# ON THE STRUCTURE OF ALISAMYCIN, A NEW MEMBER OF THE MANUMYCIN CLASS OF ANTIBIOTICS

SUGATA CHATTERJEE,* E. K. S. VIJAYAKUMAR, CHRISTOPHER M. M. FRANCO, JURGEN BLUMBACH† and BIMAL N. GANGULI††

Microbiology Department, Research Centre, Hoechst India Limited, Mulund (W), Bombay 400 080, India

H.-W. FEHLHABER and H. KOGLER*

Pharmasynthese, Hoechst AG, 6230 Frankfurt (M)-80, Germany

(Received for publication November 30, 1992)

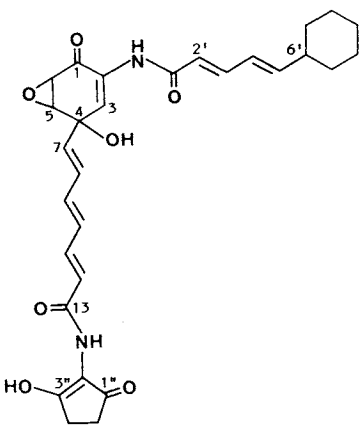
We have recently reported the taxonomy, production, isolation and biological properties of a new antibacterial antibiotic alisamycin (**1**) by the fermentation of *Streptomyces actuosus* (culture number HIL Y-88,31582)[1]. In this communication we report the structure elucidation of alisamycin which is a new member of the manumycin group of antibiotics.

The physico-chemical properties of alisamycin (**1**) are shown in Table 1. High resolution FAB mass spectrometry of alisamycin (**1**) showed an $(M+H)^+$ ion of mass 521.2276 which corresponded to a formula of $C_{29}H_{33}N_2O_7$. This indicated a molecular formula of $C_{29}H_{32}N_2O_7$ for alisamycin (**1**) and an unsaturation number of fifteen. Two UV absorption maxima at 276 and 315 nm (MeOH) indicated extended unsaturation. Although alkaline pH had only a marginal effect on the UV spectrum, acidification shifted the maxima at 315 nm to 325 nm and increased the absorption intensities at both the wavelengths by about 14%, indicating a keto-enol tautomeric system[2]. IR frequencies at 3400, 1710 and 1670 $cm^{-1}$ indicated hydroxyl, carbonyl and amide groups and a broad band at 1610 $cm^{-1}$ indicated a polyene moiety.

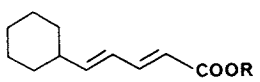
Table 2 summarizes the $^1H$ and $^{13}C$ NMR spectra of alisamycin (**1**). The proton resonances were analyzed by double quantum filtered HH shift-correlated COSY spectrum and the carbon re-

Table 1. Physico-chemical properties of alisamycin (**1**).

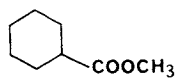
| | |
|---|---|
| Appearance | Yellow crystalline solid |
| MP | >250°C (dec.) |
| Solubility | $CHCl_3$, EtOAc, $CH_3OH$, DMSO |
| $[\alpha]_{578}$ | −122° (*c* 0.1, $CHCl_3$) |
| FAB-MS (*m/z*) | 521 $(M+H)^+$ |
| HRFAB-MS (*m/z*) | |
| Found: | 521.2276 $(M+H)^+$ |
| Calcd: | 521.2288 for $C_{29}H_{33}N_2O_7$ |
| Molecular formula | $C_{29}H_{32}N_2O_7$ |
| UV (MeOH) nm | 278, 315 |
| | 275, 308 (alkali) |
| | 278, 325 (acid) |
| IR (KBr) $cm^{-1}$ | 3400, 3300, 2930, 2860, 1710, 1670, 1610 (broad), 1520, 1370, 1325, 1280, 1255, 1210, 1175, 1160, 1125, 1100, 1000, 885, 810, 740, 660 |
| HPLC Rt | 3.5 minutes (4 × (30 + 250) mm ODS-Hypersil 10 μ column; $CH_3CN$-$H_2O$ containing 0.1% TFA; 1 ml/minute; UV 220 nm.) |



**1**



**2** R=H
**3** R=$CH_3$



**4**

† Present address: Pharmaceutical Research, Hoechst AG, 6230 Frankfurt (M)-80, Germany.
†† Departmental Head.

