A2315, NEW ANTIBIOTICS PRODUCED BY *ACTINOPLANES PHILIPPINENSIS*. 2. STRUCTURE OF A2315A

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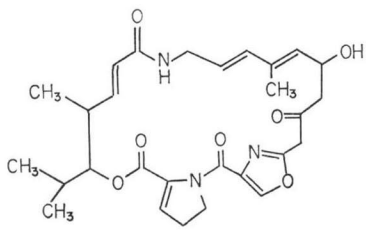
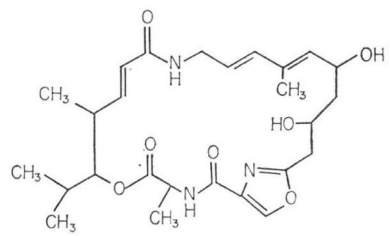
Antibiotic A2315A is a new member of the virginiamycin family of antibiotics. Physical and chemical data indicate that it is related to ostreogrycin A. Structure **II** is proposed for A2315A.

From a strain of *Actinoplanes philippinensis* COUCH, a new antibiotic has been isolated and designated A2315A.[1] It is active mainly against Gram-positive organisms. Consideration of the physical and chemical data suggested that it might belong to the virginiamycin family of antibiotics.[2] Closer scrutiny of its properties revealed a strong resemblance to ostreogrycin A (**I**).[3] The latter is identical to virginiamycin M and several other antibiotics. The nomenclature in this area has been reviewed.[4] We propose structure II for A2315A from results set forth below.



I



II

A2315A is a neutral compound with $[\alpha]_D^{27}$ −132° (*c* 0.375, MeOH), ν_{max} ($CHCl_3$) 3623, 3413, 1730, 1672, 1639 (infl.), 1626, 1600 cm^{-1}, and λ_{max} (EtOH) 214 nm (log ε 4.55). No change in the UV spectrum was observed upon adding acid or base to the solution. All attempts to crystallize A2315A have failed thus far. Purified samples of the antibiotic were obtained by column chromatography (silica gel, EtOAc - EtOH, 95: 5). The mass spectrum indicated a molecular weight of 503, which corresponded to $C_{26}H_{37}N_3O_7$ (Found: 503.26380; required: 503.26315). This formula requires the presence of ten double bonds or rings. Amino acid analysis indicated ammonia (1.02 moles), alanine (0.85 moles) and a trace amount of glycine (0.021 moles). The NMR spectrum (Fig. 1A) exhibited several C-methyl groups, one of which (δ 1.73) appeared to be on a double bond; several protons in the olefinic region, a low-field doublet centered at δ 7.42; and a sharp singlet at δ 8.08.

A2315A reacted with acetic anhydride-pyridine to form a diacetate derivative. It did not react with $NaBH_4$ in ethanol, suggesting that the carbonyl band at 1730 cm^{-1} in the IR was due to an ester or lactone group. Catalytic hydrogenation gave a hexahydro derivative.

Amino acid analysis of the hexahydro derivative gave ammonia (0.83 moles), alanine (1.02 moles),

glycine (0.021 moles), and serine (0.006 moles).

Mild base hydrolysis (0.05 N methanolic NaOH, room temperature) gave an acid, pKa (66% DMF) 5.35 (Apparent Molecular Weight 552); ν_{max} (Nujol) 3322, 1724, 1656, 1626 (infl.), 1595 cm^{-1}; λ_{max} (EtOH) 227 nm (ε 16,300/200 M.W.) Treatment of the acid with trimethyloxonium fluoroborate-diisopropylethylamine in CH_2Cl_2[5] for 2 hours gave a methyl ester, $\delta(CDCl_3)$ 3.78 (CH_3O_2C) ppm. Neither the acid nor the methyl ester gave a satisfactory mass spectrum. More vigorous hydrolysis (2.5 N methanolic NaOH, reflux) gave a mixture of products. TLC (cellulose, *n*-PrOH - CH_3CN - H_2O, 1:1:1) revealed the presence of two ninhydrin-positive spots with the same Rf values as alanine and glycine.

