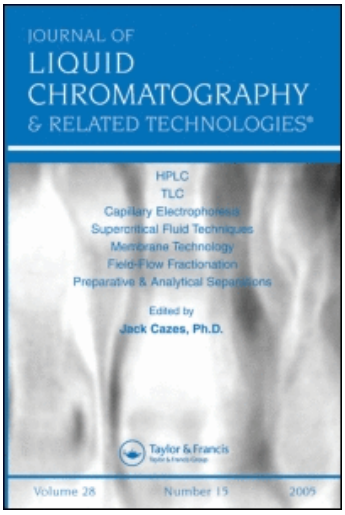

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SEPARATIONS OF THREO-ERYTHRO AMINOALCOHOLS BY PREPARATIVE HPLC

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ABSTRACT

A liquid chromatographic method which enables the separation of the threo/erythro diastereoisomers obtained from the reduction of Wellbutrin™ brand bupropion using a ternary eluent system is described. This has been achieved on a preparative scale.

INTRODUCTION

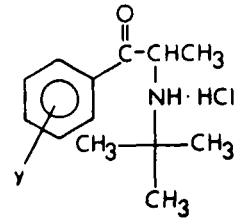
The rapid and facile separation of a mixture of isomers has always been the hope of the bench chemist. The separation of a mixture of threo and erythro isomers, particularly aminoalcohols, has not been easy (1). The major obstacle has been the great similarity in physico-chemical properties such as polarity, melting points, and solubility. Thin-layer chromatography (TLC) using silica gel does not give complete separation. A study was therefore initiated to explore the possibility of using preparative HPLC to bring about a quantitative separation. This has now been

achieved. Our primary interest, as reported herein, was to obtain the pure threo isomer as usually it is not easily accessible by chemical separations.

The aminoketone, 3-chlorophenyl-2-tert-butylaminopropiophenone, 1, (Wellbutrin™ brand bupropion, y=Cl) on reduction gave a mixture of diasteriomeric threo/erythro aminoalcohols, 2, in varying proportions depending upon the reducing agent and conditions employed. Reduction with Adam's catalyst, known to give pure erythro isomer, could not be employed because of reductive dehalogenation of the aromatic ring (1a). Of the several reducing agents attempted, it was found that sodium borohydride in aqueous ethanol at room temperature gave predominately the erythro (ca 80%) isomer. Reduction with diborane in THF resulted in a threo/erythro isomer ratio of ca 80:20 respectively.

Initially, efforts at establishing a TLC system which could effectively separate the two isomeric aminoalcohols using acetonitrile/toluene or chloroform/ethyl acetate were not successful. Complete separation was not achieved due to tailing of the spots. The use of ammonia vapors in the TLC chamber was employed (2). This resulted in a definite separation on silica plates giving a ΔR_f value (3) of 0.09. The use of ammonia to deactivate (4) the silica of the prep 500 cartridge was inadvisable; however, the use of 0.1% diethylamine in the mobile phases, mentioned above, gave desirable conditions for the effective separation of the two isomers. Triethylamine appeared to be equally useful.

In the course of these studies, it was observed that when large amounts (50 g) of the isomeric mixture were to be separated, the first load of 10 g on the fresh cartridge eluted slowly (long retention times) and showed a long tailing effect for the second



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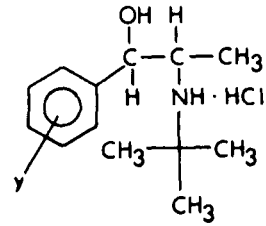


2

component, the erythro isomer (see Fig. 1). However, if the new silica cartridge was initially equilibrated with a different eluent solution having a higher concentration (1.0%) of the amine, deactivation was sufficient to make the subsequent separation of the two isomers very facile and the tailing effect, observed earlier, minimized.

