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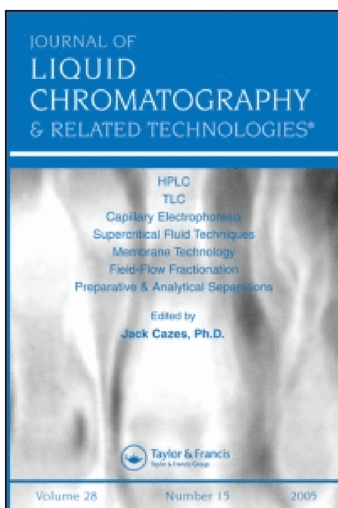

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Enantiomeric Separation of Rotenone and Rotenolone on Chiral Stationary Phases

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ENANTIOMERIC SEPARATION OF ROTENONE AND ROTENOLONE ON CHIRAL STATIONARY PHASES

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ABSTRACT

Analytical and preparative high-performance liquid chromatographic procedures have been developed for separation of optical antipodes of rotenone and rotenolone on three chiral stationary phases. Rotenone enantiomers were resolved on (+)-poly (triphenylmethylmethacrylate)-bonded silica, whereas optical resolution of rotenolone enantiomers was accomplished by using a (R)-N-3,5-dinitrobenzoylphenylglycine silica column (DNBPG). In experiments where resolution of enantiomers was achieved, each antipodal pair in both rotenone and rotenolone series was sufficiently resolved, although simultaneous resolution of all four isomers of rotenone and rotenolone was not accomplished. In all cases, the elution of (-)-(6aβ, 12aβ)-enantiomers preceded that of their antipodes. Separation factors (α) obtained with the covalent DNBPG stationary phase were slightly higher than those observed with the ionic valiant. The method has been applied to the resolution of racemates of deguelin and tephrosin.

INTRODUCTION

Rotenone (IA, Fig. 1) is a naturally occurring substance present in abundance in derris roots of Leguminosae plants. It is a useful agricultural insecticide and has been widely used as a

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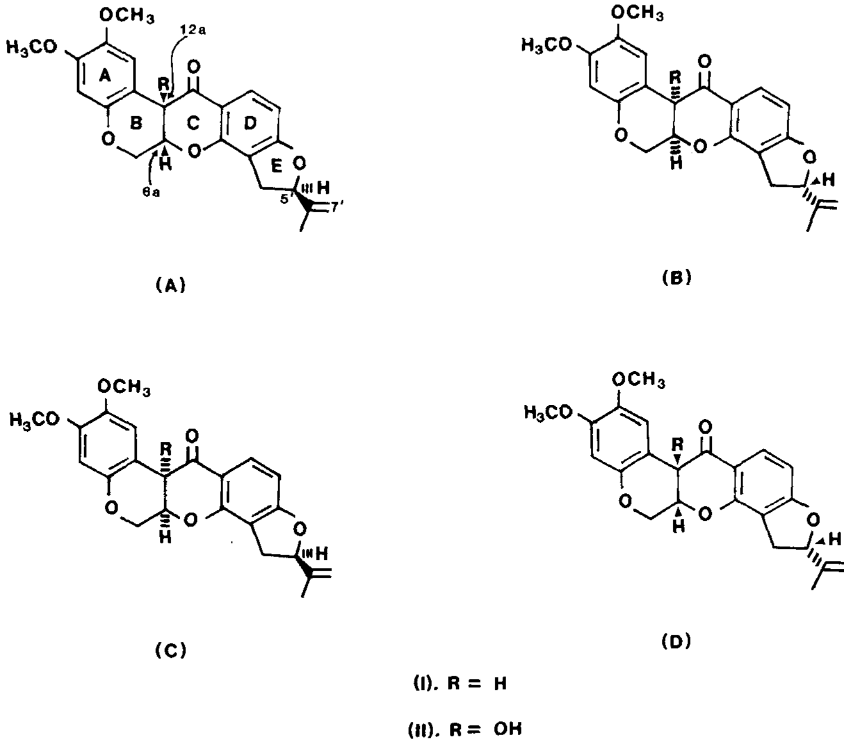

Fig. 1. Optical isomers of rotenone (I) and rotenolone (II).

