STRUCTURE OF AWAMYCIN, A NOVEL ANTITUMOR ANSAMYCIN ANTIBIOTIC

Sir:

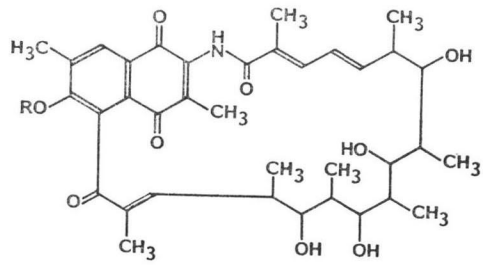
In the course of a continuing search for novel antitumor antibiotics of microbial origin, a new antitumor antibiotic awamycin was isolated from the culture broth of *Streptomyces* sp. No. 80-217 isolated from a soil sample collected in Chiba Prefecture, Japan[1]. The taxonomy of the producing organism, fermentation, isolation and physico-chemical and biological properties of this antibiotic have been reported in the preceding paper[1]. This communication deals with the structure elucidation of awamycin.

As described in the preceding paper, physicochemical characteristics of awamycin suggested that this antibiotic belongs to the group of naphthalenoid ansamycins having a sulphur atom in the molecule. Through the combination of elemental analysis and mass spectrometry, the molecular formula of awamycin was established as $C_{38}H_{49}O_{12}NS$ (MW 743)[1].

In the ^{13}C NMR spectrum of awamycin (in $CDCl_3$), 15 singlets including two carbonyl signals [δ_C 180.4 (s) and 183.1 (s)] characteristic for the quinone carbonyls[2], 13 doublets and 10 quartets were observed. On the other hand, in the ^1H NMR spectrum of awamycin (in $CDCl_3$), signals corresponded to 49H were observed which were classified into 10 methyl signals, 13 methine signals and 6 D_2O exchangeable signals.

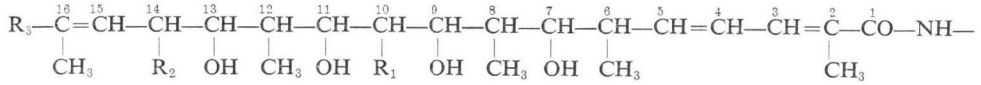
The structure of the ansa moiety of awamycin was established as shown in Fig. 1 through ^1H NMR homo-decoupling study and by comparison of ^1H NMR data of awamycin with those of rifamycin W[3] and streptovaricin D[4,5] (Fig. 1, Table 1). The existence of a carbomethoxy group was suggested by IR (ν_{max}^{KBr} 1721 and 1209 cm^{-1}), ^1H NMR [δ_H 3.715 (3H, s)] and ^{13}C NMR [δ_C 51.8 (q) and 172.8 (s)] spectral data and the positions of this moiety and the ansa moiety were elucidated through the ^1H NMR and ^{13}C NMR comparison with those of rifamycin W[3] and streptovaricin D[4,5] (Fig. 1, Table 1). Configurations of the double bonds at C2-C3 (*cis*), C4-C5 (*trans*) and C15-C16 (*trans*) and the transoid conformation at C3-C4 have been elucidated because of the similarity of ^1H NMR spectral data of awamycin [δ_H 6.440 (1H, d, J=11 Hz, C3-H), 6.945 (1H, dd, J=16 and 11 Hz, C4-H), 5.793 (1H, dd, J=16 and 9 Hz, C5-H) and 5.726 (1H, d, J=9 Hz, C15-H)] with those of rifamycin S [δ_H 6.19 (1H, d, J=9.5 Hz, C3-H), 6.60 (1H, dd, J=15.0 and 9.5 Hz, C4-H), and 5.93 (1H, dd, J=15.0 and 7.0 Hz, C5-H); C2-C3 (*cis*), C4-C5 (*trans*) and C3-C4 (transoid)][6] and protostreptovaricin I (**1**) [δ_H 5.66 (1H, d, J=10 Hz, C15-H; C15-C16 (*trans*)][5], respectively.

Because $C_{25}H_{39}O_5N$ out of $C_{38}H_{49}O_{12}NS$ of awamycin has been assigned to the ansa moiety (Fig. 1), it is obvious that the rest ($C_{13}H_{10}O_4S$) can be attributed to the naphthoquinone moiety. In the ^1H NMR spectrum of this region, three methyl signals [δ_H 2.281, 2.377 and 3.854 (each



1 R=H
2 R=CH_3

Fig. 1. Structures of the ansa regions of awamycin, rifamycin W[3] and streptovaricin D[4].



