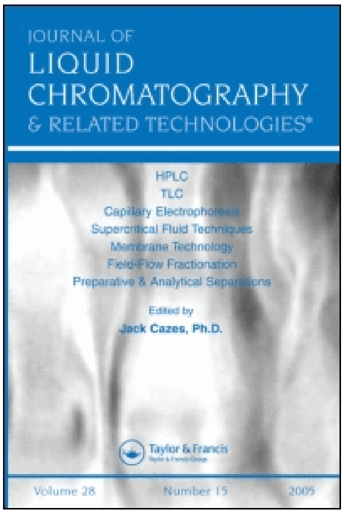

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A COMPARISON OF RADIALLY-COMPRESSED AND STAINLESS STEEL COLUMNS FOR THE REVERSED-PHASE ION-PAIR SEPARATION OF PHENCYCLIDINE SYNTHETIC MIXTURES

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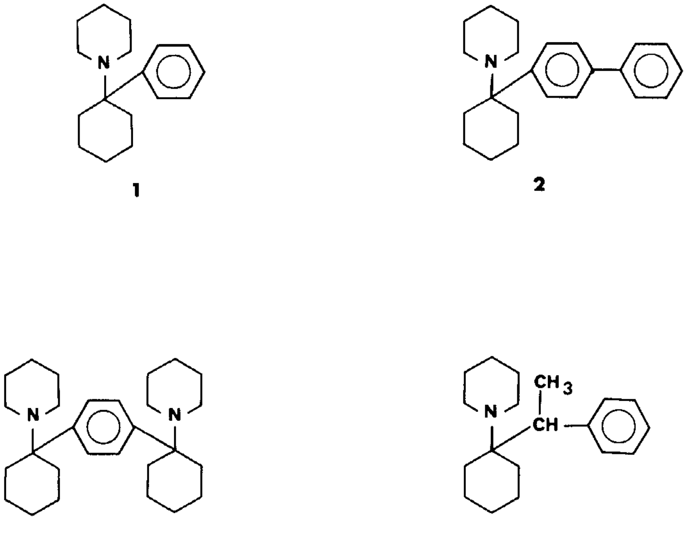
ABSTRACT

The reversed-phase ion-pair HPLC separation of phencyclidine synthetic mixtures was optimized utilizing Radial-Pak radially compressed columns. Variables examined in the optimization included column type (C-18, C-8, or CN), pairing ion (methane-, pentane-, hexane-, or octane sulfonates) and mobile phase composition (varying concentrations of methanol or acetonitrile in water). The chromatographic behavior of the phencyclidine mixtures in the various systems utilizing radially compressed columns is compared and contrasted to a similar previous study which examined similar variables on stainless steel columns. The optimum system for radially compressed columns was found to consist of a Radial-Pak C-18 column and a mobile phase of 85:15 MeOH:H_2O, 2.5% acetic acid, 1% triethylamine and 5mM sodium hexane sulfonate.

INTRODUCTION

In a previous study (1), an optimized HPLC separation for crude synthetic mixtures of phencyclidine (1-(1-phenylcyclohexyl) piperidine, PCP, 1) was reported. In that study, the separation of 1 and cosynthetics 1-[1-(1,1'-biphenyl-4-yl)cyclohexyl]piperidine (2), 1-[1-phenylethyl)cyclohexyl]piperidine (3), and 1,1'-(1,4-phenylenedicyclohexylidine)bis[piperidine] (4) (see Figure 1



1 2 3 4

FIGURE 1

Structures of PCP (1) and some identified co-synthetics.

for structures) was optimized utilizing stainless steel analytical (SSA) columns and a reversed-phase paired ion method. The success of this study prompted us to investigate the efficacy of radially-compressed columns for a similar analysis, and we wish to now report the results of this study and compare them with the previously reported results for SSA columns (1).

In the original study (1), compounds 1 and 2 were used as the probe compounds for the determination of the selectivity factor, α, since they constituted the major components of the mixture (2). A total of 11 peaks were observed using the optimized conditions (C-18 column, 70:30 $MeOH:H_2O$, 5mM $C_6H_{13}SO_3Na$, pH 3.5 with CH_3COOH and a flow rate of 2.5 ml/min); the later eluting peaks (larger k's) being small and not well-resolved. In the studies reported herein, it was noted that these strongly retained compounds were not observed except in organic-rich mobile phase mixtures, suggesting a high degree of hydrophobic character for these later eluting compounds. Since these compounds were of minor consequence, our study was limited to the compounds eluting before that peak attributed to compound 2.

