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Journal of Chromatography A, 906 (2001) 91–103

JOURNAL OF CHROMATOGRAPHY A

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High-performance liquid chromatographic and capillary electrophoretic enantioseparation of plant growth regulators and related indole compounds using macrocyclic antibiotics as chiral selectors

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Abstract

Enantioseparation of plant growth regulators, such as 3-(3-indolyl)-butyric acid, abscisic acid and structurally related molecules including a variety of substituted tryptophan compounds, has been achieved by HPLC and/or CE. The covalently bonded macrocyclic antibiotics, teicoplanin, ristocetin A and vancomycin, were used as chiral stationary phases (CSPs) in HPLC. Most of the racemates were baseline resolved in the reversed-phase mode (EtOH-H_2O) using the teicoplanin CSP. The chiral recognition mechanism is discussed in regard to the structure of the analytes. In CE, the three aforementioned macrocyclic antibiotics were used as chiral additives in a phosphate run buffer. The effect of pH and the concentration of the organic modifiers were considered. The results obtained by HPLC and CE were compared.

Keywords: Enantiomer separation; Plant growth regulators; Indole compounds; Teicoplanin; Ristocetin A; Vancomycin

1. Introduction

Basic research on plant growth regulation is becoming important, particularly in order to increase plant productivity. Plant growth regulators are naturally occurring or synthetic substances, effective in very small amounts (micromolar to submicromolar concentration range). Plant growth regulators are generally small organic molecules, with molecular mass under 1000. They act as chemical signals to control the physiological processes of plants such as: the acceleration of abscission, the induction of cell division, dormancy, and stem elongation in seedings; inhibition of rooting; stimulation of stomatal closure; and defense against environmental stresses such as excess heat, cold, salinity, etc. [1–5]. Many plant hormones are chiral compounds which contain indole rings. Moreover, the activity of the hormone enantiomers often is different. For example, the activity of the (*S*)-(+)-4,4,4-trifluoro-3-(indol-3-)butyric acid (TFIBA) is 10-fold greater than that of the (*R*)-(−) enantiomer in promoting root growth in the Chinese cabbage [6]. Consequently, enantioseparation and testing are becoming necessary to ensure accurate biological studies of these compounds. We describe herein the enantiomeric separation of several chiral

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0021-9673/01/$ – see front matter
PII: S0021-9673(00)00954-7

plant growth regulators and related compounds by HPLC and CE using macrocyclic glycopeptide antibiotics as chiral selectors.

1.1. Structure and morphology of the three macrocyclic glycopeptide antibiotics

The macrocyclic antibiotics vancomycin, teicoplanin and ristocetin A are produced as fermentation products of *Streptomyces orientalis, Actinoplanes teichomycetus* and *Nocardia lurida,* respectively [7]. These antibiotics are primarily active against aerobic and anaerobic Gram-positive microorganisms both in vitro and in vivo. They are known to inhibit cell wall synthesis [8]. Fig. 1 gives the structure of the three macrocyclic antibiotics. All three of these related compounds consist of an aglycon ''basket'' made of fused macrocyclic rings and pendant carbohydrate moieties [9–12]. The macrocycles contain both ether and peptide linkages. The aglycons of vancomycin and teicoplanin contain two chloro-substituted aromatic rings, while the analogous portion of ristocetin A contains no chloro substituents. Vancomycin is the smallest of the three compounds (MS 1449, 18 stereogenic centers). It consists of three macrocyclic rings and an attached disaccharide consisting of D-glucose and vancosamine. The other two glycopeptides (teicoplanin MW 1877, with 23 stereogenic centers and ristocetin A MW 2066, with 38 stereogenic centers) are somewhat larger. They have four, rather than three, fused macrocyclic rings and a great number of different types of pendant sugar moieties [12]. For example, teicoplanin has three attached monosaccharides, two of which are D-glucosaniine and one of which is D-mannose. Ristocetin A has a pendant tetrasaccharide and two monosaccharide moieties. These saccharides include D-arabinose, D-mannose, D-glucose and D-rhamnose. Teicoplanin has one unique characteristic: it has a hydrophobic acyl side chain (hydrophobic tail) attached to a 2-amino-2-deoxy-β-D-glycopyranosyl moiety. Consequently, teicoplanin is surface active and aggregates to form micelles. Neither of the other two glycopeptides has shown this type of behavior under the experimental conditions used in this study.

All three glycopeptides have analogous ionizable groups which control their charge and are thought to play a role in their association with and chiral recognition of chiral analytes. For example, there is an amine on the aglycon portion of each compound. Vancomycin has a secondary amine, while the other two macrocyclic compounds have primary amine groups. All three compounds also have aminosaccharide moieties. However, teicoplanin is unique in that it has two aminosaccharides, both of which are *N*-acylated. There is a carboxylic acid moiety on the glycon of both vancomycin and teicoplanin, while the equivalent group on ristocetin A is esterified. The only other ionizable groups on these compounds are the phenolic moieties. At operational pH values (~3.5–7.5), these are generally protonated and probably serve mainly as hydrogen bonding sites.

In HPLC, the three antibiotics are covalently bonded on silica and used as a chiral stationary phase (CSP). In CE, the native macrocyclic antibiotics used are desolved in the run buffer solutions.

