NEW ANTIBIOTICS FROM *ACTINOPLANES* STRAINS
STRUCTURE OF A 17002 C

E. MARTINELLI, L. F. ZERILLI, G. VOLPE, H. PAGANI and B. CAVALLERI

Research Laboratories Gruppo Lepetit S.p.A., Via, Durando, 38–20158 Milano, Italy

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A 17002 C is a new metabolite produced by *Actinoplanes* strains, structurally related to the virginiamycin factor M. On the basis of physico-chemical data, MS, IR, ^{1}H and ^{13}C NMR, structure **I** is assigned to A 17002 C.

In the course of our screening program for new antibiotics from the genus *Actinoplanes* we isolated from soil samples collected in the Indore region (India) two strains, designated A 17002 and A 15104, which produce antibiotics active mainly against Gram-positive bacteria. Taxonomic studies showed that the two strains are new species of *Actinoplanes* differing between them and from previously described strains[1].

From the fermentation broths of each of the two strains crude materials were obtained which, after purification, gave the two series of active compounds listed in Table 1. The correspondence between the four compounds in each series was achieved by comparing their chromatographic behavior in the different solvent systems and their physico-chemical properties. After a preliminary investigation, A 17002 F (A 15104 V) was found to be identical to the previously described macrocyclic lactone antibiotic madumycin II[2] or A 2315 A[3,4]*, while A 17002 C (A 15104 II) was found to be a new antibiotic. This paper deals with the structure determination of A 17002 C, while no studies have been carried out on the structure of A 17002 A (A 15104 I) and A 17002 B (A 15104 X).

Table 1. Chromatographic behavior of antibiotics produced by A 17002 and A 15104 strains*

Solvent system	A 17002 A** A 15104 I	A 17002 B** A 15104 X	A 17002 C A 15104 II	A 17002 F A 15104 V
$CHCl_3 - CH_3OH$ (9:1)	0.63	0.53	0.43	0.19
Benzene – CH_3COCH_3 (1:1)	0.28	0.14	0.29	0.11
Benzene – CH_3COCH_3 (3:2)	0.25	0.10	0.22	0.08
THF – $CHCl_3$ (1:1)	0.31	0.16	0.44	0.23
$CHCl_3 - CH_3COCH_3$ (1:1)	0.30	0.15	0.36	0.27

* TLC performed on Silica-gel Merck F_{254} plates. Spots were detected under UV light at 254 nm or bioautographed on *Staphylococcus aureus*.

** Spots corresponding to these compounds appear blue at 254 nm and light blue at 360 nm.

Structure Determination

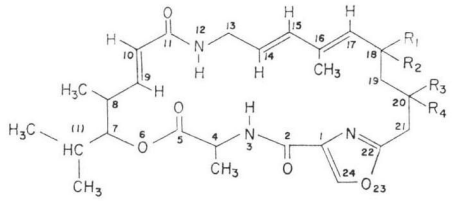
A 17002 C is a white crystalline substance, hygroscopic. By thermal analysis it shows 2.7% of imbibition water and decomposes at 158°C. Elemental analysis gives C=62.65%, H=7.90%, N=8.62%, which accounts for the molecular formula, $C_{26}H_{37}N_3O_6$ (calc. %: C=64.04, H=7.64, N=8.61).

* We thank E. Lilly and Co. (Indianapolis) U.S.A. for providing us a sample of A 2315A.

This elemental composition is confirmed by high resolution mass spectroscopy, ^{1}H and ^{13}C nuclear magnetic resonance. It shows $[\alpha]_D^{20} -21°$ (c 0.95, MeOH) and λ_{max} (95% EtOH): 214 nm, log ϵ= 4.12. The UV maximum does not change upon adding acid or base to the solution. The compound gives negative results to $FeCl_3$, FEHLING, MILLON and 2,4-DNPH tests.

The structure of A 17002 C was investigated by comparing its physicochemical properties, IR, MS, ^{1}H and ^{13}C NMR with those of the reference compound A 17002 F. Fig. 1 shows the structure assigned to A 17002 C (**I**) and that of A 17002 F (**II**).

