OLIVOMYCIN AND RELATED ANTIBIOTICS

XVI. The Antibiotics NSC A-649 and Aburamycin*

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Of the group of antitumoral antibiotics, the first of which has been assumed to be aureolic acid [2], only two antibiotics, olivomycin [3] and chromomycin [4], have been well studied and are used in oncologic practice at the present time. They have been studied in detail chemically, as a result of which the individual olivomycins A, B, C, and D and chromomycins A_2, A_3, and A_4 have been isolated, and their structural and spatial formulas have been elucidated completely [5–7]. In spite of the fact that they are likewise of considerable interest, the other antibiotics of this group have hitherto remained little studied chemically. Consequently, developing our investigations in the field of olivomycin and related compounds, we have studied two other antibiotics of this group, namely aburamycin [8] and NSC A-649 [9]. The preparation of the aburamycin and the antibiotic NSC A-649 was carried out by the normal aerobic fermentation of the previously-described producing agents, Streptomyces aburaviensis and Streptomyces NSC A-649 [8, 9]. At the end of fermentation, the culture liquid was freed from mycelium and acidified with dilute HCl to pH 3–3.5, and the antibiotics were extracted with ethyl acetate; the extracts were dried with Na_2SO_4 and evaporated, and the residues were carefully washed with petroleum ether. Liquid preparations of the antibiotics obtained in this way were subjected to preparative thin-layer chromatography on silica gel of the grade "vodnaya kremnevaya kislota" [aqueous silicic acid] type in the benzene–acetone (1 : 1) system. It was found that aburamycin and antibiotic NSC A-649 each consisted of a mixture of four substances, and in each case these were denoted by the letters A, B, C, and D. Components A (60–70%) and B (20–30%) substantially predominated, so that aburamycins C and D could be obtained only in small amounts, and the antibiotics NSC A-649 C and D were hardly available for study.

The individual aburamycins and NSC A-649s that we isolated are optically active substances; they possess feebly acidic properties and have UV and IR spectra characteristic for the antibiotics of the aureolic acid group. Under the action of acetic anhydride in pyridine (72 hr at 20° C) each of them formed a mixture of two acetates (in a ratio of approximately 4 : 3) possessing similar UV absorptions, the main acetylation product being a chromatographically less mobile acetate retaining in the IR spectrum the band of a hydroxyl group at about 3500 cm^{-1}. An exception is formed by aburamycin D which, under these conditions, formed predominantly the product of exhaustive acetylation containing no free hydroxyls. All these antibiotics exhibit a high activity against gram-positive bacteria, and their acetates are completely inactive. Information on the antibacterial properties and the main physicochemical constants of these substances are given in Tables 1 and 2, where they are compared with the corresponding characteristics of the olivomycins and chromomycins. In respect of the $[\alpha]$ values given in Table 2, it must be mentioned that the optical activity of the antibiotics of this group depends strongly on many factors (in particular, on the adsorbent used in their isolation) and therefore the $[\alpha]$ values of the acetates form more reliable characteristics.

As can be seen from Table 1 and 2, the NSC A-649 antibiotics and the aburamycins have properties scarcely distinguishable from those of the corresponding olivomycins and chromomycins. From these results it was possible to assume that they are actually identical with one another, but this assumption required further confirmation, at least in respect of the NSC A-649 antibiotics, since other workers have previously found a difference between olivomycin and the total NSC A-649 preparation in paper chromatography and have concluded that these antibiotics are not identical [10]. Consequently, for a definitive proof we undertook the acid dehydration of all the components of NSC A-649 and of aburamycin that we had isolated.

It was found that, like the olivomycins and chromomycins, each of the NSC A-649 antibiotics and the aburamycins is readily decomposed by the action of acids, forming a mixture of sugars and an aglycone containing all the chromophoric system of the initial antibiotic. On complete hydrolysis by heating with 50% acetic acid (4 hr at 75° C),

*For part XV, see [1].

Table 1. Antibacterial Activity of the NSC A-649 Antibiotics, the Olivomycins, the Aburamycins and the Chromomycins

Antibiotic		Minimum concentration suppressing*				
		St. aureus	B. mycoides	Sarcina lutea	B. subtilis	
NSC A-649	A	0.1	0.01	0.01	0.01	1000
	B	0.2	0.03	0.02	0.01	400
Olivomycin	A	0.1	0.01	0.02	0.01	1000
	B	0.2	0.02	0.02	0.03	500
	C	0.5	0.2	0.2	0.2	150
	D	2	0.5	0.5	0.5	20
Aburamycin	A	0.01	0.01	0.02	0.02	900
	B	0.02	0.01	0.02	0.02	1100
	C	0.2	0.1	0.1	0.1	800
	D	1	0.5	1	0.5	100
Chromomycin	A_2	0.01	0.01	0.01	0.01	1000
	A_3	0.02	0.02	0.01	0.01	1000

*The figures given in the last column were found by the agar diffusion method and are expressed in olivomycin units (activity of 1 μg of olivomycin A taken as the unit of activity); the remaining figures were obtained by the serial dilution method and are expressed in μg/ml.

