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STRUCTURE OF HERBIMYCIN, A NEW ANSAMYCIN ANTIBIOTIC

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Herbimycin is a new potent herbicidal antibiotic[1] isolated from the cultured filtrate of Streptomyces (Sm.) hygroscopicus AM-3672. We assign structure 1 to herbimycin based on ^{1}H- and ^{13}C-nmr spectral analyses and biosynthetic means using ^{13}C-labeled precursors.

The molecular formula, $C_{30}H_{42}N_2O_9$ for herbimycin (1) was established from the elemental analysis and high-resolution mass (M^+, m/e 574) in the early report[1]. The UV spectrum (MeOH) of 1 shows characteristic absorption maxima at 270 nm (ε 20090) and 392.5 nm (ε 1650), indicating the presence of benzoquinone nucleus[2] as a chromophore. The ^{1}H- and ^{13}C-nmr spectra of 1 indicate that 1 is structurally similar to geldanamycin (2), ansamycin antibiotic, the structure of which has been determined by Rinehart et al.[3] The presence of the following functional groups in 1 was deduced from the ^{1}H- and ^{13}C-nmr ($CDCl_3$) and ir ($CHCl_3$) spectra: $OCONH_2$ [δ 4.87 (broad s), exchange with D_2O, 1730 cm^{-1}], CONH [δ 8.78 (s), exchange with D_2O, 1655 cm^{-1}], four OCH_3 (δ 56.0, 57.6, 58.4 and 59.8), benzoquinone carbonyls (δ 187.7 and 183.9, 1690 cm^{-1}), two sec-methyl protons (δ 0.82 and 1.09), two methyl protons (δ 1.65 and 2.01) attached to double bond, a methylene carbon (δ 34.0), a conjugated diene protons [δ 6.95 (broad d), 6.48 (t) and 5.84 (q)] and an isolated double bond proton [δ 5.50 (broad d)]. The substitution pattern of benzoquinone nucleus was deduced from the ^{1}H-nmr spectral evidence; two protons observed at δ 6.60 (t, J=2.7 Hz) and 7.30 (d, J=2.7 Hz) can be assigned to those at α- and α'-positions of the quinone carbonyl[4] as shown in partial structure A. Further evidence for structure A was obtained from the off-resonance ^{13}C-nmr spectrum in which the quinone carbonyl carbon at δ 183.9 shows up as a triplet due to the long-range (vicinal) ^{13}C-^{1}H coupling ($^{3}J_{CCCH}$=10.0 Hz) to the two protons at δ 6.60 and 7.30. The functional groups connecting the ansachain moiety to the benzoquinone unit were identified to be $>CH-OCH_3$ [δ 4.50 (broad s)] from the observation that the proton signal at δ 6.60 (t) on the benzoquinone nucleus collapses into a doublet upon

