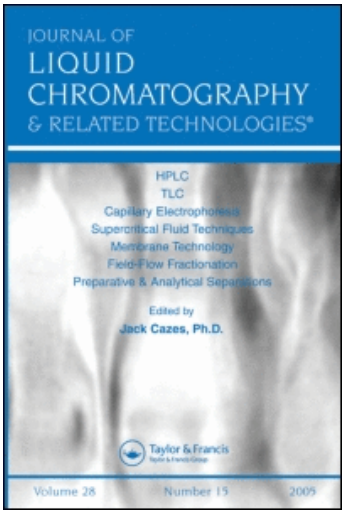

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Enantioresolution of Substituted 2-Methoxy-6-oxo-1,4,5,6-tetrahydropyridine-3-carbonitriles on Macrocyclic Antibiotic and Cyclodextrin Stationary Phases

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ENANTIORESOLUTION OF SUBSTITUTED 2-METHOXY-6-OXO-1,4,5,6-TETRAHYDRO-PYRIDINE-3-CARBONITRILES ON MACROCYCLIC ANTIBIOTIC AND CYCLODEXTRIN STATIONARY PHASES

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ABSTRACT

Nine different 4-, or 5- substituted racemic pyridones were synthesized and resolved by reversed phase LC. Seven of the compounds showed complete or partial resolution on the vancomycin bonded phase column, while five compounds each were separated on both the teicoplanin and the β-cyclodextrin chiral stationary phase (CSPs). No enantioselective separations were obtained on α- or γ-cyclodextrin stationary phases. The antineoplastic agent methotrexate also was resolved. Structural factors that significantly altered enantioselectivity included: changing the pyridone substituent from the 4 to the 5 position or vice versa, changing the size of the substituent, changing the degree of unsaturation of the substituent and changing the nature and length of the substituent "tether". The enantioselectivity of the two related macrocyclic antibiotic CSPs are somewhat similar but not identical. This provides a highly useful optimization approach for these columns. Frequently, when partial enantioresolution is obtained on one antibiotic CSP, a complete resolution is obtained on the related column using

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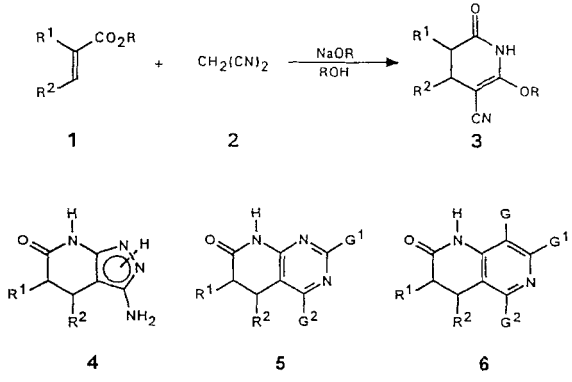
identical elution conditions. It is apparent that these separations (and CSPs) are highly complementary to each other.

INTRODUCTION

The synthesis of 2-methoxy-4-dimethoxymethyl-6-oxo-1,4,5,6-tetrahydropyridine-3-carbonitrile was reported in 1978 by Victory and Diago (1,2). The reaction of an α, β-unsaturated ester (see structure **1** below) and malononitrile (compound **2** below) provides a straight-forward route to the related 2-methoxy-6-oxo-1,4,5,6-tetrahydropyridine-3-carbonitriles (structure **3** below) (3). Since α,β-unsaturated esters, **1**, can have a wide range of substituents, they are versatile starting materials and have been used in the general synthesis of pyrazolo[3,4-b]pyridines (structure **4** below) (4), pyrido[2,3,-d]pyrimidines (structure **5** below) (5-10), and 1,6-naphthyridines (structure **6** below) (11,12). When there is a substituent (R^1 or R^2) other than hydrogen on the α, β-unsaturated ester, a stereogenic-center is created upon addition to the nitrile (see compounds **3**,**4**,**5** and **6** below). These products are racemic and must be resolved if any stereoselective studies are to be done. A series of racemic pyridones related to compound **3** were synthesized, but not yet resolved by any analytical or preparative techniques.