For reasons obvious from Figure 1, recycling was not practical as the first component, the threo isomer, would be out of phase in

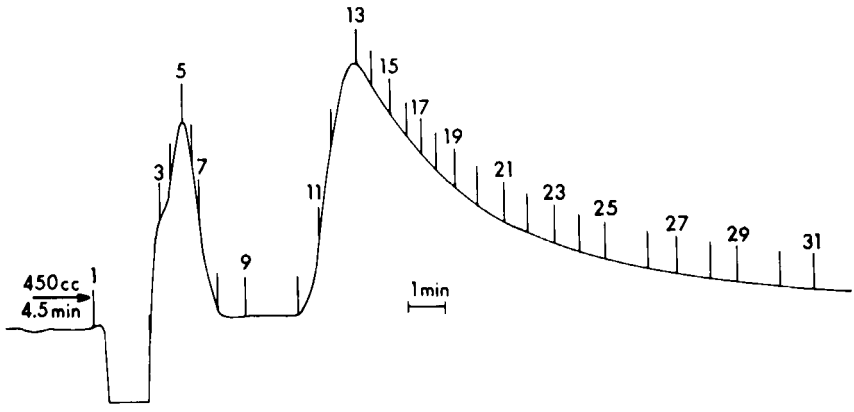


Figure 1. Preparative HPLC chromatogram of threo/erythro (79:21) Separation.

the eluting process and would precede the tail of the erythro isomer. However, the intermediate fractions between the pure threo and pure erythro fractions could be concentrated and rechromatographed. Using this approach, it was possible to obtain in a single run 47.2% of pure threo, 13.9% of a middle fraction having a threo/erythro ratio of 64:36, respectively and 2.8% of a final fraction which was 85% erythro/15% threo. This accounted for a total recovery (5) of 63.9%.

Subsequently, several other examples of the threo/erythro aminoalcohols of this class have been separated using essentially the same techniques. It should be mentioned that for the large scale separations, regular reagent grade solvents were satisfactory. Also, the availability of a continuous feeding type syringe (6) could allow the loading of 30 to 40 g of the mixture without disengaging the needle from the injector plate.

EXPERIMENTAL

Apparatus

a) Chromatographic equipment: (i) preparative LC - The apparatus consisted of a Waters Associates Liquid Chromatography Preparative 500 system equipped with a differential refractive index detector. (ii) Analytical LC-Water Associates Model 244 equipped with a Model 6000A pump, U6K universal injector and a R401 differential refractometer. (iii) RCSS-LC-Waters Radial Compression Separation System (RCSS) equipped with a Model 6000A pump, U6K injector and a R401 differential refractometer.

b) General equipment: The NMR spectra were recorded on a Perkin-Elmer R24A or a Varian XL 100 spectrometer. Results are reported on the δ scale in parts per million (ppm) downfield from TMS internal standard.

The gas chromatographic analyses were performed on a Varian 1800 chromatograph with a flame-ionization detector.

Reagents

The following solvents were used without further purification (7): Fisher HPLC grade acetonitrile and ethyl acetate, Mallinckrodt analytical reagent grade toluene and chloroform, diethylamine purchased from Aldrich Chemical Co.

The aminoalcohols were synthesized by procedures published earlier (8a-c). The isomeric mixtures of 3-10 g of threo (T)/ erythro (E) isomers were dissolved in 10 milliliters of the mobile phase (S1).

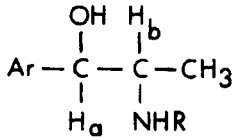
Procedures

The solvent reservoirs, injection port and the columns were maintained at ambient temperature. The mobile phase (S1) consisted of acetonitrile, toluene and diethylamine 20:80:0.1 (V:V:V). For the preparative LC separations, the "Prep-Pack"® cartridge was equilibrated using two different procedures. The first procedure (A) consisted of flushing the new cartridge with one liter (two column volumes) of the mobile phase (S1) which was discarded. The mobile phase (S1) was then recirculated for one-half hour prior to the separation. In the second procedure (B), the new cartridge was pretreated with one liter of a different solvent system (S2) consisting of acetonitrile, toluene and diethylamine 20:80:1.0 (V:V:V), that is, enhanced ten fold in diethylamine concentration. This latter eluent (S2) was discarded after which the original mobile phase (S1) was recirculated as before for one-half hour prior to the separation. The flow rate for the preparative separations was 100 mL per minute and the relative response of the refractive index detector was ten. Normally, the fractions collected were between 40 and 100 mL of eluent.