2. Experimental

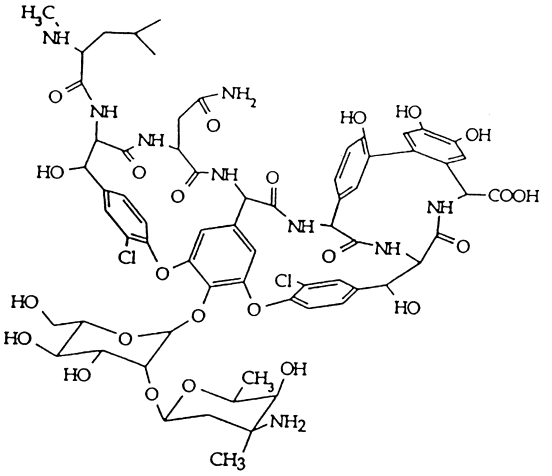
2.1. Materials

All chiral compounds analyzed in this study were purchased from Sigma (St. Louis, MO) except 3-(3-indolyl)-butyric acid which was synthesized in our laboratory. Methanol, acetonitrile, ethanol (containing 4.7% methanol and 4.6% isopropanol sold as ''reagent alcohol''), triethylamine, glacial acetic acid were obtained from Fischer Scientific (St. Louis). Sodium dihydrogenphosphate, sodium hydroxides were purchased from Sigma (St. Louis, MO). Chirobiotic V, T and R columns (250×4.6 mm I.D.) and vancomycin, teicoplanin, as well as ristocetin A were obtained from Advanced Separation Technologies (Whippany, NJ). Fused silica capillaries (50 μm I.D. and 375 μm O.D.) were purchased from Quadrex Corporation (New Haven, CT). Disposable nylon filters (0.45 μm) were obtained from Alltech (Deerfield, IL).

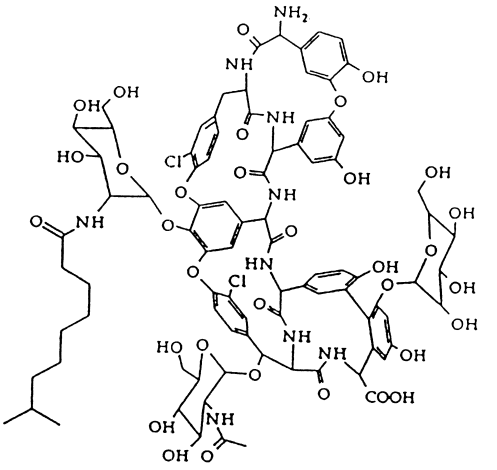
2.2. Methods

2.2.1. HPLC

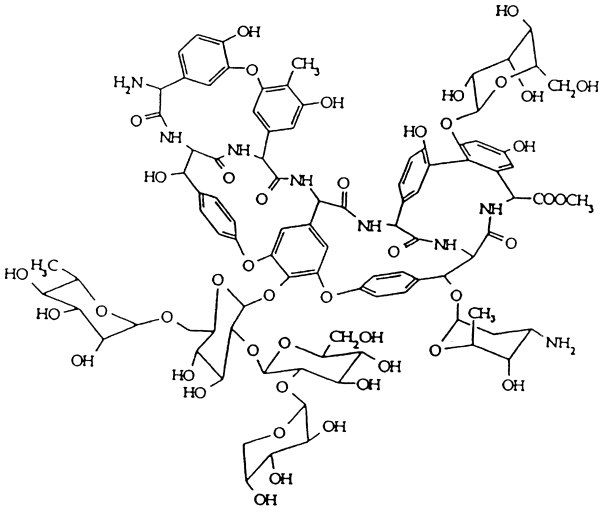
A Shimadzu LC 6A chromatograph (Shimadzu,



Vancomycin



Teicoplanin



Ristocetin A

Fig. 1. Structure of the three macrocyclic antibiotics used in this study.

Kyoto, Japan) was used to perform all separations. It included two LC-6A pumps, a SPD-6A UV detector, a SCL-6A controller, and a CR-601 integrator. The analytes were dissolved in methanol, ethanol or mobile phase solutions. Separations were carried out at a flow-rate of 1.0 or 0.5 ml/min at room temperature. The UV detection wavelength used was 254 nm.

2.2.2. CE

All separations were performed using a Quanta 4000 capillary electrophoresis apparatus provided by Waters (Marlborough, Massachusetts). It was equipped with a 254 nm lamp for UV detection. A 50 μm (I.D.)×32.5 cm (25.0 cm to detector) fused-silica capillary was used. The capillary was purged daily with 0.1 potassium hydroxide for 10 min prior to analysis. The capillary was purged with buffer between runs. These intermediate purge/cleaning runs are necessary to remove any of the macrocyclic antibiotic that attaches to the capillary wall. Vancomycin tends to bind to the capillary wall to a greater extent than ristocetin A or teicoplanin [12]. Using this protocal the reproducibility and sensitivity were good [12]. All samples were dissolved in water or methanol at concentrations ~0. 1 mg/ml and filtered prior to injection. Samples were injected for 5 s using hydrostatic mode. All separations were carried out at ambient temperature (~22°C) without temperature control. The run voltage for all separations was +5 kV.

The glycopeptide antibiotic solutions were prepared in volumetric flasks by dissolving them in buffer or buffer/organic solvent mixtures and sonicated to degas the solution and aid dissolution. Antibiotic solutions can decompose at slightly elevated temperatures. To avoid excessive heating, solutions should be sonicated only long enough to dissolve the antibiotics and remove dissolved air. Antibiotic solutions were refrigerated at 4°C when not in use.

Electrophoretic mobilities were calculated from experimental data using the following relationship:

$$\mu_e = \frac{\ell L}{V}\left(\frac{1}{t_i} - \frac{1}{t_{eof}}\right)$$

Where μ_e is the electrophoretic mobility of the solute, 1 is the capillary length to the detector, L is the total capillary length, V is the applied voltage, and t_i and t_{eof} are the measured migration times of solutes and the electrosmotic flow (*eof*). The *eof* time was measured using mesityl oxide.

