THE STRUCTURE OF VIRUSTOMYCIN A

Sir:

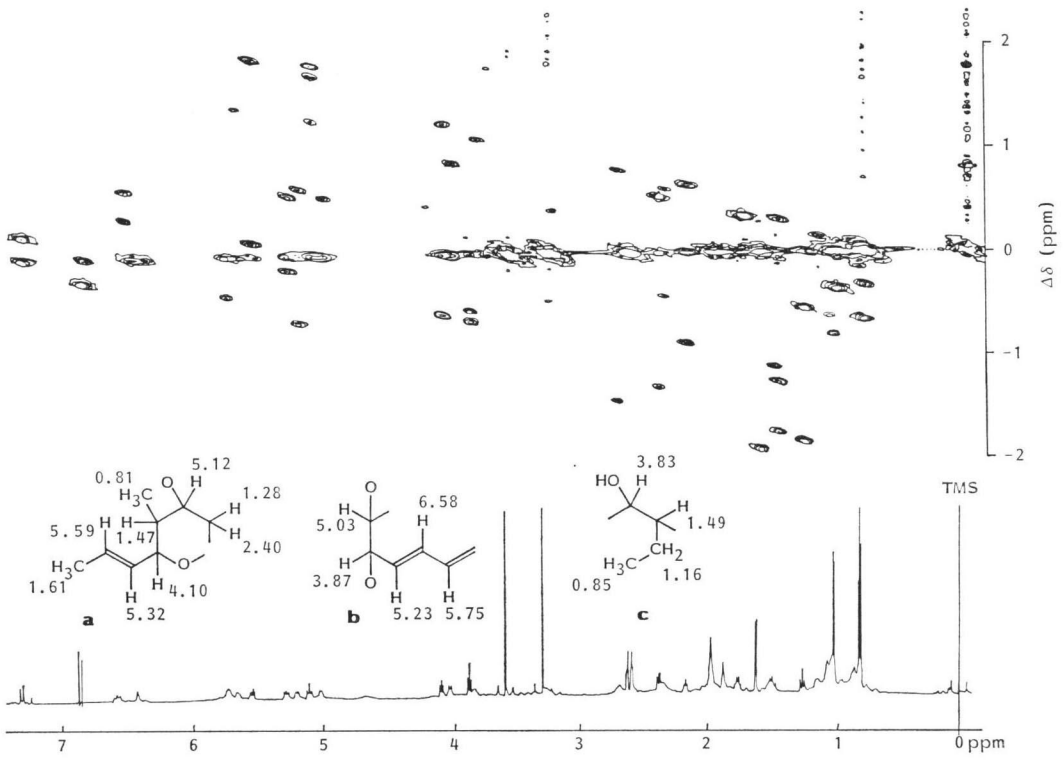
A novel antibiotic, virustomycin A (formerly, AM-2604 A), produced by *Streptomyces* sp., shows potent activity against RNA and DNA viruses and trichomonads[1]. During a study of the structure, we realized that the antibiotic is a novel 18-membered macrolide constructed from flavensomycinoic acid and the aglycone of concanamycin A. In the present paper, the structural elucidation of virustomycin A is described.

The molecular formula of virustomycin A (**I**) was estimated to be $C_{48}H_{71}NO_{14}$ by mass spectrometry. Two notable fragment ion peaks were observed in the high resolution EI-MS spectrum of **I** and whose formulas were deduced to be $C_{38}H_{54}O_6$ (*m/z* obsd 606.389, calcd 606.392) and $C_9H_9NO_5$ (*m/z* obsd 211.048, calcd 211.048). In addition, an ion at *m/z* 638 (606+MeOH) was observed in the EI-mass spectrum. On the other hand, a fast atom bombardment mass spectrometry gave the molecular ion peak at *m/z* 885, which required the addition of two mol of water to the sum of *m/z* 638 plus 211.

