CITREAMICINS†, NOVEL ANTIBIOTICS FROM *MICROMONOSPORA CITREA*: ISOLATION, CHARACTERIZATION, AND STRUCTURE DETERMINATION

GUY T. CARTER, JEANNE A. NIETSCHE, DAVID R. WILLIAMS and DONALD B. BORDERS

Medical Research Division, American Cyanamid Company, Lederle Laboratories, Pearl River, New York 10965, U.S.A.

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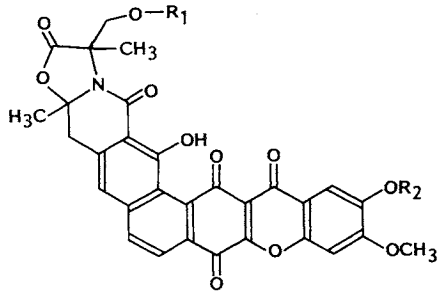
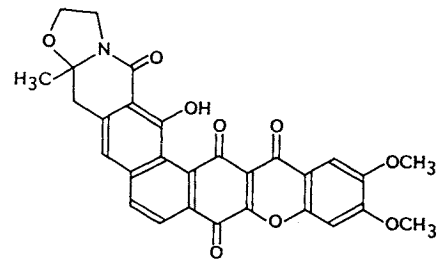
A new family of antibacterial antibiotics has been isolated from *Micromonospora citrea*. The compounds, designated citreamicins α, β, γ, ζ and η are of the polycyclic xanthone structure type. Their isolation, characterization and structure determination are presented.

The citreamicin antibiotic complex is produced by a recently described subspecies of *Micromonospora citrea*[1]. As antimicrobial agents, these compounds are effective *in vitro* against a spectrum of Gram-positive aerobic and anaerobic bacteria. Citreamicin η is the most potent congener with *in vitro* MIC values $<0.015\,\mu g/ml$ against several Gram-positive strains. The compounds are structurally related to a small family of antibiotics including cervinomycin[2], simaomicin[3], lysolipin[4], and actinoplanone[5]. The citreamicins are the first examples of this class to be reported from *Micromonospora*, and are only the second example of the structure type to have a quinone ring fused to the pyrone. In this report are presented the isolation, characterization, and structure determination of citreamicins α, β, γ, ζ and η (**1**~**5**)††.



Citreamicin α (**1**) $R_1=COCH_2CH(CH_3)_2$ $R_2=CH_3$
Citreamicin β (**2**) $R_1=COCH(CH_3)_2$ $R_2=CH_3$
Citreamicin γ (**3**) $R_1=COCH_3$ $R_2=CH_3$
Citreamicin ζ (**4**) $R_1=COCH_2CH(CH_3)_2$ $R_2=H$
Citreamicin η (**5**) $R_1=H$ $R_2=CH_3$



Cervinomycin A_2 (**6**)

Materials and Methods

NMR Experiments

The carbon detected long range heteronuclear correlation experiment (CSCMLR)[7] was performed on a GE 500 MHz spectrometer. Initial spectral acquisition parameters were: 25,641 Hz sweep width in the F_2 dimension; 512 spectra (44 scans each) were accumulated with a 1.0-second relaxation delay and 0.250 msecond increments starting with a 0.125-msecond delay, with 12.52 Hz/pt resolution in the F_2 dimension and 7.8 Hz/pt in the F_1 dimension.

† These compounds were previously designated the LL-E19085 antibiotics.

†† The structure of citreamicin α represents a revision of the original structure disclosed in our previous reports[1,6].

Tank Fermentation and Processing

Inoculum for the fermentation was developed in three stages of 48 hours each at 32°C in a seed medium consisting of (g/liter): Glucose (10), dextrin (20), yeast extract (5), NZ Amine A (5), and $CaCO_3$ (1). The final stage (300 liters) was used to inoculate 2,700 liters of sterilized fermentation medium consisting of (g/liter): Dextrin (30), glucose (5), Nutrisoy (15), corn steep liquor (5) and $CaCO_3$ (5). The tank was stirred at 110 rpm with aeration at 0.60 v/v/m at 28°C for 103 hours when it was harvested. The harvest potency of citreamicin α was 120 μg/ml by HPLC analysis. The antibiotic complex was recovered by extraction of the acidified (pH 3.0) fermentation medium with an equal volume of ethyl acetate. The resulting organic phase was concentrated to a syrupy residue under reduced pressure.