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fish toxicant. When exposed to conditionds simulating the natural enviornment, rotenone degrades rapidly giving rise to many less toxic products. The compound is, therefore, nonpersistent in the environment. Among the numerous degradation products, rotenolone constitutes the major component of the oxygenated derivatives of rotenone. It is well understood that the ready oxidation of rotenone (I) to rotenolone (II) (Fig. 1) is due to the unusually high redox potential of the 12a carbon in rotenone. This

enolizable reactive site at the 12a-position is also responsible for the facil epimerization of rotenone in mildly alkaline media leading to diastereomeric products (1-3).

Chromatographic separation and quantitative analysis of mixtures of rotenone and related compounds have been previously investigated by thin layer chromatography (TLC) (4,5), gas chromatography (GC) (6,7), and high-performance liquid chromatography (HPLC) (8). Very recently we reported on the high-efficiency HPLC resolution of epimeric rotenone compounds (9). In related studies, we were interested in structure-biological activity relationships of various isomers of rotenone derivatives including the optical antipodes. Since there are three asymmetric centers in the pentacyclic rotenone structure, one would expect two pairs of optical isomers for each rotenone derivative. Although enantiomers of both natural (-)-6aS,12aS,5'R-rotenone (IA) and its 6aR,12aR,5'R-epimer are known and have been isolated by chemical transformation (10), the corresponding rotenolone enantiomers have remained unexplored. In addition, a literature survey revealed no information concerning optical resolution of enantiomers of rotenone compounds. Hence, we embarked on this study to serve two objectives: (i) to develop HPLC methods for analytical quantification and preparative isolation of individual enantiomers of the title compounds for use in structure-biological activity studies and (ii) to ascertain the application potential of HPLC methodology for the optical resolution of racemic rotenone-like compounds.

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MATERIALS AND METHODS

Materials

Pure optical isomers of rotenone were synthesized from the natural rotenone (Aldrich Chemical Co., Milwaukee, WI.) according to published methods (1,9) with some modifications of the procedures for the purification of 6a,12a-diastereomers. After separation by recrystallization and by TLC, the individual diastereomeric components were further separated and isolated by preparative HPLC. Each of these epimerically pure materials was then subjected to epimerization reaction upon treatment with boron tribromide as described in the literature (10) . The 5'- diastereomers obtained as products from the two-step conversion were likewise separated and isolated by preparative HPLC. The optical purity of each enantiomer synthesized in this manner was checked by HPLC as described later in this section. Pure optical isomers of rotenolone were prepared from the corresponding rotenone enantiomers and potassium dichromate (Alfa Products, Danvers, MA.) following a conventional oxidation procedure (2).

All HPLC solvents were obtained from J. T. Baker (Phillipsburg, NJ.). TLC solvents were obtained from Burdick & Jackson (Muskegon, MI.). Other solvents and reagents were of high purity grade and were used as received from industrial suppliers.

Silica gel (40-140 mesh) used in the purification of large amounts of rotenolone by adsorption column chromatography was of "Baker analyzed reagent" quality. Precoated analytical and preparative TLC plates were obtained from Analtech (Newark, DE.).

Methods

In all HPLC experiments, a Varian Model LC-5020 liquid chromatograph equipped with a variable wavelength UV-VIS detector (Varian Model 110), a polarimeter (Rudolph Autopol III), and a Varian Model 9176 strip chart recorder was used. The detector output was also interfaced with a Varian Model 4270 integrator for data processing. The chiral stationary phases employed in this study consisted of a Chiralpack-(+)-OT column (CPOT) (Daicel Chemical Indusries, Tokyo, Japan), and Pirkle's ionic (PA) and covalent (PCOV) (R)-N-3,5-dinitrobenzoylphenylglycine (DNBPG) columns (Regis Chemicals, Morton Grove, IL.). Dimensions of the stainless steel columns for the analytical and preparative HPLC systems were 25 cm x 4.6 mm ID and 25 cm x 10 mm ID, respectively. All packings were made of 5 um spherical silica based materials. Unless specified otherwise, the mobile phase solvent used with CPOT column was methanol and that with the DNBPG columns was hexane-isopropanol(ISP). In normal operation, the CPOT column, housed in a water jacketed stainless steel condenser, was thermostated at 5° C by means of a constant temperature circulator(FTS Model MC-4-40-2, Stoneridge, NY.).

