STUDIES ON CIRRAMYCIN A_1. I

ISOLATION AND CHARACTERIZATION OF CIRRAMYCIN A_1

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Cirramycin A, a macrolide antibiotic, has been found to consist of one major (A_1) and several minor components (A_2, A_3, A_4 and A_5). The major active substance, cirramycin A_1 ($C_{31}H_{51}NO_{10}$), was isolated as a single component and characterized in detail. It has a strong ultraviolet absorption peak at 241 mμ and its chromophore is suggested to be an α,β-unsaturated γ,δ-epoxide carbonyl system. Cirramycin A_1 contains the basic sugar mycaminose but lacks a neutral sugar in the molecule.

In the previous publication on cirramycin[1], we have reported that cirramycin belongs to the macrolide group of antibiotics and contains at least two components, A and B. Recent studies on the cirramycin complex revealed that cirramycin A could be further separated into several components related closely each other, and the major one was isolated in crystalline form and designated cirramycin A_1. This paper reports the production, isolation and characterization of cirramycin A_1.

Fermentation Studies

As reported in a preceding paper[1], *Streptomyces cirratus* strain No. 12090 produced approximately equal amounts of cirramycins A and B. Several strains of cirramycin-producing organism were thereafter isolated in our antibiotic screening program, and a streptomyces strain, designated No. JTB-3 in our culture collection, which was isolated from a soil sample collected at Setagaya, Tokyo, was found to be suitable for the production of cirramycin A because of its rather preferential biosynthesis of the A component, yielding approximately 4 parts of A and 1 part of B in the culture broth. Therefore, strain No. JTB-3 was used for the production and isolation of cirramycin A_1 in the following studies.

On microscopic examination, the strain JTB-3 was found to produce curling aerial hyphae with open spirals bearing elliptical to oval spores of smooth surface. The cultural characteristics and biochemical properties of the strain were also very similar to those of *Streptomyces cirratus* strain No. 12090 except for a few minor differences such as milk coagulation and milk peptonization properties which were positive for the new strain, JTB-3. Therefore, strain JTB-3 was determined to be a species of *Streptomyces cirratus*. A medium of the following composition was found useful for the production of cirramycin A by strain JTB-3:

Oat meal	6.0 %	$CaSO_4 \cdot 2H_2O$	0.1 %	$MgSO_4 \cdot 7H_2O$	0.01 %
Pharmamedia	0.4	$CaCl_2 \cdot 2H_2O$	0.05	$FeSO_4 \cdot 7H_2O$	0.1
$CaCO_3$	0.2	$ZnSO_4 \cdot 7H_2O$	0.03		

Isolation of Cirramycin A_1

The fermentation broth of strain JTB-3 was filtered at slightly acidic pH and extracted with *n*-butyl acetate at pH 8.5. The activity in the solvent extract was transferred to aqueous dilute hydrochloric acid at pH 2 which was again extracted by benzene at pH 9.0. The benzene extract, which contained cirramycins A and B, was stirred repeatedly with M/10 SÖRENSEN's buffer of pH 7.0, then with dilute hydrochloric acid of pH 2.0, whereupon most of the cirramycin A was found in the buffer solution and cirramycin B in the latter acid solution. The activity in the buffer solution was extracted by benzene at pH 9.0 and the benzene extract was stirred with phosphate buffer solution at pH 6.0. Repetition of the above procedure gave cirramycin A which was free from the B component. However, when a fairly large amount of this preparation was applied on a paper strip and developed by a solvent system consisting of 0.05 N ammonia saturated with methyl iso-butyl ketone, one major zone (Rf 0.84, designated A_1) and a few minor zones (Rf 0.89, 0.75, 0.67 and 0.86, designated A_2, A_3, A_4 and A_5, respectively) were recognized by bioautography on a *Bacillus subtilis* plate.