Fig. 1. Structures of A 17002 C (**I**) and A 17002 F (**II**). The numbering system is that reported for madumycin[2] by Chemical Abstracts.



$R_1 = R_2 = R_3 = H$, $R_4 = OH$ A 17002 C (**I**)
$R_1 = R_3 = H$, $R_2 = R_4 = OH$ A 17002 F (**II**), A 2315 A

Fig. 2. MS spectra of A 17002 C (**I**) and A 17002 F (**II**) at 70 eV in DIS at 200°C.

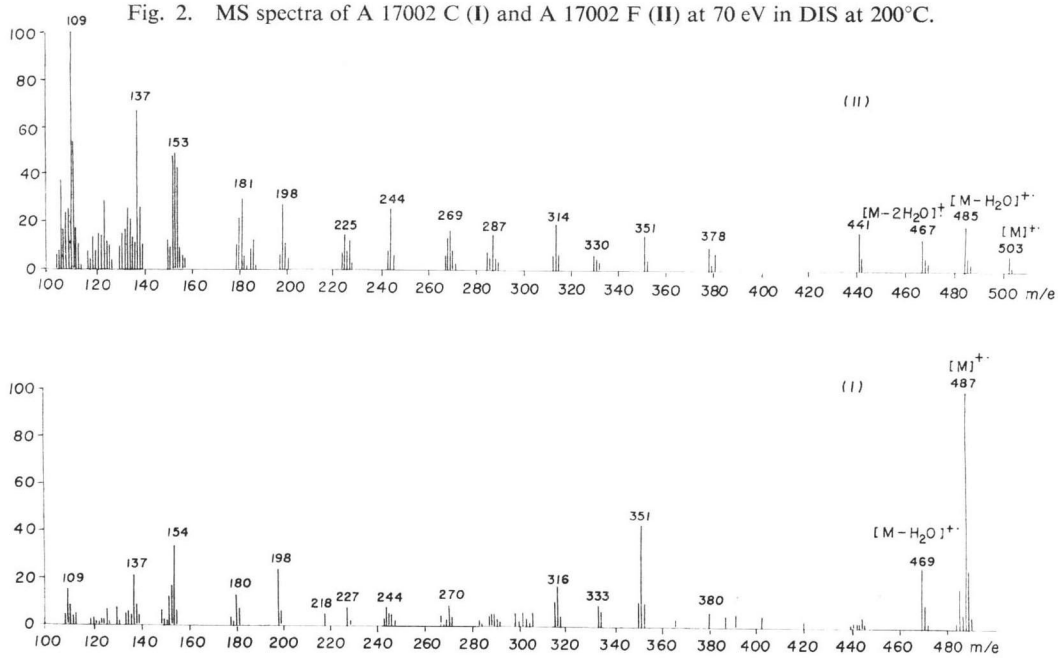


Fig. 3. IR spectrum of A 17002 C (**I**) in $CDCl_3$ solution.