For analysis of analytical samples, aliquots (10-25 ug/ml) in either methanol (CPOT column) or hexane-ISP (DNBPG columns) were injected into an analytical column through a Valco injector, which comprised a 10 ul loop and a Model CV-6-UHPa-N60 injection valve. The column effluents were monitored at 254 nm. Often it was necessary to set the detector at λ_{max} of a given analyte for trace

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analysis. Mobile phases were pumped (100-150 atm) at flow rates of 0.5 ml/min and 3 ml/min through respective CPOT and DNBPG columns. Capacity factors (k') were detemined by a standard method based on the following relationship of retention parameters: $k' = (t/t^0)-1$, where t^0 is the retention time of the unretanined compound and t is that of the solute under analysis. The void volumes were obtained by injecting water (Pirkle) and hexadecane (CPOT) as none-retained solutes. As rotenone compounds are sensitive to light and air especially in solution, all samples were freshly prepared prior to analyses and were stored in a foil -wrapped amber container in a freezer when not in use.

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In preparative HPLC, partially purified enantiomeric compounds obtained from preparative TLC and HPLC were further separated on preparative columns of 10 mm ID. Aliquot samples (0.5-5 mg) were injected into the column via a 100-200 ul loop and the mobile phase eluates were collected into 3 ml-fractions with a Buchler linear automatic fraction collector (ISCO Model 328). A refactive index detector (Varian Model 4300) was coupled between the fraction collector and the column. Fractions containing pure enantiomers were combined. Upon removal of solvent by evaporation at a reduced pressure, an analytical sample of the residue was analyzed for optical purity by HPLC on an analytical column with dual UV and polarimetric detection. Structure of each enantiomer isolated was determined by proton and carbon-13 nuclear magnetic resonance spectrometry (NMR). In all cases, the spectra of the test samples were superimposale with those of authentic materials. NMR spectra

were recorded on a JEOL FX90-Q Fourier transform multinuclear spectrometer operating at 90 MHz for protons and at 22.5 MHz for carbons.

RESULTS AND DISCUSSION

Structures of the two sets of enantiomeric pairs in the rotenone (I) and rotenolone (II) series are depicted in Fig. 1. The absolute configuration of the natural rotenone (IA) has been determined as shown [(-)-6aS,12aS,5'R] (11). Based on the outcome of chemical conversions via controlled sterochemical pathways, the respective absolute stereochemistry of IB, IC, ID has been established as (+)-6aR,12aR,5'S-rotenone, (+)-6aR,12aR,5'R-rotenone, and (-)-6aS,12aS,5'S-rotenone (10,12). Oxidation of the optical isomers of (I) with potassium dichromate in aqueous acetic acid yielded the corresponding compounds IIA, IIB, IIC, and IID with retention of configuration at 6a-, 12a-,and 5'-centers. For HPLC analysis, samples of either individual optical isomers or premixed enantiomeric pairs of variable enantiomeric compositions were employed. This facilitated unequivocal peak identification and enabled unambiguous correlation of elution order with absolute stereochemistry.

Table I summarizes the observed HPLC behavior of optical isomers of rotenone (I) and rotenolone (II) on three columns packed separately with the polymeric triphenylmethylmethacrylate (CPOT), Pirkl's covalently bonded amino acid (PCOV), and the ionically bonded amino acid (PA) stationary phases. The results clearly indicated that the resolution potential of the two types of

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Table I

HPLC Separation of Enantiomers of Rotenone (I) and Rotenolone on (II) Chiral Stationary phases

Optical isomer		Chromatographic characteristics*		
			DNBPG	
		CPOT	PCOV	PA
Rotenone				
IA (-)	k'	2.78	11.4	11.7
IB (+)	k'	5.98	11.4	11.7
	α	2.15	1.00	1.00
IC (+)	k'	3.42	11.4	11.7
ID (-)	k'	2.78	11.4	11.7
	α	1.23	1.00	1.00
Rotenolone				
IIA (-)	k'	1.50	12.7	15.9
IIB (+)	k'	1.56	15.6	19.2
	α	1.04	1.23	1.21
IIC (+)	k'	1.56	15.7	20.8
IID (-)	k'	1.50	12.7	17.4
	α	1.04	1.24	1.20

* In experiments with PCOV and PA columns, a mobile phase of hexane-ISP (85:15) was used. For other HPLC conditions, see Methods section.