Cirramycin A_1 was isolated as a single component by CRAIG's counter-current distribution using a solvent system consisting of benzene and M/10 SÖRENSEN's phosphate buffer of pH 7.0, the peak being found at tube No. 45 in 100 transfers. In this experiment, cirramycin A_2 was distributed before the A_1 peak and cirramycins A_3, A_4 and A_5 appeared after the A_1 component. A series of separatory funnels was also used for the larger scale preparation of the A_1 component.

In order to make a crystalline preparation, the above-obtained amorphous cirramycin A_1 base was dissolved in chloroform and treated with a small amount of active carbon. The filtrate was concentrated to a syrupy consistency, added to a small volume of *n*-hexane and kept in the cold room (5°C) overnight to yield colorless prisms of cirramycin A_1 chloroform solvate melting at 145~148°C. An analytically pure sample of cirramycin A_1 free base (m. p. 124~128°C) was prepared from the crystalline chloroform solvate by dissolving the latter in methanol and then azeotropically distilling the solvent.

Physico-chemical Properties of Cirramycin A_1

Cirramycin A_1 free base is insoluble in water and petroleum ether, but dissolves readily in most of the other common organic solvents. It is also soluble in aqueous acidic media and forms water-soluble salts with a variety of mineral and organic acids.

Cirramycin A_1 was titrated in 66 % aqueous dimethylformamide as a monoacidic base giving a titration equivalent of 610 ± 20 and a pKa′ of 8.0. The osmometric determination of the molecular weight gave a mean value of 606, and the microanalytical data favored a molecular formula of $C_{31}H_{51}NO_{10}$ (Mol. wt. 597.7) for cirramycin A_1 base.

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

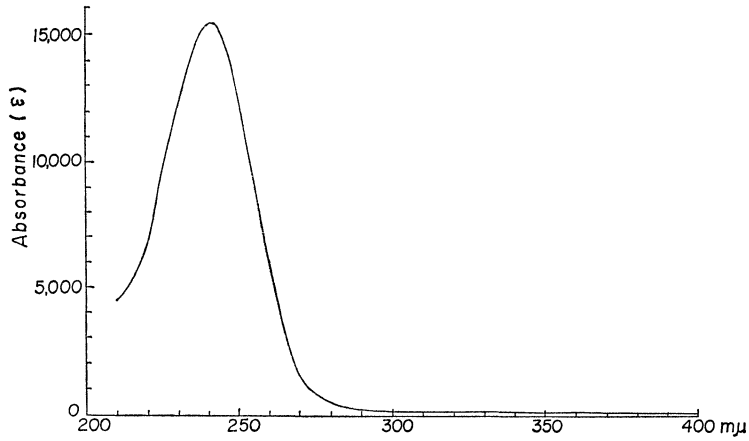


Fig. 2. IR spectrum of cirramycin A_1.

