STUDIES ON CIRRAMYCIN A_1. III

STRUCTURE OF CIRRAMYCIN A_1

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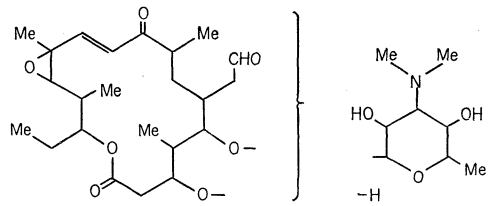
The structure of cirramycin A_1, $C_{31}H_{51}NO_{10}$, has been determined. It consists of a basic sugar, mycaminose, and a 16-membered ketolactone. Cirramycin A_1 is the first macrolide antibiotic which has been shown to have mycaminose as a sole sugar moiety.

Cirramycin A_1 is a macrolide antibiotic elaborated by *Streptomyces cirratus*[1,2]. The isolation, characterization[2] and biological activity[3] of cirramycin A_1 have been reported. This paper presents evidence that cirramycin A_1 possesses the following structure (I).



(I)

General Structural Characteristics

Cirramycin A_1 (I) is a basic compound (pKa′ 8.0) showing a titration equivalent of about 600. Elemental analysis along with the osmometric molecular weight determination (mean value : 606) established the molecular formula of $C_{31}H_{51}NO_{10}$ for cirramycin A_1, which was confirmed by the mass spectrometric data of triacetylcirramycin A_1 (II, $C_{37}H_{57}NO_{13}$, M^+ peak : m/e 723). Group analyses of I indicated the presence of six C-methyl groups but neither methoxy nor acetyl group in the molecule, which agreed with the NMR spectral data of cirramycin A_1 (Fig. 3).

The hydrolysis of cirramycin A_1 (I) with 6 N hydrochloric acid afforded a basic sugar (III), which was crystallized as the hydrochloride and analyzed as $C_8H_{17}NO_4 \cdot HCl \cdot H_2O$ melting at 111.5~113.5°C. The compound (III) was identified with the authentic sample of mycaminose by the infrared (IR) spectrum and paper chromatography. In the mass spectrum of triacetylcirramycin A_1 (II), the peaks due to diacetylmycaminosyl ion ($C_{12}H_{20}NO_5$, m/e 258) and its further decomposed fragment ions such as at m/e 198 (258 minus CH_3COOH) and m/e 156 (198 minus $COCH_2$) were recognized, which suggested

that two hydroxyl groups of mycaminose are free in the intact cirramycin A_1 molecule. The pKa' value of I, 8.0, also supports the presence of two hydroxyl groups vicinal to the dimethylamino function[4]. The amino sugar mycaminose, has also been found as a constituent of magnamycins[5], leucomycins[6], spiramycins[7] and tylosin[8], and it is interesting to note that in all of these antibiotics only one hydroxyl group of mycaminose is free.

Fig. 1. UV spectrum of cirramycin A_1 (in ethanol)

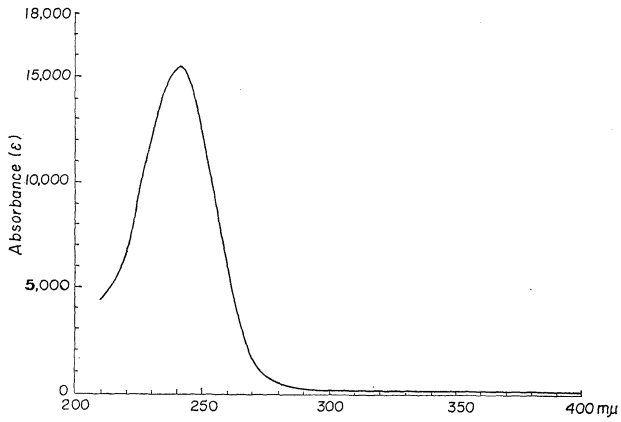


Fig. 2. IR spectrum of cirramycin A_1.

