STUDIES ON CIRRAMYCIN A_1. IV
DERIVATIVES OF CIRRAMYCIN A_1

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(Received for publication December 10, 1968)

A variety of cirramycin derivatives has been prepared by modifying the hydroxyl groups, aldehyde function, dimethylamino group of mycaminose or the conjugated carbonyl system of the cirramycin A_1 molecule. Among the derivatives evaluated, disuccinylcirramycin A_1 (III) was found to possess some improved biological properties with respect to oral absorption, local tissue irritability as well as general toxicity.

A number of chemical derivatives of macrolide antibiotics have been investigated by many research groups in order to improve the intrinsic properties of the original antibiotics, such as water solubility, acid stability and oral absorption. As reported in the previous papers[1,2], cirramycin A_1 is fairly stable in acid and it exhibits quite high *in vivo* activity especially when administered parenterally. Therefore our primary concern in the present study is to obtain derivatives capable of being well absorbed following oral administration or free from the local tissue irritation upon injection.

There are several functional groups available for chemical modification in the structure of cirramycin A_1. These are three acetylable hydroxyl groups (two in the mycaminose and one in the lactone ring), one aldehyde group and the α,β-unsaturated γ,δ-epoxy carbonyl system as well as a dimethylamino group of mycaminose. This paper reports the preparation and evaluation of various derivatives of cirramycin A_1, especially of des-epoxy and disuccinyl compounds.

Preparations and Primary Evaluations of Cirramycin A_1 Derivatives

Twenty four derivatives of cirramycin A_1 (I) listed in Table 1 were prepared. Various types of esters were made by reacting the free base of I with the appropriate acid chloride or acid anhydride in anhydrous solvent. Preparations of des-epoxy derivatives and the tetrahydro compound were reported in a preceeding paper[3]. The N-oxide compounds were obtained by a mild oxidation with hydrogen peroxide in methanol solution according to a published method[4]. The aldoxime of I was described before[3] and the preparation of the hydantoinylimino derivative is described in the experimental section. Although not all of these derivatives were isolated in analytically pure state, a purity of greater than 80% was assigned to all of the

Table 1. Derivatives of cirramycin A_1

Type of derivative	Derivative* of cirramycin A_1	Rf of PPC**				MIC *vs* *S. aureus* (mcg/ml)	CD_{50} *vs* *S. aureus* (mg/kg)	
		M-7	M-8	M-9	M-10		Subcutaneous	Oral
Esters of monobasic acids	Acetyl	0.45	—	—	0.87	1.56	15	—
	Triacetyl	0.93	—	0.98	—	1.56	18	580
	Propionyl	—	0.15	—	0.93	1.56	21	—
	Phenylacetyl	0.88	—	0.96	—	0.78	30	280
	Phenoxyacetyl	0.68	—	0.98	—	0.78	25	280
	Benzoyl	0.88	—	—	0.92	3.12	150	230
Esters of dibasic acids	Maleoyl	0	0.49	0.33	0.64	0.78	18	320
	Disuccinyl (III)	0	0.57	0.80	0.80	0.78	5.6	125
	Glutaryl	0	0.47	0.82	0.64	0.78	16	360
	Phthaloyl	0	0.48	0.33	0.68	0.78	10	200
Esters of amino acids	Leucyl	0	0.46	—		6.25	40	—
	Phenylglycyl	0	0.45	—	0.02	3.12	130	—
Esters of other acids	Methylsuccinyl (IV)	0.18	0.50	—	0.88	0.78	14	—
	β-Sulfopropionyl	0	0.46	—	0	1.56	28	190
	Methylsulfonyl	0.80	—	—	0.89	12.5	58	—
	Methylcarbamoyl	0	0.35	—	0.80	3.12	—	—
	Ethoxycarbonyl	0.61	0.30	—	0.89	0.39	22	400
Des-epoxy (II)	Depoxy (II)	—	0.35	—	0.78	1.56	8.5	140
	Depoxypropionyl	—	—	—	0.95	1.56	15	—
	Tetrahydro	—	0.88	—	0.90	12.5	—	—
N-Oxide	N-Oxide	0	0.78	0.76	—	50	18	300
	Succinyl N-oxide	0	0.78	—	0.31	50	25	400
Aldehyde-modification	Aldoxime	—	0.64	—	—	6.25	—	—
	Hydantoinylimino	—	—	—	—	3.12	27	>400
	Cirramycin A_1 (I)	0	0.51	0.28	0.73	0.78	3.2	110

* Esters are *mono*esters unless otherwise stated.

