TUNICAMYCINS, STREPTOVIRUDINS, AND CORYNETOXINS, A SPECIAL SUBCLASS OF NUCLEOSIDE ANTIBIOTICS

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ABSTRACT.—Tunicamycins, streptovirudins, and very recently, corynetoxins have been determined to be structurally related nucleoside antibiotics. Because of their special biological activity as inhibitors of protein glycosylation and their relatively complicated chemical structures, which differ from the common nucleoside antibiotics, they can be grouped together as a special subclass. A general specification system based on structural characteristics is included. The complete separation of the natural complex is still problematical, but seems to be necessary because differences in the biological activities of the individual components were observed.

Since the first report on the chemical structures of tunicamycins A, B, C, and D in 1977 (1), a number of related compounds, including new tunicamycins, streptovirudins, and corynetoxins have been described. According to their chemical structure, the tunicamycins have been ascribed to the class of nucleoside antibiotics. However, there are several differences both in chemical structure and biological activity between these and the common nucleoside antibiotics. Tunicamycins, streptovirudins, and corynetoxins contain an additional molecule of *N*-acetylglucosamine and a long-chain fatty-acid residue. The chemical structures of the ten tunicamycins, ten streptovirudins, and 14 corynetoxins known at present are shown in figure 1. It is of interest that these complicated compounds occur as metabolites of *Streptomyces* strains as well as of *Corynebacterium* belonging to relatively different taxonomic groups. Of all described nucleoside antibiotics, only septacidin (2) has structural features related to those of tunicamycins (figure 2). Mycospocidin (3), antibiotic 24010 (4), and MM-19290 (5), belonging to this group, have not been studied in detail.

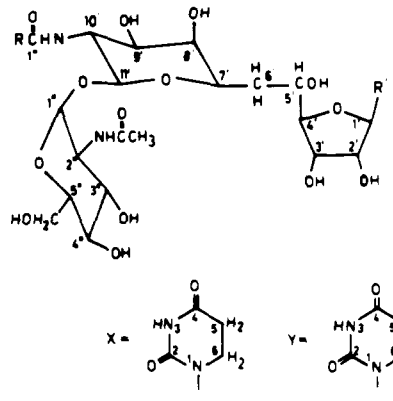
Biologically, tunicamycin and streptovirudin are of particular interest in that they are inhibitors of protein glycosylation and have proved to be valuable tools for studying the role of glycoproteins in a wide variety of biological systems (6,7). Recently, it has been shown that the separated components are biologically active, and that there are differences in the activities of the different individual compounds.

According to these special properties, tunicamycins, streptovirudins, and corynetoxins can be grouped together as an interesting, separate subclass of nucleoside antibiotics.

In all cases the antibiotics of this class were isolated as mixtures of components chemically closely related. Several reports have been published dealing with the separation of streptovirudin and tunicamycin into discrete components. Unfortunately, the investigators used different designation systems for the separated compounds. Moreover, in the case of tunicamycins, the names have been changed repeatedly in the course of the experimental work. As the result, all tunicamycin factors have more than one name and, in some cases, one and the same name is used for different compounds. Consequently, a new general classification system is needed.

In this article, the various compounds of this subclass are discussed briefly with respect to the separation, chemical structures, and biological properties.

Tunicamycin was the first detected antibiotic of this group. It was isolated in 1971 by Takatsuki *et al.* from a strain of *Streptomyces lysosuperificus* (8). Later Hamill *et al.* found that tunicamycin was also produced by strains of *Streptomyces chartreusis* (9). It was



TUNICAMYCINS

	R	R'
I	$CH_3(CH_3)CH-(CH_2)_7-CH=CH-$	Y
II	$CH_3(CH_3)CH-(CH_2)_8-CH=CH-$	Y
III	$CH_3-(CH_2)_{10}CH=CH-$	Y
IV	$C_{12}H_{25}CH=CH-$	Y
V	$CH_3(CH_3)CH-(CH_2)_9-CH=CH-$	Y
VI	$CH_3(CH_3)CH-(CH_2)_{11}-$	Y
VII	$CH_3(CH_3)CH-(CH_2)_{10}-CH=CH-$	Y
VIII	$CH_3-(CH_2)_{12}-CH=CH-$	Y
IX	$C_{14}H_{29}-CH=CH-$	Y
X	$CH_3(CH_3)CH-(CH_2)_{11}-CH=CH-$	Y