3. Results and discussion

3.1. HPLC

Chromatographic separation data for the enantioresolution of 20 compounds are presented in Table 1. They were all baseline separated (with $R_s \geq 1.5$) with exception of α-Methyl-tryptophan, tryptophan methyl ester and α-Methyl-tryptamine. Fig. 2, shows the chromatograms of some of the plant growth regulators and related indole compounds. A resolution factor as high as 5.9 was obtained for *N*-(3-indolylacetyl)-aspartic acid (Table 1, #14). Most of the separations were obtained with a very simple mobile phase: ethanol/water (EtOH/H_2O), 50/50, v/v. Hence, the isolated analytes could be easily isolated, if necessary, by evaporating the mobile phase of the collected samples. Moreover, the long-term use of ethanol/water as a mobile phase does not seem to adversely effect the column [9]. Ethanol is preferable for separations in normal-phase mode compared to other polar modifiers such as isopropanol since it produces separations of higher efficiency and better peak shapes [9–11]. The same trend has also been found for Vancomycin CSP [9–11]. A possible explanation for the beneficial effects of ethanol could be due to the fact that, on one hand, ethanol hydrogen bonds less extensively with the macrocycles glycopeptide CSPs and analytes than does methanol. Also, compared to isopropanol, ethanol is less viscous in aqueous solutions which leads to more efficient kinetics of analyte-CSP exchange, leading to faster mass transfer and better efficiency [9].

Table 2 shows the effect of ethanol concentration on retention, selectivity and resolution of some substituted tryptophans. As the concentration of ethanol increases, retention decreases while selectivity and resolution increase. This indicates that the

Table 1
HPLC data for the enantioseparation of 20 plant growth regulators and related compounds

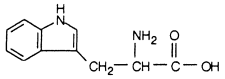
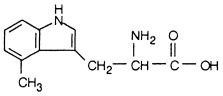
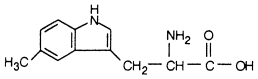
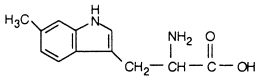
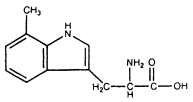
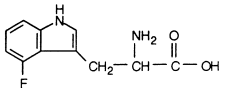
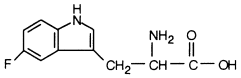
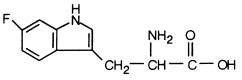
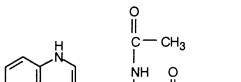
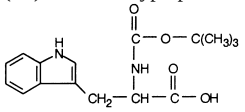
Compounds	K_1'	α	R_s	Stationary phase	Mobile phase
(1) Tryptophan 	0.9	1.5	2.1	Teicoplanin	EtOH/H_2O 50/50
(2) 4-Methyl-tryptophan 	1.3	1.4	2.3	Teicoplanin	EtOH/H_2O 50/50
(3) 5-Methyl-tryptophan 	0.85	1.62	2.2	Teicoplanin	EtOH/H_2O 50/50
(4) 6-Methyl-tryptophan 	0.94	1.63	2.1	Teicoplanin	EtOH/H_2O 50/50
(5) 7-Methyl-tryptophan 	0.93	1.55	1.78	Teicoplanin	EtOH/H_2O 50/50
(6) 4-Fluoro-tryptophan 	0.6	1.7	1.8	Teicoplanin	EtOH/H_2O 50/50
(7) 5-Fluoro-tryptophan 	0.41	2.6	3.0	Teicoplanin	EtOH/H_2O 50/50
(8) 6-Fluoro-tryptophan 	0.38	2.2	1.7	Teicoplanin	EtOH/H_2O 50/50
(9) *N*-Acetyl-tryptophan 	1.1	3.1	3.9	Teicoplanin	EtOH/0.1% TEAA 90/10 pH 4.1
(10) *N*-*t*-Boc-tryptophan 	1.4	2.0	1.5	Ristocetin A	EtOH/H_2O 60/40

Table 1. Continued

Compounds	K_1'	α	R_s	Stationary phase	Mobile phase
(11) *N*-CBZ-tryptophan	1.5	1.5	2.1	Teicoplanin	EtOH/0.1% TEAA 25/75, pH 4.1
(12) 5-Methoxy-tryptophan	0.7	1.5	2.6	Teicoplanin	EtOH/H_2O 50/50
(13) *Trans, trans*-Abscisic acid	2.2	1.3	2.5	Teicoplanin	THF/H_2O 10/90
(14) *N*-(3-Indolylacetyl)-aspartic acid	0.6	5.1	5.9	Teicoplanin	MeOH/0.1% TEAA 10/90, pH 4.1
(15) 3-Indolesuccinic acid	5.1	1.4	1.5	Ristocentin A	THF/H_2O 10/90
(16) Indole-3-lactic acid	1.7	1.9	1.7	Ristocentin A	EtOH/H_2O 60/40
(17) 3-(3-Indolyl)-butyric acid	2.9	1.4	2.8	Teicoplanin	EtOH/H_2O 30/70
(18) α-Methyl-tryptophan	0.8	1	0	Teicoplanin	EtOH/H_2O 50/50
(19) Tryptophan methyl ester			No separation		
(20) α-Methyl-tryptamine			No separation		