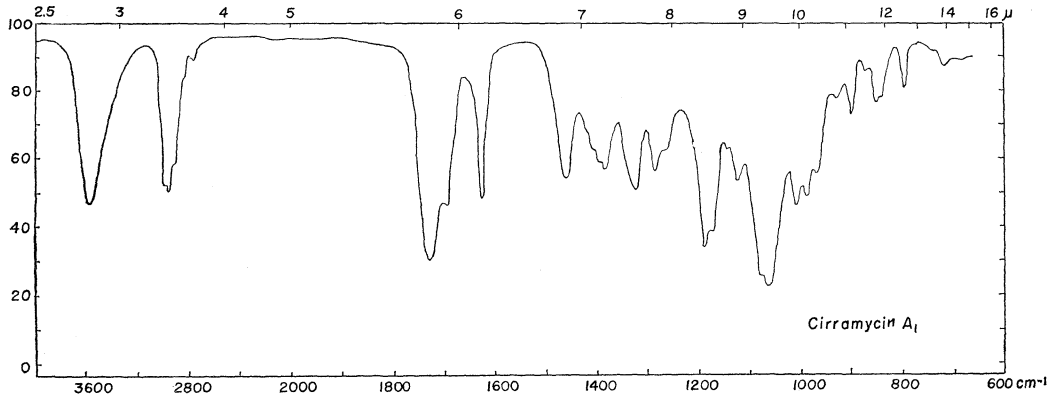
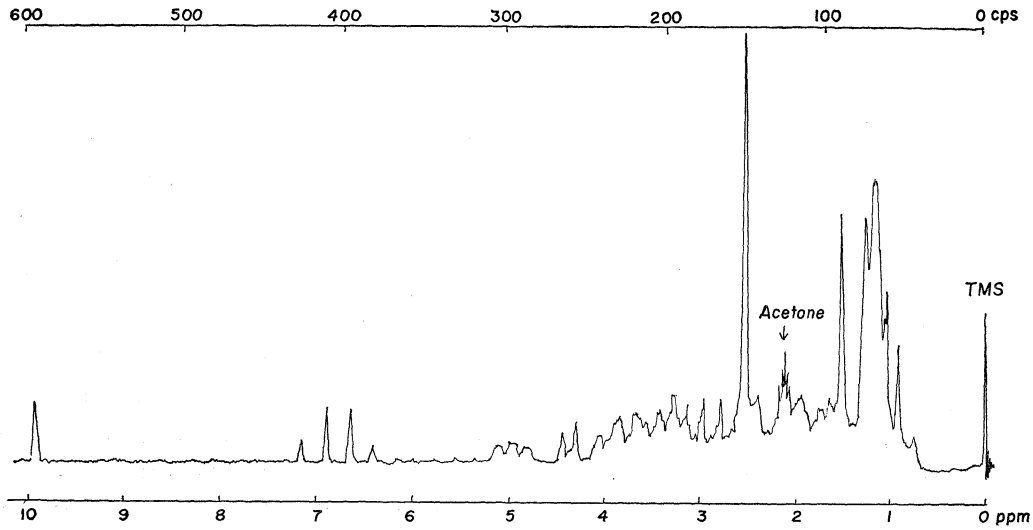


Fig. 3. NMR spectrum of cirramycin A_1. (I) (60 Mc, in acetone-d_6)



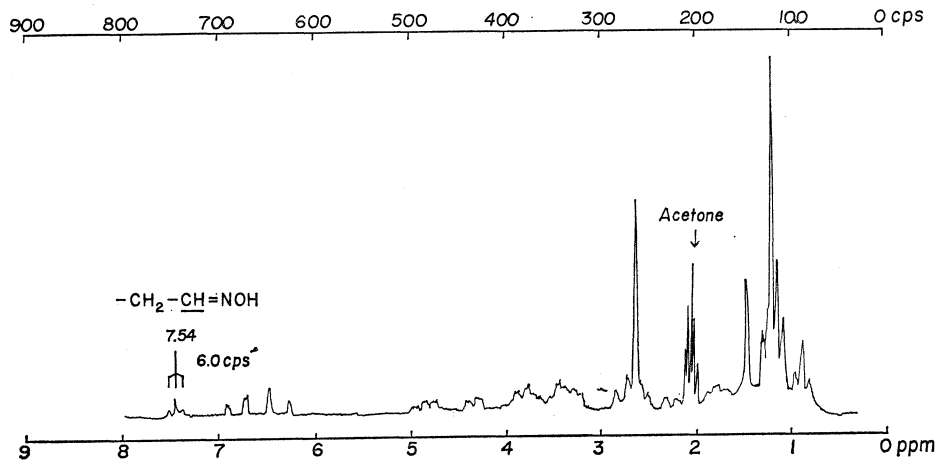
Cirramycin A_1 (I) absorbs strongly in the ultraviolet (UV) region at 241 mμ (ε: 15,400) which is attributable to an α,β-unsaturated ketone grouping (Fig. 1). The absorption bands at 1695 and 1620 cm^{-1} in the IR spectrum of I (Fig. 2) are also attributable to the above conjugate system. A strong absorption band at 1725 cm^{-1} suggests the presence of additional carbonyl group(s). Primary information obtainable from the NMR spectrum of I (Fig. 3) involves one triplet methyl (0.78 ppm), one singlet methyl (1.52 ppm), dimethylamino group of mycaminose (6 H, singlet, 2.57 ppm), trans-olefinic protons (2 H, AB-quartet, 6.45 and 6.92 ppm, J=16 cps) and one aldehyde proton (9.88 ppm).

Catalytic hydrogenation of I over palladium-charcoal gives tetrahydrocirramycin A_1 ($C_{31}H_{55}NO_{10}$, IV), which no longer shows the strong UV absorption at 241 mμ found in I but a very weak maximum at 282 mμ (ε: 72) attributed to an isolated carbonyl function, supporting a presence of the α,β-unsaturated carbonyl system in I. A treatment of I with potassium iodide in acetic acid yields, with liberation of iodine, compound V ($C_{31}H_{51}NO_9$) which shows an intense UV absorption at 284 mμ (ε: 22,350) assignable to an $\alpha,\beta,\gamma,\delta$-unsaturated carbonyl grouping. The fact is in turn suggestive of the presence of an epoxide function next to the conjugate system in the parent cirramycin A_1 molecule[9,10]. Compound V will therefore be referred to as depoxycirramycin A_1 hereafter.