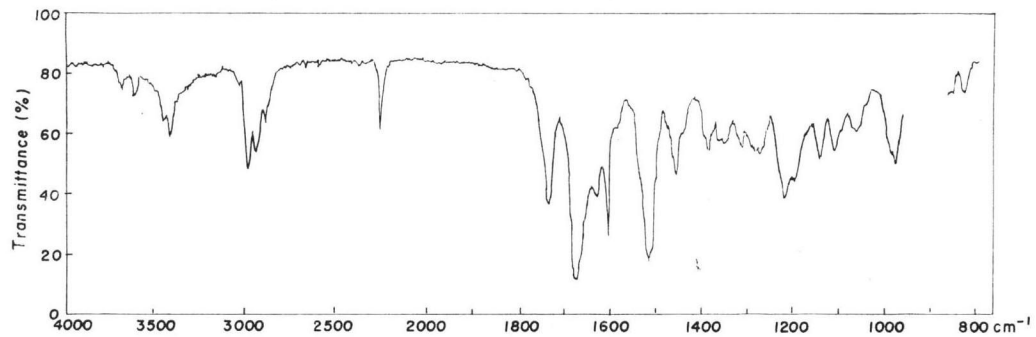


Fig. 2 shows the MS spectra of **I** and **II**; $[M]^{+\cdot}$ at *m/e* 487 is the base peak of **I**, while $[M]^{+\cdot}$ of **II** at *m/e* 503 is only 7% abundant; the difference of 16 a.m.u. between the two products may be due to the lack in **I** of one oxygen. This hypothesis is confirmed by exact mass measurement, which gives the value 487.2689, corresponding to $C_{26}H_{37}N_3O_6$ (required 487.2682), while **II** corresponds to $C_{26}H_{37}N_3O_7$.[3] Another peculiar difference is that in **II** there is loss of two molecules of water, *m/e* 485 (20%) and 467 (14%), attributable to the two OH groups, whereas **I** looses one molecule of water only, *m/e* 469 (25%). This fact and the greater stability of **I** under electron impact suggest that one of the two OH of **II** is lacking in **I**.

Table 2. Selected ions from the mass spectra of hexahydro-**I** and hexahydro-A 2315 A.

Hexahydro-**I** *m/e* (rel. int.)	Hexahydro-A 2315 A[3] *m/e* (rel. int.)	Attributions
493 (70)	509 (9)	$[M]^{+\cdot}$
475 (46)	491 (13)	$[M-H_2O]^{+\cdot}$
— —	473 (8)	$[M-2H_2O]^{+\cdot}$
449 (27)	465 (5)	$[M-CO_2]^{+\cdot}$, *a*
420 (7)	436 (3)	$[a-C_2H_5]^+$
364 (67)	380 (19)	$[a-C_4H_7NO]^{+\cdot}$
296 (100)	312 (24)	$[a-C_7H_9N_2O_2]^+$, *b*
266 (8)	282 (13)	$[b-CH_2O]^+$
252 (15)	268 (100)	$[b-C_2H_4O]^+$
238 (26)	238 (21)	$[C_{15}H_{28}NO]^+$
224 (14)	224 (14)	$[C_{14}H_{26}NO]^+$

Catalytic hydrogenation of **I** afforded a hexahydro derivative, whose selected mass spectral data are shown in Table 2 in comparison with those reported for the hexahydro derivative of A 2315A.[3] While in the latter the fragmentation of the chain induced by the OH's at C-20 and C-18 is indicated by

Fig. 4. ^1H NMR spectrum at 270 MHz of 2×10^{-2} M A 17002 C (**I**) in DMSO-d_6 solution.

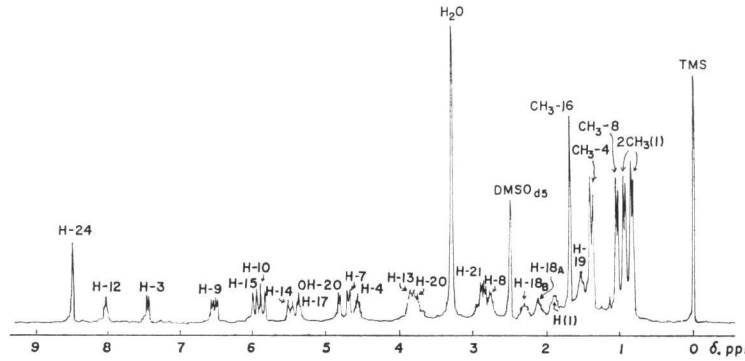


Fig. 5. ^{13}C NMR spectrum at 67.88 MHz of 1 M A 17002 C (**I**) in DMSO-d_6 ($^{12}C=99.75\%$; $D=99.9\%$).

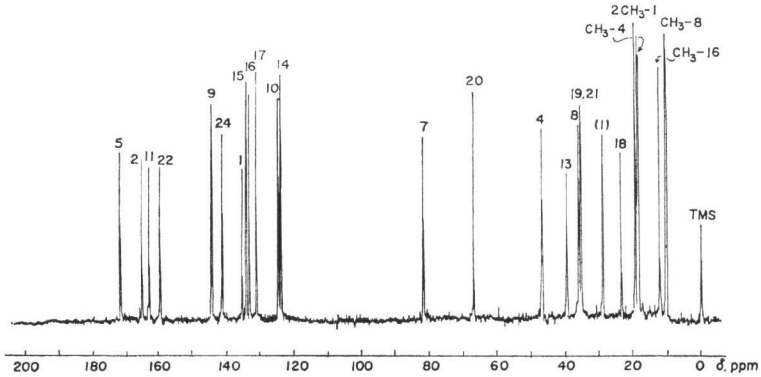


Table 3. ^{1}H NMR data at 270 MHz of A 17002 F and C in DMSO-d_6+D_2O (conc. 2×10^{-2} M, temp. 20°C).