Analytical HPLC

The analytical HPLC system consisted of a C_{18} reversed-phase column (3 μm, 2.1 × 100 mm) eluted with a 3 : 2 mixture of acetonitrile and 0.05 M pH 4.5 ammonium acetate buffer at 0.5 ml/minute. Detection was by UV absorbance at 325 nm. Retention times of the citreamicins were as follows: α, 2.9 minutes; β, 2.4 minutes; γ, 1.6 minutes; ζ, 1.9 minutes and η, 1.2 minutes.

Chromatographic Purification

The chromatographic purification of the antibiotics is outlined in the flow diagram shown in Fig. 1. A portion of the crude extract containing 9.7 g of citreamicin α was dissolved in methylene chloride (2,000 ml). To this solution was added silica gel (63 ~ 200 μm, 500 g). After stirring thoroughly, the solvent was removed under reduced pressure. The charged silica was air dried and then eluted on a coarse sintered glass funnel sequentially with hexane (3,000 ml) and methylene chloride (3,000 ml). The first 1,000 ml from methylene chloride elution contained the bulk of the antibiotics (8.4 g citreamicin α). This fraction was concentrated, redissolved in methylene chloride (500 ml) and chromatographed on a silica gel (63 ~ 200 μm, 800 g) column packed in methylene chloride. The column was eluted with methylene chloride (12,000 ml) followed by acetone - methylene chloride (1 : 9, 2,000 ml). Fractions were analyzed by analytical HPLC and combined accordingly. Citreamicin α (6.5 g) was obtained as were mixtures I, II, and III, which were enriched in the more polar minor components. Citreamicin α: $[\alpha]_D^{26}$ −58° (*c* 0.295, $CHCl_3$); *Anal* found (calcd): C 64.55 (64.57), H 4.46 (4.67), N 1.92 (2.09).

Isolation of Citreamicins β and γ

A portion of fraction I was separated by reversed-phase HPLC to obtain small quantities of citreamicins β and γ. Thus, a solution of I in acetonitrile (*ca.* 10 mg/ml) was chromatographed (2 ~ 4 ml/injection) on a C_{18} column (10 μm, 21.4 mm × 25 cm) eluted with a mixture of acetonitrile - 0.05 M pH 4.5 ammonium acetate (3 : 2). From three runs (9 ml total injected), approximately 4 mg each of citreamicins β and γ were obtained.

Isolation of Citreamicin ζ

Fraction II was dissolved in acetonitrile (100 mg/10 ml). This solution was chromatographed on a C_{18} reversed-phase column (55 ~ 105 μm, 400 g) eluted with a mixture of acetonitrile - 0.05 M pH 4.5 ammonium acetate buffer (1 : 1), to obtain citreamicin ζ (28 mg).

Isolation of Citreamicin η

Fraction III (100 mg) dissolved in acetonitrile (10 ml) was chromatographed in the 1 : 1 system described above, yielding citreamicin η (15 mg). Citreamicin η: $[\alpha]_D^{26}$ −63° (*c* 0.190, DMSO).

Basic Degradation of Citreamicin α

Citreamicin α (110 mg) was dissolved in a mixture of methanol (10 ml) and methylene chloride (2 ml). To this solution was added 1.0 N NaOH (1.0 ml), and the mixture was stirred at room temperature for 90 minutes. The reaction mixture was then acidified to pH 2.5 with 1.0 N HCl, and diluted with water (10 ml). The crude product was extracted into methylene chloride (3 × 25 ml). Upon evaporation of the solvent, the residue weighed 50 mg. This material was purified by Sephadex LH-20 chromatography. Fresh Sephadex LH-20 was treated with a solvent system consisting of methylene chloride, hexane, and methanol (10 : 5 : 2)

and after swelling of the gel was complete, a column was formed (2.8 × 39 cm). The crude product was chromatographed on this column with the same solvent system. Fractions were collected (8 ml) at 5 minutes intervals, and methyl 4,5-dimethoxysalicylate was found in fractions 12~16. The methyl 4,5-dimethoxysalicylate was identified by spectroscopic methods: EI-MS *m/z* 212 (M^+); ^{1}H NMR ($CDCl_3$) δ 10.73 (1H, s), 7.21 (1H, s), 6.48 (1H, s), 3.93 (3H, s), 3.90 (3H, s), 3.85 (3H, s); UV $\lambda_{max}^{acetonitrile}$ nm 225, 260, 319; IR ν_{max} (KBr) cm^{-1} 3400 (br), 2950, 1662, 1624, 1518, 1442, 1363, 1267, 1240.