CORYNETOXINS

	R	R'
S15a	$CH_3-CH_2(CH_3)CH-(CH_2)_{10}-$	Y
H16i	$CH_3(CH_3)CH-(CH_2)_{10}CHOH-CH_2-$	Y
U16i	$CH_3(CH_3)CH-(CH_2)_{10}-CH=CH-$	Y
H17a	$CH_3-CH_2(CH_3)CH-(CH_2)_{10}CHOH-CH_2-$	Y
S16i	$CH_3(CH_3)CH-(CH_2)_{12}-$	Y
U17a	$CH_3-CH_2(CH_3)CH-(CH_2)_{10}-CH=CH-$	Y
U17i	$CH_3(CH_3)CH-(CH_2)_{11}-CH=CH-$	Y
S17a	$CH_3-CH_2(CH_3)CH-(CH_2)_{12}-$	Y
H18i	$CH_3(CH_3)CH-(CH_2)_{12}-CHOH-CH_2-$	Y
U18i	$CH_3(CH_3)CH-(CH_2)_{12}-CH=CH-$	Y
H19a	$CH_3-CH_2(CH_3)CH-(CH_2)_{12}-CHOH-CH_2-$	Y
S18i	$CH_3(CH_3)CH-(CH_2)_{14}-$	Y
U19a	$CH_3-CH_2(CH_3)CH-(CH_2)_{12}-CH=CH-$	Y
S19a	$CH_3-CH_2(CH_3)CH-(CH_2)_{14}-$	Y

STREPTOVIRUDINS

	R	R'		R	R'
A_1	$CH_3(CH_3)CH-(CH_2)_6-CH=CH-$	X	A_2	$CH_3(CH_3)CH-(CH_2)_6-CH=CH-$	Y
B_1	$CH_3CH_2(CH_3)CH-(CH_2)_6-CH=CH-$	X	B_2	$CH_3-CH_2(CH_3)CH-(CH_2)_6-CH=CH-$	Y
B_{1a}	$CH_3(CH_3)CH-(CH_2)_7-CH=CH-$	X	B_{2a}	$CH_3(CH_3)CH-(CH_2)_7-CH=CH-$	Y
C_1	$CH_3(CH_3)CH-(CH_2)_8-CH=CH-$	X	C_2	$CH_3(CH_3)CH-(CH_2)_8-CH=CH-$	Y
D_1	$CH_3CH_2(CH_3)CH-(CH_2)_8-CH=CH-$	X	D_2	$CH_3-CH_2(CH_3)CH-(CH_2)_8-CH=CH-$	Y

FIGURE 1. Chemical structures of tunicamycins, streptovirudins, and corynetoxins.

soon discovered that tunicamycin was a family of related nucleoside antibiotics. Takatsuki *et al.* determined the chemical structures of the four major compounds designated as tunicamycins A, B, C, and D without isolating the pure substances (1). This work nicely demonstrates that even complicated structures sometimes may be determined by investigation of the unresolved complex.

Successful separation of a tunicamycin complex was first reported by Mahoney and Duksin (10). The workers used hplc, and ten fractions were obtained. More recently, the authors were able to resolve a tunicamycin sample into 16 homologues (11). In a contemporary work, Ito *et al.* isolated ten tunicamycin factors by using, independently, the same technique and determined the chemical structures (12). As shown in figure 1, all members of the tunicamycin family contain uracil to which is attached the 11 carbon aminosugar tunicamine. Attached to C-11′ is an *N*-acetylglucosamine. The ten compounds now designated in order of their elution as tunicamycin I to X differ from each

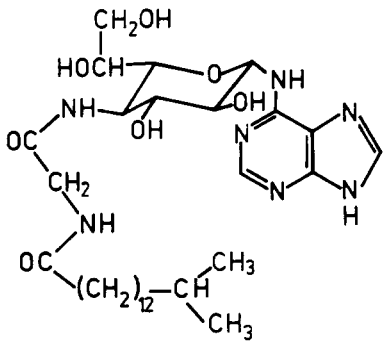


FIGURE 2. Chemical structure of septacidin.

other only in the nature of the fatty-acid residue linked to the amino group at C-10′ of the tunicamine. Recently, Keenan *et al.* have separated tunicamycin into more than 15 different biologically active fractions by hplc (13). That means there are at least five additional tunicamycin factors in the natural complex, the structures of which remain to be established. Clear resolution profiles, even with complicated mixtures, were achieved by Cockrum and Edgar (14).