For the analytical and RCSS separations the columns were equilibrated to the mobile phase (S1) for approximately one-half hour prior to the separation.

Results and Discussion

The degree of purity of the threo and erythro isomers obtained by preparative high performance liquid chromatography was verified by other methods. The identification and purity of the two isomers



was accomplished by NMR spectroscopy (9). The NMR spectrum of the threo isomer had a doublet centered at 3.9 ppm (J_{HaHb} = 8.2) as shown in Figure 2. The erythro isomer had a doublet at 4.6 ppm (J_{HaHb} = 3.5). There was also a definite difference in the chemical shifts for the doublets attributed to

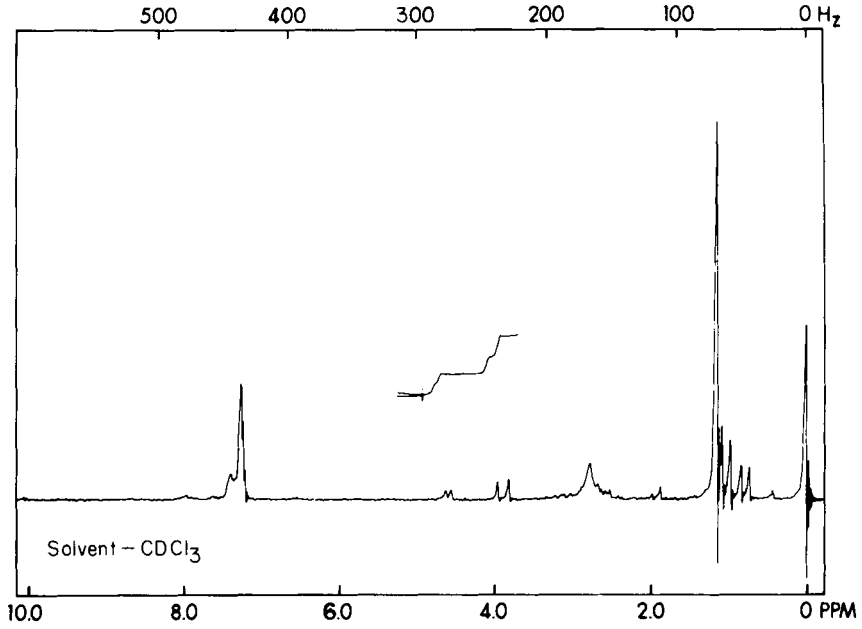


Figure 2. NMR spectrum of threo/erythro mixture (combined fractions 31-33).

the methyl protons. The threo-CH_3 doublet was found at 1.09 ppm and the erythro-CH_3 doublet at 0.8 ppm.

The threo and erythro isomers were also characterized by gas chromatography as shown in Figure 3. Using an OV-11 on Supelcoport column, the retention times for the threo and erythro isomers were 7.5 min. and 7.25 min. respectively for the conditions shown in Table 1.

The thin-layer chromatography of the threo/erythro mixture on silica gel using acetonitrile/toluene (20:80) in an ammonia atmosphere gave an R_f(T) of 0.29 and an R_f(E) of 0.20. (See Figure 4) (10). From the TLC data, the ΔR_f value (11) for this mixture was 0.09. This indicated that a load in the range of three to five