Acidic Methanolysis of Citreamicin α

Citreamicin α (100 mg) was dissolved in methanol (500 ml). A solution of 3% HCl in methanol (25 ml) was added and the solution was refluxed for 66.5 hours. The solvent was evaporated under reduced pressure and the residue was redissolved in methylene chloride (100 ml). The methylene chloride solution was washed sequentially with 5% $NaHCO_3$ (50 ml) and water (50 ml). Removal of the solvent under reduced pressure left the product alcohol **5** (70 mg), which was shown to be identical with citreamicin η in HPLC, ^{1}H and ^{13}C NMR, and FAB-MS.

Results and Discussion

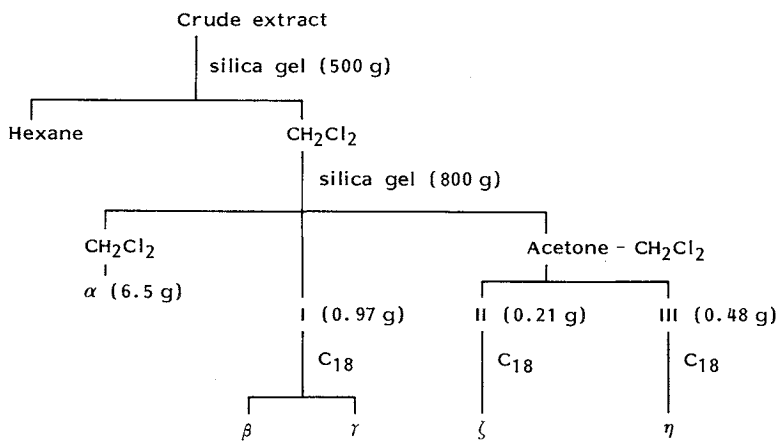
Isolation and Characterization

Whole mash from tank fermentations of the organism was adjusted to pH 3.0 and then extracted with ethyl acetate. The resulting organic extract was concentrated to a syrup under reduced pressure. This syrup was stirred with hexane and filtered to remove nonpolar impurities prior to chromatography. Two stages of silica gel chromatography (Fig. 1) yielded the major component, citreamicin α, in relatively pure form (85%). The minor components required a reversed-phase chromatographic step to complete their purification.

Selected physico-chemical properties of the citreamicins are summarized in Table 1. These compounds are highly colored pH indicators, yielding yellow to red solution under acidic or neutral conditions, which turn green when the solution is made basic. The poor solubility of these compounds was the limiting factor in their isolation and purification. The compounds are soluble in chloroform, methylene chloride, and acetone to the extent of approximately 100 mg/ml. Solubility in acetonitrile, ethyl acetate, methanol and ethanol was lower, on the order of approximately 10 mg/ml. Gel formation was common in the more polar solvents when saturated solutions were allowed to stand for several hours at room temperature.

The molecular formulas for the citreamicins were established by HRFAB-MS. The accurate mass

Fig. 1. Chromatographic purification of the citreamicins.



measurements were done on the $(M+3H)^+$ molecular adduct ions, since the antibiotics were converted to their hydroquinone analogs in the mass spectrometer under most experimental conditions. This reduction phenomenon in FAB-MS is now well documented for quinones[8]. The UV-VIS spectrum of the α component (Fig. 2) is typical of the citreamicins and shows several sharp absorption bands in the UV region with maxima at 223, 255, 320, and 384 nm plus a broad visible band centered at 410 nm. This curve is very similar to the spectrum of cervinomycin A_2[2]. The IR spectrum of citreamicin α (Fig. 3) contains significant bands attributed to hydrogen bonded OH (3440 cm^{-1}), and several carbonyl signals. The band at 1746 cm^{-1} was considered to

Fig. 2. UV-VIS absorption spectrum (CH_3CN) of citreamicin α.

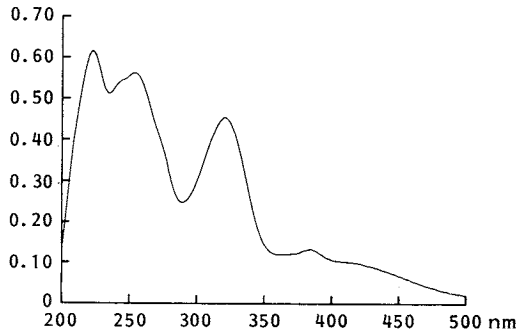


Table 1. Selected physico-chemical properties of the citreamicins.