In the comparative NMR analysis of compounds I, IV and V, a singlet methyl signal (1.52 ppm) of I shifts to higher field (0.9~1.2 ppm) in compound IV but to lower field (1.84 ppm, singlet) in V. The AB quartet of the two olefinic protons of I splits more clearly (6.43 and 7.46 ppm) in the NMR spectra of V, in which a new additional low field proton (1 H, doublet, 5.81 ppm) is seen. These observations lead to an assignment that a methyl group is substituted to the γ-position and a hydrogen atom to the δ-position of the conjugated carbonyl system of compound V.

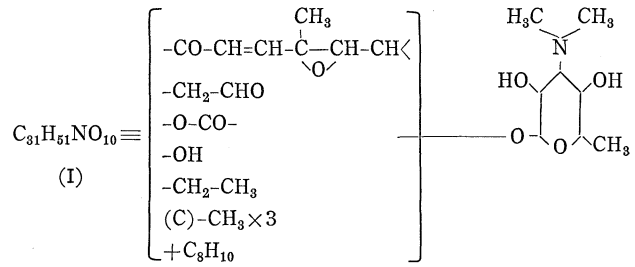
Cirramycin A_1 gives a crystalline aldoxime (VI, $C_{31}H_{52}N_2O_{10}$) by a usual procedure, which melts at 141~143°C and shows a strong UV absorption maximum at 241 mμ indicating that the conjugated carbonyl group of I remains intact. In the IR spectrum

Fig. 4. NMR spectrum of cirramycin A_1 aldoxime (VI) (100 Mc, in acetone-d_6)



of VI, the carbonyl absorption band at 1725 cm^{-1}, which is considerably weaker than that found in the spectrum of I, along with a band at 1185 cm^{-1} is assigned to the ester or lactone linkage. In the NMR spectrum of VI (Fig. 4) the methine proton next to the oximino group is observed in triplet pattern at 7.54 ppm (J=6.0 cps), which suggests that the aldehyde radical of I links to a methylene group although the original aldehyde proton appears in singlet in the NMR spectrum of cirramycin A_1. Similar NMR spectral relationship about the aldehyde proton has also been reported with magnamycin[7] and leucomycin A_3[11].

Combination of the above-described informations gives the following partial structural formula for cirramycin A_1:



As to the degree of unsaturation, the above structure involves 6 out of the 7 unsaturated groups of the cirramycin A_1 molecule and the remaining one can be assigned to the lactone ring.

Degradation Studies

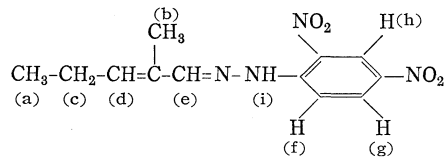
Ozonolysis of depoxycirramycin A_1 (V):

In an attempt to cleave the molecule at the γ,δ-double bond of the conjugated carbonyl system of V, a stream of ozone was introduced into a methylene chloride solution of V at −65°C. The ozonide formed was decomposed by zinc dust and water, and the volatile aldehyde mixture was distilled and precipitated as 2, 4-dinitrophenylhydrazone. The solid was purified by the preparative thin-layer chromatography (TLC) and a main component was isolated as yellow needles (VII) melting at 160~162°C. Compound VII was analyzed for $C_{12}H_{14}N_4O_4$, and showed a strong UV absorption maximum at 380 mμ (ε: 28,600) which suggested that VII is a 2, 4-dinitrophenylhydrazone of α,β-unsaturated carbonyl compound[12]. The analysis of the NMR spectrum (Table 1) suggested the following structure for VII, and accordingly indicated that

Table 1 Analysis of NMR spectrum of VII

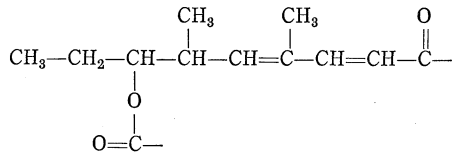
δ (ppm)	Relative intensity	Signal pattern	Coupling constant (cps)	Assignment
1.12	3H	triplet	$J_{ac}=7$	(a)
1.93	3H	singlet	—	(b)
2.37	2H	quintet	$J_{ac}=J_{cd}=7$	(c)
6.03	1H	triplet	$J_{cd}=7$	(d)
7.78	1H	singlet	—	(e)
7.95	1H	doublet	$J_{fg}=10$	(f)
8.32	1H	double doublet	$J_{fg}=10$, $J_{gh}=3$	(g)
9.12	1H	doublet	$J_{gh}=3$	(h)
11.17	1H	singlet	—	(i)

2-methyl-2-pentenal was a main volatile product after the ozonolysis of V. The identity of this product was established by synthesizing the aldehyde according to a method of DOEBNER and WEISENBORN[13].



VII

Therefore, the presence of the following partial structure was predicted in the depoxycirramycin A_1 molecule, which was further supported by a direct oxidative degradation of I as described in the next section.



Periodate-permanganate oxidation of cirramycin A_1 (I):

According to a method developed by LEMIEUX and RUDLOFF[14], I was oxidized at room temperature in an aqueous solution of periodate and permanganate. The oxidation product, which was obtained as an yellowish solid, was stirred with 1 N sodium hydroxide solution at 25°C overnight. The hydrolyzate was made acidic and extracted with ether repeatedly. The ether-soluble fraction was treated with diazomethane and the major acidic component was isolated as a methyl ester (VIII).

