CHEMICAL STUDIES ON ANTIBIOTIC SUBSTANCES.

I. PURIFICATION OF AUREOTHRICIN AND
ITS MOLECULAR FORMULA

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From the fermented broth of a new species of streptomyces Umezawa, Maeda and Kosaka (1) obtained a golden yellow crystalline antibiotic and called it aureothricin. This antibiotic inhibits the growth of gram-positive and negative bacteria and is clearly different from yellow antibiotics of streptomyces such as aureomycin (2) and xanthomycin (3). In the present paper its purification and chemical characters are described.

PURIFICATION OF AUREOTHRICIN.

During fermentation the broth and the mycelia become yellow and on the wall of the flask yellow pigment can be observed. When the aureothricin production reaches its maximum, the broth is filtered, and the filtrate is extracted with its half volume and fourth volume of ethyl acetate. Aureothricin is then transferred to the solvent. The mycelium is extracted with ethyl acetate, about tenth volume of the broth. The mycelium contains about 1/5-1/20 amount of aureothricin produced in the whole broth. Ethyl acetate solutions are combined and concentrated to its 1/5 to 1/10 volume by vacuum evaporation. The concentrated ethyl acetate solution is dehydrated with anhydrous sodium sulfate, and passed through alumina column. The upper part of the column turns to light red and the next part to golden yellow. The column is washed by passing the mixture of ethyl acetate and ether (1:1). Then the yellow part goes down and finally the passing solution turns to golden yellow. This fraction contains almost all of aureothricin in the broth. From this fraction solvents are removed by vacuum evaporation, and golden yellow needle crystals of aureothricin appear. After three times recrystallization with ethyl acetate, golden yellow thready crystals which decompose at 256°C are obtained. This authentic specimen sublimates at about 200°C under vacuum, and the sublimated crystals are platelet. However, during sublimation aureothricin does not lose its antibacterial effect and decompose at 256-257°C.

A minute quantity of aureothricin is contained also in the upper red part of the alumina column, particularly when the column is too short. The course of the purification is shown in Table 1 and 2.

CHEMICAL NATURES OF AUREOTHRICIN.

Aureothricin is very insoluble in water and only slightly soluble in organic solvents. At room temperature (20°C) its solubility is 0.02-0.03 mg/cc in water, 1.2 mg/cc in acetone, 0.7 mg/cc in ethyl acetate, 0.1 mg/cc in ether, 0.3 mg/cc in benzene, 0.8 mg/cc in ethyl alcohol, 3.9 mg/cc in chloroform and 1.5 mg/cc in ethylene dichloride. It is very stable; none of the

Table 1. Extraction process of aureothricin.

Table 2. Extraction of aureothricin.

Broth			Ethyl acetate	Extract		Effluent 2 (yellow band)	Effluent 3 (red band)	Yield	
cc	mg/cc*	Total* mg		mg/cc*	mg*	mg*	mg*	mg*	%
1,980	—	—	770	—	—	38.2	14.9	43.1	
2,660	—	—	1,365	—	—	61.7	—	61.7	
2,200	29	63.8	1,140	47	53.5	58.7	—	58.7	92
2,480	45	111.0	1,400	80	112	87.0	39.5	126.5	114
1,500	44	66.0	1,000	63	63	55.5	—	55.5	84.0
5,245	50	262	2,490	110	274	256.2	—	256.2	98.0
8,050	52	417	3,000	136	408	336.5	93.0	429.5	100.2
9,270	40	371	3,450	107	369	218.0	148.0	366.0	98.5

* Amount of aureothricin.

change of its antibiotic activity is found during one hour at 100°C in water and ethyl acetate. Particulary it is stable in strong acid solution. It is soluble in concentrated hydrochloric acid and almost none of the change is observed for two hours, though after 24 hours it turns to green.

The molecular weight of aureothricin was tested by Rast's method with camphor. As

shown in Table 3, the mean of the six measurements is 342.

Table 3. Determination of molecular weight.

Weight of sample mg	Weight of camphor mg	Temperature decrease, °C	Molecular weight
0.200	4.200	5.6	340
0.180	4.620	3.8	410
0.220	5.570	4.8	330
0.160	3.930	4.3	378
0.160	4.310	4.8	309
0.180	4.330	5.9	382
Average			342

Table 4. Elemental analysis of aureothricin.

	C	H	N	S
Specimen 1*	43.91	3.80	11.24	25.15
	43.89	3.88	10.89	25.09
Specimen 2**	44.03	3.80	11.32	23.67
	43.93	3.84	—	23.57

* Specimen 1 is obtained after three times recrystallization with ethyl acetate.

** Specimen 2 is obtained after sublimation of specimen 1.

Table 5. Deviation of the calculated value as $C_{13}H_{13}N_3S_3O_3$ (molecular formula of aureothricin from the observed value).

	Calculated %	Deviation of found %	
		Specimen 1	Specimen 2
C	43.9	0.0	+0.1
H	3.7	+0.1	+0.1
N	11.8	-0.56	-0.5
S	27.0	-1.9	-3.3
O	13.5	+2.4	+3.7

Specimens 1 and 2 are the same as shown in Table 4.

The results of elemental analysis of aureothricin is shown in Table 4. It contains carbon 43.91 %, hydrogen 3.80 %, nitrogen 11.24 % and sulfur 25.15 %. So the molecular formula of aureothricin should be $C_{13}H_{13}N_3S_3O_3$.

DISCUSSION

Here I should like to describe the differences of aureothricin from other resembling antibiotics. Aureomycin which is found by Duggar (2) is different from aureothricin from its solubility in water and its composition. Aureomycin is soluble in water and the result of elemental analysis (4) is different. Xanthomycin (3) is also soluble in water and different from aureothricin in its composition.

Recently Plattner (5) found eniatin produced by a kind of *Fusarium*. It sublimes, but its molecular formula is $C_{24}H_{42}N_2O_6$. So it is different from aureothricin.

SUMMARY

Aureothricin is produced by a new species of streptomyces and found by Umezawa and others. It is a new antibiotic and its molecular formula is $C_{13}H_{13}N_3S_3O_3$. In this paper the purification and solubilities of aureothricin are described.

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