The ^1H NMR spectrum of **I** in $CDCl_3$ with D_2O showed signals of 65 protons, and their coupling systems were studied by spin-echo-correlated spectroscopy[2] at 600 MHz as shown in Fig. 1. The method is very powerful to resolve the coupling systems in a complicated structure. Although not all coupling systems were defined, three characteristic partial structures (**a**, **b**, **c**) were deduced to be as shown in Fig. 1. In partial structure **a**, the chemical shifts of the *gem*-coupled methylene protons (δ_H 1.28 and 2.40; J=12 Hz) were attributed to an axial and equatorial proton in a 6-membered ring, respectively. No anomeric carbons were detected in the ^{13}C NMR spectrum of **I**, but a quaternary dioxygenated carbon (δ_C 99.7) was observed and this suggested the presence of a 6-membered hemiketal ring. These diagnostic NMR data suggested that **I** is similar to concanamycin A (**II**)[3].

Treatment of **I** with 0.03 N NaOH in aqueous methanol, gave two products, a macrocyclic compound and a water soluble one. The spectral

Fig. 1. The ^1H NMR and spin-echo-correlated spectra of virustomycin A at 600 MHz in $CDCl_3$, and the resulting characteristic partial structures.



data of the macrocyclic compound (**III**), *i.e.*, EI-MS *m/z* 674 (M^+); IR ν_{max}^{KBr} cm^{-1} 3600~3400, 1690, and 1620, suggested that **III** is identical with the anhydroaglycone of **II**. In order to confirm this, **III** was treated with acetic anhydride in pyridine to give its triacetate (**IV**). The ^1H and ^{13}C NMR data of **IV** were in complete agreement with the published data of the triacetylanhydroaglycone of **II**[4]. Furthermore, the EI-MS spectrum of **IV** was superimposable with that of an authentic sample.

The other alkaline degradation product, water soluble compound (**V**), yellowish needles, mp 254°C (dec.); IR ν_{max}^{KBr} cm^{-1} 3259, 3100, 1700, 1655, 1600; EI-MS *m/z* 211 (M^+); has the molecular formula of $C_9H_9NO_5$. The ^{13}C NMR spectrum at 22.6 MHz of **V** in D_2O showed overlapping peaks of two methylene carbons (δ_C 31.2), a non-protonated sp^2 carbon (δ_C 112.1), two protonated sp^2 carbons (δ_C 131.7 and 137.6), amide carbonyl and carboxylic acid carbons (δ_C 168.1 and 173.8), and two coincident carbonyl carbons (δ_C 200.8). Since the protons of the methylenes were also observed as overlapping signals (δ_H 2.17, 4H) in the ^1H NMR spectrum, the presence of a symmetrical moiety was suggested. The chemical shift of the non-protonated sp^2 carbon could be attributed to the C-2 of 2-aminocyclopentane-1,3-dione[5]. If this ring system is present, the remaining structure is assigned as -COCH=CHCOOH. These assumptions suggested that **V** was probably identical with flavensomycinoic acid[6], and this was confirmed by the EI-mass data of **V** as summarized in Fig. 2.

The presence of a flavensomycinoic acid moiety was supported by the ^1H NMR spectral data of its methyl ester (**VI**), yellowish needles, mp 233°C (dec.), obtained by the treatment of **I** in methanol with Amberlite IR 120B(H^+) under reflux followed by recrystallization from benzene - hexane. The ^1H NMR spectral data of **VI** is consistent with those of flavensomycinic acid. Therefore, **I** is constructed from flavensomycinoic acid and the aglycone of concanamycin A.

The position of substitution of the flavensomycinoic acid moiety to the macrocyclic portion was determined by comparison of the chemical shift of the C-4 proton in the pyran ring system of **I** and **II**. The signal of this proton appeared at δ_H 5.12 and 3.76 in the spectra of **I** and **II**[8], respectively. The downfield shift of 1.36 ppm indicated that the flavensomycinoic acid is attached to the C-4 oxygen since a displacement of this magnitude normally accompanies acylation of a hydroxyl group.

From all the results described above, the structure of virustomycin A (**I**) was determined to be as depicted in Fig. 3. Although two other antibiotics, flavensomycin[6] and prasinon B[7], have been known to contain a flavensomycinoic acid moiety, the structures of these antibiotics have not been determined. This is the first report which describes the gross structure of an antibiotic containing this acid moiety.

