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GAS CHROMATOGRAPHIC SEPARATION OF SUBSTITUTED PYRIDINES

J. E. PREMECZ and M. E. FORD*
Chemicals Group, Industrial Chemicals Department, Air Products and Chemicals, P.O. Box 538, Allentown, PA 18105 (U.S.A.)
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SUMMARY

Capillary gas chromatographic methodology for separation of complex mixtures of substituted pyridines has been demonstrated on polar (CAM) and non-polar (DB-5) columns. Separations are characterized by high resolution, high sensitivity, a wide dynamic detector range, and good reproducibility. For the first time, Kováts retention indices have been calculated for pyridine and substituted pyridines. Correlations of retention indices with physico-chemical properties, such as hydrogen bonding, pyridine pK_a and Hammett substituent constants are discussed.

INTRODUCTION

Pyridine is the parent of a series of compounds that are important in medicinal, agricultural, and industrial chemistry. Derivatives of pyridine are widespread, and are found in such diverse sources as vitamins, antiseptics, insecticides, herbicides, pharmaceuticals, tobacco smoke, dyes and resins, and petroleum distillates and coal liquefaction products. Most of the analytical research for determination of substituted pyridines has been carried out with packed columns. Since 1952, when the first separation of a simple mixture of pyridines was reported[1], over 40 different stationary phases have been examined[2–7]. Typically, analyses have been limited to mixtures of a few components such as the lower alkyl homologues. Regardless of column polarity, individual components are often poorly resolved; excessive tailing and residual adsorption on the support are frequently encountered. Such difficulties are minimized by pretreatment of packed columns with alkali, silanizing agents or non-ionogenic surfactants[6,7], or by derivatization of the pyridine prior to analysis[8–10]. Finally, rapid column deterioration is common.

While methodology for separation and identification of lower alkyl pyridines has been established with packed columns, there have been fewer reports on separation of functionalized pyridines by capillary column gas–liquid chromatography. A variety of liquid phases has been evaluated in capillary columns[2,5,6,9,11–13]. As with packed column chromatography, much of the capillary work was restricted to small numbers of components, such as the isomeric methylpyridines. Often the resulting chromatography is characterized by broad, tailing peaks, poor resolution, and rapid

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column deterioration. Despite the direct dependence of the efficiency and quality of chromatographic separations on partitioning of the substrate between the mobile gas and stationary liquid phases, the physical interactions and mechanisms of separation involved in pyridine chromatography have received little attention.

In this paper, we present the results of a systematic study of 50 diversely substituted pyridines on DB-5 and CAM capillary columns with linear temperature programming. For the first time, Kováts retention indices (I) for pyridine and substituted pyridines are determined. Further, I values and differences in I between the two columns are compared with physical and chemical properties of the pyridines. Correlations of retention behavior with pyridine functionality, boiling point and polarity of the stationary phase are presented and discussed.

EXPERIMENTAL

Materials

With the two exceptions noted below, pyridine and substituted pyridines were obtained from Fluka (Buchs, Switzerland). Nepera (Harriman, NY, U.S.A.) Aldrich (Milwaukee, WI, U.S.A.) and Pfaltz and Bauer (Waterbury, CT, U.S.A.) in the highest available purity. All samples were used as received. Methyl picolinimidate and N-(α-picolyl)-α-picolinamidine, representative examples of pyridyl-substituted imidates and amidines, respectively, were prepared[14]. Table I lists the pyridines used in this study and summarizes selected physical properties for each.