Proton	A 17002 F (II)			A 17002 C (I)		
	δ (ppm)	multipl.	J (Hz)	δ (ppm)	multipl.	J (Hz)
H-3	7.49	d	$J_{3,4}$ =8.5	7.63	d	$J_{3,4}$ =8.5
H-4	4.59	dq	$J_{4,CH_3\text{-}4}$ =7	4.59	dq	$J_{4,CH_3\text{-}4}$ =7
CH_3-4	1.38	d	J =7	1.42	d	J =7
H-7	4.71	dd	$J_{7,8}$ =2	4.71	dd	$J_{7,8}$ =2
H(1)	1.90	m	$J_{(1),7}$ =10	1.90	m	$J_{(1),7}$ =9
CH_3-(1)	0.84	d	J =7	0.84	d	J =7
CH_3-(1)	0.95	d	J =7	0.95	d	J =7
H-8	2.79	m	$J_{8,9}$ =5.5	2.79	m	$J_{8,9}$ =5.5
CH_3-8	1.05	d	J =7	1.05	d	J =7
H-9	6.57	dd	$J_{9,10}$ =15	6.58	dd	$J_{9,10}$ =15
H-10	5.88	bd	$J_{all\ 8,10}$ =1	5.88	bd	$J_{all\ 8,10}$ =1
H-12	8.16	t	$J_{12,13}$ =5	8.16	t	$J_{12,13}$ =7
H-13*	ν_A=3.80	2ddd	J_{gem} =16	ν_A=3.76	2ddd	J_{gem} =16
	ν_B=3.86		$J_{all\ 13,15}$ =1	ν_B=3.81		$J_{all\ 13,15}$ =1
H-14	5.64	ddd	$J_{13,14}$ =3	5.56	ddd	$J_{13,14}$ =4
H-15	6.02	d	$J_{14,15}$ =15	6.02	d	$J_{14,15}$ =15
CH_3-16	1.76	bs	$J_{all\ 16,17}$ =1	1.72	bs	$J_{all\ 16,17}$ =1
H-17	5.31	bd	$J_{17,18}$ =9	5.41	bt	$J_{17,18}$ =7
H-18	4.55	dt	$J_{18,19}$ =7	ν_A=2.14	2m	J_{gem} =14
				ν_B=2.31		$J_{18,19}$ =n.d.
OH-18*	4.6	b	—			
H-19	1.67	m	$J_{19,20}$ =n.d.	1.57	m	$J_{19,20}$ =n.d.
H-20*	3.73	m	$J_{20,21A}$ =6.5	3.92	m	$J_{20,21A}$ =6.5
OH-20*	4.83	d	$J_{OH\text{-}20,20}$=5.5	4.83	d	$J_{OH\text{-}20,20}$=5.5
H-21	ν_A=2.86	2dd	J_{gem} =16	ν_A=2.86	2dd	J_{gem} =16
	ν_B=2.95		$J_{21B,20}$ =5	ν_B=2.95		$J_{21B,20}$ =5
H-24	8.38	s	—	8.38	s	—

* Values determined before D_2O exchange.

the couples of ions at *m/e* 312, 282 and 268, 238, respectively, in hexahydro-**I** the presence of the couple at *m/e* 296, 266 and the absence of the ion at *m/e* 268 suggest that the OH should be localized at C-20.

Fig. 3 shows the IR spectrum of **I** in $CDCl_3$, which is similar to that of **II** and already reported for madumycin II.[2] This means that the main functional groups of **II** (amide, lactone, conjugated double bonds) are maintained in **I**. The only difference is the lack in **I** of the bands at 3330 cm^{-1} (ν OH) and 1065 cm^{-1} (ν C-O) attributed to the 1,3 diol system at C-18 and C-20 intramolecularly H-bonded, present in **II**.