Compound VIII, $C_{10}H_{18}O_4$, is a colorless oil and showed the IR absorptions at 3580 cm^{-1} (hydroxy), 1745 and 1170 cm^{-1} (ester). The UV absorption maximum at 215 mμ (ε : 3100) suggested a presence of an α,β-epoxy carbonyl grouping in VIII. Analysis of the NMR spectrum of VIII (Fig. 5 and

Fig. 5. NMR spectrum of (VIII) (60 Mc, in CCl_4)

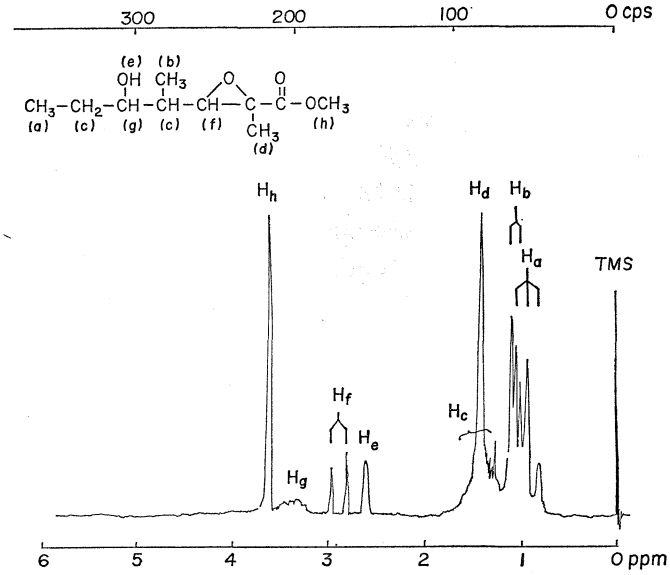
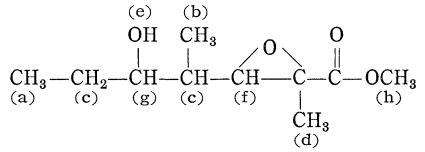


Table 2. Analysis of NMR spectrum of VIII (in CCl_4, 60 Mc)

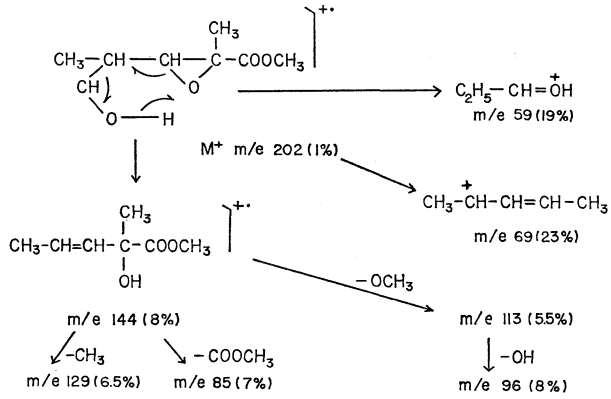
δ (ppm)	Relative intensity	Signal pattern	J (cps)	Assignment
0.94	3H	triplet	6.5	(a)
1.04	3H	doublet	6.0	(b)
1.2~1.5	3H	multiplet		(c)
1.42	3H	singlet	—	(d)
2.62	1H	singlet	—	(e)
2.91	1H	doublet	8.0	(f)
3.1~3.5	1H	multiplet		(g)
3.62	3H	singlet	—	(h)

Table 2) has led to the following structure for compound **VIII** which was confirmed by mass spectroscopy (Chart 1).



VIII

Chart 1. Fragmentations of **VIII** in mass spectroscopy



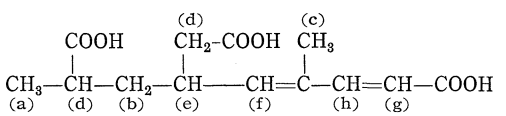
The aqueous layer after the above ether extraction was concentrated to dryness, and the residue was extracted with a mixture of methanol-benzene (1 : 1). Evaporation of the solvent gave a solid which appeared to be the fragment containing mycaminose (or a material derived from it) as evidenced by a positive ninhydrin reaction. Therefore, the solid was subjected again to an alkaline hydrolysis under more drastic condition. The hydrolyzate was made acidic and extracted with ether repeatedly. Evaporation of the ether extracts gave an acidic solid which was crystallized from an acetone-benzene mixture to give colorless needles (**IX**) melting at 195.5~196.5°C.

Compound **IX**, $C_{13}H_{18}O_6$, shows the IR absorption bands due to carboxyl groups at 3000~2500 (broad), 1725 (shoulder), 1710 and 1685 (partially resolved) along with a sharp band at 1615 cm^{-1}. It absorbs strongly in the UV region at 263.5 mμ (ε : 29,400) in ethanol and, upon treatment with diazomethane, gives a trimethyl ester ($C_{16}H_{24}O_6$, **X**). These characteristics suggested that **IX** is a tricarboxylic acid having conjugated unsaturations.