TABLE I

PHYSICAL PROPERTIES OF PYRIDINE BASES

Compound	*Melting point (°C)**	*Boiling point (°C)**	*Dipole moment (D)***	*pK_a***	*Source*
Pyridine	−42.0	115.5	2.20	5.25	Aldrich P5,750-6
2-Methylpyridine	−66.8	128.8	1.92	5.96	Aldrich 10,983-5
3-Methylpyridine	−18.3	143.9	2.40	5.63	Aldrich 23,627-6
4-Methylpyridine	3.7	144.9	2.60	5.98	Aldrich 23,961-5
2-Ethylpyridine	−63.1	148.6	1.96	5.89	Aldrich 11,242-9
3-Ethylpyridine	−76.9	165.0	2.41		Aldrich 14,239-5
4-Ethylpyridine	−90.5	168.3	2.25		Aldrich 11,243-7
2,6-Dimethylpyridine	−5.0	143.0	1.66	6.77	Aldrich L390-0
2,4-Dimethylpyridine		159.0	2.30	6.99	Aldrich L360-9
3,5-Dimethylpyridine		171.5	2.58	6.15	Aldrich L420-6
3,4-Dimethylpyridine	−12.0	178.8	1.87	6.48	Aldrich L400-1
2-Cyanopyridine	27.0	222.0	5.24		Aldrich C9,460-2
3-Cyanopyridine	49.6	206.2	3.46		Aldrich C9,480-7
4-Cyanopyridine	78.5	195.4	1.63		Aldrich C9,500-5
2,4,6-Collidine	−43.0	175.0	1.95	7.43	Aldrich 14,238-7
2,3,6-Collidine	−11.0	176.0	2.11	7.24	Aldrich C8,418-6
2-Aminopyridine	57.0	204.0	2.04	6.82	Aldrich A7,799-7
3-Aminopyridine	64.5	252.0	3.12	5.98	Aldrich A7,820-9
4-Aminopyridine	158.0	273.0	3.95	9.11	Aldrich A7,840-3
2-(Aminomethyl)pyridine	−40.0	203.0	2.25		Aldrich A6,520-4
3-(Aminomethyl)pyridine	−21.1	226.0	2.52		Aldrich A6,540-9

TABLE I (*continued*)

Compound	*Melting point (°C)**	*Boiling point (°C)**	*Dipole moment (D)***	*pK_a***	*Source*
4-(Aminomethyl)pyridine	−7.6	230.0	2.84	9.65	Aldrich A6,560-3
2-Amino-6-methylpyridine	41.0	208.0	1.65		Aldrich A7,570-6
2-Amino-3-methylpyridine	33.0	221.0	2.17		Aldrich A7,563-3
2-Amino-5-methylpyridine	76.0	227.0	2.02		Aldrich A7,568-4
2-Amino-4-methylpyridine	100.0	230.0	2.27		Aldrich 12,308-0
Methyl picolinate	14.0	232.0			Pfaltz & Bauer M26200
Methyl isonicotinate	16.1	207.0			Aldrich M5,295-0
Methyl nicotinate	42.0	204.0			Aldrich M5,920-3
Ethyl 2-picolinate	0.0	243.0			Aldrich E4,541-4
Ethyl nicotinate	8.0	224.0			Aldrich E4,060-9
Ethyl isonicotinate	23.0	219.0	2.49		Aldrich 10,473-6
2-Picolinamide	107.0				Pfaltz & Bauer P18760
Isonicotinamide	155.7		3.91		Aldrich I-1,745-1
Nicotinamide	129.0		4.20		Aldrich 24,020-6
2,2′-Dipyridylamine	89.0	307.0			Aldrich D,640-2
2,2′-Dipyridyl	71.0	272.5	0.68		Aldrich D,630-5
4,4′-Dipyridyl hydrate	114.0	305.0	0.31		Aldrich 17,729-6
2,3′-Dipyridyl		295.0	1.97		Aldrich 19,888-9
2,4′-Dipyridyl	61.5	280.0	3.84		Aldrich 19,889-7
3,3′-Dipyridyl	68.0	291.0	0.70		Aldrich 26,912-3
2,2′:6′,2″-Terpyridine	88.0				Aldrich 23,467-2
2-Dimethylaminopyridine		196.0			Aldrich 85,255-4
2-(2-Aminoethyl)pyridine					Aldrich A5,530-6
2-Pyridylacetonitrile					Fluka 82900
5-Amino-2-methoxypyridine	29.0				Aldrich A6,120-9
1-(3-Pyridyl)-2-(4-pyridyl)ethylene					Pfaltz & Bauer P31230
Methyl picolinimidate					Synthesized***
N-Picolyl-2-picolinamidine					Synthesized***
Bis-(2-picolyl)amine				7.30	Nepera

* From refs. 15 and 16.
** From refs. 17–19 and references cited therein.
*** By procedure of ref. 14.

Individual hydrocarbons and commercial mixtures of *n*-alkanes were obtained from Analabs (North Haven, CT, U.S.A.), PolySciences (Warrington, PA, U.S.A.) and Chem Services (West Chester, PA, U.S.A.), and were used as received.

