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STRUCTURES OF MILBEMYCIN β1, β2, AND β3

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The metabolite, produced by Streptomyces B-41-146 strain, is a mixture of macrolides, designated milbemycins. Milbemycins exhibit a remarkable pesticidal activity against mites such as the two-spotted spider mite and citrus red mite, and insects such as a rice leaf beetle and a tent caterpiller, without any phytotoxicity towards many varieties of crops at effective dosages.

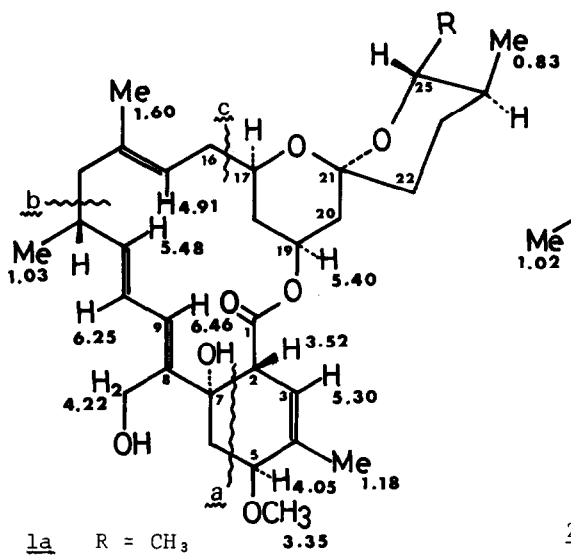
The acetone soluble fraction, obtained from the concentration of the culture filtrate, was extracted with hexane and the extract was evaporated in vacuo to give a viscous oil. The oil was repeatedly chromatographed on columns of silica gel and alumina, and the columns were eluted gradiently with various organic solvent mixtures. The chromatographic separation was monitored by tlc. Finally, each sample was purified by preparative tlc.

From the oil we isolated thirteen pure components, of which ten (milbemycin α1, 2, 3, 4, 5, 6, 7, 8, 9, and 10) are apparently novel macrolides containing a tetrahydrofuran ring as a common structural component.[1] We now report investigations leading to structures 1a, 1b, and 2 respectively for milbemycin β1, 2, and 3.

Milbemycin β1, $C_{32}H_{48}O_7$ (M^+ 544.3423), amorphous powder, $[\alpha]_D^{20}$ +160° (acetone, c=0.25), shared the spectroscopic features characteristic of a primary hydroxyl group. Treatment with p-bromophenylisocyanate afforded a crystalline p-bromophenylurethane derivative, $C_{39}H_{52}O_8NBr$. Crystals, mp. 256-8° (from MeOH), are tetragonal, $P4_1$, a, c=15.31, b=17.91 Å. A total 1844 independent reflexions were collected on a Rigaku auto-diffractometer using Mo-Kα radiation up to $2\theta \leq 50^\circ$. The structure was solved by a usual heavy-atom technique, and was

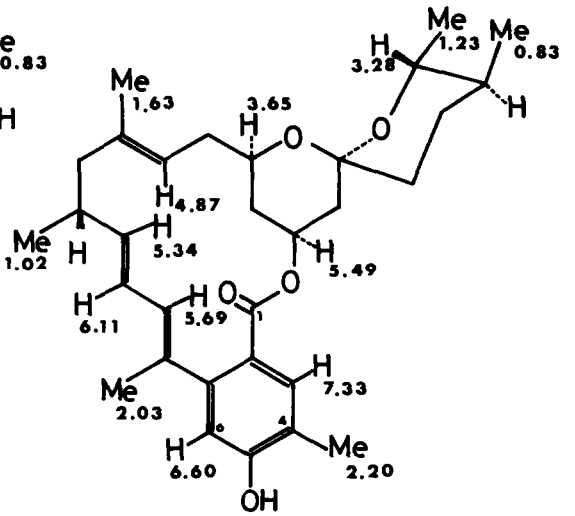
currently refined by a block-diagonal least-squares method. The final R factor is 0.134. The crystallographic X-ray analysis defined the structure and stereochemistry of M. β1 as 1a.

The assignments of the spectral data are: λ_{max}^{EtOH} 245 nm (ϵ 26,500) (conjugated diene); ν_{Nujol} 1707 cm^{-1} (sixteen-membered lactone); the chemical shifts in the ^1H nmr spectrum (100 MHz) are shown in 1a as numerals.



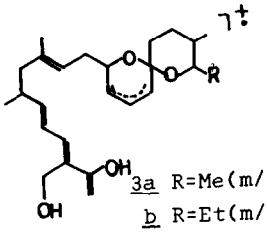
1a R = CH_3

b R = C_2H_5

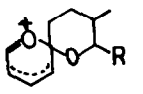


2

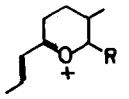
The mass spectrum shows a few intense peaks of fragment ions with a molecular ion peak. It became possible to interpret the mass fragmentation and three representative ionic structures are shown as 3a, 4a, and 5a, since there are some mass-spectrometrically fragile bonds in the structure attributed to (i) a retro Diels-Alder reaction (wavy line a in 1a), (ii) an allyl fission (b, c), (iii) an α-cleavage of an oxygen atom (c), and (iv) an ester elimination by the McLafferty rearrangement accompanied by a β-hydrogen transfer.