	1	2	3	4	5
MW	669	655	627	655	585
HRFAB-MS $(M+3H)^+$ (Calc value)	672.2117 (672.2081)	658.1925 (658.1925)	630.1596 (630.1612)	656.1778[a] (656.1768)	588.1479 (588.1506)
Molecular formula	$C_{36}H_{31}NO_{12}$	$C_{35}H_{29}NO_{12}$	$C_{33}H_{25}NO_{12}$	$C_{35}H_{29}NO_{12}$	$C_{31}H_{23}NO_{11}$
UV $\lambda_{max}^{CH_3CN}$ nm (ε)	223 (4.24×10^4), 255 (3.89×10^4), 320 (3.25×10^4), 384 (9.03×10^3), 410 (6.75×10^3)	223, 255, 320, 384, 410	223, 255, 320, 384, 410	223, 255, 320, 384, 410	223, 255, 320, 384, 410
IR ν_{max} KBr (cm^{-1})	3440, 2959, 2928, 1804, 1746, 1694, 1623, 1509, 1426, 1274	3434, 2924, 1804, 1752, 1693, 1621, 1426, 1272	3433, 2925, 1805, 1745, 1694, 1621, 1426, 1272	3429, 2960, 1805, 1745, 1693, 1626, 1421, 1279	3437, 2923, 1798, 1691, 1427, 1276

[a] $(M+H)^+$.

Fig. 3. IR absorption spectrum (KBr) of citreamicin α.

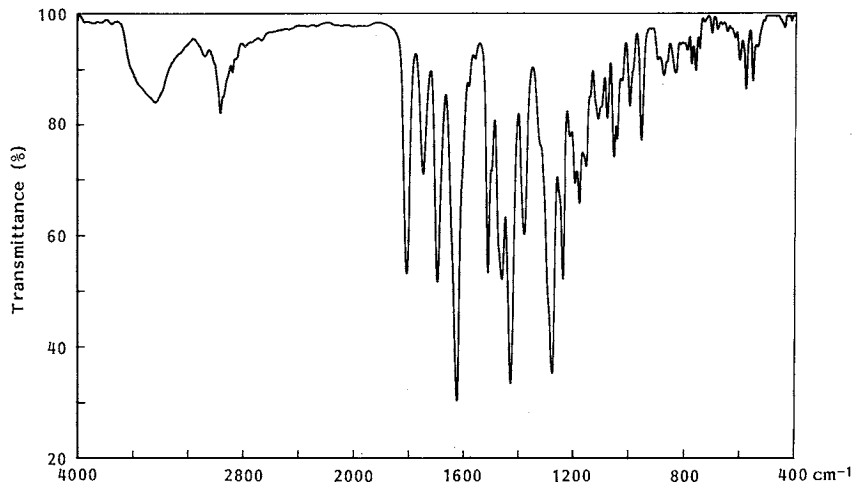


Table 2. NMR data for citreamicins.