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stationary phases, CPOT versus DNBPG (PCOV or PA), was heavily dependent on the structure type and was in sharp contrast with respect to the two series of compounds under consideration (I versus II). Thus, separation of the optical antipodes in series I was attained in various degrees with the CPOT column (α = 2.15 for the IA-IB pair, α = 1.23 for the IC-ID pair), but not with the Pirkle's DNBPG columns (α =1.00) . Conversely, the enantiomeric pairs (IIA-IIB) and (IIC -IID) were resolved with the latter column (α > > 1.00 for both pairs of IIA-IIB and IIC-IID with either PCOV or PA column under all HPLC conditions evaluated), but remained virtually inseparable with the former CPOT column. Analysis of the data (not shown here) obtained with the DNBPG columns showed that separation of the optical antipodes tended to be favored by an increase in the hexane content of the mobile phase used; but a high hexane content (> 95 %) in the mobile phase caused severe peak distortion due to low solubility of analytes in the solvent systems employed . Separation factors (α) observed in the PCOV system were only slightly higher than those found in the PA system (Table I).

Examples of HPLC chromatograms showing HPLC separation of the enantiomeric pairs of rotenone (I) and rotenolone (II) are presented in Fig. 2 and Fig. 3, respectively. It is noteworthy that the (-)-isomers in both series I and II eluted invariably earlier than their antipodes. They were less retained by all three chiral stationary phases investigated : $k'_{IA} < k'_{IB}$; $k'_{ID} < k'_{IC}$; $k'_{IIA} < k'_{IIB}$; $k'_{IID} < k'_{IIC}$ (Table I). This general trend of

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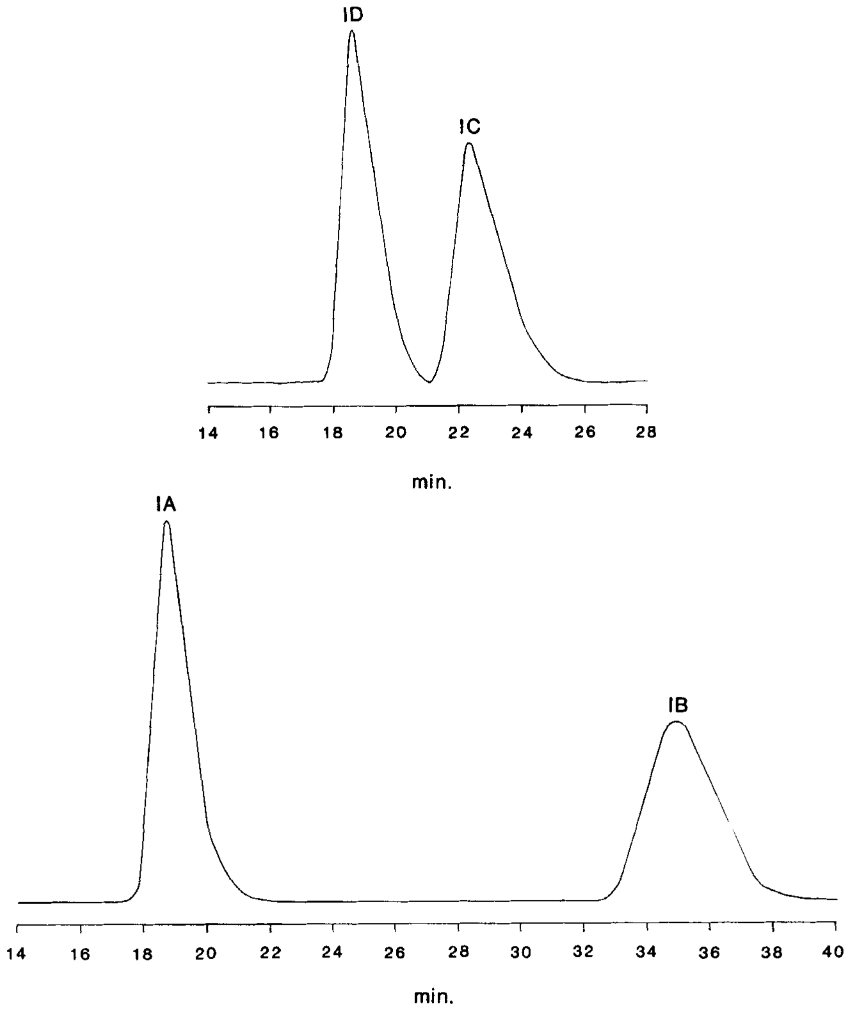