Table 2. $^{13}C$ (67.5 MHz) and $^{1}H$ NMR (400 MHz) spectral data of alisamycin (**1**) ($CDCl_3$, 303°K)[a].

| Position | $\delta_C$[b] | $^{1}H$ | | |
|---|---|---|---|---|
| | | $\delta$ (multiplicity, $J$ in Hz) | HMBC partner $^{2}J_{CH}$ | HMBC partner $^{3}J_{CH}$ |
| 1 | 188.63 | — | | |
| 2 | 128.08 | — | | |
| 3 | 126.36 | 7.40 (d, 2.6) | C-2 | C-1 |
| 4 | 71.20 | — | | |
| 5 | 57.41 | 3.70 (dd, 2.6, 3.6) | C-4 | C-7 |
| 6 | 52.93 | 3.65 (d, 3.6) | C-1, C-5 | C-2 |
| 7 | 136.29 | 5.86 (dd, 14.5, 0.3) | | C-3, C-9 |
| 8 | 131.58 | 6.58 (dd, 11.3, 14.5) | | |
| 9 | 139.52 | 6.58 (dd, 14.8, 11.3) | | |
| 10 | 131.74 | 6.42 (ddd, 11.2, 14.8, 0.3) | | |
| 11 | 143.45 | 7.32 (dd, 11.2, 14.7) | | C-13 |
| 12 | 121.59 | 6.05 (d, 14.7) | C-13 | C-10 |
| 13 | 165.48 | — | | |
| 1′ | 165.16 | — | | |
| 2′ | 120.95 | 5.84 (d, 14.8) | C-1′ | |
| 3′ | 144.16 | 7.22 (ddm, 14.8, 10.5) | | |
| 4′ | 125.52 | 6.12 (dd, 10.5, 15.5) | C-3′ | |
| 5′ | 150.76 | 6.12 (m) | | C-3′ |
| 6′ | 41.13 | 2.10 (m) | | |
| 7′,11′ | 32.25 | 1.76 (m) (eq), 1.13 (m) (ax) | | |
| 8′,10′ | 25.80 | 1.73 (m) (eq), 1.28 (m) (ax) | | |
| 9′ | 26.00 | 1.67 (m) (eq), 1.18 (m) (ax) | | |
| 1″ | 197.39 | — | | |
| 2″ | 115.01 | — | | |
| 3″ | 174.15 | — | | |
| 4″ | 32.14 | 2.61 (m) | | |
| 5″ | 25.65 | 2.53 (m) | | |
| 3″-OH | — | 13.52 (s) | | |
| 4-OH | — | 3.25 (s) | | |
| 2-NH | — | 7.54 (s) | C-1′ | C-1, C-3 |
| 13-NH | — | 7.58 (s) | C-13 | C-3″ |

[a] The $^{1}H$ and $^{13}C$ chemical shifts are in ppm from $(CH_3)_4Si$ and $CDCl_3$ as internal standards respectively.

[b] The carbon multiplicities were determined by DEPT-135 experiment.

sonances were assigned by a proton-detected CH shift-correlated multiple quantum coherence (HMQC) NMR experiment. The spectral properties showed strong similarities to those reported for the manumycin group of antibiotics. From the COSY spectrum recorded in $CDCl_3$, four spin systems could be extracted, a conjugated diene moiety attached to a methine multiplet (H-6′, $\delta$ 2.10) being part of a cyclohexane unit, one isolated triene moiety, three signals from the 5-epoxy-cyclohex-2-enone and two strongly coupled signals representing four protons. In $CDCl_3$ alisamycin (**1**) also revealed the presence of four $D_2O$ exchangeable singlets at $\delta$ 13.52, 7.58, 7.54 and 3.25 corresponding to one enolic hydroxyl, two amides and a hydroxyl proton respectively. On addition of DMSO-$d_6$ as co-solvent, the first three signals underwent large downfield shifts to $\delta$ 14.00, 9.60 and 8.45 respectively and the fourth one was not observed. The amide singlet at $\delta$ 7.54 showed COSY correlation to H-2′ ($\delta$ 5.84) and also to the H-3 ($\delta$ 7.40) which in turn showed coupling ($J$=2.6 Hz) to the epoxy proton H-5. All these observations were suggestive of a carboxamide group linking the diene unit to the epoxycyclo-

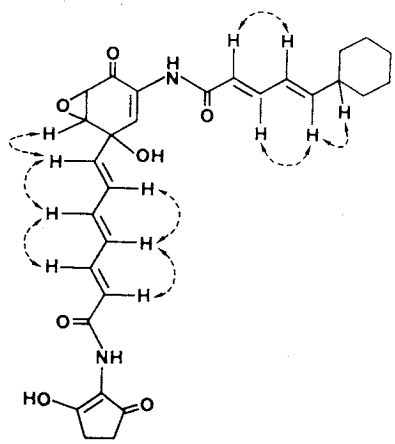
hexenone. A full confirmation was obtained by a proton-detected long-range CH shift correlation (HMBC) NMR experiment (Table 2). Thus this amide proton showed $^3J_{CH}$ correlation to C-3 ($\delta$ 126.36), C-1 ($\delta$ 188.63); $^2J_{CH}$ correlation to C-1′ ($\delta$ 165.16), and could thereby be assigned to the 2-NH proton.