Table 3. Analysis of NMR spectrum of **IX** (in $(CD_3)_2CO$, 100 Mc)

δ (ppm)	Relative intensity	Signal pattern	J (cps)	Assignment
1.28	3H	doublet	7	(a)
1.4~1.8	3H	multiplet		(b)
1.88	3H	singlet	—	(c)
2.2~2.5	3H	multiplet		(d)
2.9~3.3	1H	multiplet		(e)
5.60	1H	doublet	9	(f)
5.82	1H	doublet	15	(g)
7.28	1H	doublet	15	(h)

The NMR spectrum of **IX** (Fig. 6) was analyzed as shown in Table 3 to assign the following structure to compound **IX**.



IX

Fig. 6. NMR spectrum of (IX) (100 Mc, in acetone-d_6)

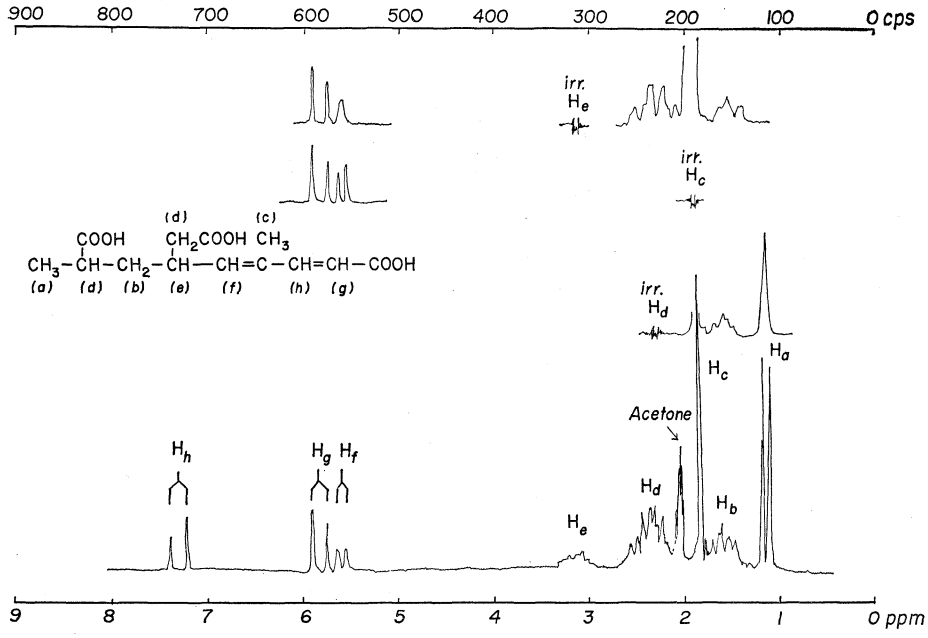
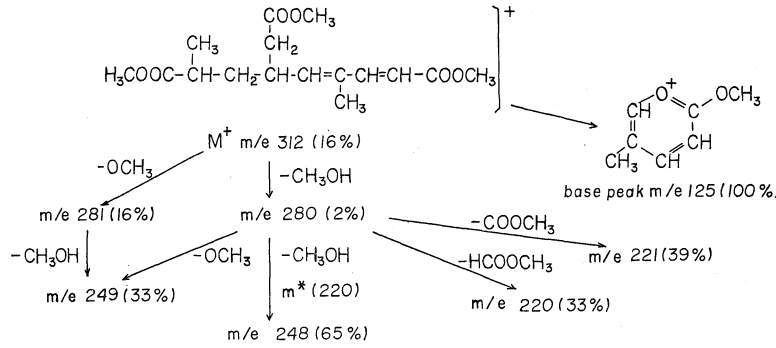


Chart 2. Fargmentations of X in mass spectroscopy

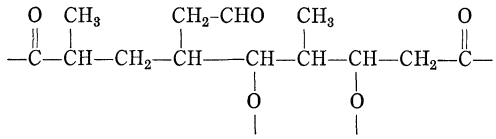


The following spin-decoupling experiments confirmed the above proton assignments: Upon irradiation at the multiplet protons (d), the doublet methyl signal (a) became a singlet. When the methyl protons (c) was irradiated, the broad doublet of (f) was sharpened indicating a presence of long-range coupling between protons (c) and (f). Irradiation at the 3.3 ppm proton (e) caused multiplicity changes of three signals: the complex signal of methylene protons (b) became much simpler, the proton (f) changed from doublet to singlet, and the protons (d) from multiplet to quartet-like pattern suggesting an overlap of the AB quartet of non-equivalent methylene protons (d) and the multiplet of methine proton (d).

The mass spectrum of the trimethyl ester (X) also supports the proposed structure of IX as shown in the following fragmentation chart (Chart 2).

Since the tricarboxylic acid, IX, was isolated from a degradation fragment to which the mycaminose was glycosidically linked and since one hydroxy group should be

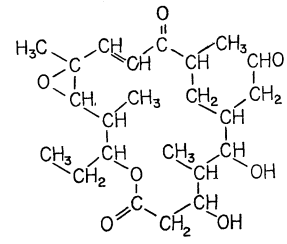
included in the lactone part of cirramycin A_1, the two C=C double bonds in **IX** are presumed to have been formed during the drastic alkaline hydrolysis of the ninhydrin-positive fraction which was obtained after the oxidative degradation of I. The acid, **IX**, might therefore represent the following partial structure of cirramycin A_1:



Thus, the empirical formula of I, $C_{31}H_{51}NO_{10}$, could be accounted for by combining the above-obtained structural informations about the two acid fragments, the α,β-epoxy ester (**VIII**) and the tricarboxylic acid (**IX**). The determination of which of the three carboxyl functions of acid **IX** is to be assigned to the original lactone carbonyl group of I, then leads to an elucidation of the lactone ring structure, and this has been done by the following sequence of reactions.