Chromatographic solvents were obtained from Fisher Scientific (Springfield, NJ, U.S.A.) in high-performance liquid chromatographic grade and were used without further purification.

Methods

Pyridine samples were prepared in methanol as 5% (w/w) solutions. For determination of relative retention times, pyridine was included as an internal standard in all samples of substituted pyridines. High-molecular-weight alkane mixtures (C_{20} and above) were diluted with *n*-heptane to 5% (w/w) solutions.

All samples and standards were analyzed with a Varian Model 3700 gas chro-

matograph equipped with a capillary injection port for use with fused-silica columns, Model 8000 autosampler, and flame ionization detector. Columns used were a fused-silica bonded and cross-linked DB-5 wall-coated open-tubular (WCOT) column (30 m × 0.32 mm I.D.) and a fused-silica base-treated CAM WCOT column (15 m × 0.24 mm I.D.); both were supplied by J&W Scientific (Rancho Cordova, CA, U.S.A.). Injection and flame ionization detection temperatures were 300°C and 290°C, respectively, for the DB-5 column; both temperatures were 240°C for the CAM column. To optimize reproducibility, all injections (0.7 μl) were made with the Model 8000 autosampler. Simultaneous sample injection and initiation of temperature programming was assured by control of the autosampler and the chromatograph with a Varian VISTA 402 chromatography data system. Chromatography was carried out under the following conditions: helium carrier gas flow-rate, 3 ml min^{-1} (determined by measurement of the retention of methanol at 60°C and correction for gas compressibility); head pressure, 13 p.s.i.g. at 60°C; splitting ratio 1:200; and chart speed, 5 mm min^{-1}. The temperature of the DB-5 column was programmed from 60 to 280°C at 10°C min^{-1} after an initial 10-min hold at 60°C. The upper temperature was maintained for 3 min. Owing to upper temperature limitations associated with polyethylene glycol liquid phases, the CAM column temperature was programmed from 60 to 240°C at 5°C min^{-1} after an initial 5-min hold at 60°C. The maximum temperature was maintained for 21 min.

Retention times were measured from the time of sample injection with the VISTA 402 system. Each sample was injected five times on the appropriate column, and absolute and relative retention times were determined by averaging the resulting values. *I* values were calculated off-line with RS/1 software in a VAX 785 minicomputer by application of the Van den Dool equation[20] to data for the two appropriate *n*-alkanes.

To determine the linear dynamic range of the flame ionization detector, mixtures of 2-(aminomethyl)pyridine and an internal standard, diglyme (reagent grade, obtained from Fisher Scientific) were analyzed on the DB-5 column. Quantitative analyses are based on peak area measurements, which are averages of at least five determinations. The linear response range was found by plotting the ratio of 2-(aminomethyl)pyridine/diglyme peak area *vs.* the ratio of 2-(aminomethyl)pyridine/diglyme concentration (%, w/w) for eleven standard solutions. Statistical calculations to evaluate the linear response range of the detector, and for correlations of retention behavior with pyridine properties, were carried out by a least squares method with RS/1 software in the VAX 785 minicomputer.

RESULTS AND DISCUSSION

Separations of a mixture of the 50 pyridines on the DB-5 and CAM capillary columns are shown in Figs. 1 and 2, respectively. The corresponding retention data, with relative retention times (pyridine = 1.000), are presented in Table II. Overall, resolution of this complex mixture was very good. Most of the components were separated by the DB-5 column; complete resolution was attained with the CAM column.

I values of each compound studied are shown in Table III. Differences in *I* (ΔI) are calculated for each component and included in Table III. Surprisingly, this

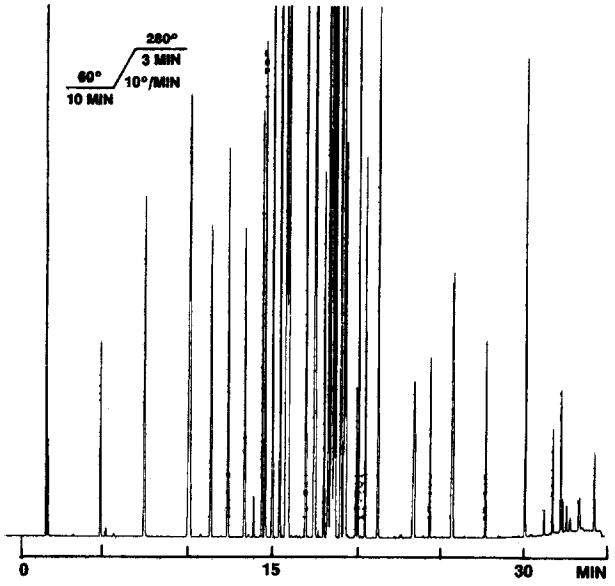

Fig. 1. Chromatogram of a mixture of 50 pyridines separated on a DB-5 capillary column with temperature programming as indicated. The initial peak is methanol solvent; the order of pyridine elution is given in Table II.