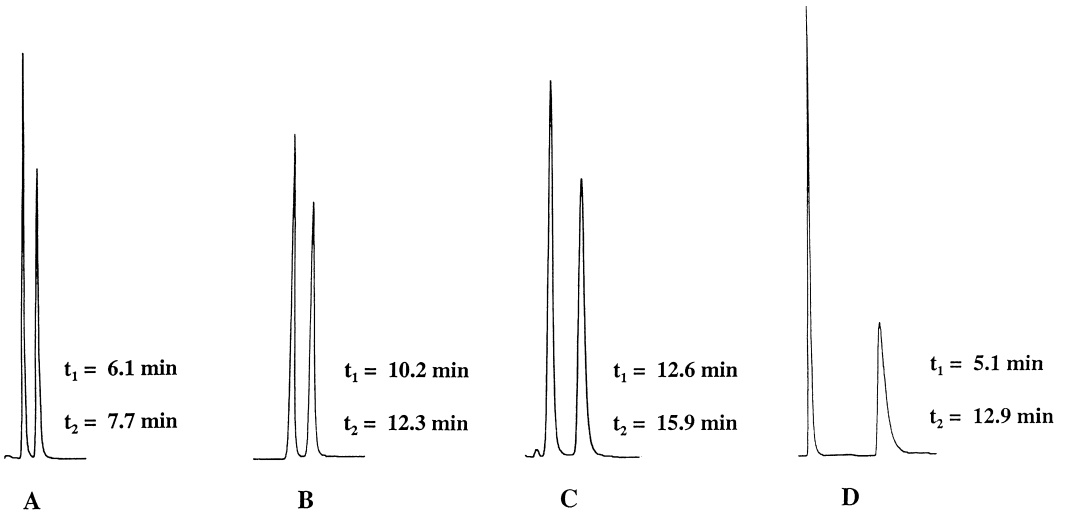


Fig. 2. Chromatograms showing typical reversed-phase enantioseparations using Chirobiotic T column: (A) tryptophan, mobile phase: ethanol/water, 50/50, v/v; (B) *trans, trans*-abscisic acid, mobile phase: tetrahydrofuran/water, 10/90, v/v; (C) 3-(3-indolyl)-buteric acid, mobile phase: ethanol/water, 30/70, v/v; (D) *N*-(-3-indolylacetyl)-aspartic acid, mobile phase: methanol/0.1% triethylammonium acetate, 10/90, v/v, Ph 4.1. The flow-rate was 1.0 ml/min at ambient temperature (~22°C). UV absorbance detection at 254 nm was used.

studied compounds are better solvated in EtOH than in H_2O, and that the increase in ethanol concentration enhances the enantioselective interactions relative to the nonenantioselective interactions. This leads to an unusual situation where decreased retention results in enhanced selectivity (α) and resolution (Table 2). It is interesting to note that for the methyl-tryptophans, the position of the substituent $-CH_3$ group on the aromatic ring has little effect on selectivity and resolution when the ethanol concentration is low (20–30%). However, in the case of 50% EtOH, the selectivity and resolution values of the methyl-tryptophans are proportional to the distance between the methyl group and the chiral center (see the structure of these compounds in Table 1). In other words, resolution of a compound on the teicoplanin CSP (Chirobiotic T) is sensitive to the ''fine'' structure of the studied analyte. A substituent group, even situated far from the chiral center, can produce some impact on selectivity and resolution of

Table 3
Effect of substituents of tryptophan on retention, selectivity and resolution. Column: Chirobiotic T; flow-rate: 0.5 ml/min

Compounds	k_1'	k_2'	α	R_s
Tryptophan	0.94	1.47	1.56	1.92
4-methyltryptophan	1.36	1.91	1.40	2.06
5-methyltryptophan	0.99	1.57	1.59	2.22
6-methyltryptophan	1.00	1.65	1.65	2.29
4-fluorotryptophan	0.61	1.03	1.69	1.50
5-fluorotryptophan	0.40	1.07	2.68	2.13
6-fluorotryptophan	0.37	0.80	2.16	1.75

Table 2
Effect of mobile phase composition on retention, selectivity and resolution of tryptophan and methyl-trytophans. Column: Chirobiotic T; flow-rate: 1 ml/min

Compounds	20% EtOH			30% EtOH			50% EtOH		
	k_1'	α	R_s	k_1'	α	R_s	k_1'	α	R_s
Tryptophan	1.38	1.25	1.00	1.26	1.27	1.34	0.94	1.52	1.51
4-methyltryptophan	2.24	1	0	1.99	1.06	0.45	1.32	1.39	1.68
5-methyltryptophan	1.66	1.16	0.95	1.34	1.29	1.43	0.96	1.57	2.06
6-methyltryptophan	1.79	1.19	0.96	1.55	1.32	1.43	0.94	1.64	2.17
7-methyltryptophan	1.69	1.17	0.95	1.39	1.27	1.25	0.93	1.55	1.78

these compounds. In order to understand the mechanism of interactions between the analytes and the CSPs, a further investigation was done, comparing two different substituents (methyl- and fluoro-groups) and their position on the indole moiety of the tryptophan molecule. The results are listed in Table 3. Generally, a methyl-group releases electrons to the aromatic (indole) ring; whereas a fluoro-group withdraws electrons from the aromatic ring. Clearly, these effects as well as the position of the groups on the indole ring can influence enantioselectivity (i.e., α-values in Table 3). There also is an effect on retention and efficiency as reflected by the resolution in Table 3. The fluoro substituents (relative to methyl groups in the same position) decrease retention and increase enantioselectivity (α's). However the resolution of the fluorotryptophan enantiomers were somewhat less than those of the corresponding methyl tryptophans. Thus the more retained, and somewhat more hydrophobic, methyltryptophans produced more efficient separations.