** Paper chromatography.

System M-7	Stationary phase:	formamide-methanol (1 : 1)
	Mobile phase:	benzene-cyclohexane (1 : 1) sat'd with formamide
System M-8	Paper:	treated with 2 % liquid paraffin
	Stationary phase:	butyl acetate
	Mobile phase:	M/15 SÖRENSEN'S buffer (pH 8) sat'd with butyl acetate
System M-9	Ascending PPC.	methyl isobutyl ketone-methyl ethyl ketone (4 : 1)
System M-10	Descending PPC.	methyl isobutyl ketone

compounds based on paper chromatography and IR spetra. The Rf values of the compounds obtained with a few solvent systems are included in Table 1.

The derivatives were primarily evaluated for antibacterial activity both *in vitro* and *in vivo*. The minimum inhibitory concentrations (MIC) of the compounds were determined against *Staphylococcus aureus* Smith by the serial tube dilution method. The *in vivo* activity was tested against an experimental infection with *S. aureus* Smith by the same technique as reported before[2], and the compounds were administered either subcutaneously or orally to determine the median curative dose (CD_{50}). The results are summarized and shown in Table 1.

The data suggest that the hydroxyl groups of cirramycin A_1 have little relation with the *in vitro* antibacterial activity since most of the esters are nearly as active as the parent antibiotic. The N-oxide derivatives showed higher *in vivo* effect than expected from the rather low *in vitro* activity, suggesting a reduction to the original antibitics in animal body. Among the present series of derivatives, depoxycirramycin A_1 (II) and disuccinylcirramycin A_1 (III) were studied further because of the indications of favorable *in vivo* activity.

Depoxycirramycin A_1 (II)

The chemistry of depoxycirramycin A_1 (II) has been discussed before[3]. The absorption and excretion of II were studied in rats by determining the urine levels following oral or intramuscular administration using a reported method[2]. The local tissue toxicity of II was assessed by the rat paw method[2], and cirramycin A_1 (I) was comparatively tested in these experiments.

Although compound II demonstrated CD_{50} values comparable to those of I, a lesser degree of urine recovery was observed with II by both oral and intramuscular administrations (Table 2). This fact suggests that II is less absorbed by or less stable in the animal organs than I. Upon injection into the plantar site of rat paws, II produced greater swelling than I did (Table 3).

Table 2. Comparative rat urine recovery test (dose: 25 mg/kg, 3 rats/group)

	No. of groups tested	% recovered in urine (mean)		
		i. m. dose	p. o. dose	ratio i. m./p. o.
Cirramycin A_1 (I)	6	4.42%	0.80%	5.5
Depoxycirramycin A_1 (II)	3	1.66	0.31	5.4
Disuccinylcirramycin A_1 (III)	6	3.19	0.98	3.3

Table 3. Assessment of local tissue irritation by rat paw method

Antibiotic concentration	Average volume increase of foot swelling (ml)		
	Cirramycin A_1 (I)	Depoxycirramycin A_1 (II)	Disuccinylcirramycin A_1 (III)
0.5%	0.43	0.82	0.10
1.0	0.89	1.05	0.12
2.0	1.12	1.17	0.25
4.0	—	—	0.60

Disuccinylcirramycin A_1 (III)

Compound III was prepared by reacting a free base of I with 2 molar equivalent of succinic anhydride in acetone solution at room temperature. It analyzes for $C_{39}H_{59}NO_{16}$ and gives a dimethyl ester ($C_{41}H_{63}NO_{16}$, IV). By analyzing the mass spectrum of IV, it was determined that the two succinyl residues esterify both of the two hydroxy group of mycaminose, as evidenced by peaks due to di-methylsuccinyl-mycaminosyl ion ($C_{18}H_{28}NO_9$, m/e 402) and its further decomposed fragment ions. The citrate salt of compound III is readily soluble in water and was used for the parenteral *in vivo* experiments.