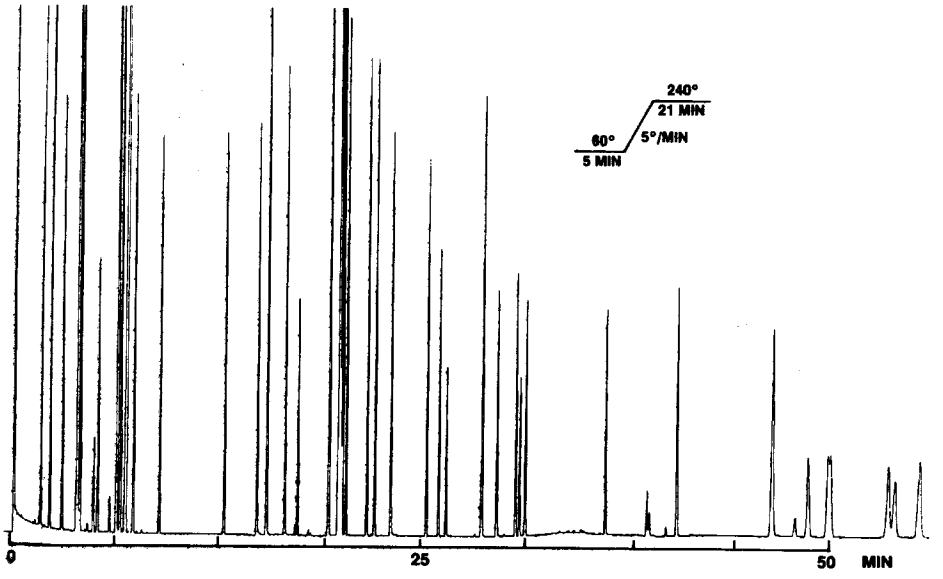

Fig. 2. Chromatogram of a mixture of 50 pyridines separated on a CAM capillary column with temperature programming as indicated. The initial peak is methanol solvent; the order of pyridine elution is given in Table II.

TABLE II

RETENTION DATA FOR PYRIDINE AND SUBSTITUTED PYRIDINES OBTAINED ON DB-5 AND CAM FUSED-SILICA CAPILLARY COLUMNS WITH TEMPERATURE PROGRAMMING

Conditions as in Figs. 1 and 2.

