METABOLITES OF MICROORGANISMS. 229†

ABSOLUTE CONFIGURATION OF NAPHTHOMYCIN A DETERMINED BY X-RAY ANALYSIS AND CHEMICAL DEGRADATION

WALTER KELLER-SCHIERLEIN* and MICHAEL MEYER

Organisch-chemisches Laboratorium der Eidgenössischen Technischen Hochschule, CH-8092 Zurich, Switzerland

LUCIANO CELLAI, SILVIO CERRINI, DORIANO LAMBA and ANNALAURA SEGRE

Istituto di Strutturistica Chimica "Giordano Giacomello", CNR I-00016 Monterotondo Stazione, Roma, Italy

WALTER FEDELI

Università dell'Aquila, I-67100 L'Aquila, Italy

MARIO BRUFANI

Gruppo di Chimica Biologica e Strutturistica Chimica, Università "La Sapienza" Piazza A. Moro 5, I-00185, Roma, Italy

(Received for publication June 19, 1984)

The relative and absolute configurations of naphthomycin A were elucidated by an X-ray structural analysis of a methylation product, 25-*O*-methylnaphthomycin A iminomethyl ether. The absolute configuration was confirmed by degradation (O_3, $NaBH_4$) to (*S*)-butane-1,2,4-triol.

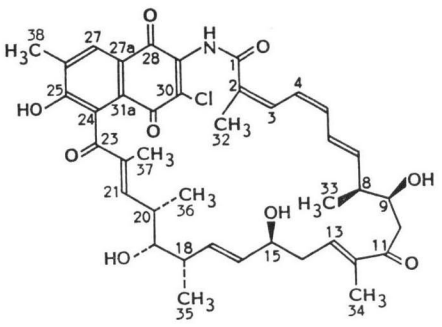
The constitutional formula of the ansamycin antibiotic naphthomycin A (**I**)[2] follows from a detailed NMR study[3] and chemical degradation[4,5]. The configurations of most of the double bonds were elucidated by NMR spin decoupling experiments[3,6]. However, nothing was known up to now of the configuration at the six centers of chirality.

Naphthomycin A itself gave very thin needles of wax-like consistency, unsuitable for an X-ray investigation. Finally we succeeded in the preparation of a dimethyl derivative, 25-*O*-methylnaphthomycin A iminomethyl ether (**II**), which gave suitable crystals from water-acetonitrile at $-10°C$. Crystal data are as follows: $C_{42}H_{50}NO_9Cl \cdot 2H_2O$, formula weight 784.34; orthorhombic, space group $P2_12_12_1$, $a=12.565$, $b=14.410$, $c=24.239$ Å, $Z=4$, $D_c=1.19$ gcm^{-3}; 3337 unique intensity data for $2\theta \leq 116.0°$ were collected on an automatic four-circle diffractometer, with graphite-monochromated CuKα radiation, using the θ–2θ scanning technique.

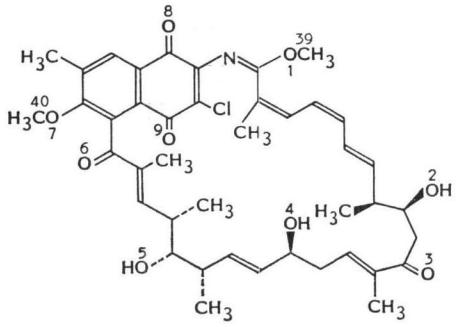
The crystal used for the data collection did not undergo any appreciable decay nor X-ray damage. Lorentz and polarization corrections, but no absorption correction, were applied. Of the unique intensity data 2898 reflections with $I \geq \sigma(I)$ were used in the structure refinement.

The structure was solved by direct methods[7~9]. Refinement was carried out by least-squares calculations in the block diagonal (9×9) approximation. The oxygen atoms of the two co-crystallized

† For preceding paper see ref[1].



I



II

The numbering of the ansa system of **II** is the same as for **I**. In addition the numbering of the extra CH_3 groups and of the O atoms as used in the X-ray analysis are given.

water molecules were located in the difference Fourier map. The contributions of 20 hydrogen atoms having stereochemically constrained positions were included in the final refinement calculations, but with their positional and isotropic thermal parameters (equal to that of the carrier atoms) fixed.

In order to assign the absolute configuration of the molecule, least-squares refinements were carried out for the two enantiomeric forms, taking into account the anomalous dispersion effect of the chlorine and oxygen atoms. The ratio of the Rw values is 1.044 which allows one to assign the absolute configuration at a very high level of confidence[10]. The final conventional discrepancy indices are R= 0.085 (Rw=0.087). The final atomic coordinates, bond distances, and valence angles are deposited at the Crystallographic Data Center, Cambridge, England.