The *in vitro* antibacterial activity and spectrum of III are essentially the same as those of I (Table 4). The absorption and excretion was primarily evaluated by the urine recovery test in rats. In this experiment, the percent recoveries were determined following both intramuscular and oral administrations of the test materials at an equal dose of 25 mg/kg. Therefore, the ratio of the two figures might reflect the relative oral absorbability of the compound. As can be seen in the right column of Table 2, there was a certain difference in the intramuscular/oral recovery ratio

Table 4. Antibacterial spectrum of disuccinylcirramycin A_1 (III)

Test organisms	Minimum inhibitory concentration	
	Disuccinyl-cirramycin A_1 (III)	Cirramycin A_1 (I)
Staphylococcus aureus FDA 209P	0.78 mcg/ml	0.39 mcg/ml
// FDA 209P (ST, SM-R)*	0.78	0.78
// FDA 209P (Novobiocin-R)	12.5	12.5
// #193 (PC, TC-R)*	1.56	0.78
// #193 (PC, TC, EM, CRM-R)*	100	100
// Smith strain	0.78	0.78
// #52-34 (PC, TC, EM, CRM-R)*	100	100
Micrococcus flavus	0.2	0.2
Corynebacterium xerosis 53-K-1	0.2	0.1
Bacillus mycoides strain "O"	0.78	0.39
Bacillus sphericus strain #122	0.78	0.39
Bacillus cereus ATCC 10702	0.78	0.39
Bacillus subtilis PCI-219	0.39	0.2
Bacillus anthracis : 115	0.39	0.2
Streptococcus hemolyticus Dick	1.56	1.56
Streptococcus pyogenes Dig 7 Type 3	0.78	1.56
Diplococcus pneumoniae Type II	0.78	0.39
Escherichia coli NIHJ	25	12.5
// PO 1495	50	50
// PO 1495 (CP, TC-R)*	100	100
Pseudomonas aeruginosa	25	25
Klebsiella pneumoniae julianelle type A	100	25
Shigella dysenteriae A	25	12.5
Shigella flexneri	6.25	12.5

* ST=Streptothricin, SM=Streptomycin, PC=Penicillin G, TC=Tetracycline, EM=Erythromycin, CRM=Carbomycin, CP=Chloramphenicol, -R=resistant.

between I (ratio, 5.5) and III (3.3), suggesting a relatively higher oral absorption of the latter.

The oral absorption was also studied with dogs. A group of 8 healthy dogs was given orally 50 mg/kg of III in gelatin capsules, and the blood levels were determined at 1, 3 and 6 hours after administration. The peaks were mostly attained after 3 hours and the antibiotic levels ranged from 0.46 mcg/ml to 1.98 mcg/ml with a mean value of 0.95 mcg/ml which is considerably higher than that obtained with I (mean, 0.38 mcg/ml)[2].

Table 5. Intramuscular injection of disuccinylcirramycin A_1 (III) into dogs (volume of dose, 5 ml)

Observations	Time after administration	Antibiotic concentration		
		1%	2%	4%
Painful response by palpation	1 hr	−	−	+
	3	−	−	+
	6	−	−	−
	24	−	−	−
Swelling	1 hr	−	±	+
	3	−	−	+
	6	−	−	+
	24	−	−	−
Induration	1 hr	−	−	−
	3	−	−	−
	6	−	−	−
	24	−	−	−

As was anticipated from the increased water solubility, III produced a much lesser degree of local tissue irritation than did the parent antibiotic when injected into rat paws (Table 3). The actual pain liability of III was further evaluated by intramuscular injection into dog thighs. Three groups of two dogs were given intramuscularly 5 ml of aqueous solution of the compound (1, 2 and 4%) into one

hind leg, and the opposite leg served as control which received the same volume of saline. The injection sites were palpated at 1, 3, 6 and 24 hours after treatment for evidence of painful reaction, swelling or induration. As shown in Table 5, somewhat painful response was observed with 4% solution until after 3 hours and the swelling lasted for 6 hours. With 2% solution, however, no painful reaction was recognized except for a slight swelling at 1 hour examination.

Compound III was found to have remarkably reduced toxicity showing subcutaneous LD_{50} of 1,400 mg/kg in comparison with that of the parent antibiotic (280 mg/kg)[2].

Experimental

Hydantoin-1-yl-imino-cirramycin A_1

To a solution of 96 mg (0.83 mM) of 1-aminohydantoin (prepared from hydrazinoacetic acid by a method of UODA *et al*[5]) in 32 ml of N/20 hydrochloric acid was added with stirring 398 mg (0.66 mM) of cirramycin A_1. After stirring for one hour at room temperature, the aqueous solution was extracted with *n*-butanol. Evaporation of the solvent gave 380 mg of crude product which was crystallized from carbon tetrachloride to yield 216 mg of colorless needles melting at 176~178°C. UV λ_{max}^{EtOH} 241 mμ (ε: 14,900).