Peak No.	*Compound*	*DB-5 column**		*CAM column*	
		t_R**	*RRT***	t_R**	*RRT****
1	Pyridine	4.751	1.000	3.226	1.000
2	2-Methylpyridine	7.398	1.567	3.815	1.226
3	3-Methylpyridine	10.073	2.134	5.714	1.833
4	4-Methylpyridine	10.120	2.140	5.891	1.877
5	2,6-Dimethylpyridine	11.418	2.418	4.666	1.505
6	2-Ethylpyridine	12.445	2.632	5.686	1.817
7	2,4-Dimethylpyridine	13.402	2.836	6.921	2.231
8	3-Ethylpyridine	14.429	3.059	8.302	2.665
9	4-Ethylpyridine	14.689	3.102	8.611	2.756
10	4-Cyanopyridine	15.019	3.176	16.403	5.247
11	3,5-Dimethylpyridine	15.089	3.197	9.110	2.913
12	2,4,6-Collidine	15.543	3.273	7.965	2.590
13	3,4-Dimethylpyridine	15.833	3.362	10.665	3.416
14	3-Cyanopyridine	15.900	3.373	18.069	5.764
15	2-Aminopyridine	15.948	3.380	21.371	6.761
16	2,3,6-Collidine	16.039	3.385	7.888	2.906
17	2-(Aminomethyl)pyridine	17.107	3.622	17.062	5.422
18	2-Amino-6-methylpyridine	17.617	3.687	21.687	6.960
19	Methyl picolinate	17.675	3.723	21.322	7.018
20	2-Cyanopyridine	17.770	3.759	21.501	6.856
21	2-Amino-3-methylpyridine	18.209	3.858	21.527	6.884
22	3-Aminopyridine	18.343	3.869	25.943	9.566
23	2-Dimethylaminopyridine	18.472	3.888	14.528	4.661
24	2-Amino-4-methylpyridine	18.520	3.893	23.414	7.548
25	2-Amino-5-methylpyridine	18.599	3.898	22.952	7.363
26	3-(Aminomethyl)pyridine	18.621	3.939	20.781	6.654
27	Methyl isonicotinate	18.753	3.955	16.322	5.258
28	4(Aminomethyl)pyridine	18.845	3.975	21.356	6.819
29	2-(2-Aminoethyl)pyridine	19.174	4.038	18.927	6.049
30	Methyl nicotinate	19.227	4.048	18.013	6.767
31	4-Aminopyridine	19.428	4.099	29.191	10.752
32	2-Pyridylacetonitrile	19.439	4.101	24.349	7.939
33	Methyl picolinimidate	19.872	4.189	19.382	6.210
34	Ethyl isonicotinate	20.297	4.277	18.541	6.075
35	5-Amino-2-methoxypyridine	20.634	4.372	27.184	8.881
36	Ethyl nicotinate	20.707	4.419	19.044	7.048
37	2-Picolinamide	21.362	4.483	30.832	9.854
38	Ethyl 2-picolinate	21.456	4.509	30.669	10.003
39	Isonicotinamide	23.572	4.976	39.955	12.823
40	Nicotinamide	23.671	5.011	39.806	12.816
41	2,2'-Dipyridyl	24.546	5.153	27.759	8.874
42	4,4'-Dipyridyl hydrate	25.816	5.442	32.524	10.418
43	2,3'-Dipyridyl	25.913	5.477	32.011	10.310
44	3,3'-Dipyridyl	25.931	5.480	32.518	10.379
45	2,4'-Dipyridyl	25.966	5.497	32.178	10.323
46	2,2'-Dipyridylamine	27.904	5.886	37.325	11.955
47	Bis-(2-picolyl)amine	29.310	6.248	37.502	13.803

TABLE II (*continued*)

Peak No.	*Compound*	*DB-5 column**		*CAM column*	
		t_R**	*RRT***	t_R**	*RRT****
48	N-Picolyl-2-picolinamidine	30.274	6.273	48.135	15.344
49	1-(3-Pyridyl)-2-(4-pyridyl)ethylene	31.937	6.371	41.572	13.621
50	2,2′:6′,2″-Terpyridine	34.437	7.270	50.425	16.229

* Identities of closely eluting peaks checked by injection of specific components.
** t_R = Absolute retention times (min), measured from sample injection (Figs. 1 and 2).
*** RRT = Relative retention time (relative to pyridine = 1.000).

TABLE III

KOVÁTS RETENTION INDICES (*I*, RELATIVE TO *n*-ALKANES) FOR PYRIDINE AND SUBSTITUTED PYRIDINES ON DB-5 AND CAM FUSED-SILICA CAPILLARY COLUMNS WITH TEMPERATURE PROGRAMMING

Conditions as in Figs. 1 and 2.