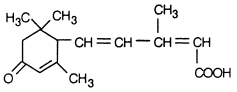
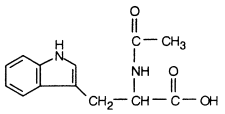
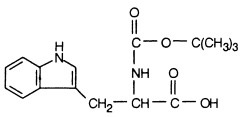
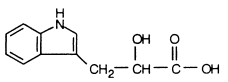
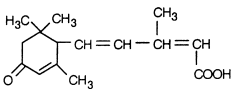
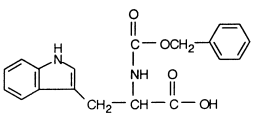
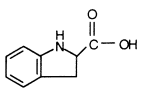
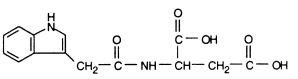
If substituents on the indole ring (which are relatively far from the stereogenic center) have an effect on selectivity and resolution, a substituent on or near the stereogenic center can have an even more pronounced influence on these factors. This is illustrated by the results of α-methyl-tryptophan (Table 1, #18). Compared to tryptophan, which is baseline resolved with EtOH/H_2O (50/50, v/v) on the Chirobiotic T column, α-methyl-tryptophan is not resolved under the same conditions (however a partial separation is obtained with EtOH/H_2O: 90/10, v/v). Substitution of the hydrogen on the *a*-amino group of tryptophan by CH_3CO– and $C_6H_5OCH_2CO$– gives *N*-acetyltryptophan (Table 1, #9) and *N*-CBZ-tryptophan (Table 1, #11), respectively. Like α-methyl-tryptophan, neither are resolved on the Chirobiotic T CSP with E*t*OH/H2O (50/50, v/v) as the mobile phase. Nevertheless, they are both baseline separated on the same column using E*t*OH/0.1% TEAA (90/10. V/v, pH 4.1) and EtOH/0.1% TEAA (25/75, v/v, pH 4.1), respectively as the mobile phase. Indole-3-lactic acid (Table 1, #16) is obtained if the α-amino group of tryptophan is replaced by an –OH group. This compound was retained, but not resolved on the Chirobiotic T column with a mobile phase consisting of EtOH/H_2O: 50/50, v/v. However, a baseline separation of this compound was obtained on the Chirobiotic R CSP with EtOH/H_2O, 60/40, v/v [11]. This result indicates that, on one hand, the interaction mechanisms between indole-3-lactic acid and the Chirobiotic T and R are somewhat different, but on the other hand, the analytes carboxy-group is apparently the main interaction site for these compounds. This conclusion is confirmed by the baseline separation of 3-(3-indolyl)-butyric acid (Table 1, #17) in which there is no amino group but the requisite carboxy group remains. Another way to evaluate the relative interaction activity of the $-NH_2$ and –COOH groups of this set of analytes, with the CSPs, is to keep the $-NH_2$ group at its original position and esterify or remove the –COOH group. This leads to the two following compounds: tryptophan methyl ester (Table 1, #19) and α-methyl-tryptamine (Table 1, #20). Neither of these compounds are separated by any of the macrocyclic glycopeptide CSPs (Chirobiotic T, R and V) with any of the three types of mobile phases (normal-phase, reversed-phase and polar organic mobile phase). This result confirms again that the carboxylate group (–COOH) is the principal site for association and also is important for chiral recognition between these particular analytes and the glycopeptide CSPs. The amino group, if present, may sometimes provide secondary interactions.

3.2. Enantioseparation by capillary electrophoresis (CE)

As a complementary method to HPLC enantiomeric separations, CE also has been used. Three glycopeptide antibiotics (vancomycin, teicoplanin, and ristocetin A) have been used as chiral run buffer additives in CE, to resolve the chiral plant growth regulators and related indole compounds previously examined by HPLC.

Table 4 lists eight racemates that were baseline resolved by CE. As is noted, most racemates are resolved by two or more chiral selectors. A resolution factor as high as 8.9 is obtained with 2 m*M* Ristocetin A + 20% methanol in 0.1 *M* NaH_2PO_4 buffer for *N-t*-Boc-tryptophan (Table 4, #3). Fig. 3 shows the electropherograms obtained for *trans, trans*-abscisic acid using teicoplanin and vancomycin

Table 4
CE data for enantioseparation of eight plant growth regulators and related compounds

Compounds	Time (1) (min)	Time (2)	μ_e (1) ($cm^2\ kV^{-1}\ min^{-1}$)	μ_e (2)	R_s	Run buffer (in 0.1 *M* NaH_2PO_4)	pH
(1) *Trans, trans*-Abscisic acid	15.3	18.0	−6.3	−7.6	4.4	5 m*M* Teicoplanin +10% ACN	6
	20.0	27.5	−6.9	−8.8	8.6	2 m*M* Vancomycin	6
	37.0	38.8	−10.8	−10.9	1.6	2 m*M* Ristocetin A	6
							
(2) *N*-Acetyl-tryptophan	20.8	32.0	−5.3	−7.7	7.8	5 m*M* Teicoplanin +30% ACN	6
	18.5	22.4	−7.4	−8.6	4.5	2 m*M* Vancomycin	7
							
(3) *N-t*-Boc-tryptophan	17.6	18.5	−6.2	−6.6	1.8	5 m*M* Teicoplanin +20% ACN	6
	18.0	30.0	−4.9	−7.9	8.9	2 m*M* Ristocetin A +20% MeOH	6
	31.7	35.7	−8.8	−9.2	2.3	2 m*M* Vancomycin	6
							
(4) Indole-3-lactic acid	47.8	52.3	−12.4	−12.6	1.8	2 m*M* Vancomycin	6
	21.7	41.5	−5.6	−8.6	8.8	2 m*M* Ristocetin A	6
							