Fig. 2. Enantiomeric separation of rotenone (I) on CPOT.

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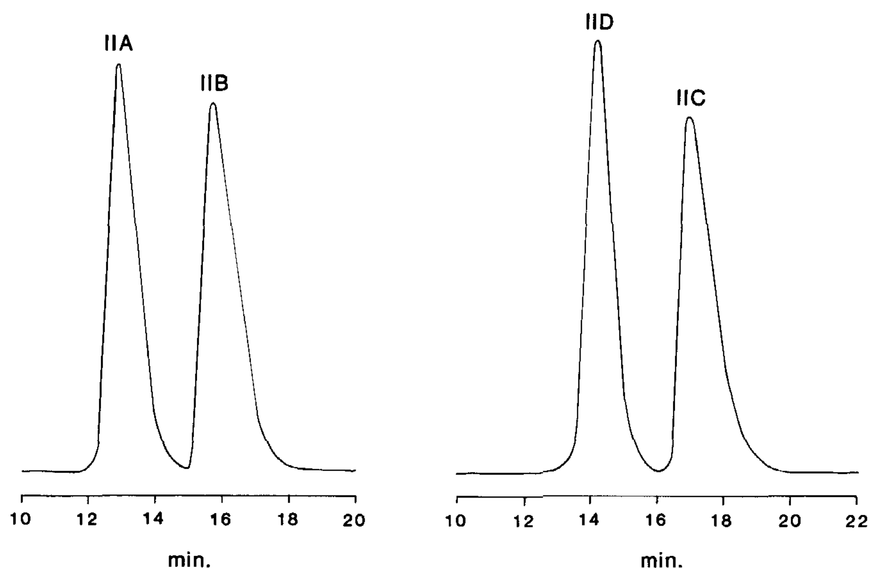

Fig. 3. Enantiomeric separation of rotenolone (II) on PA.

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elution order is analogous to that reported by Pirkle and coworkers on HPLC resolution of other class of optically active compounds on DNBPG columns (13). As demonstrated in our recent work (9), diastereomeric compounds that are epimeric at the B/C ring junction (IA-IC and IIA-IIC) can be separated with relative ease by normal-phase and reversed-phase HPLC . The results of the present study also demonstrated that the same above sets of epimers were separated on the suitable chiral phases without much difficulty (Table I) . For enantimeric separation of (I) on CPOT where a relatively high degree of chiral recognition involved in the chromatographic process was evidently operative ,the magnitude of α value for IA-IB was considerably greater than that for IC-ID (Table

I and Fig. 2). This is illustrative of a notable differentiability in molecular chirality between structures IA and IB for specific chiral interactions with the CPOT phase. While the retention data for the enantiomers in the rotenone series I obtained with the PCOV column were comparable with those found in HPLC with the PA column, the rotenolone compounds in series II were more strongly retained by the PA phase than by the PCOV phase (Table I).