The foregoing properties are similar in many respects to those described for ostreogrycin A (**I**).[3] Substitution of alanine for the dehydroproline moiety of ostreogrycin A accounted for several apparent differences. In addition, the UV absorption properties of A2315A and ostreogrycin A differ in that the former lacks the longer wavelength inflection at 272 nm, and its spectrum does not change with the addition of base. To accommodate this fact and the observation that A2315A formed a diacetate, as well as to arrive at the observed molecular formula, we postulated that A2315A contains a hydroxyl group in place of the keto group of ostreogrycin A. Thus, structure **II** was considered for A2315A.

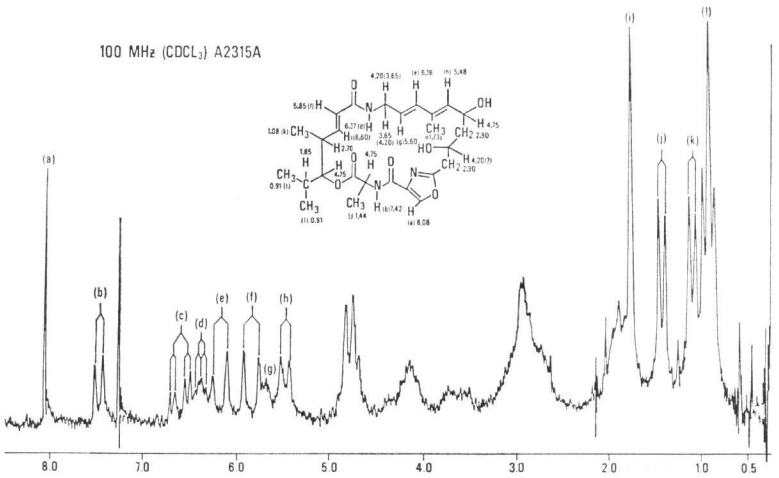
To provide further evidence for the proposed structure, a detailed analysis of the NMR spectrum of A2315A was undertaken. The one-proton singlet at δ 8.08 (a) (Fig. 1A) was assigned to the proton on the oxazole ring. This peak appears at δ 7.84 in the spectrum of ostreogrycin A.[3b] The doublet at δ 7.42 (J=8 Hz) (b) was assigned to the amide proton of the alanine moiety. As such it would be expected to undergo exchange. Addition of D_2O or CD_3OD caused little if any change in appearance of the doublet. However, addition of a trace of DCl resulted in its complete disappearance. Irradiation at δ 4.75 caused this doublet to collapse to a singlet and, in addition, collapsed the methyl doublet at δ 1.44 (J=7 Hz) (j) to a singlet. Thus, the alanine methine proton is one of the protons contributing to the group of peaks centered at δ 4.75.

The proton appearing as a quartet at δ 6.60 (J=16, J=6 Hz) (c) is coupled to an olefinic proton at δ 5.85 (doublet, J=16 Hz) (f). Irradiation at δ 2.70 removed the small coupling (J=6 Hz) and collapsed the methyl doublet at δ 1.08 (J=7 Hz) (k) to a singlet. These observations are consistent with the presence of a double bond flanked by a carbonyl group and a carbon atom bearing a methyl group. The methine proton attached to the carbon atom bearing the methyl group must contribute to the broad, unresolved absorption in the region of δ2.70.

An absorption pattern which appears to be centered at δ 6.37 (d), and which is partially obscured by a doublet centered at δ 6.18 (e), was shifted downfield to δ 7.01 by the addition of DMF-d_7-D_2O. Under these conditions, a triplet (J=5 Hz) was evident. Addition of CD_3OD resulted in disappearance of the signal. Accordingly, this triplet was assigned to the second amide proton. Irradiation at δ 6.37 caused a change in multiplicity of the multiplet centered at δ 3.65 and of the signal at δ 4.32 which is believed to be part of a second multiplet centered at about δ 4.20. Conversely, irradiation at δ 3.65 or δ 4.20 resulted in collapse of the δ 6.37 triplet. The multiplets at δ 3.65 and approximately 4.20 are assigned to the protons of the methylene adjacent to the amide nitrogen. The δ 4.20 multiplet is partially obscured by another signal at δ 4.12.