	R_1	R_2	R_3
Awamycin	$COOCH_3$	CH_3	C=O
Rifamycin W	CH_3	CH_2OH	C=O
Streptovaricin D	$COOCH_3$	CH_3	C—O

Table 1. NMR data of ansa moieties of awamycin (in $CDCl_3$), rifamycin W [in $CDCl_3$ - DMSO-d_6 (3: 1)] and streptovaricin D (in $CDCl_3$)*.

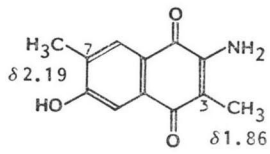
	Awamycin		Rifamycin W[3]	Streptovaricin D[4,5]	
	δ_H	δ_C	δ_H	δ_H	δ_C
NH	8.36 1H, s	—	8.55 1H, s	***	—
1	—	164.6 s	—	—	169.4 s
2	—	131.7 s[a]	—	—	127.5 d
2-CH_3	2.191 3H, s	12.1 q	2.08 3H, s	***	12.9 q
3	6.440 1H, d	128.9 d[b]	6.31 1H, d	7.66 1H, d	134.7 d
4	6.945 1H, dd	128.8 d[b]	7.00 1H, dd	6.52 1H, t	124.0 d
5	5.793 1H, dd	142.7 d[c]	6.32 1H, dd	5.83 1H, t	144.1 d
6	2.328 1H, m	42.0 d	2.61 1H, m	3.00 1H, m	41.9 d
6-CH_3	1.240 3H, d	20.6 q	0.96 3H, d	1.26 3H, d	22.2 q
7	3.445 1H, t	82.7 d	4.46 1H, dd	3.54 1H, q	83.6 d
7-OH	4.397 1H, d	—	**	***	—
8	2.10 1H, m	38.7 d	2.06 1H, m	2.30 1H, m	38.8 d
8-CH_3	0.890 3H, d	15.7 q[d]	1.23 3H, d	0.99 3H, d	15.7 q
9	4.212 1H, dt	76.5 d	4.81 1H, dd	4.25 1H, q	77.6 d
9-OH	4.635 1H, d	—	**	***	—
10	2.838 1H, s	46.9 d	2.54 1H, m	2.94 1H, d	47.4 d
10-$COOCH_3$	3.715 3H, s	172.8 s	—	3.78 3H, s	173.0 s
	—	51.8 q	—	—	51.9 q
10-CH_3	—	—	0.96 3H, s	—	—
11	4.146 1H, t	73.1 d	3.94 1H, dd	4.25 1H, d	73.4 d[a]
11-OH	4.089 1H, d	—	**	***	—
12	2.05 1H, m	38.3 d	2.00 1H, m	1.95 1H, m	38.5 d[b]
12-CH_3	0.801 3H, d	9.1 q	1.03 3H, d	0.80 3H, d	9.1 q
13	3.515 1H, td	69.9 d	5.26 1H, dd	3.45 1H, d	70.4 d[a]
13-OH	2.167 1H, d	—	**	***	—
14	2.675 1H, m	37.0 d	3.12 1H, m	2.57 1H, m	37.5 d[b]
14-CH_3	0.746 3H, d	17.4 q[d]	—	0.73 3H, d	15.1 q
14-CH_2OH	—	—	3.78 1H, dd	—	—
	—	—	4.08 1H, dd	—	—
	—	—	**	—	—
15	5.726 1H, d	144.4 d[c]	7.18 1H, d	5.51 1H, d	153.6 s
16	—	136.9 s[a]	—	—	***
16-CH_3	2.038 3H, s	16.2 q[d]	2.34 3H, s	***	12.7 q
17	—	195.3 s	—	—	169.0 s

* δ ppm from TMS.
** δ 3.4~4.5 (5H).
*** Data not given in the paper.
[a~d] Assignments may be interchanged.

3H, s)] and a phenolic OH signal [δ_H 11.98 (1H, s, D_2O exchangeable)] were observed.