Scheme 1

Recently macrocyclic antibiotics have been proposed as novel chiral selectors in LC, TLC, CE, foam flotation, etc. (13-18). Both ansa compounds (13,14) and particularly oligophenolic glycopeptides (13,16-18) have been used to successfully resolve a large number of enantiomers. We found that vancomycin, teicoplanin and ristocetin A make excellent LC chiral stationary phases when covalently attached to a silica gel support (13,19). In this work, we examine the chromatographic resolution of several racemic 2-methoxy-6-oxo-1,4,5,6-tetrahydropyridine-3-carbonitriles and the compound methotrexate on both the macrocyclic antibiotic stationary phases and the more traditional β-cyclodextrin bonded phase.

EXPERIMENTAL

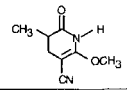
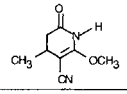
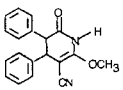
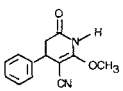
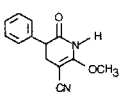
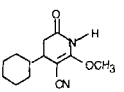
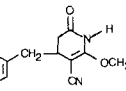
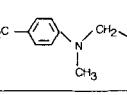
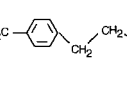
Chemicals

All of the racemic substituted pyridone compounds used in this study were synthesized by Dr. J. I. Borrell and Associates of the Institut Químic de Sarrià, Barcelona, Spain. Methotrexate and its (+)-enantiomer were purchased from Sigma (St. Louis, MO). Teicoplanin was the generous gift of Marian Merrill Dow (Kansas City, MO). All HPLC solvents (acetonitrile, methanol, triethylamine, glacial acetic acid, methyl-*tert*-butyl-ether) were obtained from Fisher Scientific (Pittsburgh, PA). The doubly filtered distilled water was used to prepare triethylammonium acetate buffer.

Methods

The HPLC was performed with a Waters model 590 HPLC with a 745B data module and a UV detector with a fixed wavelength of 280 nm or a Shimadzu LC6A with a variable wavelength SPD6A UV-detector (254 nm) and a CR601 Chromatopac recorder. All separations were carried out at a flow rate of 1.0 ml/min and at room temperature (~ 22°C). Mobile phase compositions are listed in Table I. The vancomycin-bonded stationary phase was prepared according to the recent work of Armstrong (13). It is now available commercially as the

Table I. Chromatographic Data for the Enantioresolution of 4 or 5 Substituted 2-Methoxy-6-oxa-1,4,5,6-tetrahydropyridine-3-carbonitriles.

Compound	Code	k'[a]	α	R_s	Mobile Phase[b]	Column[c]
	L_{Me}	0.85	1.23	0.14	A	Van
	L_C	2.37 0.51 2.30	1.14 1.06 1.34	1.90 0.60 1.56	A A D	β Van Tei
	L_{PhPh}[d]	3.62[d] 1.91[d]	8.50[d] 1.91[d]	9.35[d] 2.52[d]	A E	β Tei
	L_{Ph}	4.00 0.53 3.31	1.08 1.14 1.38	1.25 1.50 2.21	A C D	β Van Tei
	L_A	3.30 1.18	1.12 1.12	1.66 0.90	A A	β Van
	L_{CH}	0.59 4.10	1.13 1.44	0.91 1.72	C D	Van Tei
	L_{BZ}	6.37 3.00	1.10 1.20	1.70 0.96	A D	β Tei
	L_{M1}	1.43	2.19	5.40	A	Van
	L_{M2}	2.40 1.46	1.38 1.23	2.50 1.45	C A	Van Van

[a] Capacity factor for the first eluted enantiomer.

[b] Mobile phases are: A=90% buffer I/10% acetonitrile; B=495/5/1/1 (acetonitrile/methanol/acetic acid/triethylamine by volume); C=4ml methyl-*tert*-butyl ether/200 ml mixture of 90% buffer I and 10% methanol; D=90% buffer II/10% methanol; E: 70% buffer II/30% methanol. Buffer I contains 1% triethylammonium acetate, pH 7.0. Buffer II contains 1% triethylammonium acetate, pH 4.1.

[c] Column β, Van and Tei stand for β-cyclodextrin, vancomycin and teicoplanin bonded stationary phases, respectively.