Hamill *et al.* have found by hplc and fdms studies that mycospocidin and antibiotic MM-19290 contain factors common to the tunicamycin and streptovirudin complexes, with mycospocidin ranging in molecular weight from 802 to 858 and with MM-19290 ranging in molecular weight from 774 to 858.

Streptovirudin has been isolated from fermentations of a strain of *Streptomyces griseoflavus* (15). The separation of the antibiotic complex by column chromatography using Sephadex LH_{20} afforded eight biologically active components designated in order of their elution as streptovirudin A_1, A_2, B_1, B_2, C_1, C_2, D_1, and D_2 (16). The chemical structures have been published recently (17) (figure 1). Further studies of the ^1H- and ^{13}C-nmr spectra have shown that all streptovirudins were pure compounds, except B_1 and B_2, which contained about 20% of their isomeric homologues containing the iso-C_{13}-fatty-acid residue in place of the anteiso-C_{13}-fatty-acid side chain (figure 1). According to these results, of all streptovirudins, only B_{2a} and C_2 are suggested to be identical with tunicamycin factors. The other streptovirudins differ from the tunicamycins in the fatty-acid residues that have shorter chain lengths and, in some cases, anteiso forms. In addition, in streptovirudins of series I (A_1, B_1, B_{1a}, C_1, D_1), uracil is replaced

by dihydrouracil. At present it is not clear whether the series I streptovirudins are precursors of the series II components. Dihydrouracil is usually found by reduction of uracil by NADPH during catabolism. Corresponding tunicamycins or corynetoxins containing dihydrouracil have not been detected yet. In our experiments, the presence of series I streptovirudins in an authentic tunicamycin sample produced by *Streptomyces chartreusis* was excluded by using Sephadex LH_{20} chromatography (18).

Recently, Vogel *et al.* (19) reported on a group of toxic compounds isolated from galled seed heads of annual ryegrass (*Lolium rigidum*) occupied by *Corynebacterium rathayi*. The compounds, named corynetoxins 1-8, were separated from each other by reversed-phase hplc. Detailed investigations have shown that the extracts contained at least 14 individual factors closely related to tunicamycins (20). The structures of the two main compounds (corynetoxins H 17a and U 17a) have been elucidated and are as shown in figure 1. The structures of the other purified or partially purified corynetoxins (figure 1) have been postulated on the basis of molecular weight measurements, hydrolysis, and conversion of the unsaturated acid corynetoxins into the saturated series by selective hydrogenation of the fatty-acid double bond. Two of the members (U 16i and U 17i) were found to have structures and retention times identical to tunicamycins VII and X. As shown in figure 1 the corynetoxins differ from tunicamycins and streptovirudins of series I in that they contain fatty-acid residues which have slightly longer chain lengths and a β-hydroxy series in addition to the common β-unsaturated and saturated series. Interestingly, some of the corynetoxins contain the anteiso fatty acids previously found in streptovirudins.

As mentioned before, some confusion exists about the designation of tunicamycin factors. Mahoney and Duksin proposed a simple system to designate the tunicamycins (11). This system is useful for the classification of the known tunicamycins. However, it does not allow the inclusion of related compounds such as streptovirudins and corynetoxins. Recently, a useful system to designate the corynetoxins was used by Edgar *et al.* (20). The individual factors have been specified by a term indicating the acid series, carbon number, and methyl branching. This system may serve, in a slightly modified way, to classify all described, chemically defined members of this class of antibiotics, as shown in table 1.