Table 2. Physicochemical Properties of the NSC A-649 Antibiotics, the Olivomycins, the Aburamycins, and the Chromomycins

Antibiotic		Constants of the antibiotic			Constants of the acetate of the antibiotic			
		R^*_{oA}	$[\alpha]_D$ (EtOH)	λ^{EtOH}_{max}, mμ (lg ε)	R^{2*}_{aoA}	Mp, °C (micro, from EtOH)	$[\alpha]_D$ ($CHCl_3$)	λ^{EtOH}_{max}, mμ (lg ε)[3*]
NSC A-649	A	1.00	−28	227, 279, 317, 332, 407 (4.35; 4.68; 3.69; 3.46; 4.14)	1.00	211–212	−22	222, 258i, 267, 330, 360i (4.45; 4.67; 4.81; 4.05; 3.69)
	B	0.89	−31	222, 279, 317, 332, 407 (4.35; 4.65; 3.80; 3.66; 4.05)	0.85	167–169	−15	222, 250i, 258i, 267, 329, 360i (4.50; 4,54; 4.69; 4.81; 4.02; 3.77)
Olivomycin	A	1.00	−35	227, 277, 318, 333, 408 (4.33; 4.71; 3.78; 3.68; 4.16)	1.00	212–214	−22	223, 249i, 258i, 267, 330, 360i (4.47; 4.47; 4.62; 4.75; 3.99; 3.63)
	B	0.89	−26	227, 278, 318, 332, 405 (4.27; 4.68; 3.72; 3.69; 4.15)	0.85	167–169	−16	223, 249i, 258i, 267, 330, 360i (4.44; 4.45; 4.61; 4.74; 3.92; 3.71)
Aburamycin	A	1.03	−48	228, 281, 317, 332, 412 (4.45; 4.74; 3.94; 3.79; 4.08)	1.05	220–222	−21	223, 250i, 259i, 267, 320i, 329, 360i (4.49; 4.52; 4.69; 4.82; 4.01; 4.06; 3.57
	B	0.93	−46	226, 282, 318, 331, 415 (4.35; 4.61; 3.86; 3.67; 3.95)	0.95	211–213	−18	223, 259i, 267, 320i, 329, 360i (4.47; 4.71; 4.82; 4.02; 4.06; 3.78)
	C	0.80	−17	228, 282, 318, 331, 415 (4.32; 4.60; 3.87; 3.71; 3.94)	1.05	219–221	−18	223, 250i, 258i, 267, 320i, 328, 360i (4.46; 4.50 4.63; 4.75; 4.01; 4.02; 3.61)
	D	0.69	−17	228, 282, 318, 331, 415 (4.35; 4.55; 3.86; 3.69; 3.93)	1.22	—	+1	223, 250i, 268i, 267, 320i, 328, 360i (4.39; 4.45; 4.59; 4.72; 3.96; 3.97; 3.59)
Chromomycin	A_2	1.03	−46 −61[4*]	229, 279, 317, 331, 412 (4.22; 4.71; 3.90; 3.79; 3.93)	1.05	223[4*]	−44[4*]	267, 315, 328 (4.81; 4.00; 4.03)[4*]
	A_3	0.93	−47 −57[4*]	230, 281, 304, 318, 330, 412 (4.39; 4.72; 3.85; 3.92; 3.84; 4.07)	0.95	214[4*]	−26[4*]	267, 329 (4.82; 4.05)[4*]
	A_4^{4*}	–	−47	230, 279, 318, 332, 415 (4.27; 4.67; 3.85; 3.77; 3.93)	—	189–191	+4	224, 266, 318, 328 (4.47; 4.79; 3.98; 4.02)

*Ratio of the R_f value to the R_f value of olivomycin A on silica gel in the benzene-acetone (1 : 1) system

[2*]Ratio of the R_f value to the R_f value of the acetate of olivomycin A on silica gel in the benzene-acetone (5 : 1) system

[3*]i) Inflection.

[4*]According to the literature [14].