Anal. Calcd. for $C_{34}H_{54}N_4O_{11}\cdot\frac{1}{2}CCl_4$: C 53.69, H 7.05, N 7.26.
Found: C 53.90, H 7.28, N 7.44.

Disuccinylcirramycin A_1 (III)

A solution of 7.0 g (11.7 mM) of cirramycin A_1 in 45 ml of anhydrous acetone was added to 2.7 g (27 mM) of succinic anhydride and stirred for 5 hours at room temperature. After evaporation of the solvent, the residue was dissolved in 120 ml of ethyl acetate, washed with two 50 ml portions of acidic water (pH 4) and three 50 ml portions of water, successively. The solvent layer was dried over anhydrous sodium sulfate and concentrated *in vacuo* to 20 ml. Addition of 80 ml of *n*-hexane to the concentrate gave 7.01 g of the desired product. UV λ_{max}^{EtOH} 240 mμ (ε: 15,100).

Anal. Calcd. for $C_{39}H_{59}NO_{16}$: C 58.70, H 7.45, N 1.76.
Found: C 58.03, H 7.85, N 2.05.

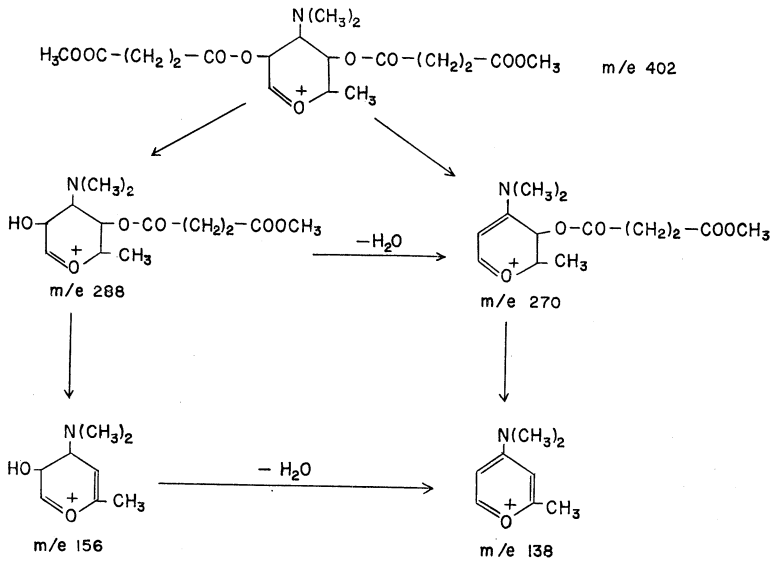
The citrate salt of III was prepared as follows: To a stirred solution of 5.3 g (6.65 mM) of III in 40 ml of ethyl acetate was added 750 mg (3.57 mM) of citric acid in 40 ml of ethyl acetate, and the mixture was kept in the cold for two hours. The resultant precipitate was collected by filtration, washed with ethyl acetate and dried *in vacuo* to give 4.4 g of white powder.

Dimethylsuccinylcirramycin A_1 (IV)

To a solution of 385 mg (0.5 mM) of III in 3 ml of dioxane and 1 ml of acetone was added under ice-cooling 110 mg (1.1 mM) of triethylamine and 110 mg (1.0 mM) of ethyl chloroformate in dioxane solution to obtain a mixed anhydride. The reaction mixture was decomposed with 5 ml of methanol to yield 171 mg of the desired product. An analytical sample was obtained by column chromatography on Florisil.

Anal. Calcd. for $C_{41}H_{63}NO_{16}$: C 59.62, H 7.69, N 1.70.
Found: C 58.80, H 7.46, N 1.97.

The mass spectrum of IV shows ion peaks at m/e 402, 288, 270, 156 and 138, which were consistent with the following fragmentation of disuccinylmycaminose:



Acknowledgements

The authors wish to express their thanks to Dr. KOICHI IWADARE and Mr. EIJI IWADARE of Banyu Pharmaceutical Co. and Dr. JOSEPH LEIN of Bristol Laboratories for their advice and encouragement throughout the present series of works.

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