OLIVOMYCIN AND RELATED ANTIBIOTICS

XXVIII.* MONOMERIC COMPOSITION OF AUREOLIC ACID AND PROOF THAT THE ANTIBIOTICS LA-7017 AND MITHRAMYCIN ARE IDENTICAL TO IT

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Continuing investigations on the chemistry of the olivomycins and chromomycins, we have undertaken a study of the parent substance of this group – aureolic acid [3, 4] – and also the antibiotics LA-7017 [5] and mithramycin [6]. It has been found that each of the three actinomycetes that produce these antibiotics form only one biologically active substance; i.e., in contrast to the olivomycins-chromomycins all three of these antibiotics are single-component compounds. When carefully purified samples of aureolic acid, of LA-7017, and of mithramycin were compared, it was found that in their physicochemical and biological properties, and in those of their acetates, they scarcely differed (Tables 1 and 2). These results show the probable identity of the antibiotics mentioned which, however, is contradicted by some results of investigations published previously in which a definite difference between aureolic acid, LA-7017, and mithramycin with respect to biological activity and some other properties was reported [4-6].

In view of this, for a definitive answer to the question of the identity of the antibiotics compared we

TABLE 1. Physicochemical Properties of Aureolic Acid, Mithramycin, and Antibiotic LA-7017

Antibiotic and its acetate	R_f^*	$[\alpha]_D^{20}$ (c 0.3 in ethanol), deg	λ_{max} nm (log ε)†
Aureolic acid	0,36	−51	229, 280, 317, 330, 417 4,39 4,68 3,92 3,77 3,98
Mithramycin	0,36	−50	229, 279, 317, 330, 415 4,33 4,68 3,93 3,74 3,97
Antibiotic LA-7017	0,36	−60	229, 280, 317, 330, 415 4,38 4,68 3,94 3,79 3,98
Aureolic acid acetate	0,54	−32	224, 259i, 266, 319i, 326, 360 4,39 4,65 4,80 3,95 3,99 3,57
Mithramycin acetate	0,54	−30	224, 259i, 266, 319i, 326, 360 4,41 4,65 4,79 3,94 3,99 3,56
Acetate of antibiotic LA-7017	0,54	−29	224, 259i, 266, 319i, 326, 360 4,40 4,66 4,79 3,95 4,03 3,58

*On thin-layer chromatography in silica gel in the benzene-acetone (1 : 1) system (for the antibiotics) and in the benzene–acetone (3 : 1) system (for the acetates).
†i – inflection.

* For Communication XXVII, see [1], and for a preliminary communication see [2].

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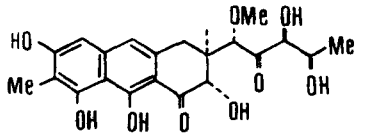
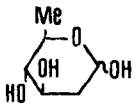
TABLE 2. Antibiotic Properties of Aureolic Acid, Mithramycin, and Antibiotic LA-7017

Antibiotic	Minimum concentration (μg/ml) to suppress			
	S. aureus	B. mycoides	S. lutea	B. subtilis
Auroelic acid	0,05	0,005	0,005	0,005
Mithromycin	0,05	0,005	0,005	0,005
Antibiotic LA-7017	0,05	0,005	0,005	0,005

investigated the monomeric composition of each of them. It was found that the acid hydrolysis of all three antibiotics formed chromomycinone (I) [7]* (Table 3), olivose (II) [9, 10], oliose (III), [9, 10], and mycarose (IV) [1]. The results of a quantitative analysis of the monosaccharides in the hydrolyzate using triphenyltetrazolium chloride (see [11]) showed that the olivose (II) and the oliose (III) were present in a ratio of 3 : 1. To determine the mycarose we used reduction by $NaBH_4$ followed by exhaustive acetylation and gas-liquid chromatography (see [12]). The results of carbohydrate analyses and also of a comparison of the $E^{1\%}_{1cm}$ values for aureolic acid, mithramycin, and antibiotic LA-7017, on the one hand, and of chromomycinone, on the other hand, showed that each of these antibiotics contains one residue of chromomycinone (I), one of D-oliose (III), and one of D-mycarose (IV), and three of D-olivose (II).