(5) *Cis, trans*-Abscisic acid	29.0	30.1	−10.9	−11.1	1.5	2 m*M* Ristocetin A	6
							
(6) *N*-CBZ-tryptophan	20.9	21.8	−8.2	−8.5	1.5	2 m*M* Teicoplanin +20% ACN	6
	16.8	17.8	−7.1	−7.6	2.3	2 m*M* Vancomycin	7
							
(7) Indoline-2-carboxylic acid	28.0	36.5	−8.4	−9.6	6.2	5 m*M* Teicoplanin +30% CAN	6
	34.1	50.1	−11.6	−12.9	6.8	2 m*M* Ristocetin A	6
							
(8) *N*-(3-Indolylacetyl)-aspartic acid	36.7	49.7	−11.5	−12.5	8.7	5 m*M* Teicoplanin +20% ACN	6
							

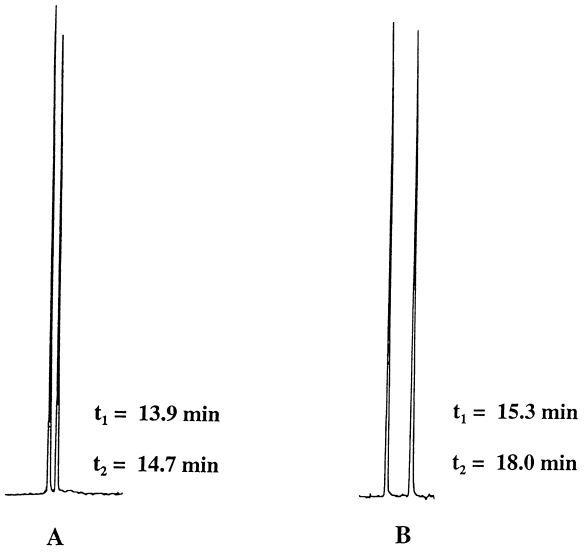


Fig. 3. Electropherograms showing the resolution of *trans, trans*-abscisic acid. The running buffer was (A) 0.1 *M* phosphate buffer-5 m*M* teicoplanin-10% acetonitrile, pH 6.0; (B) 0.1 *M* phosphate buffer-2 m*M* vancomycin, pH 6.0. The run voltage was +5 kV and the analytes were detected at 254 nm at ambient temperature. The capillary was 50 μm I.D.×32.5 cm (25 cm to the detector).

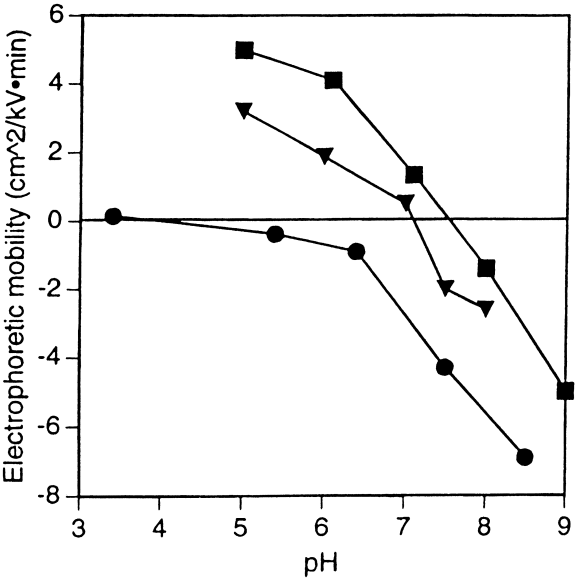


Fig. 4. Plot showing the effect of solution pH on the electrophoretic mobility of ristocentin A (■), vancomycin (▲), and teicoplanin (●) macocyclic antibiotics using 0.1 *M* phosphate buffer. The capillary for ristocetin A and vancomycin studies was 32.5 cm×50 μm I.D. (25 cm to the detector window). The voltage was +5 kV. The electrophoretic mobility of teicoplanin was obtained using a 44 cm×50 μm I.D. capillary (36.5 cm to the detector) and a run voltage of +10 kV. Either acetone or methanol was used as the electroosmotic flow marker.

as chiral run buffer additives, respectively. Compared to the chromatogram for the same compound (see Fig. 2), the peaks obtained by CE are much more narrow, indicating the greater efficiency of CE.

3.2.1. pH effects

Glycopeptide antibiotics are ionizable chiral selectors and their electrophoretic mobility is dependent on the pH of buffered solutions [12]. This is shown in Fig. 4. Note that the pI value for teicoplanin is approximately 3.8 under these experimental conditions. However, a p*I* value of 6.5 has previously been reported [13,14]. This significant difference of p*I* measurement is undoubtedly related to the surface activity and structure of teicoplanin. Self-association is known to affect apparent p*I* values and other physico-chemical properties of a wide variety of compounds [8,13]. Therefore, the pH of the running buffer governs not only the charge and migration behavior of the chiral analytes but also those of the chiral selectors. Fig. 5 shows the pH effect on the separation of *N*-*t*-Boc-tryptophan with vancomycin as a chiral additive. As can be seen, a decrease in pH of the run buffer causes an increase in resolution of this racemate. This confirms the results previously obtained for other racemates [12]. Apparently, the interactions between the test solute and vancomycin are enhanced when low pH buffers are used. However, more information is needed in order to more accurately elucidate the separation mechanism.