3a R=Me(m/e 402)
b R=Et(m/e 416)



4a R=Me(m/e 181)
b R=Et(m/e 195)



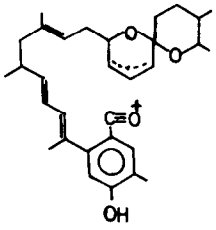
5a R=Me(m/e 153)
b R=Et(m/e 167)

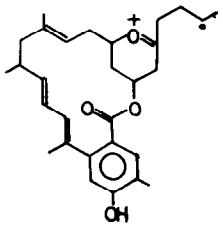
Milbemycin β2, $C_{33}H_{50}O_7$ (M^+ 558.3509), is extremely similar to M. β1, since the uv, ir, and nmr spectra are substantially identical with those of M. β1. The comparison of the mass spectrum of M. β2 with that of M. β1 showed changes consistent with an increase of 14 mass units (CH_2) in the corresponding fragment ions (e.g., 3b, 4b, and 5b) and the parent ion. This indicates that an ethyl group is attached at position 25 in this molecule instead of the methyl group in M. β1. The ^1H nmr spectral evidence (3H triplet at 0.82 ppm) also supports the structural assignment of M. β2. Therefore, the structure is formulated as 1b.

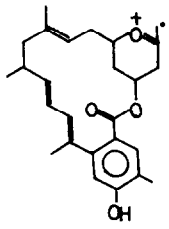
Milbemycin β3, $C_{31}H_{42}O_5$ (M^+ 494.3066), mp. 185-7° (from hexane), is another congener containing an aromatic moiety, λ_{max}^{EtOH} 247 nm (ϵ 29,200). The ir spectrum shows absorption bands due to an aromatic ring at 1670, 1608, and 1582 cm^{-1}. Chemical shift (158.7 ppm) of the carbon at position 5 was assigned to the aromatic carbon bearing a hydroxyl group.

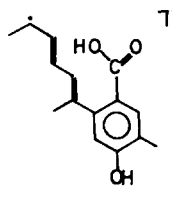
The principal structural of features were deduced from correlations of high resolution mass spectra, 100 MHz ^1H, and 25.2 MHz ^{13}C nmr spectral data.

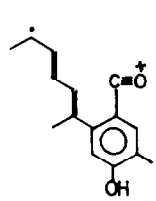
The fragmentation patterns of the mass spectrum in M. β3 are virtually identical with that in M. β1 except the fragment ions based on the retro Diels-Alder reaction. In the case of M. β3, the fragment ions stabilized by an aromatic ring system gave rise to more intense peaks (m/e 494, 476, 450, 408, 245, and 227)


m/e 476


m/e 450

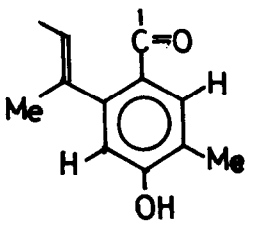

m/e 408


m/e 245

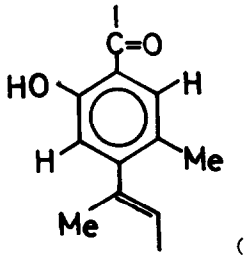

m/e 227

The ^{13}C nmr spectrum shows signals of an ester carbonyl carbon (172 ppm), 12 sp^2 carbons, and 18 sp^3 carbons, of which three are C-O tertiary carbons (72.60, 69.62, and 69.00 ppm) and one is an O-C-O quarternary carbon (99.56 ppm). Of the six aromatic carbons, only two are hydrogen substituted, which are in a 1,4-relationship since the signals (7.33 and 6.60 ppm) of these two aromatic protons occur as two singlets of a para-coupled pair. The signals of these protons are enhanced by nuclear Overhauser effects by the aromatic methyl group

and the olefinic methyl group, respectively. Double irradiation experiments showed: (i) one proton and an aromatic methyl group at C_4 are in an ortho relationship (ii) the other ring-proton at C_6 and an olefinic methyl group at C_8 are in close proximity. As the chemical shift at higher field must be assigned to the proton in the ortho-position to the hydroxyl group, the arrangement of aromatic substituents should be represented as either (A) or (B):



(A)



(B)

The partial structure (B) was excluded mainly for the following two reasons: (i) from biogenetic considerations that (B) requires an eighteen-membered lactone while all other congeners are sixteen-membered macrolides, and (ii) from the observation that the hydroxyl group is not intramolecularly hydrogen bonded. If the partial structure (B) is included in M. β3, hydrogen bonding would be expected between the hydroxyl group and the ester carbonyl, since the former must be in sterically crowded environment with respect to the latter. The chemical shifts of ^1H nmr spectrum are shown in 2 as numerals. Other spectral data are consistent with the structure of 2.

The characteristic features of metabolites reported here consist of three basic units: a sixteen-membered lactone, a spiro-ketal ring system consisting of two six-membered rings,[2] and cyclohexenediol or phenol.

Reference

1 Symposium papers of the 18th symposium on the chemistry of natural products, at Kyoto in Japan, 1974, pp. 309.

2 A similar ring system containing two six-membered rings was found in an antibiotics, A23187. M. O. Chaney, P. V. Demarco, N. D. Jones, and J. L. Occolowitz, J. Am. Chem. Soc., 96, 1932 (1974).