Fig. 1 shows a perspective drawing of the molecular structure of **II**. The absolute configuration is reported in formula **II** for the iminomethyl ether and in formula **I** for naphthomycin A. The absolute

Fig. 1. Perspective view of the molecule of **II**.

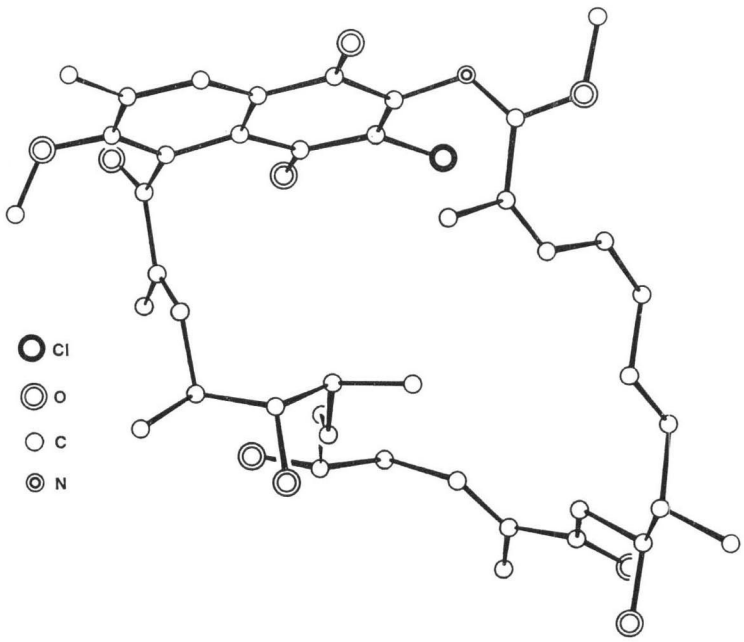
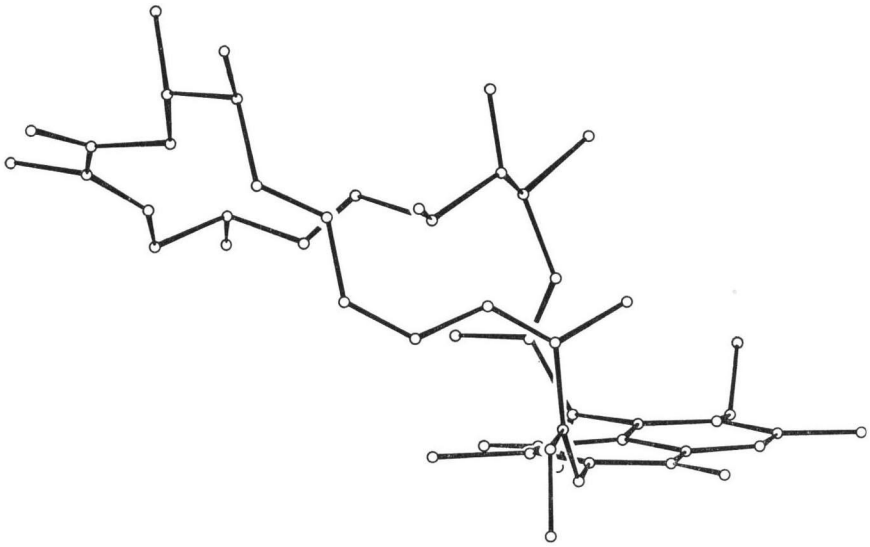


Fig. 2. Conformational view of **II** showing the chair-like arrangement of the ring systems.

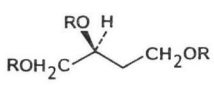


configuration is *S* for all six centers of chirality, *i.e.* C(8), C(9), C(15), C(18), C(19) and C(20). The C(2) and C(4) double bonds have the *Z* configuration, whereas the C(6), C(12), C(16) and C(21) double bonds as well as the C=N double bond in **II** have the *E*-configuration. This structure is fully consistent with that deduced from ^{1}H NMR[3,6] and degradation studies[4,5].

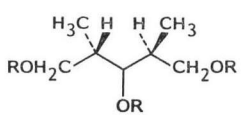
The sequences N=C(1)−C(2)=C(3) and C(20)−C(21)=C(22)−C(23) at the junction of the ansa chain with the chromophore are almost parallel to one another, and both are nearly perpendicular to the mean plane of the naphthoquinone moiety. The central part of the ansa ring spanning from C(4) to C(19) takes a semicircular shape. The mean plane of this sequence makes an angle of 16° with that of the chromophore ring system. As a result of these geometrical features, the molecule of **II** displays a chair-like shape (Fig. 2).