Peak No.	*Compound*	*I*		*ΔI**
		DB-5 column	*CAM column*	
1	Pyridine	736.336	1182.744	446.408
2	2-Methylpyridine	813.546	1215.677	402.131
3	3-Methylpyridine	861.043	1283.549	422.506
4	4-Methylpyridine	861.933	1289.327	427.394
5	2,6-Dimethylpyridine	884.219	1243.134	358.915
6	2-Ethylpyridine	904.622	1281.668	377.047
7	2,4-Dimethylpyridine	931.842	1325.263	393.421
8	3-Ethylpyridine	962.034	1368.730	406.696
9	4-Ethylpyridine	968.129	1378.019	409.890
10	4-Cyanopyridine	978.315	1665.250	686.936
11	3,5-Dimethylpyridine	979.874	1390.814	410.940
12	2,4,6-Collidine	992.836	1357.967	365.131
13	2-Aminopyridine	1002.282	1873.146	870.864
14	3,4-Dimethylpyridine	1003.375	1444.850	441.475
15	3-Cyanopyridine	1007.252	1733.301	726.049
16	2,3,6-Collidine	1008.944	1366.165	357.221
17	2-(Aminomethyl)pyridine	1053.573	1689.738	636.164
18	2-Amino-6-methylpyridine	1073.752	1893.189	819.437
19	Methyl picolinate	1075.217	1879.466	804.250
20	2-Cyanopyridine	1079.158	1880.069	800.911
21	2-Amino-3-methylpyridine	1098.770	1883.106	784.337
22	3-Aminopyridine	1104.704	2111.372	1006.668
23	2-Dimethylaminopyridine	1110.030	1590.230	480.200
24	2-Amino-4-methylpyridine	1112.558	1952.430	839.873
25	3-(Aminomethyl)pyridine	1117.352	1849.218	731.866
26	2-Amino-5-methylpyridine	1117.563	1974.693	857.131
27	Methyl isonicotinate	1125.115	1663.121	538.006
28	4-(Aminomethyl)pyridine	1130.341	1879.410	749.069
29	Methyl nicotinate	1145.426	1742.815	597.388
30	2-(2-Aminoethyl)pyridine	1146.730	1768.389	621.658
31	2-Pyridylacetonitrile	1157.091	2020.354	863.263

(*Continued on p. 30*)

TABLE III (*continued*)

Peak No.	*Compound*	*I*	*ΔI**	
		DB-5 column	*CAM column*	
32	4-Aminopyridine	1158.084	2287.177	1129.093
33	Methyl picolinimidate	1181.334	1788.510	607.176
34	Ethyl isonicotinate	1201.311	1754.507	553.196
35	Ethyl nicotinate	1223.558	1771.482	547.924
36	5-Amino-2-methoxypyridine	1226.384	2165.233	938.849
37	2-Picolinamide	1267.907	2358.245	1090.338
38	Ethyl 2-picolinate	1269.395	2352.916	1083.520
39	Isonicotinamide	1407.598	2894.250	1486.652
40	Nicotinamide	1426.473	2898.561	1472.088
41	2,2′-Dipyridyl	1471.741	2192.835	721.094
42	4,4′-Dipyridyl hydrate	1565.304	2453.298	887.994
43	3,3′-Dipyridyl	1572.781	2453.746	880.965
44	2,4′-Dipyridyl	1574.012	2435.166	861.153
45	2,3′-Dipyridyl	1575.745	2427.782	852.038
46	2,2′-Dipyridylamine	1730.874	2738.672	1007.797
47	Bis-(2-picolyl)amine	1853.793	2769.289	915.497
48	1-(3-Pyridyl)-2-(4-pyridyl)ethylene	1936.364	3014.073	1077.709
49	N-Picolyl-2-picolinamidine	2088.178	3297.927	1209.749
50	2,2′:6′,2″-Terpyridine	2303.630	**	**

* $\Delta I = I_{CAM} - I_{DB\text{-}5}$.
** This compound eluted after the highest boiling *n*-alkane (C_{30}).

tabulation constitutes the first report of *I* values for pyridine and substituted pyridines on polar and non-polar capillary columns. *I* values for the 50 pyridines range from 736.3 to 2303.6 on the DB-5 column and from 1182.7 to 3297.9 on the CAM column, and can be used for identification of specific compounds. Huber *et al.*[21] have stated that identification by means of *I* values measured on two stationary

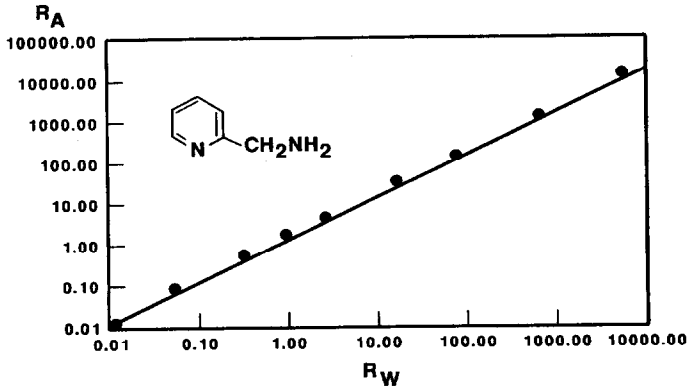

Fig. 3. Plot of R_A/R_W for 2-(aminomethyl)pyridine and diglyme on the DB-5 capillary column. R_A = [Area of 2-(aminomethyl)pyridine peak]/(area of diglyme peak). R_W = [wt.% of 2-(aminomethyl)pyridine]/(wt.% of diglyme).