From table 1, it can easily be learned that streptovirudins contain fatty acids with shorter chain lengths than those found in tunicamycins and corynetoxins. Dihydrouracil has only been found in streptovirudins. Corynetoxins contain fatty-acid residues, which have slightly longer chain lengths than those found in tunicamycins. Interestingly, as can be expected from the biosynthesis (21) anteiso methyl branching has been found only in members containing long-chain fatty acids with odd numbers of carbon atoms. This finding may be useful for the identification of new members of this class of antibiotics. Furthermore, the relations between the chemical structures and the reported relative retention times give early information with respect to the structure elucidation.

BIOLOGICAL ACTIVITIES OF ISOLATED TUNICAMYCIN AND STREPTOVIRUDIN COMPONENTS.—Tunicamycin and streptovirudin have been ascribed to the nucleoside antibiotics because of the presence of a nucleoside part in their molecule (22). They are active against Gram-positive bacteria, yeasts, fungi, a number of viruses, and mammalian cells in culture. The corynetoxins were found to be responsible for the toxicity of annual ryegrass (*Lolium rigidum*) to grazing animals.

However, tunicamycin and streptovirudin have found considerable interest because of their ability to act as inhibitors of protein glycosylation in a wide variety of biological systems. Such compounds are valuable tools for biochemical and physiological studies

TABLE 1. List of nucleoside antibiotics of the tunicamycin type. Specification according to Edgar *et al.* (20) is slightly modified in order to include tunicamycins and streptovirudins. Terms are listed in order of the number of carbon atoms (C-12 to C-19) of the side-chain fatty acid.

General terms	Synonyms	General terms	Synonyms
u 12 i U	streptovirudin A_2	u 16 i U	tunicamycin VII
u 12 i DU	streptovirudin A_1		=corynetoxin U16i
u 13 a U	streptovirudin B_2	u 16 n U	tunicamycin VIII
u 13 a DU	streptovirudin B_1	h 16 i U	corynetoxin H16i
u 13 i U	streptovirudin B_{2a}	s 16 i U	corynetoxin S16i
	=tunicamycin I	U 17 (?) U	tunicamycin IX
	=tunicamycin A_o?	u 17 a U	corynetoxin U17a
u 13 i DU	streptovirudin B_{1a}	u 17 i U	tunicamycin X
u 14 n U	tunicamycin III		=corynetoxin U17i
u 14 i U	streptovirudin C_2	s 17 a U	corynetoxin S17a
	=tunicamycin II	h 17 a U	corynetoxin H17a
u 14 i DU	streptovirudin C_1	u 18 i U	corynetoxin U18i
u 15 a U	streptovirudin D_2	s 18 i U	corynetoxin S18i
u 15 a DU	streptovirudin D_1	h 18 i U	corynetoxin H18i
u 15 (?) U	tunicamycin IV	u 19 a U	corynetoxin U19a
u 15 i U	tunicamycin V	s 19 a U	corynetoxin S19a
s 15 a U	corynetoxin S15a	h 19 a U	corynetoxin H19a
s 15 i U	tunicamycin VI		

s=saturated fatty acid
u=unsaturated fatty acid
h=β-hydroxy fatty acid
n=normal
i=iso
a=anteiso
U=uracil
DU=dihydrouracil.

on the role of carbohydrates in the various glycoprotein functions (6,7). Preliminary studies have shown that the corynetoxins share these properties (20).

In biological and biochemical experiments, usually the purified natural complex was used. As mentioned above, the composition of tunicamycin from different sources was relatively constant. However, differences to early enriched tunicamycin samples are not excluded (23). This might be important because the individual components were found to be not equally active. Thus, all of the ten separated streptovirudin homologues were highly active against a variety of Gram-positive bacteria and viruses. But the antibacterial activity increased in the order A_1 to D_2 (16). Thus, the antimicrobial activity depends to a certain degree on the length of the fatty-acid side chain. On the other hand, when we compared streptovirudin homologues containing identical fatty-acid side chains, for example A_1 and A_2, only slight differences were observed, indicating that uracil can be replaced by dihydrouracil without significant loss of activity.