I



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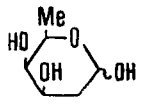
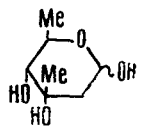


III



IV

*The identity of the aglycones of chromomycin and of mithramycin was first reported by M. G. Brazhnikova et al [8].

TABLE 3. Physicochemical Properties of Chromomycinone (I) and the Aglycones of Aureolic Acid, Mithramycin, and Antibiotic LA-7017

Substance	R_f*	mp, °C (micro)†	$[\alpha]_D^{20}$ (c 1 in EtOH), deg	λ_{max}^{EtOH}, nm (log ε)	Empirical formula‡
Chromomycinone	0,63	176–178	+76	232, 282, 326, 340, 412 (4,42; 4,69; 3,83; 3,76; 4,09)	$C_{21}H_{24}O_9$
Aglycone of aureolic acid	0,63	176–178	+76	232, 281, 326, 340, 412 (4,38; 4,65; 3,80; 3,77; 4,04)	$C_{21}H_{24}O_9$
Aglycone of mithramycin	0,63	174–176	+74	232, 281, 326, 340, 412 (4,43; 4,68; 3,83; 3,77. 4,08)	$C_{21}H_{24}O_9$
Aglycone of antibiotic LA-7017	0,63	176–177	+74	232, 281, 326, 340, 412 (4,42; 4,67; 3,83; 3,78; 4,06)	$C_{21}H_{24}O_9$
Acetate of chromomycinone	0,65	184	—	260, 302, 364 (4,80; 3,87; 3,46)	$C_{33}H_{36}O_{15}$
Acetate of the aglycone of aureolic acid	0,65	180–182	—	260, 302, 364 (4,80; 3,89; 3,49)	$C_{33}H_{36}O_{15}$
Acetate of the aglycone of mithramycin	0,65	180–182	—	260, 302, 364 (4,79; 3,87; 3,48)	$C_{33}H_{36}O_{15}$
Acetate of the aglycone of LA-7017	0,65	180–182	—	260, 302, 364 (4,82; 3,88; 3,48)	$C_{33}H_{36}O_{15}$

*On silica gel in the benzene–acetone (3 : 2) system (for the aglycones) and the (5 : 1) system (for the acetates).

†The aglycones were recrystallized from acetic acid and the acetates from ethanol.

‡For the aglycones, found, %: C 55.4-56.0; H 5.5-6.0; mol. wt. (m/e) 420. $C_{21}H_{24}O_9 \cdot 2AcOH$. Calculated, %: C 55.6; H 6.0; mol. wt. (without AcOH) 420. For the acetates, found, %: C 58.8-59.1; H 5.3-5.4; mol. wt. (m/e) 658. $C_{33}H_{36}O_{15}$. Calculated, %: C 58.9; H 5.4; mol. wt. 658.

The facts given above lead to the conclusion that aureolic acid, antibiotic LA-7017, and mithramycin are one and the same substance, which it is proposed in future to call aureolic acid, since it was first described under this name.

EXPERIMENTAL

For general information on the experiments, see [13].

1. Isolation of Aureolic Acid, Mithramycin, and Antibiotic LA-7017. The producing agent of aureolic acid (Streptomyces sp.) was used to ferment a medium containing soya flour (1.5%), starch (2.5%), NaCl (0.3%) and $CaCO_3$ (0.3%) with ph 6.9-7.0 at 28°C for 96 h; the producing agent of mithramycin (A. atroolivaceus)* was grown in the same medium for 144 h; and the producing agent of antibiotic LA-7017 (Streptomyces sp. LA-7017) was grown for 96 h on a medium containing maize extract (0.5%), starch (1.5%), glucose (1%), $(NH_4)_2SO_4$ (0.4%), NaCl (0.5%), and $CaCO_3$ (0.5%). In each case, the mycelia were filtered off, and the filtrate was acidified with dilute HCl to pH 3 and extracted with ethyl acetate. The extract was washed with water, dried, and evaporated, the residue was triturated with ether, and the resulting crude preparation was purified by thin-layer chromatography in silica gel. The properties of the antibiotics and their acetates (obtained by the action of Ac_2O + Py, 72 h at 20°C) are given in Table 1; by potentiometric titration in 10% ethanol it was found that $pH_a = 7.6$.