TABLE IV

REGRESSION PARAMETERS FOR CORRELATIONS OF I AND ΔI WITH PYRIDINE PROPERTIES

*Figure**		*r*	*Slope*	*Intercept*
3		0.999	2.19	**
4		0.978	***	***
5	DB-5	0.992	4.15	271.03
	CAM	0.960	3.71	740.81
6		0.982	−66.94	796.30
7		0.927	316.66	460.78

* All correlations had levels of significance $\leqslant 0.05$.

** Not reported, owing to non-linearity of this relationship for R_A, $R_W < 0.01$.

*** The data were best fit to a third order expression: $-2.51x^3 + 0.17x^2 - 28.89x + 2630.70$.

phases of different polarities has the same reliability as one from low-resolution mass spectra.

Using 2-(aminomethyl)pyridine as a model, and diglyme as the internal standard, the linear dynamic range of the flame ionization detector was determined. As shown in Fig. 3, detector response was linear (Table IV) over six orders of magnitude of R_A/R_W. In absolute terms, detector response was linear from 190 pg to more than 600 ng of 2-(aminomethyl)pyridine injected on the column. Below 190 pg, effects of adsorption on the column were shown by increased scatter of R_A/R_W values and curvature of the R_A/R_W plot. Owing to the structural similarities among the pyridines used in this study, comparable sensitivity and wide range linearity are expected for the remaining pyridine derivatives.

Retention is a phenomenon that depends primarily on interactions between the stationary liquid phase and absorbed analyte molecules. Such interactions depend upon the nature of both the solute and stationary phase, and include simple intermolecular attraction (Van der Waals forces), hydrogen bonding, and, with pyridines, acid–base interactions. In addition, depending on the substitution pattern of the pyridine ring, these interactions may be modified by steric effects.

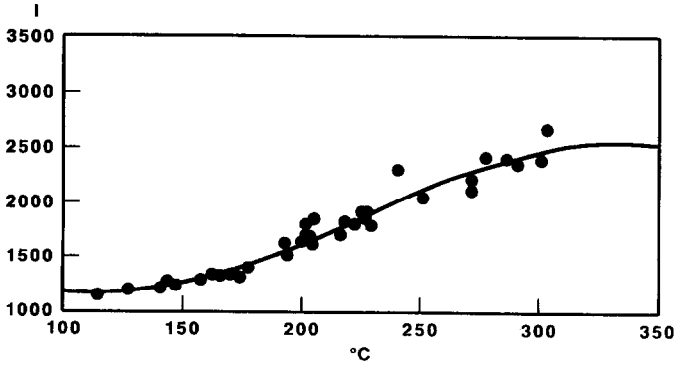

Fig. 4. Plot of I of pyridine and substituted pyridines on the CAM capillary column as a function of analyte boiling point.

With the DB-5 column, the 50 pyridines generally eluted in order of increasing boiling point, as would be expected with a low-polarity column. The non-polar liquid phase of the DB-5 column, dimethyldiphenylpolysiloxane, cannot interact strongly (*e.g.*, via hydrogen bonding) with the various pyridines. Consequently, separations on this column are primarily the result of differences in the relatively weak Van der Waals forces between the pyridine and the polysiloxane liquid phase. As anticipated, compounds with similar boiling points, such as 3-methylpyridine (b.p. 143.9°C) and 4-methylpyridine (b.p. 144.9°C) were not resolved.

In contrast, a statistically significant, but non-linear, correlation between I and pyridine boiling point was found for chromatography of the mixture with the polar CAM column (Fig. 4; Table IV). As discussed below, this curvature reflects the occurrence of additional intermolecular interactions between various pyridine solutes and the uncapped polyethylene glycol liquid phase of the CAM column.

Although some scatter is observed if I on either column is plotted *vs.* boiling point for all 50 components, subsetting the retention data by functional group improves the quality of the correlations. For example, good linear correlations between I and boiling point are obtained for alkylpyridines on both DB-5 and CAM columns (Fig. 5; Table IV). However, as expected for chromatography on the higher-polarity column, greater I values are obtained for alkylpyridines on the CAM, rather than on the DB-5, column.