During the determination of the k'- and α-values of 1 and 2 as a function of solvent strength, it was observed that as the concentration of the organic component of the mobile phase decreased, the peak previously attributed to 2 began to separate into two peaks, the smaller being the more retained (*vide infra*). Some preliminary evidence that this peak did indeed consist of two compounds was previously obtained by collecting the peak semi-preparatively from a 5/8" C-18 column and, following workup, comparing the mass and NMR spectra. Slight differences in these spectra were observed compared to the synthetic 2, and it is suspected that the second peak represents an isomer of 2. Important, however, is the demonstrated increased resolution of the Radial-Pak columns over that of the SSA columns.

EXPERIMENTAL SECTION

Apparatus

A Waters Associates (Milford, MA) ALC 244 Liquid Chromatograph was used for all analyses. Mobile phase delivery was accomplished by twin M6000A pumps interfaced through the Waters model 660 solvent programmer. Detection was by UV at 254 nm at a sensitivity of 0.02 AUFS. Chromatograms were obtained using a Houston Instruments Omniscribe strip chart recorder (Houston, TX). Columns were radially compressed in the Waters Radial Compression Module. Columns examined included the 10cm x 8mm id Radial-Pak C-18, C-8, and CN (all Waters).

Reagents and Samples

All solvents were prepared exactly as described previously (1) except that 1% triethylamine (TEA) was added. Crude PCP was synthesized according to the method of Kalir (3).

Analysis and Calculations

The standard solution used for the determination of k' and the selectivity ratio, α, was 0.30 μg/μL of crude PCP in MeOH. Each injection was 10.0 μL at a mobile phase flow rate of 2.5 mL/min. Column void times (to) were obtained by injection of pure MeOH or H_2O. The k' values for PCP and **2**, and $\alpha_{\mathbf{2}/\mathbf{1}}$ (the selectivity between **1** and **2**), were calculated in the usual manner (4).

RESULTS AND DISCUSSION

Table 1 contains the values of least-square slopes and intercepts for log $k'_{\mathbf{1}}$ vs. % organic in the mobile phase for each of the pairing agents examined on the Radial-Pak C-18, C-8 and CN columns. With MeOH as the organic modifier, both the absolute value of the slope (|slope|) and the y-intercept decrease in the order C-18 > C-8 > CN column for a given pairing agent. The value of |slope| is an indication of the sensitivity of log k' to changes in %MeOH, i.e. the steeper the slope (larger |slope|) the more effect a small change in %MeOH will have on the retention of the solute (1). The y-intercept, corresponding to log k' at 0% MeOH, is an indicator of the ability of a particular column (or pairing agent) to retain the analyte (i.e. large values for y-intercept mean greater retention). Since both y-intercept and |slope| decrease as column polarity is increased, it is obvious that the more non-polar column (i.e., C-18) permits more control of analyte retention for a given pairing agent with MeOH containing mobile phases.

TABLE 1

Least Squares Equations[a] for log k'_1 vs. % MeOH and vs. % MeCN in Mobile Phase.[b]

Pairing Ion	MeOH/H_2O			MeCN/H_2O		
	C-18 Slope/Intercept	C-8 Slope/Intercept	CN Slope/Intercept	C-18 Slope/Intercept	C-8 Slope/Intercept	CN Slope/Intercept
C_1SO_3Na	-0.0293/2.59	-0.0277/2.17	-0.0210/1.34	-0.0119/1.10	-0.0148/1.06	-0.0132/0.769
C_5SO_3Na	-0.0300/2.66	-0.0290/2.28	-0.0193/1.19	-0.0144/1.21	-0.0146/1.02	-0.00910/0.499
C_6SO_3Na	-0.0299/2.68	-0.0315/2.53	-0.0186/1.13	-0.0148/1.28	-0.0147/1.05	-0.0133/0.767
C_8SO_3Na	-0.0317/2.85	-0.0310/2.49	-0.0202/1.28	-0.0132/1.19	-0.0157/1.11	- .0208/1.33

[a] Correlation coefficients in all cases were >0.96. The equation is of the form log $k'_1 = m$ (% organic) + b where m = slope and b = y-intercept.

[b] These equations were linear over the range of 50-90% MeOH.