Table 3. Physicochemical Properties of the Aglycones of the NSC A-649 Antibiotics, of the Olivomycins, of the Aburamycins, and the Chromomycins

Substance	$R^*_{o(ao)}$	Mp, °C (micro)	[α] (c[2]* 1)			λ^{EtOH}_{max}, mμ (lg ε)[3]*	Empirical formula	Found (calculated)		
			589 mμ	578 mμ	546 mμ			C, %	H, %	M[4]*
Aglycone of NSC A-649	1.00	180—181 (from MeCN)	+66	+73	+99	229, 276, 324, 340, 408 (4.25; 4.58; 3.63; 3.51; 4.08)	$C_{20}H_{22}O_9$	58.8 (59,)	5.7 (5.5)	406 (406)
Aglycone of the olivomycins (olivin, I)	1.00	182—183 (from MeCN)	+60	+67	+99	230, 276, 324, 340, 408 (4.27; 4.56; 3.64; 3.56; 4.03)	$C_{20}H_{22}O_9$	58.8 (59.1)	5.6 (5.5)	406 (406)
Aglycone of the aburamycins	1.07	175—177 (from AcOH)	+77	+80	+113	232, 28:, 326, 340, 415 (4.43; 4.68; 3.87; 3.84; 4.06)	$C_{21}H_{24}O_9 \cdot 2CH_3COOH$	55.9 (55.6)	5.7 (6.0)	420 (420)
Aglycone of the chromomycins (chromomycinone, XI)	1.07	176—178 (from AcOH)	+77	+81	+115	232, 282, 326, 340, 415 (4.42; 4.69; 3.83; 3.76; 4.09)	$C_{21}H_{24}O_9 \cdot 2CH_3COOH$	55.45* (55.6)	6.05* (6.0)	420 (420)
Acetate of the aglycone of NSC A-649	1.00	198—200 (from EtOH)	−9	−10	−12	251i, 259, 305i, 316i, 360i (4.71; 4.83; 3.88; 3.83; 3.58)	$C_{32}H_{34}O_{15}$	58.2 (58.3)	5.4 (5.2)	658 (658)
Hexaacetate of olivin	1.00	197—199 (from EtOH)	−7	−8	−9	251i, 260, 304i, 316i, 360i (4.64; 4.74; 3.88; 3.77; 3.58)	$C_{32}H_{34}O_9$	58.3 (58.3)	5.2 (5.2)	658 (658)
Acetate of the aglycone of the aburamycins	1.05	178—180 (from EtOH)	−14	−15	−17	251i, 259, 302i, 316i, 364i (4.71; 4.86; 3.89; 3.72; 3.53)	$C_{33}H_{36}O_{15}$	58.8 (58.9)	5.3 (5.4)	672 (672)
Hexaacetate of chromomycinone[5]*	—	184 (from MeOH)	—	—	—	260, 302, 364 (4.80; 3,87; 3.46)	$C_{33}H_{36}O_{15}$	59.1 (58.9)	5.4 (5.4)	(672)

*R_o) ratio of the R_f value of the aglycone to the R_f value of olivin on silica gel in the benzene-acetone (3 : 2) system, R_{ao}) ratio of the R_f value of the acetate of olivin on silica gel in the benzene-acetone (3 : 1) system.

[2]*For the aglycones, in alcohol, for the acetates, in chloroform.

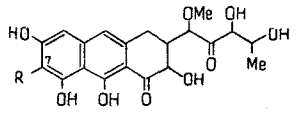
[3]*i) Inflection.

[4]*Determined by mass spectrometry

[5]*According to the literature [12].

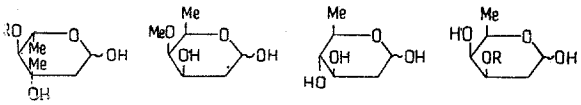
all the NSC A-649 antibiotics gave the same aglycone with mol. wt. 406, while all the aburamycins formed another aglycone with mol. wt. 420. The two aglycones are very similar in their spectral characteristics, the second of them possessing UV absorption of somewhat longer wavelengths and, judging from its mass spectrum, being a higher homolog of the first. A detailed study of these substances led to the conclusion that the NSC A-649 antibiotics have as their aglycone the olivin (I) previously isolated from the olivomycins [11], while the aglycone of the aburamycins consists of chromomycinone (II), which is also the aglycone of the chromomycin [12]. A direct comparison of the properties of the corresponding aglycones and their acetates (Table 3) confirmed the correctness of this conclusion.