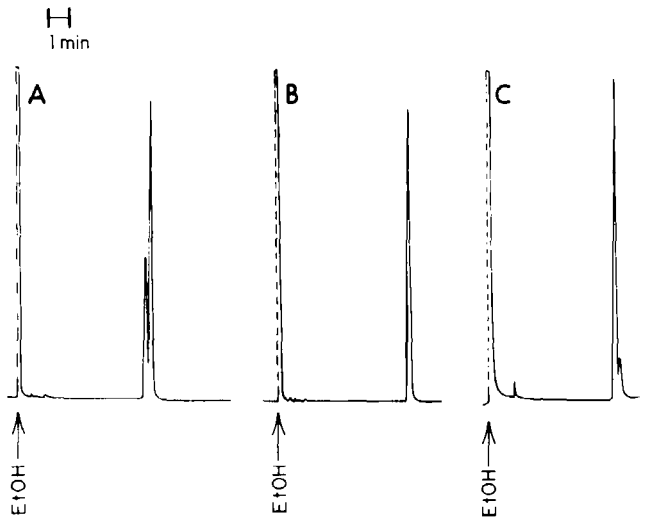


Figure 3. GC Analysis of threo/erythro mixture (A) Combined fractions 31-33; (B) combined fractions 11-30; (C) fraction 34 (See Table I for GC conditions).

Table 1

Conditions for Gas Chromatography

instrument:	Varian 1800 GC with FID
column:	10% OV-11 on 100/120 supelcoport (6 ft. x 2 mm glass)
column temp:	180-260°C at 8°/min
injector temp:	240°C
detector temp:	275°C
carrier gas:	Helium at 40 cc/min
chart speed:	0.5 inch/min
solvent:	ethanol

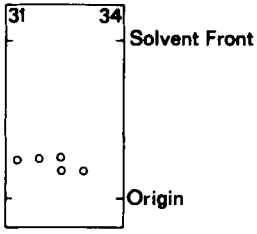


Figure 4. TLC of threo/erythro mixture on silica gel using acetonitrile/toluene (20:80) in an ammonia atmosphere (Fractions 31-34)

grams could be statisfactorily separated by a single pass using one Prep-Pak® cartridge (3).

The mixture prior to separation, as determined by NMR, consisted of the threo and erythro isomers in a ratio of 79:21 respectively. Three and six-tenths grams of the mixture was dissolved in 10 mL of the previously described eluent to load the cartridge. Figure 1 shows the preparative chromatogram for this separation. The individual fractions were scanned by TLC. Similar fractions were combined and concentrated. These were analyzed by NMR and gas

chromatography. Fractions 1-10 consisting of approximately 1100 mL of eluent were discarded since thin-layer chromatography showed no fluorescent material (12). The chromatogram asymptotically returns to the baseline, therefore fractions 31 to 34 are not shown. Fraction 32 contained 500 mL of eluent while fractions 31, 33 and 34 were approximately one liter each. Fractions 11 to 30 gave 1.7 grams of pure threo isomer as shown by NMR (see Figure 5). Fractions 31 to 33 gave 0.5 grams of a mixture consisting of 36% erythro and 64% threo (Figure 2). The last fraction gave 0.1 grams of a mixture which was 85% erythro and 15% threo (Figure 6).