3.2.2. Effect of organic modifiers

As can be seen in Table 4, when teicoplanin is used as chiral selector, a certain amount of acetonitrile (10–30%) is required to achieve baseline separation. Conversely, vancomycin-based enantioseparations do not need any organic modifier. For ristocetin A based separations, it seems that beneficial effects of organic modifiers are analyte dependent. For most of solutes tested, baseline separation is obtained without adding any organic modifiers. However, when 20% Methanol is added to 0.1 *M* NaH_2PO_4 buffer containing 2 m*M* ristocetin A, it gives rise to a

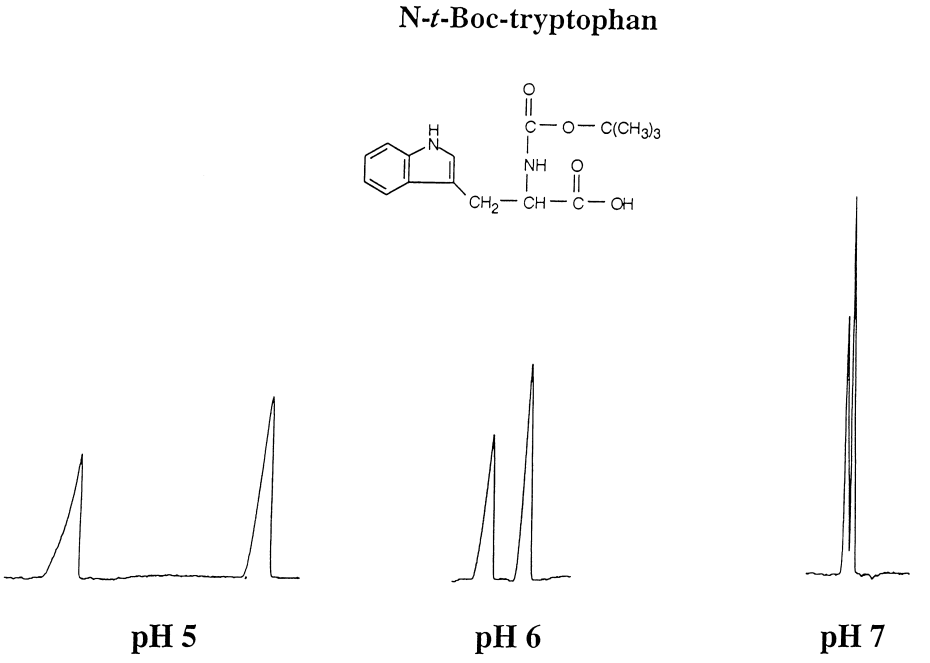


Fig. 5. Electropherograms showing the pH effect on the enantioseparation of N-t-Boctryptophan. The separation was carried out with 0.1 *M* phosphate buffer-2 m*M* vancomycin with indicated pH values. See Fig. 3 for further experimental details.

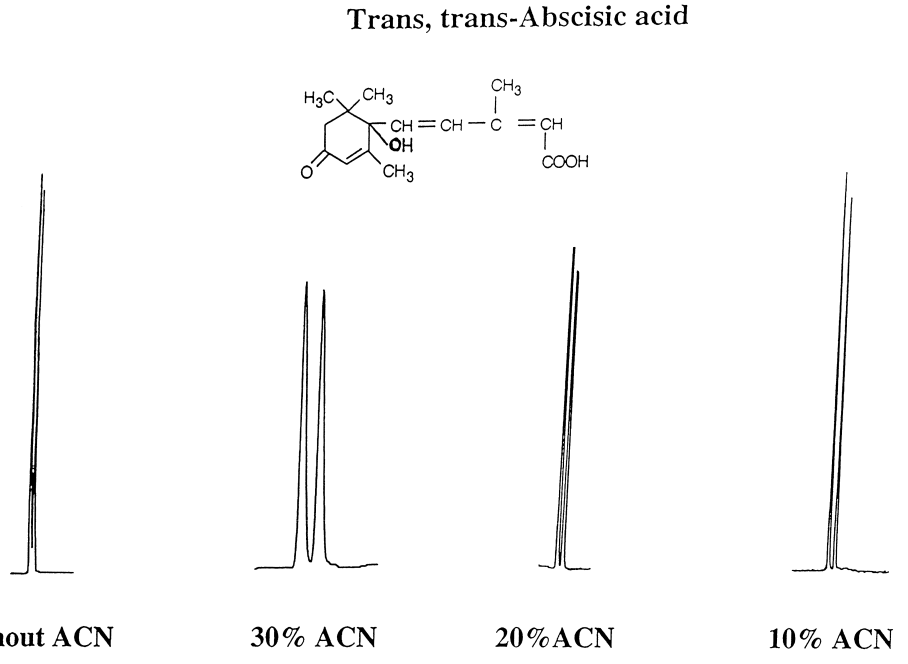


Fig. 6. Electropherograms showing the effect of organic modifier (acetonitrile) concentration on the enantioseparation of *trans, trans*-abscisic acid. The separation was carried out with 0.1 *M* phosphate buffer-5 m*M* teicoplanin at pH 6.0 with indicated volume percent of acetonitrile. See Fig. 3 for further experimental details.

baseline separation of *N-t*-Boc tryptophan with a resolution factor of 8.9 which is the greatest resolution value obtained in this study. The fact that organic modifiers produce different effects for different glycopeptide antibiotics is directly related to the molecular structure of these chiral selectors. Teicoplanin, which contains a hydrophobic ''tail'', is the most surface active of glycopeptides and self-associates in buffered solutions. Vancomycin and ristocetin A, on the other hand, do not aggregate under the same conditions. As has been reported previously, self-association can sometimes hinder or alter chiral recognition and the addition of acetonitrile can inhibit the aggregation of teicoplanin [12]. Consequently, better separations are generally obtained with addition of acetonitrile for the teicoplanin based CE system. Fig. 6 shows the effect of acetonitrile concentration on the resolution of *trans, trans*-abscisic acid. Generally, only a small amount of acetonitrile (10%) is needed to inhibit aggregation and obtain a baseline separation.