TABLE 2

Least Squares Equations[a] for log k'_2 vs. % MeOH and vs. % MeCN in Mobile Phase as a function of Pairing Ion and Column Type.[b]

Pairing Ion	MeOH/H_2O			MeCN/H_2O		
	C-18 Slope/Intercept	C-8 Slope/Intercept	CN Slope/Intercept	C-18 Slope/Intercept	C-8 Slope/Intercept	CN Slope/Intercept
C_1SO_3Na	-0.0445/4.30	-0.0565/5.01	-0.0394/3.27	-0.0235/2.31	-0.0233/2.00	-0.0280/2.04
C_5SO_3Na	-0.0445/4.32	-0.0427/3.84	-0.0303/2.36	-0.0241/2.27	-0.0237/1.96	-0.0180/1.26
C_6SO_3Na	-0.0463/4.50	-0.0484/4.37	-0.0302/2.35	-0.0265/2.50	-0.0252/2.10	-0.0226/1.58
C_8SO_3Na	-0.0470/4.56	-0.0430/3.85	-0.0313/2.45	-0.0246/2.34	-0.0247/2.05	-0.0270/1.88

[a] Correlation coefficients in all cases were >0.94. The equation is of the form log k'_1 = m (% Organic) + b where m = slope and b = y-intercept.

[b] These equations were linear over the range of 50-90% MeOH in all cases.

As Table 1 shows, log k'_1 varies less predictably when MeCN-containing mobile phases are used. For the C_1, C_5, and C_6SO_3Na pairing agents, the y-intercept decreases as column polarity is increased, but a sharp increase in intercept is noted for C_8SO_3Na on the CN column. It is to be noted that the y-intercepts are different on the same column for MeOH and MeCN mobile phases. Since the y-intercept represents k' at 0% organic, this difference would appear to be a contradiction to the concept that k' should be the same in 100% aqueous mobile phase. However, in mobile phase compositions of less than 30% organic, log k' vs. % organic begin to deviate from linearity. The extrapolation to give the log k' values shown in Tables 1 and 2 utilized the linear range of values. The change in |slope| is even less predictable in MeCN mobile phases with no systematic trend being noted. At this time, no explanation presents itself to account for the apparent randomness of analyte retention in MeCN mobile phases. However, similar differences in the behavior of log k' in MeOH- and MeCN- containing mobile phases were observed in the previous study using SSA columns (1).

The least-square slopes and intercepts were determined for compound 2 on each column and with each pairing agent (Table 2), and for any given column type and pairing agent combination, both |slope| and y-intercept were larger for 2 than for 1 under the same conditions. This is not unexpected, since 2 contains an additional (relatively non-polar) phenyl group.

The experimental and predicted values for k'_1 on both the SSA C-18 column (from the previous study (1)) and on the Radial-Pak C-18 column are compared in Table 3. It is interesting to note that for each pairing agent/% MeOH combination, k'_1 is smaller for the SSA column than for the Radial-Pak column. A possible explanation for the greater retentivity of the Radial-Pak column relative to the SSA column lies in the fact that the

TABLE 3

Experimental and Predicted[a] k'_1-Values as a Function of % MeOH and Pairing Ion Using SSA[b] and Radial-Pak C-18 Columns.

	SSA C-18		Radial-Pak C-18	
% MeOH	Exptl. k'_1	Pred. k'_1	Exptl. k'_1	Pred. k'_1
		C_5SO_3Na		
45	4.35	3.80	- -	- -
50	2.68	2.55	15.24	- -
60	1.07	1.14	7.18	7.31
65	0.72	0.77	- -	- -
70	- -	- -	3.48	3.69
80	0.17	0.23	1.84	1.86
90	- -	- -	0.95	1.06
		C_6SO_3Na		
45	7.19	6.81	- -	- -
50	- -	- -	- -	- -
55	2.60	2.75	- -	- -
60	1.60	1.75	7.80	7.82
65	1.10	1.11	- -	- -
70	0.71	0.71	3.92	3.89
80	- -	- -	1.95	1.94
85	- -	- -	1.41	1.37
90	- -	- -	0.98	1.04
		C_8SO_3Na		
55	8.45	7.12	- -	- -
60	3.49	4.09	9.13	8.93
70	- -	- -	4.11	4.33
75	0.75	0.77	- -	- -
80	0.41	0.44	2.23	2.09
85	- -	- -	1.39	1.46
90	0.16	0.15	1.01	1.01

[a] Predicted values are as predicted using equation (1).