A catalytic hydrogenation of **V** gave depoxytetrahydrocirramycin A_1 (**XI**) which was treated with sodium borohydride to yield depoxyoctahydrocirramycin A_1 (**XII**, $C_{31}H_{59}NO_9$). Compound **XII** was hydrolyzed with 50 % potassium hydroxide under similar conditions to those used for preparation of acid **IX**, and yielded an acidic material which, although not isolated in crystalline state, showed a UV absorption maximum at 263.5 mμ ($E^{1\%}_{1cm}$ 84). The spectrum of this material is characteristic of an $\alpha,\beta,\gamma,\delta$-unsaturated carboxylic acid which in turn suggets that two hydroxy groups are present at the β and δ positions to the lactone carbonyl. Therefore, coupled with the fact that the aldehyde function should be connected with a methylene group, the following structure can be given to the lactone part of cirramycin A_1 and accordingly the structure (I), shown earlier to cirramycin A_1.

Structure of lactone ring of cirramycin A_1



Experimental

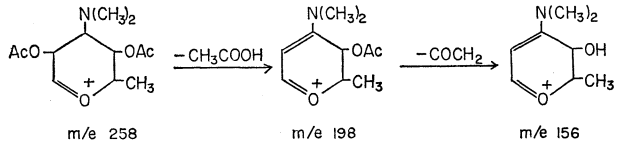
Cirramycin A_1 base (I)

Amorphous cirramycin A_1 base (I) or crystalline chlroform solvate of I used in the present study was prepared by method described in the preceeding paper[2]. NMR $\delta^{CO(CD_3)_2}_{TMS}$: 0.9~1.25 (15H, m), 1.52 (3H, s, CH_3-C(O)-C), 2.57 (6H, s, $(CH_3)_2N$-), 6.45 and 6.92 (2H, AB quartet, J=16 cps, H>C=C<H), 9.88 (1H, s, -CHO).

Triacetylcirramycin A_1 (II)

A solution of I (1.0 g) in 10 ml of acetic anhydride and 5 ml of pyridine was stirred at room temperature overnight and then poured into 50 g of crushed ice. The mixture was extracted at pH 6 with three 20-ml portions of ethyl acetate. The combined extracts were washed with water, dried over anhydrous sodium sulfate and concentrated *in vacuo* to 10 ml. Addition of 15 ml of *n*-hexane to the concentrate precipitated 290 mg of solid which was crystallized from *n*-hexane. Colorless needles. M. p. 111~113°C. UV λ^{EtOH}_{max} 240.5 mμ (ε : 14,600). MS m/e : 723 (M^+), 695 (M^+ -CO), 663 (M^+ -CH_3COOH), 635 (663 -CO),

449 (M^+-diacetylmycaminosyl), 421 (449 -CO), 389 (449 -CH_3COOH), 361 (389 -CO), 258, 198 and 156 (fragment ions from diacetylmycaminose as shown below).



Anal. Calcd. for $C_{37}H_{57}NO_{13}$: C 61.39, H 7.94, N 1.95
Found : C 60.95, H 7.95, N 2.08

Isolation of mycaminose (III)

One gram of I was refluxed with 15 ml of 6 N hydrochloric acid for 2 hours. The reaction mixture was cooled and decanted to remove some tarry materials. The aqueous solution was shaken with chloroform and *n*-butanol and then concentrated *in vacuo* to dryness. The residue, 280 mg, was dissolved in 1 ml of water, chromatographed on a column of Dowex 50W-X8 (H-type) and eluted with 0.5 N hydrochloric acid. The fractions which gave a positive anthrone reaction were collected and evaporated to dryness. The solid, 50 mg, was crystallized from aqueous *iso*-prapanol. M. p. 111.5~113.5°C. $[\alpha]_D^{27.5}$ +29° (*c* 2, H_2O). Identical IR spectrum with an authentic mycaminose hydrochloride. Identical Rf value (0.66) with mycaminose by paper chromatography with a solvent system of *n*-propanol - pyridine - acetic acid - water (8 : 8 : 1 : 4).

Anal. Calcd. for $C_8H_{17}NO_4 \cdot H_2O \cdot HCl$: C 39.10, H 8.20, N 5.70, Cl 14.43
Found : C 39.23, H 8.30, N 5.79, Cl 14.67

Tetrahydrocirramycin A_1 (IV)

Hydrogen was introduced under atmospheric pressure to a stirring suspension of 500 mg (0.87 mM) of I and 160 mg of 5 % palladium-charcoal in 20 ml of ethanol at room temperature. An equivalent to 1.90 millimolar hydrogen gas was absorbed during 16 hours. The reaction mixture was filtered and the filtrate was evaporated *in vacuo* to leave 450 mg of white amorphous solid which was crystallized from *n*-hexane. M. p. 95~98°C. UV λ_{max}^{EtOH} 282 mμ (ε : 72). NMR $\delta_{TMS}^{CDCl_3}$: 0.9~1.27 (18H, m), 2.50 (6H, s, $(CH_3)_2N$-).