The carbohydrate composition of the antibiotics studied was elucidated by the same method as we have used previously in determining the structure of the olivomycins and chromomycins [5, 13]. With this aim, the reaction solutions after the acetic acid hydrolysis of the antibiotics described above were chromatographed on Whatman No. 2 paper in the butanol–ethanol–water (4 : 1 : 5) system. In the qualitative determination of the sugars, the reagent for revealing the spots was a 1% solution of $SbCl_3$ in chloroform. For quantitative analysis, the chromatograms were treated with a mixture of equal volumes of 4% ethanolic triphenyltetrazolium chloride and 1 N methanolic NaOH, and were then dried at 65° C for 1 hr. The spots were cut out, the triphenylformazan which they contained was eluted with a mixture of ethanol and acetic acid (10 : 1), and it was determined in the eluate from the optical density of the solution at 480 mμ. The formulas of the sugars detected, III–VIII, are given below



I R=H

II R=Me



III R=^{i}PrCO

IV R=Ac

V

VI

VII R=Ac

VIII R=H

and the results of the quantitative determination of the carbohydrates are given in Table 4. It can be seen from this table that in respect of their carbohydrate composition the NSC A-649 antibiotics and the aburamycins are completely identical with the analogous olivomycins and chromomycins.

Table 4. Carbohydrate Composition of the NSC A-649 Antibiotics, the Olivomycins, the Aburamycins, and the Chromomycins

Antibiotic		Sugars found, mole/mole					
		olivomycose		olivomose (V)	olivose (VI)	olivose	
		4-O-CPr^{i} (III)	5-OAc (IV)			3-OAc (VII)	3-OH (VIII)
NSC A-649	A	1	0	1	2	1	0
	B	0	1	1	2	1	0
olivomycin	A	1	0	1	2	1	0
	B	0	1	1	2	1	0
	C	1	0	1	2	0	1
aburamycin	A	1	0	1	2	1	0
	B	0	1	1	2	1	0
	C	1	0	1	2	0	1
	D	0	0	1	2	1	0
chromomycin	A_2*	1	0	1	2	1	0
	A_3*	0	1	1	2	1	0
	A_4*	0	0	1	2	1	0

*According to the literature [14].

The results obtained, as a whole, definitively show the identity of aburamycins A, B, and D with chromomycins A_2, A_3, and A_4, and of the NSC A-649 antibiotics A and B with olivomycins A and B. We also confirmed this conclusion by a direct comparison of the physicochemical and antimicrobial properties of the samples of aburamycin

A and chromomycin A_2, aburamycin B and chromomycin A_3, NSC A-649 A and olivomycin A, and NSC A-649 B and olivomycin B that we had available and also by a comparison of the properties of aburamycin D with those described in the literature for chromomycin A_4 [14]. So far as concerns aburamycin C and the C and D components of the antibiotic NSC A-649, isolated in small amounts, the chromatographic properties of the two latter substances showed their probable identity with olivomycins C and D, and the results on the monomer composition of aburamycin C (and the coincidence of the properties of its acetate with those of the acetate of aburamycin A) permitted it to be assigned the structure of the previously unknown 7-methylolivomycin C. The undoubted biogenetic relationship of all the antibiotics of this group make any difference in the configuration of their glycosidic bonds extremely unlikely.

Thus, aburamycin is a mixture of chromomycins A_2, A_3, and A_4 and 7-methylolivomycin C, and antibiotic NSC A-649 is a mixture of olivomycins A, B, C, and D.

The culture of the producing agent of NSC A-649 and a sample of this antibiotic was kindly given to us by Dr. H. Schmitz (Bristol Laboratories, Syracuse, New York), a culture of the producing agent of aburamycin by Dr. G. A. de Vries (CBS, Baarn), and by Dr. A. Tseino (Kaken Chemical Co., Tokyo), a sample of aburamycin by Prof. A. S. Khokhlov (Institute of the Chemistry of Natural Compounds, AS USSR, Moscow), and samples of chromomycins A_2 and A_3 and chromomycinone by Prof. K. Nakanishi (University of Tohoku, Sendai) and by Dr. K. Morita (Takeda Chemical Industries, Osaka).

CONCLUSIONS

1. The antibiotics NSC A-649 and aburamycin have each been separated by adsorption chromatography on silica gel into the individual components A, B, C, and D.

2. By a determination of the monomeric composition of these components and by a direct comparison of physicochemical and antimicrobial properties it has been shown that the antibiotic NSC A-649 is a mixture of olivomycins A, B, C, and D, and aburamycin is a mixture of chromomycins A_2, A_3, and A_4 and 7-methylolivomycin C.

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