[b] Data taken from Ref. 1

Radial-Pak packing material is not end-capped after the reaction which bonds the octadecysilane moiety to the silica-gel (the C-18 SSA column utilized in (1) was end-capped). The lack of end-capping exposes the solute to free silanol groups present on the silica base. Interaction of the positive, protonated nitrogen in the piperidyl ring of **1** with these silanol sites could result in increased retention. As noted in the Experimental Section, triethylamine (TEA) was added to the mobile phases used on Radial-Pak columns in an attempt to minimize these secondary "polar" interactions. Mobile phases which did not contain TEA were found to be unacceptable due to severe peak tailing. The fact that addition of TEA significantly improved peak shapes is indicative of these proposed "polar" interactions. It should be noted that the non-end-capped silica and the addition of TEA to the mobile phases results in a fundamentally different system for the Radial-Pak columns as opposed to the SSA columns. The comparisons between Radial-Pak and SSA columns made herein reflect not only the differences due to the compression of the Radial-Pak columns, but primarily the differences due to mobile phase and packing material chemistry.

The log k' values for **1** and **2** were fitted to an equation of the form

$$\log k' = a\,(\%\,\mathrm{MeOH}) + b\,(nC) + c\,(\%\mathrm{MeOH})\,(nC) + d \qquad (1)$$

using the GLM procedure of SAS (5), where %MeOH is the concentration of MeOH in the mobile phase and nC is the chain-length of the sulfonate pairing-ion (i.e. for hexane sulfonate nC=6). The derived a, b, c, and d parameters are tabulated in Table 4 for each of the columns examined.

In order to determine the optimum system for the separation of PCP and its cosynthetics, $\alpha_{2/1}$ (the selectivity ratio between **2** and **1**) was maximized since our previous study (1) had shown that as the separation of **1** and **2** increased, all components were

TABLE 4

Coefficients for Equation 1 for PCP (1) and 2 on Three Different Radially Compressed Columns.[a]

For PCP(1) -

Column	a	b	c	d
C-18	-0.0267	0.0646	-0.000598	2.32
C-8	-0.0259	0.0722	-0.000718	1.97
CN	-0.0172	0.0359	-0.000354	0.980

For 2 -

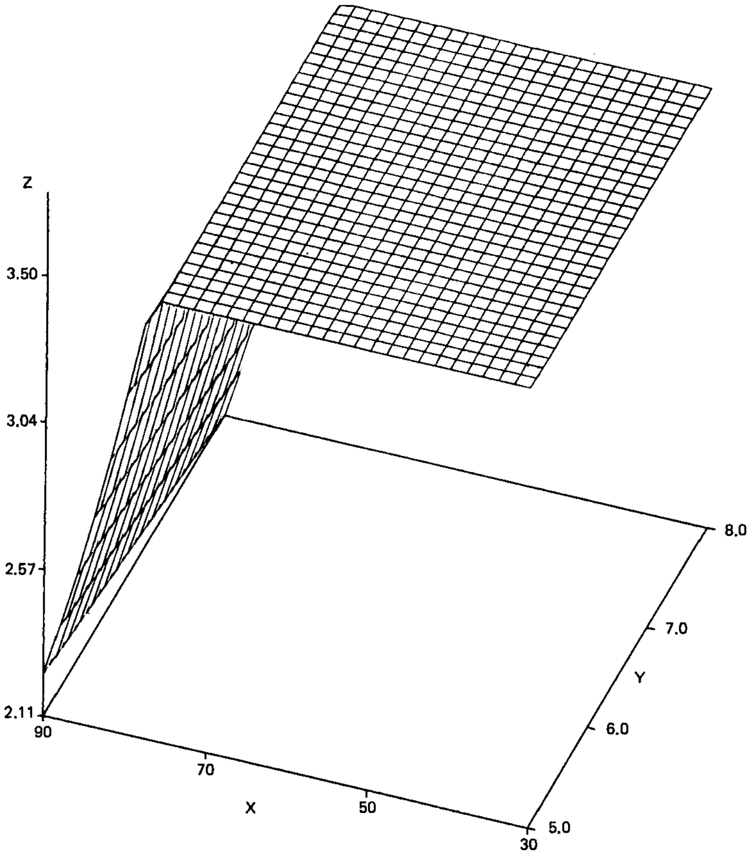
Column	a	b	c	d
C-18	-0.0413	0.0678	-0.000733	4.03
C-8	-0.0410	0.00764	-0.000224	3.77
CN	-0.0298	0.0223	-0.000159	2.26