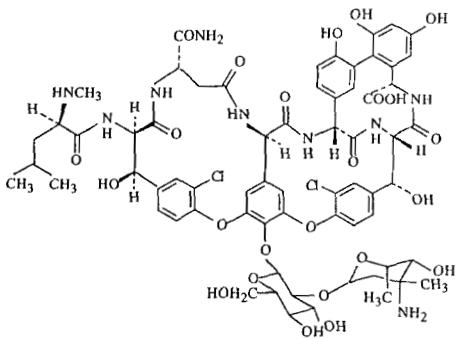
[d] This compound has two stereogenic centers and consists of two pairs of enantiomers. While the diastereomeric separation was adequate on all columns, only a partial resolution of the enantiomers was obtained. In the case of the β-cyclodextrin column the second pair of enantiomers was partially resolved ($R_s \cong 0.6$); while in the case of the teicoplanin column, the first pair of enantiomers was partially resolved. ($R_s \cong 0.5$).

CHIROBIOTIC V column from Advanced Separation Technologies (Whippany, NJ). The teicoplanin column was prepared in the same way as previously reported for vancomycin (13). The β-cyclodextrin bonded stationary phase (Cyclobond I, 250 x 4.6 mm i.d.) was obtained from Advanced Separation Technologies as were the γ- and α-cyclodextrin bonded phase columns. The 1%, pH = 4.0 ~ 7.0 triethylammonium acetate buffer was prepared by dissolving 10 ml of HPLC grade triethylamine in HPLC grade water and diluting it to a volume of l liter. Glacial acetic acid was used to adjust the solution to the desired pH.

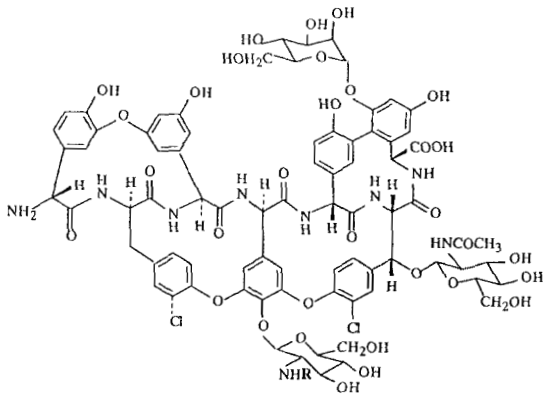
RESULTS AND DISCUSSION

A series of pyridones (based on 2-methoxy-6-oxo-1,4,5,6-tetrahydropyridine-3-carbonitrile) were synthesized with various substituents at either the 4 or 5 positions. All of these compounds are racemic mixtures. They also are considered precursors for potential antineoplastic agents. Given current regulatory concerns (20) and the fact that enantiomers often produce difficult biological responses (21,22), it was necessary to have an analytical means to monitor individual enantiomers. Other secondary considerations were that the method be compatible with water containing solvents (in view of the synthetic process and work-up, as well as future *in vitro* and *in vivo* studies) and that analogous preparative scale separations be feasible. To our knowledge these racemic substituted pyridones have not been resolved previously by any means.

Recently vancomycin (a chiral, macrocyclic, oligophenolic, glycopeptide antibiotic) was attached to 5μ silica gel and subsequently shown to be an effective chiral stationary phase (CSP) (13). Native vancomycin is ionizable and has a molecular weight of 1449. The aglycone portion of the molecule consists of three small, fused macrocyclic rings. Together they form a "basket-shaped moiety" (13,15). A freely rotating disaccharide is attached to the "basket". More extensive details on the chemical and physical properties of vancomycin and its molecular recognition properties have been published (15). Teicoplanin A_2 also is a chiral, macrocyclic, oligophenolic glycopeptide antibiotic that is related to



Vancomycin



Teicoplanin A_2

A_2-1: R=(Z)-4-decanoic acid; A_2-2: R=8-methylnonanoic acid;
A_2-3: R=n-decanoic acid; A_2-4: R=8-methyldecanoic acid;
A_2-5: R=9-methyldecanoic acid.

Figure 1. Structures of the two oligophenolic, glycopeptide, macrocyclic antibiotics used in this study. Note that the aglycone portion of vancomycin consists of three fused rings while that of the teicoplanin A_2 consists of four fused rings. Both contain attached sugar moieties that are free to rotate. The teicoplanin A_2 also has a hydrophobic "tail" group attached to one of the sugars.

vancomycin (23,24). It differs from vancomycin in that the aglycone "basket" contains four rather than three small, fused macrocyclic rings; and that there are three attached monosaccharides. In addition there is a C_9 or C_{10} hydrocarbon "tail" attached to one of the monosaccharides (23,24). Hence, teicoplanin is a mixture of very closely related compounds with molecular weights between 1876 and 1892. Teicoplanin A_2 was attached to a 5μ silica gel support in a manner identical to that for vancomycin (19). The structures of vancomycin and teicoplanin are shown in Figure 1.