So, it was shown that the naphthoquinone moiety of awamycin is substituted with OH, CH_3, OCH_3 and SCH_3 groups in addition to the ansa moiety at C-18 and C-26.

The position of a phenolic OH (δ_H 11.98) was estimated to be *peri*- (C-21) to the carbonyl group of naphthoquinone because of its characteristic low field shift in the ^1H NMR spectrum. A signal at δ_C 195.3 and an IR absorption maximum at 1665 cm^{-1}, attributed to the carbonyl (C-17) of the ansa moiety of awamycin, are quite similar to those of protostreptovaricin II (**2**) [δ_C 195.8 and ν_{max} 1670 cm^{-1} (nonhydrogen bonded), respectively] and are different from those of protostreptovaricin I (**1**) [δ_C 204.4 and ν_{max} 1640 cm^{-1} (hydrogen bonded), respectively][5]. This fact suggests that awamycin also has an OCH_3 group at C-19 and an OH group



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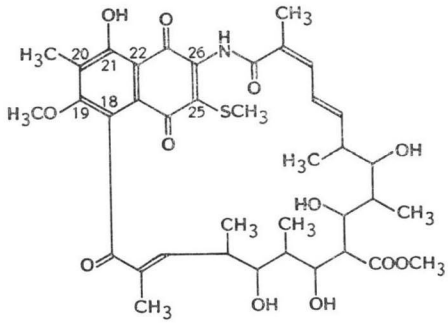

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at the C-21 position, like protostreptovaricin II (**2**). The *meta* substitution of the OCH_3 (C-19) and OH (C-21) groups is also supported by the ordinary ^{13}C NMR chemical shift of C-19 and C-21 (δ_C 161.9 and 162.6).

Because positions C-18 and C-26 (ansa moiety), C-19 (OCH_3) and C-21 (OH) are occupied by the substituents, the position of the methyl group is either C-20 or C-25 and is concluded to be C-20 because the chemical shift of the methyl group (δ_H 2.281) of awamycin is similar to that of the aromatic (and not to the olefinic) methyl group of 2-amino-3,7-dimethyl-6-hydroxy-1,4-naphthoquinone (**3**)[7]. The positions of the methyl group and the ansa moiety at C-20 and C-18 and C-26, respectively are also supported from the biosynthetic point of view of various ansamycins[2,8]. Because other positions of the naphthoquinone moiety of awamycin have been determined, the position of SCH_3 group is C-25.

From all the accumulated data described above, the structure of awamycin is concluded to be **4**.

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References

1) Umezawa, I.; H. Oka, K. Komiyama, K. Hagiwara, S. Tomisaka & T. Miyano: A new antitumor antibiotic, awamycin. J. Antibiotics 36: 1144~1149, 1983
2) Rinehart, K. L., Jr. & L. S. Shield: Chemistry of the ansamycin antibiotics. Fortschr. Chem. Org. Naturst. 33: 231~307, 1976
3) Martinelli, E.; G. G. Gallo, P. Antonini & R. J. White: Structure of rifamycin W. A novel ansamycin from a mutant of *Nocardia mediterranei*. Tetrahedron 30: 3087~3091, 1974
4) Rinehart, K. L., Jr.; M. L. Maheshwari, F. J. Antosz, H. H. Mathur, K. Sasaki & R. J. Schacht: Chemistry of the streptovaricins. VIII. Structures of streptovaricins A, B, D, E, F, and G. J. Am. Chem. Soc. 93: 6273~6274, 1971
5) Deshmukh, P. V.; K. Kakinuma, J. J. Ameel & K. L. Rinehart, Jr.: Protostreptovaricins I~V. J. Am. Chem. Soc. 98: 870~872, 1976
6) Gallo, G. G.; E. Martinelli, V. Pagani & P. Sensi: The conformation of rifamycin S in solution by ^1H NMR spectroscopy. Tetrahedron 30: 3093~3097, 1974
7) Knöll, W. M. J.; K. L. Rinehart, Jr., P. F. Wiley & L. H. Li: Streptovaricin U, an acyclic ansamycin. J. Antibiotics 33: 249~251, 1980
8) White, R. J.; E. Martinelli, G. G. Gallo, G. C. Lancini & P. Beynon: Rifamycin biosynthesis studied with ^{13}C enriched precursors and carbon magnetic resonance. Nature 243: 273~277, 1973