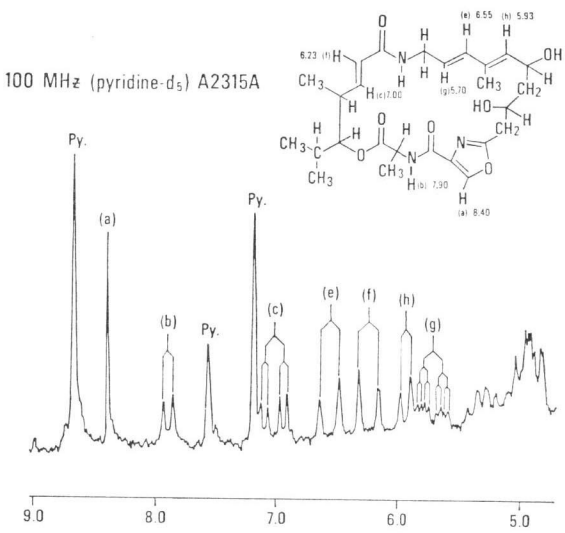
In the deuterochloroform spectrum (Fig. 1A), two doublets are evident at δ 6.18 (J=15 Hz) (e) and 5.48 (J=9 Hz) (h) and another signal underlies the high-field side of the δ 5.85 doublet (f) and the doublet at δ 5.48. In pyridine-d_5 (Fig. 1B), these peaks are separated and a doublet of quartets appears at δ 5.70

Fig. 1A. NMR Spectrum of A2315A ($CDCl_3$)



(g). Irradiation at δ 5.70 collapsed the doublet at δ 6.55 (e) (which corresponds to the δ 6.18 doublet in $CDCl_3$) to a singlet. In $CDCl_3$, irradiation at δ 3.60 and 4.15 caused a change in multiplicity of the visible portion of the doublet of quartets. Therefore, the latter and the δ 6.18 doublet are assigned to the protons of the unsubstituted double bond. The remaining olefinic proton at δ 5.48 is coupled to a proton which accounts for part of the absorption at δ 4.75 and which is assumed to be attached to the carbon atom bearing the allylic hydroxyl group.

Fig. 1B. NMR Spectrum of A2315A (pyridine-d_5)



The proton attached to the carbon atom bearing the other hydroxyl group is believed to contribute to the broad absorption at δ 4.20. The protons of the adjacent methylene groups are believed to give rise to the broad area of absorption at about δ 2.90. The proton attached to the carbon atom bearing the isopropyl group appears to contribute to the multiple peaks at δ 4.75. Irradiation at that point sharpened a multiplet at about δ 1.85 which may be assigned to the methine proton of the isopropyl group. The triplet of peaks at δ 0.91 (1) due to the latter is resolved into two doublets at 220 MHz.

The high-resolution mass spectrum of the hexahydro derivative provided additional structural evidence. Exact mass measurements are given in Table 1. Expulsion of carbon dioxide from the molecular ion with hydrogen transfer is considered to yield a ring-opened species[3b] as in **III** which appears at *m/e* 465 in the mass spectrum (Fig. 2). Of particular interest are the ions at *m/e* 224, 238, 268, 282 and 312. They correspond to those produced in the fragmentation of the "perhydro A" derivative of osterogrycin A in which the double bonds and carbonyl group are reduced and the oxazole ring opened.[3b]

Fig. 2. Mass spectrum of hexahydro-A2315A

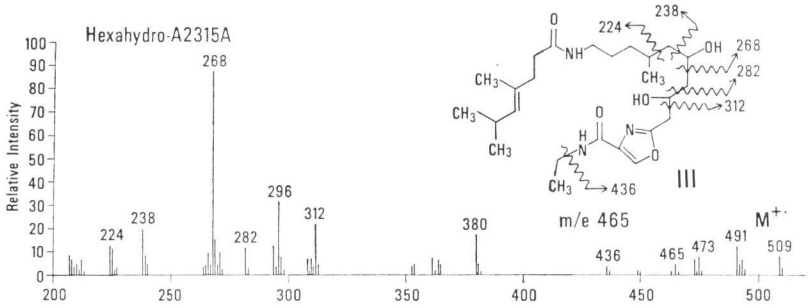


Table 1. Exact mass measurements of some peaks in the mass spectrum of hexahydro-A2315A.