Both vancomycin and teicoplanin CSPs are multimodal in that they can be used in the reversed phase mode, normal phase mode and the polar organic mode (*e.g.*, 90-99% acetonitrile plus minor additives) and give different enantioselectivities in each (13, 25). The enantioresolution capabilities of the two new antibiotic-based CSPs in the reversed phase mode were compared to the more traditional cyclodextrin columns. Table I summarizes the enantioseparation data for the nine racemic, substituted pyridone compounds. Also, listed are the "code symbols" for each compound. The best resolution was obtained in the reversed phase mode in all cases. A partial resolution for L_{CH} was obtained (data not included) using a polar organic mobile phase, but, in general, this approach was not effective for these compounds. In all but a single case (i.e., L_A) the antibiotic-based LC columns produced the highest enantioselectivities (αs). The β-cyclodextrin column gave the best resolution in three cases (i.e., L_A, L_C and L_{BZ}) largely because of efficiency considerations. No enantioresolutions were observed with either the α-, or γ-cyclodextrin columns.

Apparently hydrophobic association can be important for both the antibiotic and cyclodextrin-type of chiral selectors. This is well established for cyclodextrins in the reversed phase mode where inclusion complexation is involved (26-28). In contrast, the individual macrocyclic rings of vancomycin and teicoplanin are too small to include the compounds in this study. However when the smaller rings are fused, they form a "basket-like" structure with hydrophobic and hydrophilic regions (Figure 1). It was previously shown that

the retention on a vancomycin column shows typical reversed-phase behavior (i.e., high retention with highly aqueous mobile phases and decreasing retention as the organic modifier is increased)(13). The compounds in this study show analogous retention behavior, which is indicative of hydrophobic association. However, it should be noted that only in rare cases are hydrophobic interactions alone sufficient for chiral recognition (29). Most often additional interactions are needed, including: hydrogen bonding, steric repulsion, dipole stacking, π-π associations, and so forth.

As shown in Table I, relatively small changes in a group's substitution position, degree of saturation and chain length can affect enantioselectivity. For example the only difference between L_{ME} and L_C (Table I) is that the methyl substituent on the pyridone ring is in the 4 position in one case and the 5 position in the other. However the 4-methyl compound (L_C) is baseline resolved with both the teicoplanin and β-cyclodextrin columns, while the 5-methyl analogue (L_{ME}) is not resolved by either column. There are several other related examples where small structural changes can completely change the enantioselectivities (i.e., L_{Ph} vs L_A, L_{Ph} vs L_{CH}, L_{Ph} vs L_{BZ}, L_A vs L_{M2} and L_{M1} vs L_{M2} in Table I).

From an analytical point of view, it is interesting to compare separations on the two related macrocyclic antibiotic columns. In some cases a compound was adequately resolved on both columns (Figure 2A). However, more often a partial resolution was obtained with one macrocyclic antibiotic while complete resolution was obtained on the other, related CSP (Figures 2B and C). This behavior was observed not only for the compounds in this study but for a variety of other types of analytes as well (Figure 3). This provides a very interesting and useful means for optimizing an enantiomeric separation that does not exist with other columns. Apparently the structurally related oligophenolic, glycopeptide antibiotics have somewhat similar, but not identical enantioselectivities. Hence, if no more than a partial resolution can be obtained on one CSP, it is highly likely that a complete separation can be obtained on a near "relative" CSP using the