Position	^{13}C[a]			^{1}H[b]				
	1	4[c]	5[d]	1	2	3	4	5[d]
1	62.3	62.3	63.4					
1a	20.2	20.0	19.0	1.83, 3H, s	1.83, 3H, s	1.82, 3H, s	1.81, 3H, s	1.77, 3H, s
$1b_1$	65.0	64.9	65.8	4.46, 1H, d, 11.6	4.47, 1H, d, 11.6	4.43, 1H, d, 11.5	4.45, 1H, d, 11.5	3.90, 1H, br d, 10.1
$1b_2$				4.78, 1H, d, 11.6	4.77, 1H, d, 11.6	4.81, 1H, d, 11.5	4.76, 1H, d, 11.5	4.33, 1H, br d, 10.1
2	171.5	171.6	171.9					
4	93.4	93.4	93.9					
4a	25.8	25.6	25.3	1.80, 3H, s	1.77, 3H, s	1.75, 3H, s	1.75, 3H, s	1.71, 3H, s
5a	41.9	41.7	41.4	3.35, 1H, d, 12.8	3.36, 1H, d, 14.8	3.36, 1H, d, 14.9	3.35, 1H, d, 14.8	3.37, 1H, d, 14.7
5b				3.48, 1H, d, 12.8	3.50, 1H, d, 14.8	3.50, 1H, d, 14.9	3.48, 1H, d, 14.8	3.51, 1H, d, 14.7
6	134.8	134.6	135.2					
7	117.7	117.7	117.6	7.19, 1H, s	7.21, 1H, s	7.21, 1H, s	7.26, 1H, s	7.23, 1H, s
8	140.7	140.5	140.3					
9	132.2	132.2	132.2	7.92, 1H, d, 8.5	7.94, 1H, d, 8.5	7.94, 1H, d, 8.6	7.91, 1H, d, 8.5	7.96, 1H, d, 8.5
10	124.4	124.2	123.9	8.20, 1H, d, 8.5	8.26, 1H, d, 8.5	8.26, 1H, d, 8.6	8.22, 1H, d, 8.5	8.24, 1H, d, 8.5
11	129.7	129.7	129.6					
12	178.1	178.0	177.7					
13	153.4	153.5	153.8					
15	150.8	150.2	150.8					
16	100.4	100.2	100.7	7.09, 1H, s	7.14, 1H, s	7.14, 1H, s	7.14, 1H, s	7.17, 1H, s
17	155.5	154.7	155.6					
$17OCH_3$	56.8	56.5	56.7	4.02, 3H, s	4.04, 3H, s	4.04, 3H, s	4.06, 3H, s	4.04, 3H, s
18	148.7	146.3	148.6					
$18OCH_3$	56.5	—	56.1	3.99, 3H, s	4.02, 3H, s	4.02, 3H, s		4.02, 3H, s
19	104.9	108.4	104.4	7.58, 1H, s	7.66, 1H, s	7.66, 1H, s	7.75, 1H, s	7.63, 1H, s
20	119.5	119.6	119.3					
21	172.1	171.7	173.0					
22	120.8	120.1	120.2					
23	181.2	181.4	180.0					
24	137.7	137.5	137.3					
25	119.9	119.7	119.2					
26	162.1	161.6	161.4					
26OH				13.50, 1H, s	13.50, 1H, s	13.46, 1H, br s	13.45, 1H, s	5.28, 1H, br
27	107.3	107.1	107.4					
28	165.8	165.5	165.8					
1′	171.5	172.8						
2′	42.9	42.8		2.21, 2H, d, 6.8	2.55, 1H, septet, 7.0	2.10, 3H, s	2.20, 2H, d, 6.6	
3′	25.5	25.4		2.10, 1H, septet, 6.5	(2′a) 1.18, 3H, d, 7.0		2.10, 1H, septet, 6.6	
3a′	22.35	22.2		0.96, 3H, d, 6.5	(2′b) 1.19, 3H, d, 7.0		0.95, 3H, s	
4′	22.38	22.2		0.96, 3H, d, 6.5			0.95, 3H, s	

[a] $CDCl_3$, ppm from TMS.
[b] $CDCl_3$, ppm from TMS, No. H, multiplicity, J in Hz.
[c] $CDCl_3$-CD_3OD (1 : 1).
[d] $CDCl_3$-DMSO-d_6 (1 : 1).

Fig. 4. ^{13}C NMR spectrum of citreamicin α ($CDCl_3$).

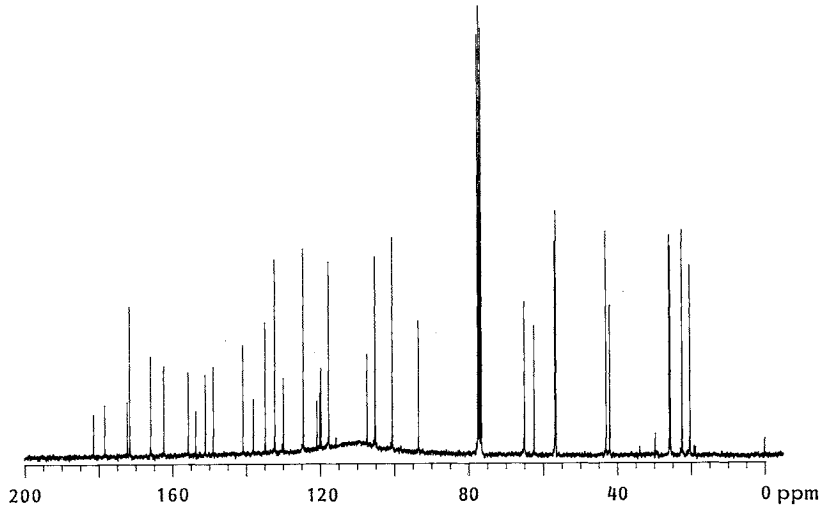
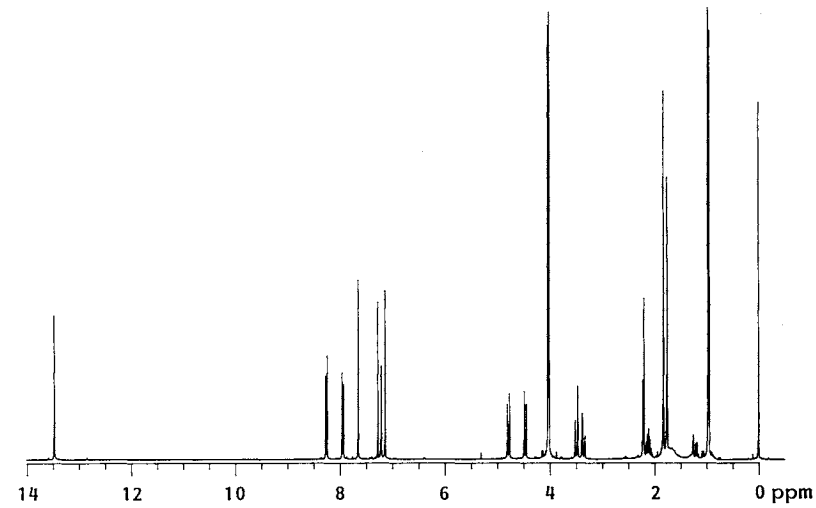