[a] Correlation coefficients for the equations on each of the columns were >0.995 for 1 and >0.993 for 2.

increasingly well-resolved. Figures 2, 3 and 4 are three-dimensional plots of $\alpha_{2/1}$ as a function of both % MeOH and nC. The plots were generated using the SAS/graph procedure (6) and solving

$$\alpha_{2/1} = \frac{10\uparrow \text{ eq 1 for compound 2}}{10\uparrow \text{ eq 1 for compound 1}}$$

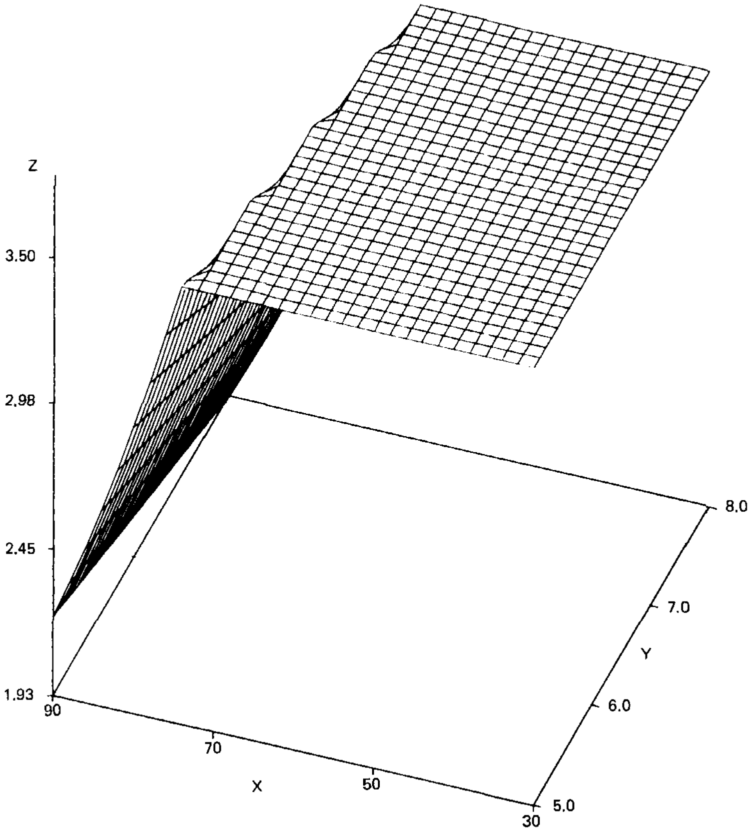
for MeOH percentages of 30-90% at 2% intervals and pairing ion chain lengths of 5 to 8 at 0.1 intervals. The $\alpha_{2/1}$ (z) axis in each plot was arbitrarily clipped at $\alpha = 3.5$ since qualitative examination of the chromatograms showed that an α value of 3.5 was more than sufficient to result in resolution of all peaks of interest while keeping analysis times relatively short.



X IS %MEOH, Y IS PAIRING-ION CHAIN LENGTH, & Z IS ALPHA
ALPHA VALUES GREATER THAN 3.5 *SET EQUAL TO* 3.5

FIGURE 2

Plot of $\alpha_{2/1}$ (z axis) as a function of % MeOH (x axis) and nC (y axis) on the radially compressed C-18 column.