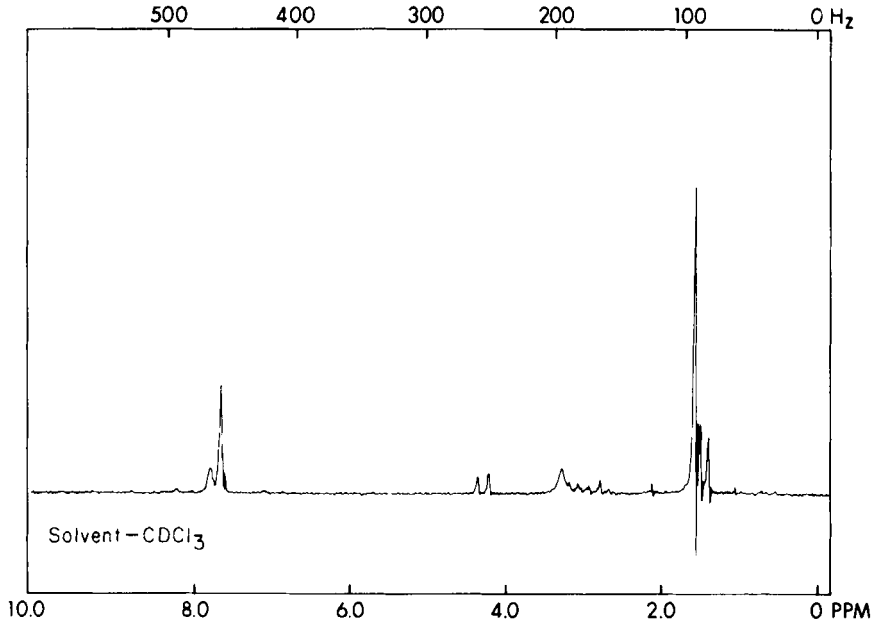


Figure 5. NMR of combined fractions 11-30 (pure threo isomer).

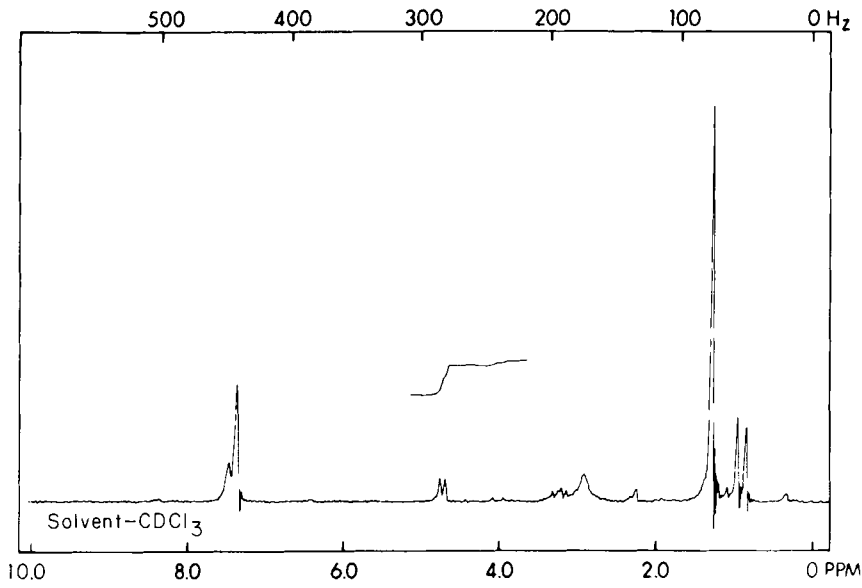


Figure 6. NMR of fraction 34 - erythro/threo mixture - 85%/15% respectively

These studies were essentially directed at obtaining the pure threo isomer. Since the initial mixture was enriched in the threo isomer, the erythro isomer was not obtained in pure form in this specific separation. As shown in Figure 1, the erythro isomer eluted in the tail of the threo isomer (Fractions 31 to 34).

Figures 7 and 8 show the analytical HPLC and the Radial Compression Separation System (RCSS) chromatograms respectively. Table 2 compares the chromatographic data of the two analytical HPLC's with the data obtained from the preparative LC. It should be noted that overloading the column as in the preparative mode

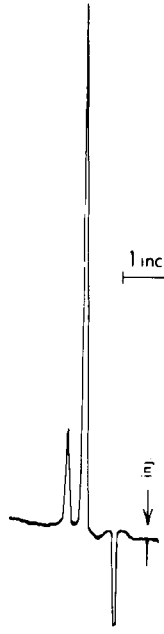
Column: Waters μ-porasil

Mobile phase: Acetonitrile/Toluene/ diethylamine - 20:80: 0.1 (V:V:V).

Chart speed: 0.2 inch/min.

Flow Rate: 2 cc/min

Figure 7 - Analytical HPLC chromatogram



Column: Waters Radial-PAK SI®
Mobile phase: Acetonitrile/Toluene/ diethylamine - 20:80: 0.1 (V:V:V)

Chart speed: 0.4 inch/min.