Other antibiotics know to contain the 2-aminocyclopentane-1,3-dione moieties are manumycin[5],

Fig. 2. The structures of **V** (flavensomycinoic acid) and **VI** (flavensomycinic acid), and the summarized EI-MS data of **V**.

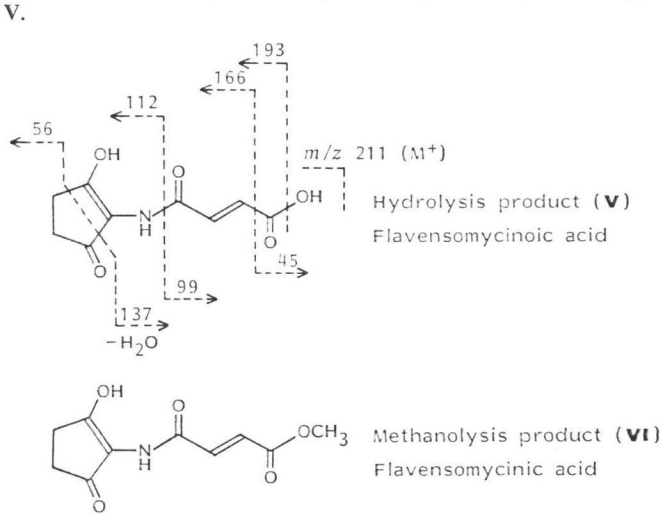
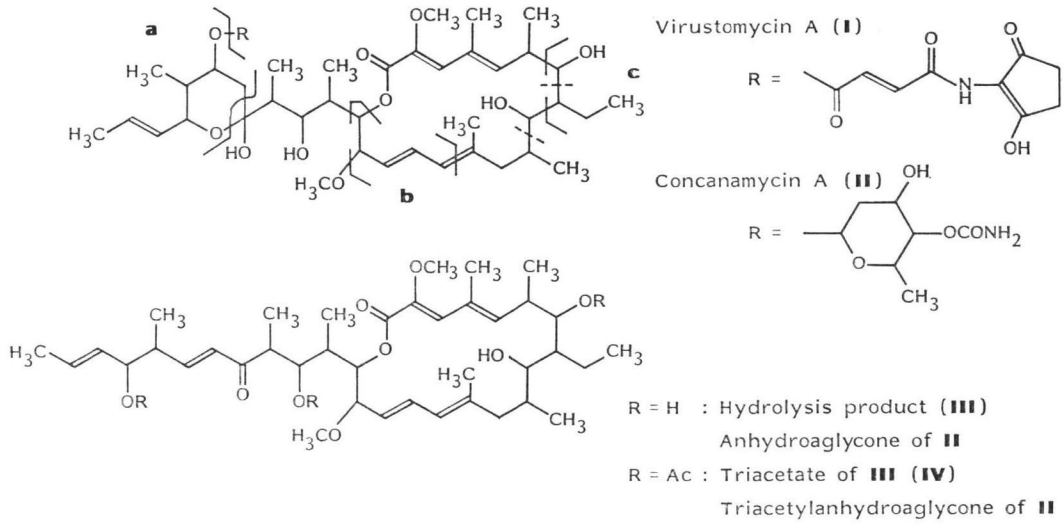


Fig. 3. The structures of virustomycin A and related compounds.



limocrocin[9], moenomycin[10], asukamycin[11], AM-6201[5] (reductiomycin) and senacarcin A[12]. Since the moiety is unusual, although found in various types of antibiotics, it is an interesting target for biosynthetic investigation.

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SATOSHI ŌMURA
NOBUTAKA IMAMURA
KIYOIZUMI HINOTOZAWA
KAZUHIKO OTOGURO
GABOR LUKACS*
RAMINE FAGHIH*
RICHARD TOLMANN**
BYRON H. ARISON**
JACK L. SMITH**

School of Pharmaceutical Sciences,
Kitasato University
and The Kitasato Institute,
Minato-ku, Tokyo 108, Japan
*Institut de Chimie des Substances Naturelles
du C.N.R.S.,
91190 Gif-Sur-Yvette, France
**Merck Sharp & Dohme
Research Laboratories,
Rahway, New Jersey, 07065, U.S.A.

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