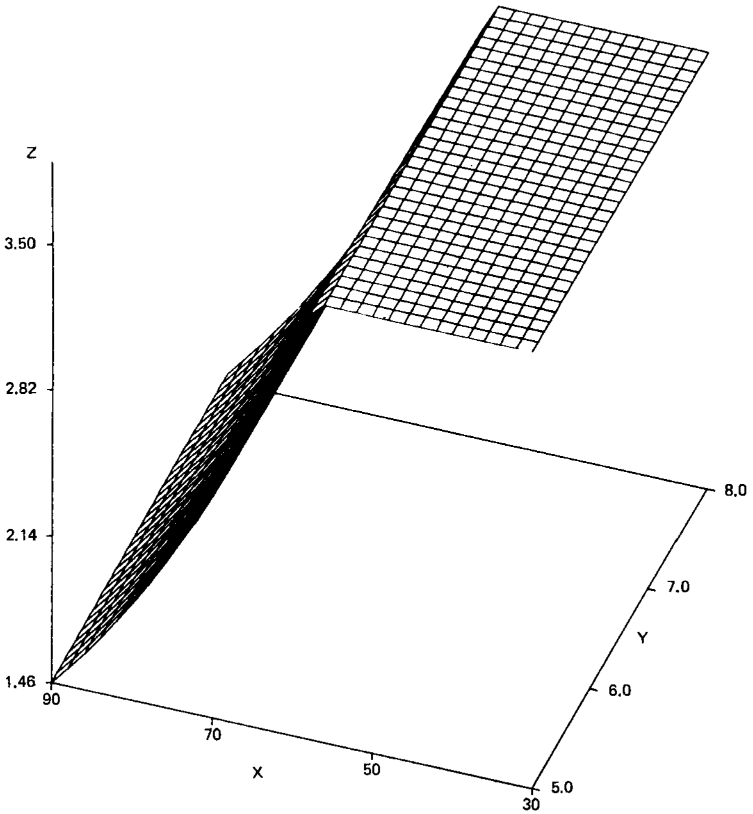


X IS %MEOH, Y IS PAIRING-ION CHAIN LENGTH, & Z IS ALPHA
ALPHA VALUES GREATER THAN 3.5 SET EQUAL TO 3.5

FIGURE 3

Plot of $\alpha_{\underset{\sim}{2}/\underset{\sim}{1}}$ as a function of % MeOH and nC on the radially compressed C-8 column.

Examination of Figs. 2-4 shows that all three columns are capable of achieving α values of 3.5 within the range of conditions examined. However, the "steepness" of the plots increases in the order C-18 (Fig 2) > C-8 (Fig 3) > CN (Fig 4). The fact that the C-18 column exhibits the steepest slope indicates that,



X IS %MEOH, Y IS PAIRING-ION CHAIN LENGTH, & Z IS ALPHA
ALPHA VALUES GREATER THAN 3.5 *SET EQUAL TO* 3.5

FIGURE 4

Plot of $\alpha_{2/1}$ as a function of % MeOH and nC on the radially compressed CN column.

for a given % MeOH, the C-18 column would yield the largest α value of the three columns examined. It is interesting to note that this steepness occurs only in the % MeOH (x) direction. The plots are all relatively flat in the nC (y) direction, indicating that pairing-ion chain length exerts little effect on the separation of **1** and **2**.

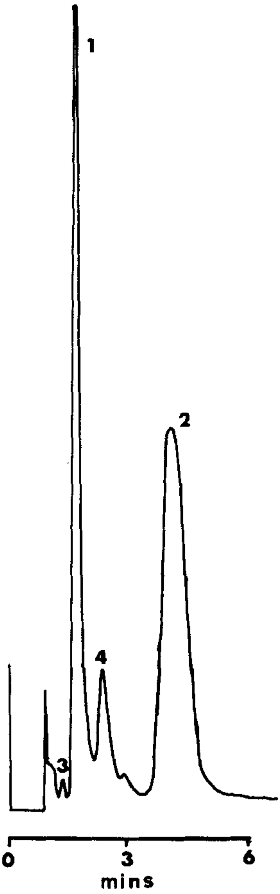


FIGURE 5

Chromatogram of crude synthetic PCP mixture. Numbers correspond to structures in Figure 1. Conditions: 85:15 MeOH:H_2O, 5m$\underset{\sim}{M}$ C_6SO_3Na + 1% TEA and 2.5% HOAc; Flow rate 3.5 mL/min; UV @ 254 nm, 0.1 AUFS; Radially compressed C-18 column.

Figure 5 illustrates the separation of a crude PCP mixture obtained on a Radial-Pak C-18 column. The chromatogram shown in Fig. 5 was obtained using conditions which were predicted from Fig. 4 to yield approximately $\alpha_{\mathbf{2}/\mathbf{1}} = 3.5$ (i.e. 85:15 MeOH:H_2O with C_6SO_3Na as pairing agent). The actual value of $\alpha_{\mathbf{2}/\mathbf{1}}$ as measured from the chromatogram is approximately 3.6. As demonstrated in Figures 3 and 4, an equivalent $\alpha_{\mathbf{2}/\mathbf{1}}$ can also be obtained on the C-8 or CN columns by decreasing the amount of MeOH in the mobile phase. However, the more MeOH-rich mobile phase is preferred since MeOH-rich mobile phases are less viscous, allowing higher flow rates at lower pressures, thereby reducing analysis time.