The ^{1}H NMR spectrum of **I** at 270 MHz in DMSO-d_6 is reported in Fig. 4; the ^{1}H NMR data of **I** and II, obtained by ^{1}H homodecoupling at 270 MHz in DMSO-d_6+D_2O, are reported in Table 3. The comparative examination of these data shows that in **I** the macrocyclic ring must have the same sequence and relative configuration of protons as that of **II**, except that in **I** C-18 is a CH_2 (2 m at δ 2.14 and 2.31), while in **II** is a CHOH (dt at δ 4.55). Additional information can be derived from the data in Table 3:

(1) The overall conformation of the macrocyclic lactone ring of **I** is substantially the same as that of **II**, as shown by the same values of the chemical shifts and vicinal interproton coupling constants.

(2) In DMSO, the amide protons (H-3 and H-12) and the common alcoholic proton (OH at C-20) are intermolecularly bound to the solvent in the same way in **I** and **II**.

(3) The isopropyl chain at C-7 in **I** and **II** is blocked in a preferred conformation with H-(1) and H-7 in *trans*.*

The ^{13}C NMR spectrum of **I** at 67.88 MHz in DMSO-d_6—^{12}C is reported in Fig. 5; the ^{13}C NMR data of **I** and **II**, obtained at 67.88 MHz in DMSO-d_6 by the appropriate ^{13}C [^1H] selective decouplings and by data of literature, are reported in Table 4. These data confirm the structure assigned to **I**, as shown *e.g.* by the chemical shifts (δ 23.7) and multiplicity of C-18. Other general considerations derived from Table 4 are:

(1) The ^{13}C NMR data of all the carbons of **II**

* A detailed analysis of the conformation of these antibiotics is beyond the scope of our work. The conformation in solution of virginiamycins, factors S, has been reported.[5]

Table 4. ^{13}C NMR data at 67.88 MHz of A 17002 F and C in ^{12}C DMSO-d_6 (conc. 1 M, temp. 20°C).

Carbon	A 17002 F (II)		A 17002 C (I)	
	multipl. (^1J)	δ (ppm)	multipl. (^1J)	δ (ppm)
C-1	s	135.8	s	135.4
C-2	s	165.0	s	165.0
C-4	d	47.0	d	47.0
CH_3-4	q	18.5	q	18.3
C-5	s	171.5	s	171.5
C-7	d	81.6	d	81.6
C-(1)	d	29.2	d	29.2
CH_3-(1)	q	19.4	q	19.4
CH_3-(1)	q	18.5	q	18.5
C-8	d	35.2	d	36.0
CH_3-8	q	10.6	q	10.6
C-9	d	144.5	d	144.5
C-10	d	124.8	d	124.4
C-11	s	162.7	s	162.7
C-13	t	39.6	t	39.6
C-14	d	126.0	d	123.7
C-15	d	134.2	d	134.1
C-16	s	133.4	s	133.3
CH_3-16	q	13.0	q	12.4
C-17	d	135.6	d	131.1
C-18	d	65.0	t	23.7
C-19	t	44.2	t	35.4
C-20	d	66.2	d	67.1
C-21	t	35.3	t	35.3
C-22	s	159.5	s	159.5
C-24	d	141.1	d	141.1

Table 5. Summary of the relationships between producing strains and structure of macrocyclic lactones. For the basic structure see Fig. 1.

Producing strain	Name	R_1	R_2	R_3	R_4
Actinoplanes philippinensis NRRL 5462[3,4]	A 2315 A	H	OH	H	OH
Actinoplanes auranticolor Routien[6,7,8] sp. nov. (ATCC 31011)	CP-35,763 (Plauracin)				
Actinoplanes azureus (ATCC 31157)[6,7,8]					
Actinomadura flava[2]	Madumycin II				
Actinoplanes A 15104 (ATCC 33002)	A 15104 V				
Actinoplanes A 17002	A 17002 F				
Actinoplanes philippinensis NRRL 5462[3,4]	A 2315 B	H	OH	O	
Actinoplanes auranticolor Routien[6,7,8] sp. nov. (ATCC 31011)	CP-36,926				
Actinoplanes azureus (ATCC 31157)[6,7,8]					
Actinomadura flava[2]	Madumycin I				
Actinoplanes A 15104 (ATCC 33002)	A 15104 II	H	H	H	OH
Actinoplanes A 17002	A 17002 C				

fully support the structure previously assigned[2,3] to this antibiotic.