The more downfield amide proton showed an exchange cross peak with the enolic proton in the NOESY spectrum and it also showed long-range COSY correlation with the H-11 proton ($\delta$ 7.32). In the HMBC spectrum this NH proton exhibited $^3J_{CH}$ correlation to the C-3″ ($\delta$ 174.15) and $^2J_{CH}$ interaction to the C-13 ($\delta$ 165.48) carbonyl, the latter in turn showing $^2J_{CH}$ interaction with H-12 ($\delta$ 6.05). Thus it became manifest that the conjugated triene and the cyclopentenone unit were linked by a carboxamide group. A $^2J_{CH}$ coupling of the triene terminus H-7 to C-4 ($\delta_H/\delta_C$ 5.86/71.20) established the point of attachment of the triene unit to C-4†. An observed NOE interaction between the H-7 and H-5 lent further support to this attachment and was suggestive of proximal orientation of the *trans*-$\Delta^7$ bond to the epoxy unit in the most preferred conformation. The absolute configuration at C-4 was not established.

The double bond geometries were determined by coupling constant measurements as well as NOE studies. Large coupling constant values (14 ~ 15 Hz) observed for H-12 and H-2′ established *E*-configuration of the corresponding double bonds. The olefinic protons H-8/H-9 and H-4′/H-5′ were isochronous appearing at $\delta$ 6.58 and 6.12 respectively, and their coupling constant values could not be measured by simple analysis of the $^1$H NMR spectrum. The problem of strong coupling could be resolved by simulating all the olefinic signals with the LAOCOON program and the best fitting values were taken. These values confirmed *E*-configuration for all the five disubstituted double bonds of alisamycin (**1**). Most of the olefinic protons also exhibited long range couplings (Table 2). The *E*-configurations of the double bonds were further corroborated by the NOE network (Fig. 1) as revealed in a phase-sensitive 2D NOESY spectrum (300 MHz, $CDCl_3$ - DMSO-$d_6$, 500 ms mixing time with 4% random variation).

The number of double bonds in the individual side chains of alisamycin (**1**) was unequivocally established by chemical degradation. Thus, a mild alkaline hydrolysis following the procedure reported[3] for manumycin afforded a dienic acid (**2**) which was converted to its methyl ester (**3**) and subsequently identified by spectral analysis to be 5-cyclohexyl-pent-2*E*,4*E*-dienylcarboxylic acid (**2**). These findings established a dienic upper side chain. Finally any ambiguity in the structure assignment of the terminal cycloalkyl substituent (*i.e.* cyclohexyl vs. methylcyclopentyl) was ruled out by $HNO_3$ oxidation[4] of alisamycin (**1**). The resulting acid on methylation afforded methylcyclohexanecarboxylate (**4**) which was found identical with an authentic sample by GC-MS analysis. The occurrence of a cyclohexyl residue is in agreement with the biosynthetic pathways proposed for this class of antibiotics[5]. In the case of asukamycin in particular, which has a 7-cyclohexyl-hept-2*E*,4*E*,6*E*-trienoyl moiety as the upper side chain, the starter unit is reported to be a cyclohexyl-carbonyl-CoA, derived from the shikimate pathway, with chain extension by three molecules of malonyl-CoA. By direct analogy, it can be suggested that the biogenesis of the upper side chain of alisamycin (**1**) involves a starter unit of the shikimate derived cyclohexyl-carbonyl-CoA, with chain extension by two malonyl-CoA molecules.

Fig. 1. NOE network of alisamycin (**1**).




Acknowledgments

We thank Dr. P. K. INAMDAR for elemental analysis and recording some of the spectra and Dr. G. SUBBAIAH for the GC-MS analysis. The technical assitance of Mr. RAJKUMAR MAURYA is acknowledged.

## References

1) FRANCO, C. M. M.; R. MAURYA, E. K. S.

† A $^3J_{CH}$ coupling of the 4-OH proton to the epoxy carbon C-5 was observed.

VIJAYAKUMAR, S. CHATTERJEE, J. BLUMBACH & B. N. GANGULI: Alisamycin, a new antibitoic of the manumycin group. I. Taxonomy, production, isolation and biological activity. J. Antibiotics 44: 1289～1293, 1991

2) BRODASKY, T. F.; D. W. STROMAN, A. DIETZ & S. MIZSAK: U-56,407, a new antibiotic related to asukamycin: Isolation and characterization. J. Antibiotics 36: 950～956, 1983

3) ZEECK, A.; K. SCHRÖDER, K. FROBEL, R. GROTE & R. THIERICKE: The structure of manumycin. I. Characterization, structure elucidation and biological properties. J. Antibiotics 40: 1530～1540, 1987

4) KAKINUMA, K.; N. IKEKAWA, A. NAKAGAWA & S. OMURA: The structure of asukamycin, a possible shunt metabolite from 3-dehydroquinic acid in the shikimate pathway. J. Am. Chem. Soc. 101: 3402～3404, 1979

5) THIERICKE, R.; A. ZEECK, A. NAKAGAWA, S. OMURA, R. E. HERROLD, S. T. S. WU, J. M. BEALE & H. G. GLOSS: Biosynthesis of the manumycin group antibiotics. J. Am. Chem. Soc. 112: 3979～3987, 1990