Close examination of the peak attributed to compound **2** in Fig. 5 shows a slight deformation on the trailing edge of the peak. This shoulder is attributed to the possible presence of an isomer of **2** (*vide supra*). Further separation of this shoulder can be achieved by further decreasing the % MeOH in the mobile phase at the cost of substantially increased analysis time. Figure 6 illustrates a chromatogram in which the shoulder is more completely resolved utilizing %MeOH = 75. However, the increase in resolution required an approximate two-fold increase in analysis time, substantially increased tailing for the compound **1** peak, and resulted in a greatly decreased detection efficiency for **3**, **4**, and other (unidentified) peaks in the mixture.

In summary, the liquid chromatographic behavior of **1** has been determined under reversed-phase paired ion conditions on radially compressed columns. An optimized system (consisting of a C-18 Radial-Pak column and a mobile phase of 85:15 MeOH:H_2O, 5 m$\underline{M}$ hexane sulfonate, 2.5% acetic acid and 1% TEA) is presented for the separation of **1** and cosynthetics **2**, **3**, and **4** on radially compressed columns and the method demonstrated on a crude synthetic PCP mixture. It was shown that the Radial-Pak C-18 column could provide superior resolution (relative to the SSA

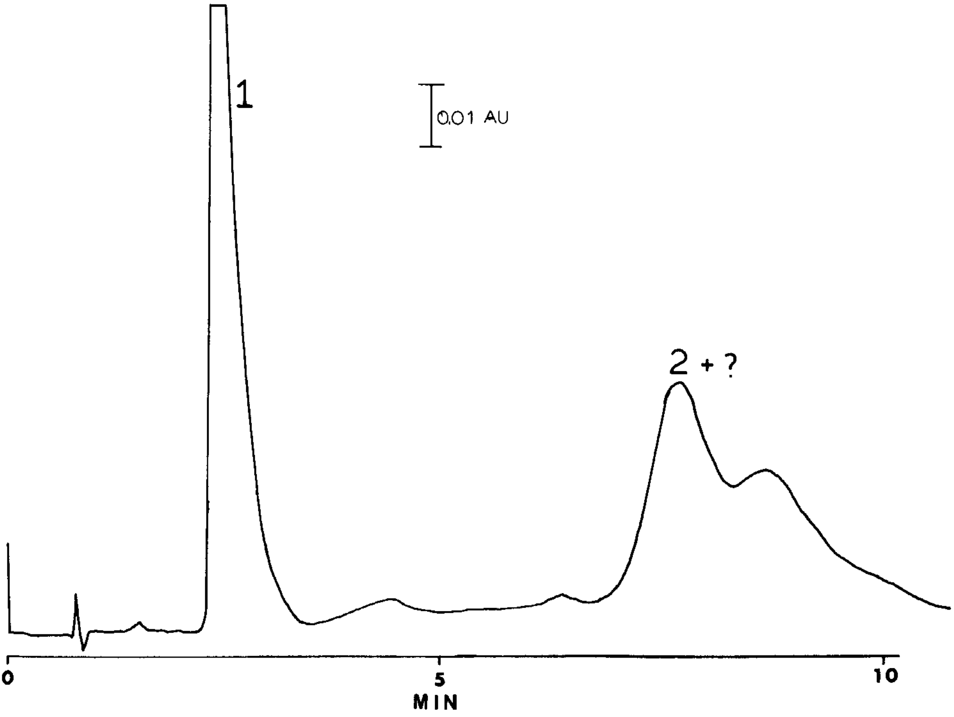


FIGURE 6

Chromatogram of crude synthetic PCP mixture illustrating increased resolution of shoulder from peak attributed to 2. Conditions: same as Fig. 1 except 75:25 MeOH:H_2O.

C-18 column) for a suspected isomer of 2. Comparisons of log k'_1 vs. % MeOH plots for the Radial-Pak C-18 column and the SSA C-18 column showed that PCP has a much higher affinity for the Radial-Pak C-18 column. Whether this increased affinity is due to hydrophobic or polar interactions is not clear at the present time. However, we suspect that the differences in affinity are due to polar interactions since it was necessary to dope the mobile phases used on Radial-Pak columns with TEA to obtain acceptable peak symmetries.

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