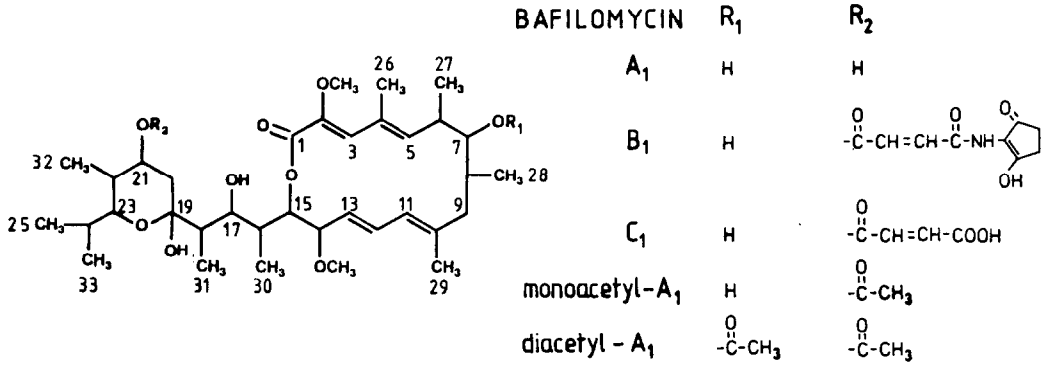


Fig. 1. Structures of bafilomycins

Bafilomycin **B_1** and **C_1** are C21 substituted derivatives of bafilomycin **A_1**.

<u>Bafilomycin</u> **B_1** is the N-(3-hydroxy-2-cyclopentenone-2-yl)-fumarylester monoamide. (Yellow amorphous powder; m. p. 89 - 96°C (d); $C_{44}H_{65}NO_{13}$; FD-MS $(M+H+Na)^+$ m/z 839; λ_{max}^{MeOH} 248 nm (ε 35000), 285 nm (sh) (ε 18500) and 355 nm (ε 12500); ν_{max}^{KBr} 3450, 3200, 2900, 1720, 1690 and 1240 cm^{-1}). Hydrolysis of bafilomycin **B_1** yielded N-(3-hydroxy-2-cyclopentenone-2-yl)-fumaric acid monoamide. (Yellow needles; m.p. >235°C (d); FD-MS: m/z 211; HR-EIMS: 211.048069 for $C_9H_9NO_5$; ^{13}C NMR (100.62 MHz in $^{12}CD_3OD$) and ^1H NMR (400 MHz in CD_3OD) in ppm: C1' 167.9

(s); C2' 135.4 (d), H2' 7.35 (d,15.3 Hz),C3' 137.7 (d), H3' 6,78 (d,15.3 Hz), C4' 165.5 (s), C6' 115.6 (s), C7' and C10' 189 (s), C8' and C9' 30.0 (t), H8' and H9' 2.57 (s, 4H)).

Bafilomycin C_1 is the fumarylester of bafilomycin A_1. (Colorless amorphous powder; m. p. 145 - 150°C (d); $C_{39}H_{60}O_{12}$; λ_{max}^{MeOH} 245 nm (ε 22000), 280 nm (sh) (ε 12000), 335 nm (sh) (ε 4800) and 350 nm (sh) (ε 2500). ν_{max}^{KBr} 3450, 2900, 1715, 1690 and 1250 cm^{-1}; ^{13}C NMR (100.62 MHz in $^{12}CD_3OD$) and ^1H NMR (400 MHz in CD_3OD) in ppm: C1' 166.1 (s), C2' 133.8 (d), H2' 6.95 d (15.4 Hz), C3' 134.2 (d), H3' 6.70 d (15.4 Hz) and C4' 170.1)

Products of acetylation: Acetylation of bafilomycin A_1 with acetic anhydride in pyridine leads to a mono- and a diacetyl derivative. The resonance for H-21 changes from a broad signal (δ=3.69 ppm) in bafilomycin A_1 to a clear ddd (δ=4.95 ppm; ^3J=11.0 Hz, ^3J=10.5 Hz, and ^3J=4.7 Hz) in the monoacetyl derivative with a concomitant downfield shift of δ=1.26 ppm.

In the diacetyl derivative the broad signal for H-7 (δ=3.29 ppm) of bafilomycin A_1 becomes a dd (^3J=7.0 Hz and ^3J=2.5 Hz) at δ=4.73 ppm.

The procedure described for the isolation of bafilomycins A_1,B_1 and C_1 also yielded in minor amounts the corresponding ketals with C19-OCH_3. C19-OH disappears and a new methoxygroup is located at δ_H=3.05 ppm (s, 3 H). These compounds are called bafilomycin A_2, B_2 and C_2.

The bafilomycins are new 16-membered macrolide antibiotics consisting of an α-methoxy-α,γ-unsaturated lactone ring. This partial structure is also present in concanamycin A, B and C[7,8]. Hygrolidin shows similarity to bafilomycin C_1, but differs in the substituents at C2 and C23 as well as in the conformation of the tetrahydropyranring [9].

ACKNOWLEDGMENT

This work was supported by Deutsche Forschungsgemeinschaft (Sonderforschungsbereich 76), and Fonds der Chemischen Industrie.

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(Received in Germany 23 March 1983)