Flow Rate: 3 cc/min

Figure 8 - RCSS chromatogram

Table 2

Comparison of Analytical and Preparative LC Data

	Analytical HPLC	RCSS	Prep LC
k'(T)	0.6	1.0	1.7
k'(E)	1.2	1.5	4.8

reduces the k' ($k' = (V_1-V_o)/V_o$, where V_1 = the retention volume of a particular compound and V_o = one column volume) of the threo isomer relative to the erythro isomer.

Several other mixtures which differed from 85% threo/15% erythro to 10% threo/90% erythro have been separated. These studies in varying percentage composition are shown in Table 3 and a discussion of their results follows.

When the threo isomer predominated, as in examples 1 and 2, it was obtained in excellent yield by a single pass through one "Prep-Pak"® cartridge using the standard eluent (S1) acetonitrile, toluene and diethylamine 20:80:0.1 (V:V:V). The remaining fractions were enriched in the erythro isomer. These enriched fractions from examples 1 and 2 could presumably be rechromatographed to obtain the pure erythro isomer as illustrated in example 4.

It has been our observation that when the erythro isomer predominates in the original mixture, it can be obtained in a high degree (>99%) of purity as illustrated by example 4. However, the yield is rather poor. Several factors could be attributed to this low yield. First, since the erythro (i.e., the second elution component) isomer was predominant, the cartridge was overloaded

Table 3

Examples of Mixtures of Varying Compositions

Example	Amount of Mixture, g	% Composition[a]	Equilibration Method[b]	Results[a,c]
1	12.5 g[d]	85%T/15%E	A	8.9 g T 1.16 g 46%T/54%E 1.12 g 16%T/84%E
2	9.9 g	85%T/15%E	B	6.8 g T 1.9 g 61%T/39%E 0.8 g 5%T/95%E
3	5.9 g	13%T/87%E	A	3.6 g E(>97%) 2.3 g 29%T/71%E
4	8.2 g	10%T/90%E	B	2.6 g E(>99%) 4.5 g 20%T/80%E
5	3.4 g	59%T/41%E	A	1.5 g T 0.5 g 43%T/57%E 0.6 g 9%T/91%E
6	2.0 g[e]	43%T/57%E	A	0.5 g T 0.4 g 37%T/63%E 0.7 g 6%T/94%E

[a] % composition determined by NMR.
[b] See procedure section for description of method A and B.
[c] Single pass through one "Prep-Pack"® cartridge. Results are given for various combined fractions.
[d] Two separations of 6.25 g each (second separation was performed on same column as the first).
[e] This separation was done using the mobile phase (S3) ethyl acetate, chloroform and diethylamine 20:80:0.1 (V:V:V).

with respect to this isomer which caused it to elute faster than normal. At the same time, the cartridge was not overloaded with respect to the threo (the primary elution component) isomer which continued to come off the cartridge at its normal elution rate. This explains why the erythro isomer "had caught up with" the threo

isomer giving a poor yield of the second component - the highly pure erythro isomer.

Also, deactivation of the column through pretreatment with an amine, makes both isomers elute faster which could enhance this effect. The tailing of the threo (i.e. the first component) isomer was also a factor which reduced the amount of the pure erythro product obtained.

Example 3 demonstrated that the use of a non-pretreated cartridge gave a higher yield of the erythro isomer but with a lower degree of purity (>97%). This again reflects the tailing of the threo component which is more pronounced when a non-pretreated cartridge is used. Rechromatography of the combined first fraction in example 3 could give the pure erythro isomer.

The fifth example in Table 3 illustrates that when the percent composition was nearly equal, a fair separation can be obtained. The threo isomer was obtained pure, while the erythro isomer was obtained having about 91% purity. Again, rechromatography of the remaining fractions could give the pure erythro isomer. It is important to note that the reversal of the composition of the threo/erythro mixture does not alter the order of elution of the two isomers. The threo isomer is always followed by the erythro component.