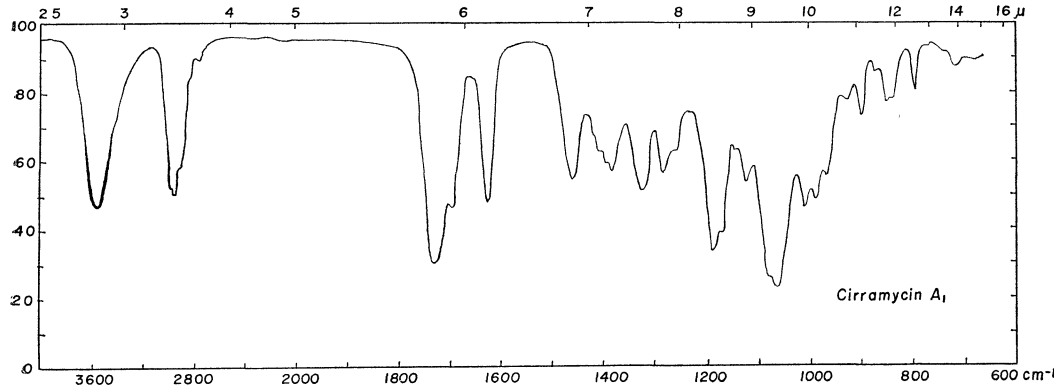
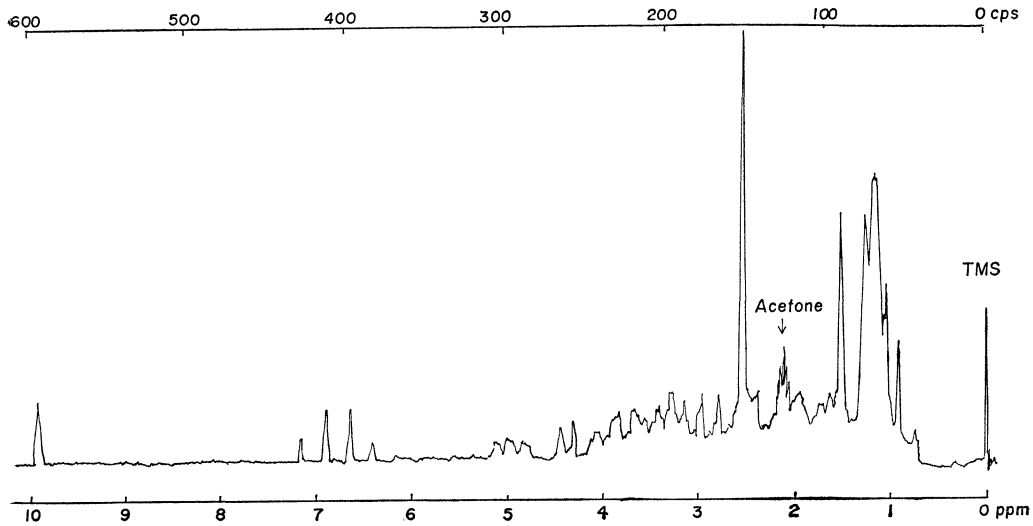


Fig. 3. NMR spectrum of cirramycin A_1 (I) (60 Mc, in acetone $-d_6$)



Calc'd : C 62.29, H 8.60, N 2.34
Found : C 62.42, H 8.49, N 2.31

The specific optical rotation $[\alpha]_D^{23}$ of the free base in chloroform is $-28°$ (c 1.0). It absorbs strongly in the ultraviolet region at 241 mμ ($E_{1cm}^{1\%}$ 258) which is attributable to a conjugated carbonyl grouping. The infrared absorption bands at 1695 and 1620 cm^{-1} are also indicative of the α,β-unsaturated carbonyl system in the molecule. The presence of an aldehyde function is apparent from the NMR spectrum at 9.88 ppm. The UV, IR and NMR spectra of cirramycin A_1 are shown in Figs. 1, 2 and 3, respectively.

Cirramycin A_1 gives positive FEHLING, TOLLENS and MOLISCH reactions but is negative to SELIWANOFF, biuret, ninhydrin and ferric chloride reagents. It gives a reddish orange color by the erythromycin test[3] and yellowish brown by the carbomycin test[3]. On acid hydrolysis* cirramycin A_1 yields a basic sugar which was identified with mycaminose[4]. A neutral sugar was not recognized in the hydrolyzate.

Group analysis of the compound indicates no acetyl, no methoxy and about six C-methyl groups in the molecule. The presence of 3 acylatable hydroxy groups was shown by the formation of triacetylcirramycin A_1*. On catalytic hydrogenation two moles of hydrogen were absorbed to yield tetrahydrocirramycin A_1*, which showed a very weak absorption maximum at 286 mμ. Treatment of cirramycin A_1 with potassium iodide in acetic acid gave a compound* having a strong UV absorption maximum at 284 mμ ($E_{1cm}^{1\%}$ 384), attributed to an $\alpha,\beta,\gamma,\delta$-unsaturated carbonyl system which is in turn suggestive of the presence of an epoxide function next to the conjugate system in the parent cirramycin A_1 molecule. The preparation and biological activity of some of the above derivatives will be reported in a separate paper[5].

References

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* Experimental details will appear in a forthcoming paper[2].