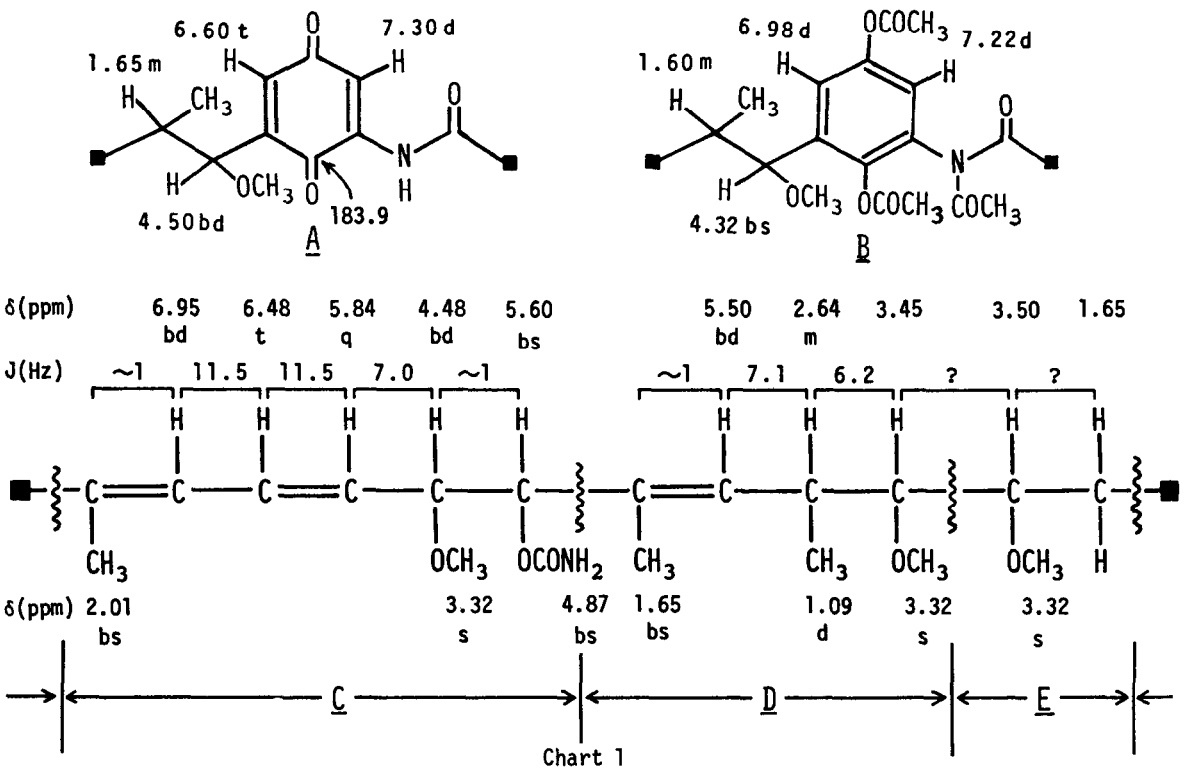


Chart 1

irradiating the methyne proton at δ 4.50, and from the fact that the ^{13}C chemical shift values of the nitrogen-substituted carbon (δ 138.2) and the amide carbonyl (δ 168.7) in 1 (in $CDCl_3$) are virtually identical with those in 2 (δ 139.6 and δ 169.1 in DMSO-d_6, respectively).

Additional confirmation for the skeletal structure A is provided by a colorless compound, 3 [$C_{37}H_{51}NO_{12}$, M^+, m/e 701 (701.344; Calcd. for $C_{37}H_{51}NO_{12}$ 701.346), ν_{CO} 1730 and 1770 cm^{-1}], which was obtained by reductive acetylation of 1 with Zn dust in acetic anhydride. The UV absorption maximum [λ_{max} 271 nm (ε 17000)] in 3 suggests the presence of the dienamide group. The ^1H-nmr spectrum of 3 exhibited two aromatic protons (δ 6.98 and 7.22 each as a doublet with J=2.7 Hz) and four acetyl groups at δ 2.28 (two aromatic acetoxyls), δ 2.28 (N-acetyl) and δ 2.07 (acetoxyl derived from the OH group by elimination of a carbamoyl group during acetylation). In addition, the presence of partial structure B in 3 was strongly suggested by the coupling (J=2.0 Hz) between the protons at δ 4.32 (broad s) and 1.60 (m) which could be assigned to those on the carbons attached to the methoxyl and sec-methyl groups, respectively. Consequently, the sec-methyl group should be located adjacent to the methoxyl group in the partial structure A of 1.

With regard to the structure of the ansachain moiety in 1, the presence of the following

partial structures C and D were confirmed by proton spin decoupling experiment on 1, as indicated in Chart 1. In addition, the validity for the structures of fragments C and D was also supported by the remarkable similarity in the ^{13}C chemical shifts of 1 and 2. ^{13}C Chemical shift assignments[5] aided by selective ^{13}C-^{1}H decoupling of 1 and comparison of the chemical shifts of 1 with those of 2 are listed in Table 1. Two possible structures, that is, $-CH(OCH_3)CH_2-$ and $-CH_2CH(OCH_3)-$, were considered for the remaining fragment E (C_3H_6O) which contains a methoxyl group (δ 3.32). It was difficult to assign unequivocally the structure of fragment E from the ^{1}H- and ^{13}C-nmr spectral data alone. On the other hand, Rinehart et al.[6] have reported that the ansachain moiety of the antibiotic 2 is derived biosynthetically from one acetate, two glycerates or glycolates and four propionates. This biosynthetic evidence for 2 were utilized to the structural elucidation of 1 in connection with the structural similarity of 2 to 1. Namely, in order to obtain more definitive evidence for the structure of fragment E and the arrangement of the partial structures A, C, D and E in 1, biosynthetic study was carried out using ^{13}C-labeled propionate as a precursor. [1-^{13}C]Sodium propionate (90% enriched in ^{13}C) was fed to the fermentation culture[7] of Sm. hygroscopicus AM-3672, and ^{13}C-labeled herbimycin was isolated from the fermentation broth. The ^{13}C-nmr spectrum of ^{13}C-labeled herbimycin exhibited a strong

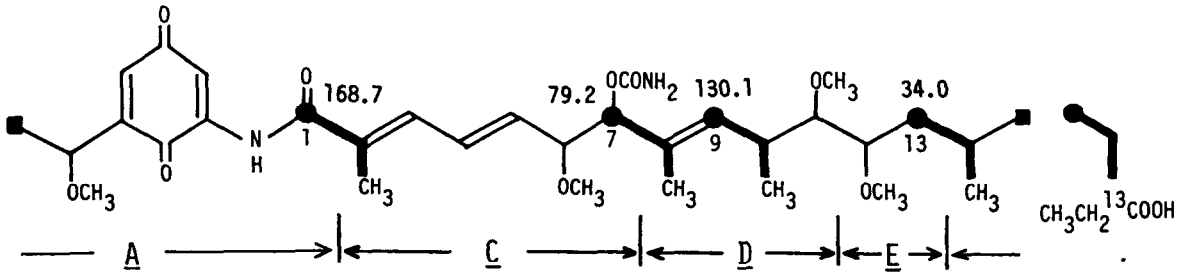
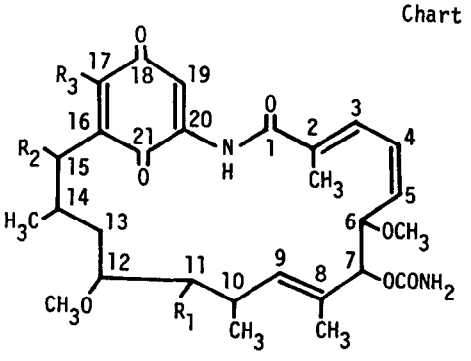
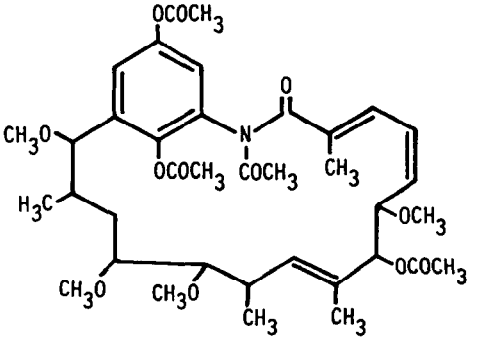