A comparison of the data in Table I for the two fundamentally different types of chiral phases (CPOT versus DNBPG columns) led to a general understanding of chiral reqiurements in these systems. For effecting chiral recognition, the hydrophobic interaction (probably of π-π origin) between the triphenylmethyl group of the chiral polymeric CPOT phase (14) and the rotenone enantiomers appeared to be important. In this case, aromatic groups and unsaturated systems with conjugated bonds in a chiral structure seemed to have favorable contributions to the chiral recognition process (15). Apparently the presence of the 12a-hydroxy group in rotenolone destablized such interactions. On the other hand, there are π-donor-, hydrogen bond donor-and hydrogen bond acceptor-sites in rotenolone enantiomers for simultaneous interactions with the DNBPG chiral phases, separation of the enantiomeric pairs can therefore be realized. The absence of the hydrogen donor in the rotenone series (the 12a-OH is replaced by a hydrogen) would render chiral recognition ineffective , because the specific requirements of essential multiple interaction sites are not fulfilled. This may partly explain why attempted separations of rotenone enantiomers on the DNBPG columns failed.

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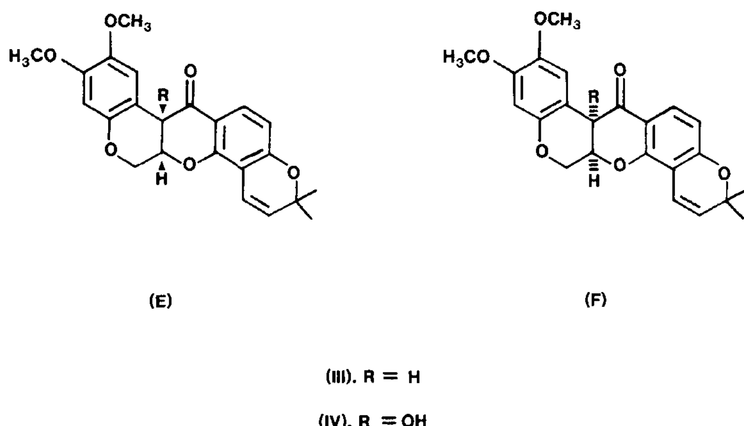

Fig. 4. Optical antipodes of deguelin (III) and tephrosin (IV).

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Application of the chiral phase HPLC method developed in this study to the separation of racemic deguelin (III) and tephrosin (IV) yielded similar results. As shown in Fig. 4, the compounds III and IV are the structural analogues of respective rotenone and rotenolone, each having chiral centers at the B/C ring junction with some structural modification of the E-ring. With DNBPG columns, a base-line resolution of the IVE-IVF pair was achieved, though the IIIE-IIIF racemate could not be separated with these columns (Table II). Nevertheless, both racemic mixtures (IIIE -IIIF and IVE-IVF) were well resolved on the CPOT column (Fig. 5 and Table II) . We presume that the π-π interactions of the E-ring system in III and IV with the tiphenylmethyl group of CPOT play more important role than that in I and II for chiral recognition during the chroamtographic separation process. Destablization of these specific interactions by the polar 12a-OH may not be

Table II

HPLC Separation of Racemates of Deguelin (III) and Tephrosin (IV) on Chiral Stationary phases

Optical isomer		Chromatographic characteristics*		
		CPOT	PCOV	PA
Deguelin				
IIIE (-)	k'	2.64	9.72	9.36
IIIF (+)	k'	5.02	9.72	9.36
	α	1.90	1.00	1.00
Tephrosin				
IVE (-)	k'	1.44	10.9	15.1
IVF (+)	k'	2.34	13.3	17.8
	α	1.63	1.22	1.18

* For HPLC conditions, see footnote to Table I.

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significant in this case. Table III shows the results of optical purity analysis on two sets (III and IV) of samples each containing five mixtures of premixed enantiomers. Coefficient of variation for three replicate analysis averaged 2.25-5.61%.