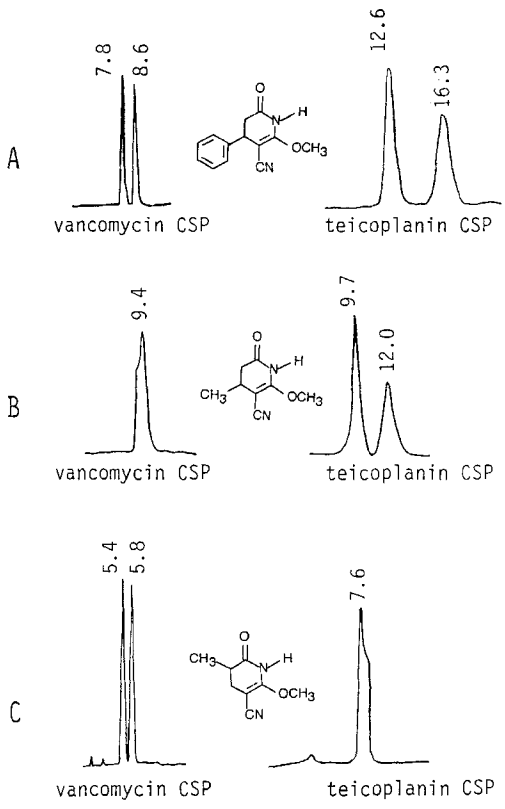


Figure 2. Enantiomeric separations of three racemic substituted pyridones that illustrate the complementary enantioselectivities of the two related antibiotic chiral stationary phases. All three racemates (A, B and C) were separated under identical conditions (i.e., mobile phase = 10:90, methanol: 1% triethylammonium acetate buffer, pH 4.1 at a flow rate of 1.0 ml/min). In each case the columns consisted of either a 25 cm x 0.44 cm (i.d.) vancomycin or teicoplanin bonded phase material (5 μ silica support).

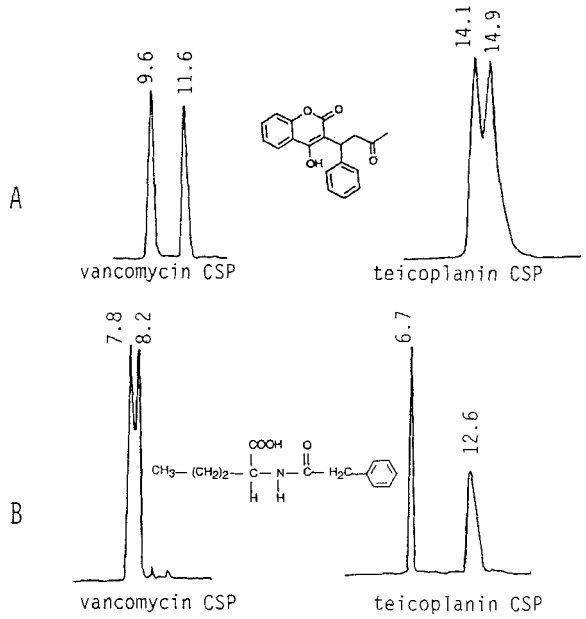


Figure 3. Chromatograms illustrating that the "principle of complementary separations" for the macrocyclic antibiotic CSPs applies to a variety of different compounds. Part "A" shows the separation of racemic warfarin on a vancomycin vs a teicoplanin CSP. In both cases the mobile phases consisted of 10:90, acetonitrile: 1% triethylammonium acetate buffer, pH 4.1; and a flow rate of 1.0 ml/min. Part "B" shows the separation of racemic N-CBZ-norvaline. In both cases the mobile phase consisted of 20:80, methanol: triethylammonium acetate buffer, pH 4.1; and a flow rate of 1.0 ml/min.

same mobile phase conditions (Figures 2 and 3). This "principle of complementary separations" can be useful when a baseline resolution must be found quickly.

As stated previously, the substituted pyridones resolved in this study can be used in the synthesis of active agents for cancer chemotherapy. One of the better known compounds of this genre is methotrexate, which is produced as a racemate

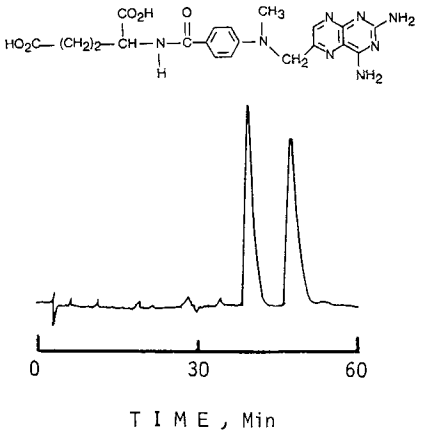


Figure 4. Enantiomeric resolution of methotrexate on a 25 cm x 0.44 cm (i.d.) Cyclobond I column. The mobile phase was 7.5:92.5, acetonitrile: 1% triethylammonium acetate buffer, pH 7.0; at a flow rate of 1.0 ml/min.

(30-33). The resolution of methotrexate is shown in Figure 4. Apparently the type of columns used in this study can be used to resolve both the synthetic intermediates and the final products for this class of compounds.

ACKNOWLEDGEMENT

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