Anal. Calcd. for $C_{31}H_{55}NO_{10}$: C 61.87, H 9.21, N 2.33
Found : C 61.41, H 8.54, N 2.29

Depoxycirramycin A_1 (V)

A solution of 1.0 g of I in 5 ml of acetic acid was added with 1.5 g of potassium iodide and stirred for half an hour at 75~78°C. The reaction mixture was cooled, poured onto 200 g of crushed ice and then extracted with three 50-ml portions of ethyl acetate at pH 8.5. The combined extract was washed with 50 ml of 2 % aqueous sodium thiosulfate solution at pH 9 then with water and dried over anhydrous sodium sulfate. Evaporation of the solvent gave 736 mg of white solid which was crystallized from *n*-hexane. M. p. 117~120°C. $[\alpha]_D^{26.5}$ −28.8 (*c* 2, $CHCl_3$). UV λ_{max}^{EtOH} 284 mμ (ε : 22,300). IR $\nu_{C=C}$ 1590 cm^{-1}. NMR : $\delta_{TMS}^{CDCl_3}$ 0.9~1.37 (15H, m), 1.84 (3H, s, CH_3-C=C), 2.52 (6H, s, $(CH_3)_2N$-), 5.81 (1H, d, J=11 cps, -CH=C(CH_3)-CH=), 6.43 and 7.46 (2H, AB quartet, J=16 cps, -CH=CH-CO-), 9.94 (1H, s, -CHO).

Anal. Calcd. for $C_{31}H_{51}NO_9$: C 64.00, H 8.84, N 2.41
Found : C 63.36, H 8.65, N 2.41

Cirramycin A_1 aldoxime (VI)

A solution of 310 mg of I in 20 ml of ethanol was added with 40 mg of hydroxylamine hydrochloride and a drop of pyridine, and heated under reflux for 3 hours. The reaction mixture was cooled and concentrated *in vacuo* to give 270 mg of solid which was dissolved in 5 ml tetrahydrofuran and filtered. Addition of 20 ml of *n*-hexane to the filtrate gave

147 mg of white precipitate which was crystallized from ether and *n*-hexane. M. p. 141~143°C. UV λ_{max}^{EtOH} 241 mμ (ε : 11,700).

Anal. Calcd. for $C_{31}H_{52}N_2O_{10}$: C 60.76, H 8.55, N 4.57
Found : C 59.74, H 8.16, N 4.80

Ozonolysis of depoxycirramycin A_1 (V) and isolation of 2, 4-dinitrophenylhydrazone of 2-methyl-2-pentenal (VII)

To a solution of 1.0 g of V in 100 ml of methylene chloride was introduced a stream of ozone gas maintaining the temperature below −65°C by acetone-dry ice. The blue-colored reaction mixture was concentrated to about 3 ml and added with 4 g of zinc dust and 80 ml of water. After heating under reflux for 10 minutes, the mixture was distilled with occasional additions of water, and the volatile distillate was introduced into a solution composed of 1.2 g of 2, 4-dinitrophenylhydrazine, 6 ml of conc. sulfuric acid and 39 ml of 75 % ethanol. About 800 mg of yellowish brown precipitate was collected, which showed several spots by silica gel TLC using a solvent system of benzene - ethyl acetate (20 : 1). The major component (Rf : 0.9) was isolated by preparative TLC using 40 silica gel plates and crystallized from ethanol to give 62 mg of fine orange needles of VII. M. p. 160~162°C. UV $\lambda_{max}^{CHCl_3}$ mμ (ε) : 260 (16,700), 280 (11,700), 290 (10,600) and 380 (28,600). MS m/e : 278 (M^+), 261 (M^+ -OH), 249 (M^+ -C_2H_5), 245 (M^+ -(CH_3+H_2O)), 243 (M^+ -(H_2O+OH)).

Anal. Calcd. for $C_{12}H_{14}N_4O_4$: C 51.79, H 5.07, N 20.14
Found : C 51.81, H 5.09, N 19.91

Periodate-permanganate oxidation of cirramycin A_1 (I)

A mixture of 4.01 g of I, 12 g of sodium metaperiodate, 876 mg of potassium permanganate and 291 mg of potassium carbonate in 2,800 ml of water was stirred at 25°C for 16 hours. The reaction mixture was filtered and the filtrate was washed with 300 ml of ethyl acetate. The aqueous layer was separated, adjusted to pH 2 by 6 N hydrochloric acid and extracted with three 500-ml of *n*-butanol. The combined extract was washed with 500 ml of water and then evaporated under reduced pressure to leave 4.03 g of yellowish solid. The oxidation product, 3.9 g, was dissolved in 100 ml of 1 N sodium hydroxide solution and stirred at 25°C overnight then at 50°C for 4 hours. After being washed with 20 ml of ethyl acetate, the reaction mixture was made acidic (pH 2) and extracted with three 50-ml portions of ether (Fraction 1) then with three 30-ml portions of *n*-butanol (Fraction 2). The aqueous layer after the above solvent extractions was adjusted to pH 6.0 and concentrated *in vacuo* to dryness. The residue was extracted with 50 ml of an 1 : 1 mixture of methanol-benzene (Fraction 3). Evaporation of the above solvent extracts gave 518 mg of oily materials from Fraction 1, 1.98 g from Fraction 2 and 1.04 g from Fraction 3. The material obtained from Fraction 2 was found by thin-layer chromatography to be a mixture of Fractions 1 and 3.