(2) The striking equality between the ^{13}C shifts of **I** and **II** is a further confirmation that the two molecules, apart the established structural difference, are stereochemically related.

Relationships between I and II and Other Described Macrocyclic Lactones

Table 5 summarizes the relationships between producing strains and macrocyclic lactones having the basic structure shown in Fig. 1, so far described. It can be seen that, out of five different strains of *Actinoplanes*[9] only three chemically different structures are produced. Two of them have the same CHOH function at C-18 and differ at C-20 having either a CHOH or a C=O; the third, described in this paper, has a CHOH at C-20 and differs from the previous having a CH_2 at C-18 instead of a CHOH. It can be noticed that compounds hydroxylated at C-18 are also produced by an *Actinomadura* strain. All these compounds are related to the virginiamycin factor M[9], produced by *Streptomyces*, in which the lactone bond is formed through a dehydroproline instead of an alanine moiety.

Experimental

Instrumentation

Differential thermal analysis was carried out with a Du Pont 990 thermoanalyzer. UV-visible spectra were measured on a Perkin Elmer 4000 and IR spectra on a Perkin Elmer model 580 spectrometer. Mass spectra were recorded on a Hitachi RMU-6L spectrometer. High resolution mass data were obtained on a Varian MAT CH 5 spectrometer by courtesy of Varian S.p.A., Italy. Both ^1H and ^{13}C NMR spectra and data were obtained on a Pulse Fourier Transform Bruker WH 270 cryo-spectrometer equipped with disk unit.

Occurrence and isolation of metabolites from the fermentation broth of A 17002

Submerged fermentation of the A 17002 strain was carried out in E/25 medium at 28°C for 96 hours. Culture broth (50 liters) was filtered after addition of Hyflo filter aid, adjusted to pH 3.5 with concd. HCl and extracted with ethyl acetate. The extracts were dried on anhydrous Na_2SO_4 and evaporated. To the oily residue ethyl ether was added (0.5 liter) followed by petrol ether up to a total volume of 2 liters. The precipitate was filtered and dried *in vacuo*, 13 g. The crude product was dissolved in 20 ml of methanol, chloroform was added up to 300 ml, and the solution was chromatographed on a Silica gel (0.06~0.2 mm) column (500 g) in chloroform. Fractions of 300 ml each were collected and checked by TLC. A stepwise elution was carried out with chloroform containing 1% v/v methanol (14 to 23); 2% methanol (24 to 30); 3% methanol (31 to 41); 4% methanol (42 to 53), and 6% methanol (54 to 62).

Fractions 25 to 26 and 26 to 29 gave by evaporation A 17002 A and A 17002 B, respectively, which were further purified.

Fractions 45 to 48 were evaporated and the residue was rechromatographed on a Silica gel column eluting with ethyl acetate. Combined eluates gave, after evaporation, a powder that by crystallization from ethylacetate – *n*-hexane yielded 0.55 g of pure A 17002 C. Fractions 54 to 61 gave a residue which was dissolved in few milliliters of methanol and chromatographed on buffered (SÖRENSEN pH 6) Silica gel eluting with benzene containing increasing amounts of methanol (from 1 to 4% v/v).

Selected fractions were evaporated and the residue was dissolved in chloroform and precipitated with petroleum ether yielding 0.6 g of A 17002 F.

Fermentation of A 15104 was done in similar conditions and the culture broth was worked up essentially as described for A 17002 C. Compounds A 15104 I, X and V were detected by TLC also in the methanol extract of the mycelium cake.

Hexahydro derivative of **I**

A solution of 25 mg of I in 10 ml of ethanol was hydrogenated at room temperature and

atomospheric pressure in the presence of 10 mg of 5% Pd on carbon. After an uptake of 3 moles of H_2, Celite was added and the catalyst filtered off. The material obtained after evaporation of the solvent was triturated with petroleum ether, filtered and dried *in vacuo*.

Acknowledgments

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