An independent determination of the absolute configuration was carried out by a chemical degradation of naphthomycin A with ozone. The crude ozonolysis mixture was reduced with sodium borohydride. Three aliphatic alcohols (**III, V** and **VII**) could be isolated from the mixture and were subsequently characterized as the respective acetylation products (**IV, VI** and **VIII**). Compound **IV** showed the spectroscopic characteristics of 1,2,4-triacetoxybutane (**IV**) and was identical with a sample prepared by reduction of dimethyl (*S*)-malate and subsequent acetylation. The coincidence of the optical rotations of the two samples confirmed the (*S*) chirality at C(15) of naphthomycin A as deduced from the X-ray analysis.

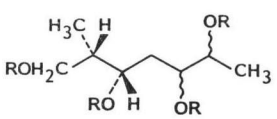
A second degradation product (**VI**) was an optically active triacetate of 2,4-dimethylpentane-1,3,5-



III R = H
IV R = CH_3CO



V R = H
VI R = CH_3CO



VII R = H
VIII R = CH_3CO

triol derived from C(17) to C(21) of naphthomycin A, the (2*S*,4*S*)-chirality of which follows from the X-ray structure.

The third degradation product was a mixture of diastereomeric 2-methyl-1,3,5,6-tetrahydroxyheptanes (**VII**, acetate **VIII**), since the reduction of the ozonide (C(12)) and the keto group (C(11) of naphthomycin A) took a non-stereospecific course. However, by careful chromatography of the tetraacetate (**VIII**), a single compound was isolated from the mixture. The chirality at C(2) and C(3) must both be *S* (according to the X-ray structure of **II**), whereas that at C(5) and C(6) is not determined. The compounds **VI** and **VIII** can serve as references for the determination of the configuration of other naphthomycin related ansamycins (see a forthcoming paper).

Experimental

General

^{1}H NMR spectra were run at 300 MHz with Fourier transformation with a Bruker WM 300 spectrometer. Optical rotations were measured with a Perkin-Elmer 141 polarimeter.

Degradation of Naphthomycin A

An O_3 containing oxygen stream was bubbled through a solution of 116 mg (0.161 mmol) naphthomycin A in 8 ml absolute EtOH at −78°C. After 2 hours the cold solution was dropped into 30 ml of H_2O containing 600 mg (15.85 mmol) $NaBH_4$. After standing at room temp overnight the solution was acidified (pH 3) with 2 N H_2SO_4 and then neutralized with 2 N NaOH. After evaporation under reduced pressure the residue was extracted with 50 ml MeOH, filtered, and the filtrate evaporated. The crude product was chromatographed on Sephadex LH-20 with MeOH as eluent, in order to remove boric acid. The mixture containing three components (TLC) was separated into 3 fractions on a silica gel column (10 g) with EtOAc - MeOH, 9: 1.

(2*S*,4*S*)-2,4-Dimethyl-1,3,5-trihydroxypentane (**V**)

The first fraction gave 13.6 mg (0.092 mmol) of a colorless oil, Rf 0.23 (EtOAc - MeOH, 9: 1); $[\alpha]_D^{24}$ +5.15° (*c* 1.4, MeOH); ^{1}H NMR (CD_3OD) 0.879 (3H, d, J=6.8), 0.881 (3H, d, J=6.8), 1.70~1.86 (3H, m), 3.48 (1H, dd, J_1=10.6, J_2=6.1), 3.57 (3H, m), 3.72 (1H, dd, J_1=10.6, J_2=5.3).

(2*S*,3*S*)-2-Methyl-1,3,5,6-tetrahydroxyheptane (**VII**)

The second fraction gave 10.5 mg crude **VII** as an oil, Rf 0.12 (EtOAc - MeOH, 9: 1), which was acetylated without further purification (see below).

(*S*)-1,2,4-Trihydroxybutane (**III**)

The third fraction, 6.4 mg of colorless liquid, contained **III** (Rf 0.08) as the main constituent and minor amounts of **VII**. The sample was acetylated without further purification.

Acetylation Products

The alcohols **III**, **V** and **VII** were acetylated each with 1 ml pyridine and 1 ml acetic anhydride at room temp overnight. After evaporation under reduced pressure the products were purified by preparative TLC.