Chart 2





	R_1	R_2	R_3
1	OCH_3	OCH_3	H
2	OH	H	OCH_3

3

Chart 3

Table 1. Comparison of the ^{13}C-nmr spectra of herbimycin (1) and geldanamycin (2)

Carbon No.	1	2	Carbon No.	1	2
-NHCO-	168.7 s	169.1 s	11	82.3 d	71.9 d
2	134.5 s	133.2 s	11-OCH_3	58.4* q	-
2-CH_3	12.4 q	12.2 q	12	83.4 d	80.2 d
3	128.2 d	128.4 d	12-OCH_3	57.6* q	56.5 q
4	125.6 d	125.7 d	13	34.0 t	31.0 t
5	136.7 d	137.8 d	14	36.7 d	26.6 d
6	78.3 d	81.6 d	14-CH_3	13.6 q	13.0 q
6-OCH_3	56.0* q	56.0 q	15	78.7 d	31.7 t
7	79.2 d	80.6 d	15-OCH_3	59.8* q	-
7-$OCONH_2$	155.9 s	156.0 s	16	144.6 s	128.1 s
8	131.6 s	132.6 s	17	132.6 d	156.4 s
8-CH_3	14.1 q	12.5 q	17-OCH_3	-	61.0 q
9	130.1 d	131.9 d	18	187.7 s	183.6 s
10	34.1 d	32.1 d	19	112.9 d	110.9 d
10-CH_3	16.3 q	23.3 q	20	138.2 s	139.6 s
			21	183.9 s	183.1 s

The chemical shifts are δ_C-values in ppm from internal TMS in $CDCl_3$ for 1 and DMSO-d_6 for 2. Multiplicities in the off-resonance decoupling spectrum, s; singlet, d; doublet, t; triplet, q; quartet. Assignment denoted with * may be interchanged.

enrichment in four carbon signals at δ 168.7 (C-1), 79.2 (C-7), 130.1 (C-9) and 34.0 (C-13).

Since the enrichment pattern for these carbons, which were derived from carboxyl carbon of a propionate, gave the result concordant with biosynthetic pattern of 2, the validity of structure E and the location of the fragments C, D and E in benzoquinone moiety A was established as shown in Chart 2. Thus, taking into consideration the nmr spectral data, biosynthetic evidence and comparison of those with 2, it was concluded that structure 1 would be the most suitable for herbimycin.

References and Footnotes

1) S. Ōmura, Y. Iwai, Y. Takahashi, N. Sadakane, A. Nakagawa, H. Ōiwa, Y. Hasegawa and T. Ikai, J. Antibiotics, 32, 255 (1979).
2) S. Natori, H. Nishikawa and H. Ogawa, Chem. Pharm. Bull., 12, 236 (1964).
3) K. Sasaki, K. L. Rinehart, Jr., G. Slomp, M. F. Grostic and E. C. Olson, J. Am. Chem. Soc., 92, 7591 (1970).
4) The long range ^{13}C-^1H and ^1H-^1H coupling constant values for heteroaromatic molecule are cited from the following reports: a) G. Govil, J. Chem. Soc. (A), 1416 (1967). b) G. Govil, ibid., 1420 (1967).
5) Detailed argument regarding the assignment of ^{13}C chemical shift in 1 will be presented in full paper.
6) A. Haber, R. D. Johnson, K. L. Rinehart, Jr., J. Am. Chem. Soc., 99, 3541 (1977).
7) The fermentation medium employed for preparation of ^{13}C-labeled herbimycin consists of the following composition: 2.0% glucose, 1.0% soybean meal, 0.4% KCl, 0.25% yeast extract, 0.1% meat extract, 0.5% $(NH_4)_2SO_4$, 0.02% K_2HPO_4, and 0.3% $CaCO_3$ (pH 7.2 before sterilization). After cultivating for 24 hr, 0.2% [1-^{13}C]sodium propionate was fed to the culture, and the fermentation was continued for an additional 81 hr. The isolation method of herbimycin from the fermentation broth has been described in the early report.

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