3.2.3. Principle of complementary separations

The glycopeptide antibiotics teicoplanin, vancomycin and ristocetin A belong to the same family of molecules. The similar, but not identical structures of the three antibiotics produce similar, but not identical enantioselectivities. Therefore, if only a partial enantioresolution can be obtained with one glycopeptide, there is a high probability that a baseline or better separation can be obtained with one of the others. In other words, the basic enantioselective retention mechanisms of the three antibiotics are related [12]. However, the secondary interactions that can affect chiral recognition, can be somewhat different. The principle of complementary separations is illustrated in Fig. 7. Note that this empirical approach is very useful in optimizing an enantiomeric separation.

3.3. Comparison of separations in HPLC and CE

It is difficult to make direct, rigorous comparisons of enantioseparations by HPLC and CE, even when using the same chiral selector and the same set of chiral analytes. This is because of differences in the composition of the separation solvents, selector/selectant ratios, mobility of the selector, and so on.

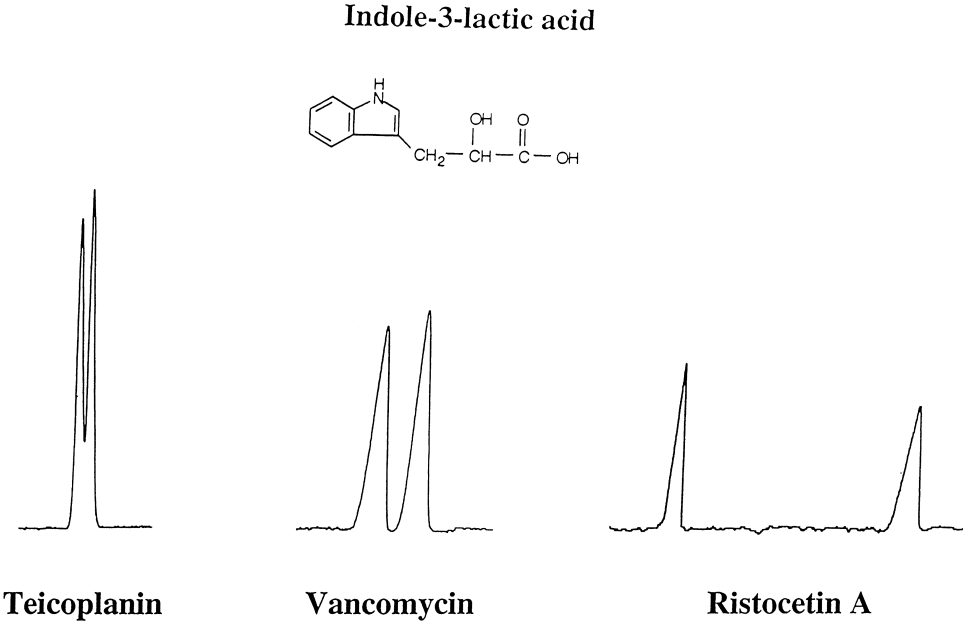


Fig. 7. Electropherograms showing the ''principle of complementary separation'' of the three macrocyclic using 0.1 *M* phosphate buffer-2 m*M* teicoplanin or vancomycin or ristocetin A at pH 6.0. See Fig. 3 for further experimental details.

However, one can easily and legitimately determine if a racemate can be separated by one approach but not by the other. CE has the advantage of greater efficiency which allows racemates to be resolved even if their selectivity factor (α) is low. HPLC can have an advantage in terms of enantioselectivity because of the variety of mobile phases that can be used (e.g., the bonded chiral selector does not have to be soluble in the mobile phase) [15].

The number of chiral compounds baseline resolved by HPLC in this study is almost twice that obtained by CE. This indicates that the covalently bonded antibiotic columns, especially Chirobiotic T, have good applicability and are easy to use. Conversely, most of the racemates studied are baseline separated on only one column (Chirobiotic T) in HPLC, whereas they are baseline resolved by at least two of the macrocyclic selectors in CE. Furthermore, the resolution factors are sometimes greater in CE than in HPLC, as a result of the greater efficiency of CE. For example, *trans, trans*-abscisic acid is baseline separated only on the Chirobiotic T column in HPLC with a resolution factor of 2.5. The same analyte is baseline separated by all three macrocyclic glycopeptides in CE, and with a resolution factor of 4.4 when teicoplanin is used as a chiral selector. Similarly, *N*-*t*-Boc-tryptophan is baseline separated only on the Chirobiotic R column in HPLC with a resolution factor of 1.5, but it is baseline resolved by all three macrocyclic antibiotics in CE with a resolution factor of 8.9 when ristocetin A is used as a chiral additive.

4. Conclusions

Chiral plant growth regulators and related indole containing compounds can be resolved by HPLC and/or CE. HPLC is easier to operate and has greater applicability than CE. Conversely, CE is more efficient and optimization of a partial separation is sometimes faster in CE than in HPLC. A small change of the molecular structure of a solute can cause tremendous effects on selectivity and resolution factors. For amino acid analytes, the amino group ($-NH_2$) appears to be of secondary importance while the carboxylate group appears to be the principal docking site that leads to enantioselective interactions between the analytes and the macrocyclic glycopeptides CSPs. The three macrocyclic antibiotics are remarkably selective and efficient chiral selectors for both HPLC and CE.

Acknowledgements

Support by the National Institute of Health, NIH RO1 GM 53825 is gratefully acknowledged.

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