Fig. 5. ^{1}H NMR spectrum of citreamicin α ($CDCl_3$).



be due to a simple aliphatic ester group. The highest frequency carbonyl absorption at 1804 cm^{-1} was identified as being characteristic of this series of compounds, and a review of the literature failed to reveal any related antibiotics with this unusual IR absorption. This was one of the earliest indications that the citreamicins were novel.

The NMR data for the citreamicins are summarized in Table 2. Citreamicin α is the major component produced by the culture and the other components represent simple structural variants of it, hence the NMR data for citreamicin α will be used to exemplify the properties of the group. In its ^{13}C NMR spectrum (Fig. 4), there are signals representing twenty-four sp^2 hybridized carbons between 100 ~ 182 ppm; nineteen of these are due to quaternary carbons. In the ^{1}H NMR spectrum (Fig. 5), only two of the five aromatic proton signals showed connectivity. These (δ 7.92 and 8.20 (J=8.5 Hz)) appeared to be *ortho* to each other. The aliphatic portions of citreamicin α are represented by ^{13}C and ^{1}H NMR signals as follows: Four *C*-methyls (δ_C 20.2, δ_H 1.83; δ_C 22.3, δ_H 0.95; δ_C 22.4, δ_H 0.95; δ_C 25.8, δ_H 1.80), two *O*-methyls (δ_C

Fig. 6. Partial structure of citreamicin α.

Arrows indicate ^{1}H-^{13}C long range couplings.

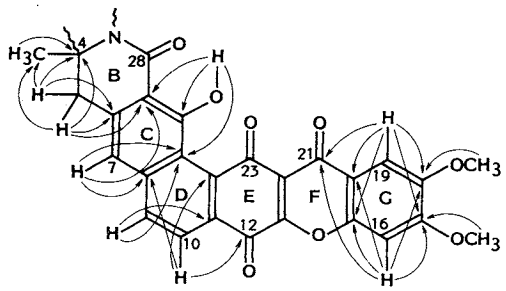


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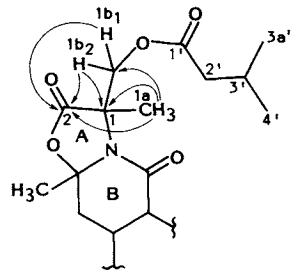
Fig. 7. A-Ring structure of citreamicin α.

Arrows indicate ^{1}H-^{13}C long range couplings.



56.5, δ_H 3.99; δ_C 56.8, δ_H 4.02), three methylenes (δ_C 41.9, δ_H 3.35, 3.48; δ_C 42.9, δ_H 2.21 (2H); δ_C 65.0, δ_H 4.46, 4.78), one methine (δ_C 25.5, δ_H 2.10), and two quaternary carbons (δ_C 62.3, 93.4).

Structure Determination of Citreamicins α and η

Given the spectroscopic similarities noted above between these antibiotics and the lysolipin class, and in particular cervinomycin A_2 (**6**), the partial structure **7** shown in Fig. 6 was proposed. Support for **7** was obtained by isolation of methyl 4,5-dimethoxysalicylate from methanolic base degradation of citreamicin α. This product is derived from fission of the pyrone (F) ring.

Partial structure **7** was confirmed by a long range 2D heteronuclear correlation experiment (CSCMLR). As indicated by the arrows in Fig. 6, crosspeaks were observed between the 4a methyl proton resonance δ 1.80 and C-4 δ 93.4. The C-4 resonance showed correlations with both the 5-H proton resonances δ 3.35 (5a-H), and δ 3.48 (5b-H). Crosspeaks were observed between both 5-H's and C-4a δ 25.8, and C-6 δ 134.8. The 5b-H signal also showed a crosspeak to δ 107.3 (C-27). No crosspeaks were observed to the C-28 signal in this experiment because of the lack of protons within 2 or 3 bonds of the amide carbonyl. The chemical shift δ 165.8 is characteristic of the amide carbonyl in related structures[2,3,5], and the nitrogen must be bonded to C-4 (in addition to an oxygen) to create the oxazolidine.