The minimum efficiencies estimated commercially for CPOT and Pirkle's columns were 2800 and 40000 plates per meter, respectively. The efficiency of separations shown in the chromatograms can be improved by lowering the mobile phase flow

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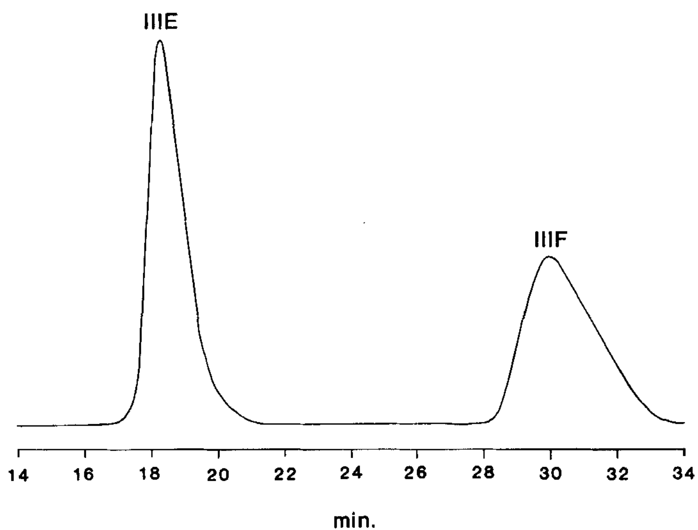

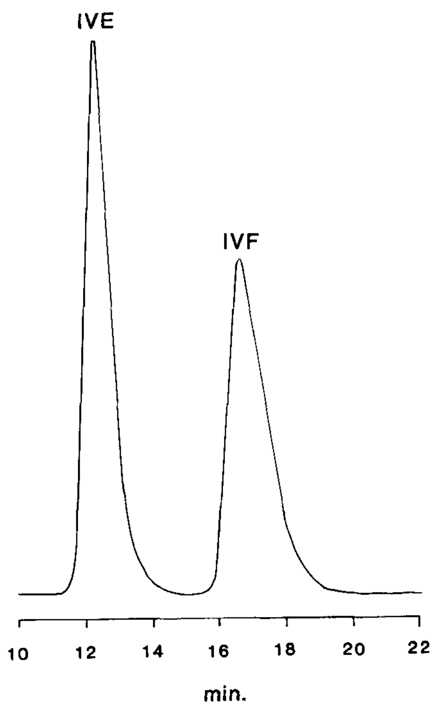

Fig. 5. Enantiomeric separation of deguelin (III) and tephrosin (IV) on CPOT.

Table III

Determination of The Optical Purity of Deguelin and Tephrosin

Sample No.	Enantiomeric composition (%)			
	E		F	
	(a)*	(b)*	(a)	(b)
Deguelin (III)				
1	50.3	50.4	49.7	49.6
2	3.78	3.69	96.2	96.3
3	20.6	20.8	79.4	79.2
4	9.51	9.49	90.5	90.5
5	83.7	83.5	16.3	16.5
Tephrosin (IV)				
6	50.1	49.9	49.9	50.1
7	43.7	43.9	56.3	56.1
8	8.55	8.61	91.4	91.4
9	72.7	72.9	27.3	27.1
10	93.0	93.2	7.00	6.80

* (a)= HPLC analysis with the CPOT column. (b)= HPLC analysis with the PCOV column for IVE-IVF antipodes; the IIIE-IIIF antipodes were converted to IV-compounds prior to analysis on PCOV.

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rate to render mass transfer more efficient. The inevitable losses in mass transfer characteristics at elevated eluent flow rates resulted in relatively broader bandwidths as seen in the present cases. However, the column selectivity data (α values) should shed some light on the chromatographic behavior of enantiomers of rotenone and rotenolone on the chiral phases of interest.

In all the experiments where optical resolution of enantiomers was achieved, each of the antipodal pairs IA-IB, IC-ID, IIA-IIB, and IIC-IID in both rotenone and rotenolone series was sufficiently resolved, although simultaneous resolution of all four isomers of rotenone and rotenolone was not accomplished. It was of interest to note that, except for the diastereomeric pair IB-IC, the isomers having the same B/C ring junction stereochemistry were not resolvable regardless of the chiral phase used. The lack of differentiability may be associated with the low molecular dissymmetry inherent in the diastereomers that bear an epimeric relationship at the 5'-asymmetric center.

In conclusion, the method presented can be utilized in a wide variety of chiral separation involving rotenone-like racemates for preparative isolation of optical isomers and optical purity determination. This method represents the first direct approach to optical resolution of naturally ocurring racemates of rotenone family.

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