Isolation of monocarboxylic acid methyl ester (VIII), $C_{10}H_{18}O_4$

The oily material obtained from the above Fraction 1, 510 mg, was dissolved in 10 ml of ether and reacted with 30 ml of ethereal solution of diazomethane (*ca.* 20 mM) at room temperature overnight. Evaporation of the solvent gave 468 mg of crude oil which was purified by the silicic acid column chromatography to yield 210 mg of colorless oil (VIII). Retention time in gas-chromatography (30 % SE-30, 201°C, He 200 ml/min) : 1.2 min. UV λ_{max}^{EtOH} 215 mμ (ε : 3100).

Anal. Calcd. for $C_{10}H_{18}O_4$: C 59.38, H 8.97
Found : C 59.89, H 8.83

Isolation of tricarboxylic acid (IX), $C_{13}H_{18}O_6$

The tarry material obtained from the above Fraction 3, 1.50 g, was heated under reflux with 40 ml of 50 % aqueous potassium hydroxide solution for 20 hours. After being washed with 20 ml of ether, hydrolyzate was adjusted to pH 2.0 by 6 N hydrochloric acid and extracted with three 20-ml portions of ether. The combined extracts were washed

with 30 ml of water, dried over anhydrous sodium sulfate and concentrated *in vacuo* to dryness. The residual solid, 471 mg, was crystallized from acetone-benzene to yield 205 mg of white needles (**IX**). M. p. 195.5~196.5°C. UV λ_{max}^{EtOH} 263.5 mμ (ε : 29,400), $\lambda_{max}^{N/10\ HCl}$ 269.5 mμ (ε : 30,200), $\lambda_{max}^{N/10\ NaOH}$ 263 mμ (ε : 29,200).

Anal. Calcd. for $C_{13}H_{18}O_6$: C 57.77, H 6.71
Found : C 57.23, H 6.48

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References

1) KOSHIYAMA, H.; M. OKANISHI, T. OHMORI, T. MIYAKI, H. TSUKIURA, M. MATSUZAKI & H. KAWAGUCHI: Cirramycin, a new antibiotic. J. Antibiotics, Ser. A 16 : 59~66, 1963.
2) KOSHIYAMA, H.; H. TSUKIURA, K. FUJISAWA, M. KONISHI, M. HATORI, K. TOMITA & H. KAWAGUCHI: Studies on cirramycin A_1. I. Isolation and characterization of cirramycin A_1. J. Antibiotics 22 : 61~64, 1969.
3) FUJISAWA, K.; K. MATSUMOTO, T. OHMORI, T. HOSHIYA & H. KAWAGUCHI: Studies on cirramycin A_1. II. Biological activity of cirramycin A_1. J. Antibiotics 22 : 65~70, 1969.
4) WOODWARD, R. B.: Struktur und Biogenese der Makrolide, eine neue Klasse von Naturstoffen. Angew. Chem. 69 : 50~58, 1957.
5) HOCHSTEIN, F. A. & P. P. REGNA: Magnamycin. IV. Mycaminose, an aminosugar from magnamycin. J. Am. Chem. Soc. 77 : 3353~3355, 1955.
6) OHMURA, S.; H. OGURA & T. HATA: The chemistry of the leucomycins. II. Structure and stereochemistry of leucomycin A_3. Tetrahedron Letters 1967-14 : 1267~1271, 1967.
7) KUEHNE, N. E. & B. W. BENSON: The structures of the spiramycins and magnamycin. J. Am. Chem. Soc. 87 : 4660~4662, 1965.
8) MORIN, R. B. & M. GORMAN: The partial structure of tylosin, a macrolide antibiotic. Tetrahedron Letters 1964-34 : 2339~2345, 1964.
9) WOO, P.W.K.; H. W. DION & Q. R. BARTZ: Partial structure of chalcomycin. II. A C_{17}-mycinosyloxy moiety. J. Am. Chem. Soc. 86 : 2724~2726, 1964.
10) BODFORSS, S.: Zur Kenntnis der aromatischen Oxidoverbindungen. Chem. Ber. 49 : 2795~2813, 1946.
11) OHMURA, S.; H. OGURA & T. HATA: The chemistry of the leucomycins. I. Partial structure of leucomycin A_3. Tetrahedron Letters 1967-7 : 609~613, 1967.
12) BRANDE, E. A. & E.R.H. JOHNES: Studies in light absorption. II. 2,4-Dinitrophenylthydrazone. J. Chem. Soc. 1945 : 498~503, 1945.
13) DOEBNER, O. & A. WEISENBORN: Über die Darstellung der Sorbinsäure-homologen, ungesättigten Säuren mit zwei Doppelbindungen, γ,ε-dimethylsorbinsäure. Chem. Ber. 35 : 1136~1147, 1902.
14) LEMIEUX, R. U. & E. V. RUDLOFF: Periodate-permanganate oxidations. I. Oxidation of olefines. Canad. J. Chem. 33 : 1701~1709, 1955.