Peak	Composition	Calculated Mass	Empirical Sum
509	$C_{26}H_{43}N_3O_7$	509.30989	509.31010
491	$C_{26}H_{41}N_3O_6$	491.29750	491.29954
473	$C_{26}H_{39}N_3O_5$	473.29007	473.28897
465	$C_{25}H_{43}N_3O_5$	465.3220	465.3203
436	$C_{23}H_{38}N_3O_5$	436.28312	436.28115
312	$C_{18}H_{34}NO_3$	312.25656	312.25387
282	$C_{17}H_{32}NO_2$	282.24559	282.24330
268	$C_{16}H_{30}NO_2$	268.22956	268.22765
238	$C_{15}H_{28}NO$	238.21715	238.21709
224	$C_{14}H_{26}NO$	224.20282	224.20144

Finally, treatment of A2315A with MnO_2 gave a product with properties consistent with the presence of a dienone moiety formed by oxidation of the allylic hydroxyl group indicated in structure **II**.

After completion of this work, the structures of madumycin I and II were reported.[6] They differ from A2315A only in regard to the hydroxyl groups. Madumycin I is the diketo analog of A2315A, and madumycin II is the monoketo analog in which the non-allylic hydroxyl group is in the higher oxidation state.

Experimental

UV spectra were measured on a Cary Model 15 recording spectrometer. IR spectra were obtained on a Perkin-Elmer 221 spectrometer. NMR spectra were recorded on a Varian Associates HA–100 spectrometer. Chemical shifts are reported as δ values in ppm relative to TMS equal to zero. Mass spectra were recorded on a CEC Model 21–110A–1 spectrometer.

Acetylation of A2315A.

A sample of the antibiotic (104 mg) was treated with acetic anhydride (1 ml) and pyridine (1 ml) for 20 hours at room temperature. The mixture was evaporated to dryness under reduced pressure. The residue was taken up several times in CH_3OH and then Et_2O and evaporated to dryness to give an amorphous residue of the diacetate derivative which could not be induced to crystallize. It appeared as a single spot on TLC (4: 1 EtOAc - CH_3OH). NMR ($CDCl_3$) δ 2.00 (s), 2.08 (s) ppm (CH_3CO). Mass spectrum: *m/e* 587.28425 (M^+); required for $C_{30}H_{41}N_3O_9$, 587.28426.

Catalytic hydrogenation of A2315A.

A solution of A2315A (250 mg) in ethanol (14 ml) was stirred in a hydrogen atmosphere with 5%

Pd on charcoal (100 mg) catalyst until the uptake of hydrogen ceased (approx. 1 hour). The mixture was filtered through Celite and the filtrate was evaporated to yield a colorless foam (221 mg). UV: λ_{max}^{EtOH} 204 nm (log ε 4.15). IR: $\nu_{max}^{CHCl_3}$ 3571, 3356, 1715, 1653, 1587 cm^{-1}. Mass spectrum: *m/e* 509.30989 (M^+); required for $C_{26}H_{43}N_3O_7$, 509.31010. The NMR spectrum ($CDCl_3$) lacked the methyl peak at δ 1.73 ppm as well as peaks in the olefinic region.

MnO_2 Oxidation of A2315A.

To a solution of A2315A (1.00 g) in $CHCl_3$ (150 ml) was added activated MnO_2 (15 g). The mixture was allowed to stir overnight at room temperature. Filtration through Celite and evaporation of the solvent gave 0.52 g of white foam which was chromatographed (Merck silica gel, 50 g). Elution with EtOAc gave 0.35 g of product. UV: λ_{max}^{EtOH} 212 (log ε 4.48) and 281 (log ε 4.29) nm. Mass spectrum: *m/e* 501.24758 (M^+); required for $C_{26}H_{35}N_3O_7$, 501.24728. In the NMR spectrum ($CDCl_3$) the peak due to a methyl group on a double bond was shifted downfield to δ 2.15 ppm.

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