Connectivity to the C ring was established through a crosspeak between C-27 and 7-H δ 7.19. The 7-H resonance showed additional crosspeaks to δ 140.7 (C-8) and 119.9 (C-25). The latter signal was correlated with the phenol proton resonance δ 13.5, as were C-26 δ 162.1 and C-27, thus completing the connections of ring C. The link between the C and D rings was made through the observed crosspeaks between 9-H δ 7.92 and C-25, as well as 10-H δ 8.20 and C-8. The signal for 9-H also showed a crosspeak to δ 129.7 (C-11) as did 10-H with δ 137.7 (C-24) and δ 178.1 (C-12), thus linking the D and E rings. Owing to the lack of protons, no direct correlations were observed between the quinone (E) and pyrone (F) rings. The orientation of the pyrone was assigned as shown, on the basis of the lower field signal δ 181.2 for the C-23 quinone carbonyl as compared to that of C-12, and by analogy to cervinomycin A_2.

The structure of the G ring was corroborated in this experiment as well. Thus, 16-H δ 7.09 shows crosspeaks to δ 150.8 (C-15), δ 155.5 (C-17), δ 148.7 (C-18), δ 119.5 (C-20), and δ 172.1 (C-21). The 19-H signal δ 7.58 showed crosspeaks to the same set of carbon signals.

The remaining portions of the structure (Fig. 7) of citreamicin α, which represent the novel features of these antibiotics, were assigned on the basis of spectroscopic and chemical evidence. Acidic methanolysis

of citreamicin α resulted in primary alcohol **5**, indicating loss of a C_5H_9O unit. This moiety was identified as an isovaleryl group by comparison of the ^1H NMR data for **5** with those for citreamicin α. In the ^1H NMR spectrum of citreamicin α (Fig. 5) signals for the isovaleryl group can be readily assigned (δ 2.21, 2H, d, $J=6.8$ Hz, 2'-CH_2; 2.11, 1H, m, 3'-H; 0.96, 6H, d, $J=6.5$ Hz, 3'a, 4'-CH_3). These signals are lacking in the spectrum of **5**, which also shows a substantial upfield shift for one set of methylene protons (δ 3.90 and 4.33, br d, $J=10.1$ Hz). Compound **5** (citreamicin η) has lost the IR band at 1746 cm^{-1} characteristic of the aliphatic ester group of citreamicin α, but still retains the high frequency carbonyl band (1798 cm^{-1}). In addition to this carbonyl group, three carbons remained to be incorporated into the final structure of **5**, one quaternary (δ 63.4), one *C*-methyl group (δ_C 19.0; δ_H 1.83, s), and the primary alcohol carbon (δ_C 65.8; δ_H 3.90, 1H, d, $J=10.1$ Hz, 4.33, 1H, d, $J=10.1$ Hz). These carbons and the remaining unsaturation required by the molecular formula are best accommodated by the oxazolidinone moiety shown in the A-ring partial structure of Fig. 7. The lactone group of this moiety should have a high frequency carbonyl stretching band in the IR[9]. For example, simple oxazolidinones synthesized from amino acid derivatives and aldehydes have been reported[10] showing this characteristic IR band near 1800 cm^{-1}. Evidence for this A-ring structure was also obtained from the CSCMLR data for citreamicin α. Thus, the C-1a proton resonance δ 1.83 showed crosspeaks with δ 62.3 (C-1), 65.0 (C-1b) and 171.5 (C-2). The C-1b proton signals (δ 4.46, 4.78) each showed crosspeaks to C-2, and one of these (δ 4.78) showed a crosspeak to C-1. This analysis allowed the formulation of structure **1** for citreamicin α and hence that of primary alcohol **5**, which was identical with the natural product citreamicin η.

Structure Determination of Citreamicins β, γ and ζ

Acidic methanolysis of citreamicins β and γ yielded primary alcohol **5**, thus confirming the identity of the core portions of their structures with that of **1**. The structures of their ester side chains were established by ^1H NMR. By subtracting the elements of **5** from their molecular formulas, the acyl side chains of citreamicins β and γ were determined to consist of C_4H_9O and C_2H_3O, respectively. The isobutyryl group of citreamicin β was assigned from the ^1H NMR data as follows: δ 2.55, 1H, septet, $J=7.0$ Hz, 2'-H; 1.19, 3H, d, $J=7.0$ Hz, 2'a-CH_3; 1.18, 3H, d, $J=7.0$ Hz, 2'b-CH_3. Therefore structure **2** was established for citreamicin β. For citreamicin γ, the three-proton singlet in the ^1H NMR spectrum at δ 2.10 clearly identified the acetyl group, and led to assignment of structure **3**.