Elbein *et al.* found only slight differences in the effectiveness of series I and series II streptovirudins when the effect of both types on the *in vitro* synthesis of dolichylpyrophosphoryl-GlcNAc by using a pig aorta solubilized GlcNAc-1-P transferase was studied (24). The differences were much more significant when the inhibition of dolichylphosphorylglucose formation was studied. While the series II compounds were similar to tunicamycin (50% inhibition at 0.5-1.5 μg/ml), the series I streptovirudins had only low activity on this reaction (50% inhibition at 50-100 μg/ml). Thus, the presence of intact uracil in the molecule is obviously much more critical for the formation of dolichylphosphorylglucose than for inhibition of GlcNAc incorporation. Some differences were also observed in the effectiveness of the separated streptovirudin components to inhibit the incorporation of [^{3}H] mannose into the lipid-linked oligosac-

charides and into glycoprotein in virus-infected cultured MDCK cells. But in these experiments, streptovirudins A_1 and A_2, independent of the base moiety, were found to be much less effective than B_1, B_2, C_1, or C_2, probably because of the rate of uptake by the cells (24).

Mahoney and Duksin (10) first attempted to separate tunicamycin into discrete components and to compare their biological activities. Two major and eight minor components have been separated, all of which possess the ability to inhibit protein glycosylation. The effects of the two major fractions on *in vitro* incorporation of *N*-acetylglucosamine and mannose into saccharide-lipids in an *in vitro* assay using microsomes as a source of the enzymes and acceptors, and on protein synthesis in chick embryo tendon fibroblasts were studied. The two components differed in the amount of antibiotic required to block glycosylation. They also differed in their ability to inhibit protein synthesis while completely blocking protein glycosylation. Although the compounds have not been characterized chemically, the results allowed the conclusion that the two major activities attributed to tunicamycin complex (*e.g.*, protein glycosylation and synthesis inhibition) can be separated.

Recently, Keenan *et al.* (13) isolated 17 active tunicamycin fractions that were found to be potent inhibitors of the formation of dolichylpyrophosphoryl-*N*-acetylglucosamine and also of the synthesis of dolichylphosphorylglucose; however, in the inhibition of glucose transfer, the tunicamycins were up to 50 times less active. These results are in line with previous findings with tunicamycin complex. However, some differences were noted between the different fractions, suggesting that the nature of the fatty-acid residue may influence the inhibition. Thus, the earlier peaks eluted from the hplc column were the least active, while the later peaks were more effective. MDCK cells in culture infected with influenza virus were used to investigate the effect on protein synthesis and protein glycosylation. The four main fractions were potent inhibitors of mannose incorporation into lipid-linked oligosaccharides and into protein. But there was only little inhibition of mannose incorporation into Dol-P-Man up to an antibiotic concentration of 10 μg/ml. [^{3}H] Leucine incorporation into protein was only affected when higher levels of the tunicamycin fractions were used.

The same authors attempted to isolate individual streptovirudins by hplc. Four fractions labeled A, B, C, and D were separated and tested for biological activity on protein synthesis and protein glycosylation in MDCK cells. However, the separation was monitored at 256 nm as the detection wave length. Under these conditions, series I streptovirudins are not detectable, and the isolated fractions were probably mixtures of series I and series II streptovirudins. This suggestion is supported by the molecular weights found for the four fractions, which are characteristic of streptovirudins containing dihydrouracil in their molecules. This could account for the greater variation in activity as compared with the tunicamycin fractions (13).

Further biochemical results using a discrete tunicamycin component have been published recently by Duksin and Mahoney (23). The compound, named A_o, was obtained from tunicamycin complex by preparative hplc. Considering the reported chemical structure, tunicamycin A_o is probably identical with tunicamycin I described by Ito *et al.* (12) (figure 1). It shares the properties of the other components of this type in that it acts as a potent inhibitor of protein glycosylation. Tunicamycin A_o also inhibits, to some degree, protein synthesis. An activity of tunicamycins and streptovirudins on protein synthesis has sometimes been observed, but this required higher levels of antibiotic than necessary to inhibit protein glycosylation. The effectiveness on protein synthesis may depend on the system of study (11) and seems to be restricted to some of the individual components (10,13), which is difficult to interpret.

In conclusion, there are, in fact, differences in the effectiveness of the tunicamycin

and streptovirudin homologues. The use of such discrete components has raised some new questions, and more work is needed to establish the exact structure-activity relationships.

From table 1 it can easily be concluded that the detection of many new members within the series listed in this table can be expected.

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