2. Acid Hydrolysis of Aureolic Acid, Antibiotic LA-7017, and Mithramycin. A solution of 1 g of aureolic acid in 50 ml of 50% aqueous acetic acid was heated at 75°C for 3.5 h, diluted with water, and extracted with ethyl acetate. The combined extracts were washed with water, dried, and evaporated, and the residue was chromatographed in the benzene–acetone (3 : 2) system. The zone with R_f 0.58-0.70 yielded 300 mg of chromomycinone (I), which crystallized from acetic acid with two molecules of the solvent (see Table 3).

After the extraction of the chromomycinone, the aqueous fraction and the wash waters were combined and evaporated to dryness, and the last traces of acetic acid were eliminated by distillation with ethanol. Then the resulting mixture of sugars was chromatographed in the benzene–acetone (1 : 1) system. The zone with R_f 0.65 {on Whatman No. 2 paper in the n-butanol–ethanol–water (4 : 1 : 5) system [1]}, and the zone with R_f 0.13-0.39 yielded 420 mg of a mixture of olivose (II) and oliose (III). After thrice-repeated chromatography in the benzene–acetone (1 : 2) system, the zone with R_f 0.42-0.53 yielded 173 mg of olivose (II) with $[\alpha]_D^{28}$ + 24° (c 1; water), R_f 0.52 (on paper), and the zone with R_f 0.26-0.36 yielded 74 mg of oliose (III) with $[\alpha]_D^{28}$ + 51° (c 0.9; water), R_f 0.46 (on paper), identified by a direct comparison with the products of the hydrolysis of the olivomycins [10].

Mithramycin and antibiotic LA-7017 were hydrolyzed similarly; the hydrolysis products were shown by direct comparison to be identical with chromomycinone (I) (see Table 3), olivose (II), oliose (III), and mycarose (VII), respectively.

3. Quantitative Determination of the Monosaccharides in Aureolic Acid, Antibiotic LA-7017, and Mithramycin. Each of the antibiotics (30 mg) was hydrolyzed under the conditions of experiment 2. The solution was evaporated to dryness, and the residue was dissolved in 50 ml of ethanol and, to eliminate the chromomycinone, the solution was filtered through 7 g of Al_2O_3 (activity grade V). The filtrate was evaporated, the residual mixture of monosaccharides was reduced in 3 ml of an aqueous solution of 45 mg of $NaBH_4$ (2 h at 20°C), the excess of reagent was decomposed with Amberlite IR-120 resin (H^+), the boric acid was eliminated by repeated distillation with methanol, and the resulting mixture of alditols was acetylated with 1 ml of Ac_2O and 1 ml of Py (15 h at 20°C). After evaporation in vacuum, the residue was subjected to gas-liquid chromatography (10% of polyethylene glycol succinate on Chromosorb W, 190°C, N_2 60 ml/min).

The ratio of the amount of mycarose (IV) to the total amount of olivose (II) and oliose (III) determined in this way was 1 : 3.7, 1 : 4.2, and 1 : 4.5 in the three respective cases. It was found by paper chromatography (see [11]) that the amounts of olivose (II) and oliose (III) in the hydrolyzates were in the proportion of 1 : 2.8, 1 : 2.9, and 1 : 3.0.

The culture of the producing agent of mithramycin was kindly given to us by Academician of the Academy of Medical Sciences of the USSR G. F. Gauze (Moscow), the culture of the producing agent of antibi-

*Antibiotic 11,294, which is formed by this actinomycete, has previously been shown to be identical to mithramycin.

otic LA-7017 by Prof. P. Sensi (Milan), the culture of the producing agent of aureolic acid by Dr. J. E. Philip and R. L. Girolami (Chicago), and the sample of chromomycinone by Dr. K. Morita (Osaka).

SUMMARY

1. It has been shown that aureolic acid, antibiotic LA-7017, and mithramycin are identical.

2. It has been established that aureolic acid is a glycoside of chromomycinone (I) and contains the monosaccharides D-olivose (II), D-oliose (III), and D-mycarose (IV) in a ratio of 3 : 1 : 1.

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