(2*S*,4*S*)-2,4-Dimethyl-1,3,5-triacetoxypentane (**VI**)

From 13.2 mg **V** 12.2 mg acetylation product **VI** was obtained as a colorless liquid, Rf 0.39 (TLC, $CHCl_3$ - EtOAc, 9: 1); $[\alpha]_D^{24}$ −3.76°; $[\alpha]_{365}^{24}$ −10.08° (*c* 1.2, CH_2Cl_2); IR ($CHCl_3$) 1735, 1465, 1382, 1240, 1210, 1040, 1025, 990, 970 (only strong and medium peaks are given); MS *m/z* 275 (M+H, 0.1), 259 (0.2), 242 (0.5), 215 (1), 173 (67), 154 (4), 145 (1), 131 (97), 113 (100), 112 (47), 103 (47), 43 (88), 28 (37); ^{1}H NMR ($CDCl_3$) 0.93 (3H, d, J=6.9), 0.99 (3H, d, J=6.9), 2.04 (3H, s), 2.05 (6H, s), 2.0~2.3 (2H, m), 3.87 (1H, dd, J_1=11.1, J_2=6.2), 3.92~4.01 (2H, m), 4.02 (1H, dd, J_1=11.1, J_2=4.5), 4.99 (1H, dd, J_1=8.6, J_2=3.7).

(2*S*,3*S*)-2-Methyl-1,3,5,6-tetraacetoxyheptane (**VIII**)

The sample prepared from 10 mg **VII** gave a single zone by preparative TLC. A slower moving and

a faster moving half of the zone were scratched out separately. The latter one was eluted with EtOAc and again purified by preparative TLC. Finally 1.4 mg of a colorless oil was obtained, Rf 0.37 (TLC, $CHCl_3$ - EtOAc, 9: 1); $[\alpha]_D^{24}$ $-8.6°$; $[\alpha]_{365}^{24}$ $-16.4°$ (*c* 0.14, CH_2Cl_2); IR ($CHCl_3$) 1735, 1460, 1435, 1375, 1240, 1025; ^1H NMR ($CDCl_3$) 0.95 (3H, d, J=6.9), 1.20 (3H, d, J=6.4), 1.82 (1H, ddd, J_1=14.8, J_2=6.8, J_3=4.2), 1.90 (1H, ddd, J_1=14.8, J_2=6.8, J_3=1.0), 2.03 (3H, s), 2.041 (3H, s), 2.045 (3H, s), 2.07 (3H, s), 2.00~2.13 (1H, m), 3.90 (1H, dd, J_1=11.0, J_2=6.2), 3.97 (1H, dd, J_1=11.0, J_2=7.2), 4.95 ~5.07 (3H, m).

(*S*)-1,2,4-Triacetoxybutane (**IV**)

From 6.4 mg of crude **III** 2.7 mg of purified **IV** was obtained as a colorless oil. $[\alpha]_D^{24}$ $-13.5°$; $[\alpha]_{365}^{24}$ $-31.9°$ (*c* 0.27, CH_2Cl_2); IR ($CHCl_3$) 1740, 1440, 1375, 1240, 1050. A reference sample[11] prepared by reduction of dimethyl (*S*)-malate and subsequent acetylation had $[\alpha]_D^{24}$ $-12.0°$; $[\alpha]_{365}^{24}$ $-31.8°$ (*c* 2.0, CH_2Cl_2). Rf (TLC) and IR coincident with those of the product **IV** from degradation.

Preparation of 25-*O*-Methylnaphthomycin A Iminomethyl Ether (**II**)

Naphthomycin A (500 mg, 0.64 mmol) was stirred for 24 hours in the dark with 22 ml of CH_3I in 20 ml absolute MeOH and 35 ml $CHCl_3$ in the presence of 2.5 g Ag_2O at room temp. The mixture was filtered through Celite and the filtrate evaporated. By chromatography on 40 g of silica gel 60 with EtOAc as the eluent the mixture was separated into two major compounds. The first one, 210 mg, was purified by a second chromatography on 10 g of silica gel (EtOAc) and crystallization from MeOH - H_2O, 8: 2 to yield 100 mg of pure **II**. ^1H NMR ($CDCl_3$) 3.73 (3H, s), 4.00 (3H, s); the phenolic OH and the NH signals are lacking. The remainder of the spectrum is very similar to that of naphthomycin A[3]. Crystals for the X-ray analysis were grown from CH_3CN - H_2O.

The second compound showed 3 OCH_3 signals in the ^1H NMR: 3.25, 3.86 and 4.14 ppm. The nature of this product was not further investigated.

Acknowledgment

NMR spectra were taken by Miss B. BRANDENBERG, MS by Mrs. L. GOLGOWSKI. Authentic (*S*)-1,2,4-Triacetoxybutane was prepared by Mr. U. BAHNMÜLLER. These contributions are gratefully acknowledged.

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