Citreamicin ζ was shown to be a lower homolog of **1** by FAB-MS analysis. The ^1H NMR spectrum of citreamicin ζ showed just one OCH_3 signal (δ 4.06), suggesting that one of the G-ring OCH_3 groups was absent. Comparison of the NMR data for citreamicin ζ with those of **1** revealed some significant differences. The 19-H signal of citreamicin ζ is shifted downfield by 0.17 ppm to δ 7.75, while the 16-H resonance shifted by only 0.05 ppm. In the ^{13}C NMR spectrum of citreamicin ζ, the C-18 signal appears at δ 146.3, a shift of 2.4 ppm upfield, and the C-19 signal has moved downfield 3.5 ppm to δ 108.4. The signals for C-17 and C-16 are only slightly shifted. These results indicate that it is the 18-OCH_3 group which is absent and therefore citreamicin ζ was assigned structure **4**.

Citreamicins α, β, γ, ζ, and η (**1**$\sim$**5**) represent highly modified A-ring variants of cervinomycin A_2. These compounds are unusual members of the lysolipin class in that they lack the methylenedioxy function and have quinone rings fused to the pyrone ring. These antibiotics are the first members of this structure class to be reported from *Micromonospora*. The culture produces a number of other components with homologous side chains which have not been fully characterized. It is noteworthy that **5** was the only

monomethoxylated product discovered in the course of this work. There was no evidence for the production of the corresponding 17-OH analogue or the didemethoxy compound.

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References

1) MAIESE, W. M.; M. P. LECHEVALIER, H. A. LECHEVALIER, J. KORSHALLA, J. GOODMAN, M. J. WILDEY, N. KUCK & M. GREENSTEIN: LL-E19085α, a novel antibiotic from *Micromonospora citrea*: Taxonomy, fermentation and biological activity. J. Antibiotics 42: 846~851, 1989
2) ŌMURA, S.; Y. IWAI, K. HINOTOZAWA, Y. TAKAHASHI, J. KATO, A. NAKAGAWA, A. HIRANO, H. SHIMIZU & K. HANEDA: Cervinomycin A_1 and A_2, new antibiotics active against anaerobes, produced by *Streptomyces cervinus* sp. nov. J. Antibiotics 35: 645~652, 1982
3) LEE, T. M.; G. T. CARTER & D. B. BORDERS: Structure determination of simaomicin α and β, extremely potent, novel anticoccidial agents produced by *Actinomadura*. J. Chem. Soc. Chem. Commun. 1989: 1771~1772, 1989
4) DOBLER, M. & W. KELLER-SCHIERLEIN: Metabolites of microorganisms. 162nd communication. The crystal and molecular structure of lysolipin I. Helv. Chim. Acta 60: 178~185, 1977
5) KOBAYASHI, K.; C. NISHINO, J. OHYA, S. SATO, T. MIKAWA, Y. SHIOBARA & M. KODAMA: Actinoplanones A and B, new cytotoxic polycyclic xanthones from *Actinoplanes* sp. J. Antibiotics 41: 502~511, 1988
6) CARTER, G. T.; J. A. NIETSCHE, D. R. WILLIAMS & D. B. BORDERS: LL-E19085 alpha, a novel antibiotic from *Micromonospora citrea* ssp. nov. Isolation and structure determination. Program and Abstracts of the 28th Intersci. Conf. on Antimicrob. Agents Chemother., No. 314, p. 164, Los Angeles, Oct. 23~26, 1988
7) MARTIN, G. E. & A. S. ZEKTZER: Two-Dimensional NMR Methods for Establishing Molecular Connectivity. pp. 238~240, VCH Publishers, Inc., 1988
8) DASS, C.; R. SESHADRI, M. ISRAEL & D. M. DESIDERIO: Fast atom bombardment mass spectrometric analysis of anthracyclines and anthracyclinones. Biomed. Environ. Mass Spectrom. 17: 37~45, 1988
9) BELLAMY, L. J.: The Infrared Spectra of Complex Molecules, 3rd Ed. p. 204, Chapman and Hall, 1975
10) FREIDINGER, R. M.; J. S. HINKLE, D. S. PERLOW & B. H. ARISON: Synthesis of 9-fluorenylmethyloxycarbonyl-protected N-alkyl amino acids by reduction of oxazolidinones. J. Org. Chem. 48: 77~81, 1983