Other eluents can also be employed advantageously for this separation. For example, ethyl acetate, chloroform and diethylamine 20:80:0.1 (V:V:V) (S3) gave similar results as shown in

example 6. This solvent system gave higher R_f values of 0.52 and 0.39 for the threo and erythro isomers respectively in the TLC (10) as compared with the earlier eluent system (S1). Although the higher R_f values would imply that the isomers would not be retained as long on the cartridge, the actual observation was that the threo isomer was retained longer relative to the erythro isomer. However, the order of elution remained unchanged. This resulted in a lower yield of the pure threo component. Consequently, almost one and one-half as much of the nearly pure (94%) erythro isomer was realized than was obtained by the eluent system (S1). Ethyl acetate/dichloromethane 20:80 (V:V) (10) gave similar results by TLC (R_f (T) = 0.59 and R_f (E) = 0.46). While this eluent system (S4) was not employed for the preparative LC separations, it would be expected to give results similar to those obtained using eluent system (S3).

Pretreatment of the fresh cartridge with a different solvent system (S2) containing 1% diethylamine as described earlier in the procedure section, resulted in a duration of separation of one-half to one-fourth the time required for a non-pretreated cartridge. Also, the solvent consumption, as would be expected, was reduced proportionately.

Using the same set of conditions as described above for the 3.6 g sample, separations of large amounts (ca 50 g) of the isomeric aminoalcohols of this class using five to ten gram portions per injection, have been successful. For a given set of conditions, the separation was repeatable.

These studies in separations of the two diastereoisomers seem to indicate that it would be more advantageous to use two different sets of solvent systems if the recovery of both of the isomers, in high yield, is desired. The fractions enriched in the erythro isomer after chromatography with eluent (S1) could be rechromatographed using the latter two solvent systems (S3 or S4).

Conclusion

Preparative HPLC has been found to be a satisfactory method for the separation of threo/erythro mixtures resulting in high yields of the individual isomers having excellent purity.

Acknowledgment

The authors are indebted to Suzette R. Medlin for her excellent technical assistance. Also thanks are due to Dr. B. Stuart Hurlbert for analytical and spectral data and its interpretation and to Mr. James Cichetti for the gas chromatography. Appreciation is also expressed to Mr. Don Harris and Dr. Warren Beverung of Waters Associates for their excellent technical suggestions.

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2. Uchytil, B. Thin-layer and High-Speed Liquid Chromatography of the Derivatives of 1,4-Phenylenediamine, Journal of Chromatography, 93, 447-455 (1974).

3. Waters Associates "TLC to Prep" manual copyright 1979 (Part No. 82185) page 8.

4. Deactivation of the "Prep-Pak"® cartridge was accomplished using diethyl or triethylamine. Aqueous ammonium hydroxide dissolves the silica gel and shortens the column life.

5. No systematic study to optimize the recovery has been attempted. Several parameters such as activity of the silica gel, concentration of the diethylamine, etc. could effect the total recovery.

6. A three-way valve which can be placed between the syringe and the needle is available from Popper and Sons, Newhyde Park, New York, N.Y. (Part No. 6017).

7. For the analytical LC, the solvents were filtered through a 0.45 μM millipore filter and degassed by a steady stream of helium.

8 a) See Reference 1a.
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9.a) Tucker, H. Stereospecific Synthesis of threo- and erythro-1-(aryloxy)-3-(alkylamino) butan-2-ols, J. Org. Chem., 44 (16), 2943 (1979).
b) The threo-erythro compounds shown in Table V Reference 1a were studied in cooperation with Dr. S. Hurlbert using 100 MHz NMR to determine the NMR shifts.

10. The TLC plates used were purchased from MC/B Manufacturing and were silica gel 60 F_{254} on aluminum support. Layer thickness was 0.2 mm. The ammonia atmosphere was accomplished by placing a beaker of concentrated aqueous ammonia in the TLC chamber and lining the walls of the chamber with filter paper.

11. The ΔR_f value can be corelated to the load which can be separated by one Prep-Pak® cartridge on a single pass through the column.

12. The negative deflection could possibly be due to some soluent carried over from the preparation of the aminoalcohols or from displacement of the amine modifier, diethylamine, by the aminoalcohols.