With the CAM column, the non-linear dependence of I on boiling point (Fig. 4) and the preferential retention of pyridines (Fig. 5) suggest that additional interactions influence the rate of elution. To evaluate this possibility, we examined the dependence of ΔI on physical and chemical properties of the pyridines. This approach was chosen for the following reason. Since retention on the DB-5 column depends primarily on Van der Waals forces (see above), subtraction of $I_{DB\text{-}5}$ from I_{CAM} yields a quantity, ΔI, that estimates the magnitude of the remaining interactions between the pyridines and the CAM column. Such interactions may include hydrogen bonding and acid–base interactions.

Consideration of ΔI for the isomeric pyridine methyl and ethyl esters revealed that the 2-substituted esters had substantially higher retention indices than the corresponding 3- and 4-substituted analogues (Table III). With the presence of the ring nitrogen and the ester group, both polar hydrogen bond acceptors, strong absorption

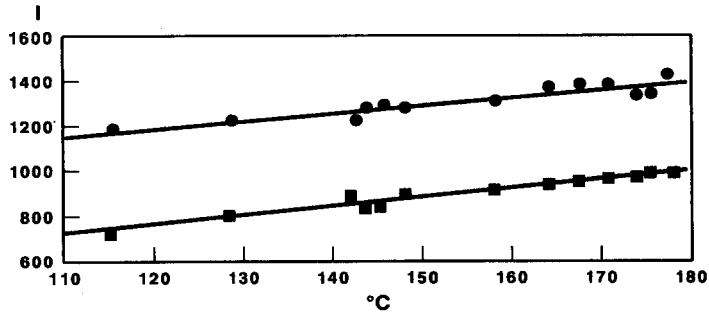

Fig. 5. Plot of I of alkylpyridines on the CAM (●) and DB-5 (■) capillary columns as a function of analyte boiling point.

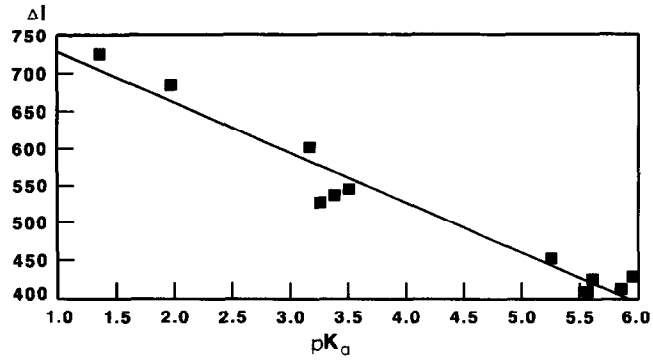

Fig. 6. Plot of ΔI as a function of pK_a for pyridine and 3- and 4-substituted alkyl, carbalkoxy, and cyanopyridines.

of pyridine esters on the CAM column would be anticipated. However, for the 2-substituted esters, the close proximity of the two heteroatomic functionalities permits chelation with the uncapped polyethylene glycol liquid phase via hydrogen bonding. As a consequence, retention times for 2-substituted pyridine esters are anomalously high on the CAM column.

Strong chelation with the polar liquid phase might similarly be expected for 2-substituted pyridine amides. However, this was not observed. Instead, picolinamide eluted more rapidly than either nicotinamide or isonicotinamide (Table III). Pyridine amides have both hydrogen bond donors (the hydrogen atoms of the amide functionality) and hydrogen bond acceptors (the carbonyl oxygen, and ring and amide nitrogen atoms). For picolinamide, intramolecular hydrogen bonding of the ring nitrogen with the amide hydrogen competes with intermolecular hydrogen bonding to the stationary phase. In this case, formation of an intramolecular hydrogen bond reduces intermolecular interactions with the liquid phase. On the other hand, intramolecular hydrogen bonding is not possible with 3- and 4-substituted pyridine amides; exclusive association of the ring nitrogen and amide group with the stationary phase explains the longer retention times of these isomers on the CAM column.

In addition to pyridine substitution, the basicity of the pyridine ring affects retention behavior. For 3- and 4-substituted pyridines in which hydrogen bonding and chelation effects are absent (specifically, pyridine, alkylpyridines, pyridine esters, and the cyanopyridines), linear correlations of ΔI with pK_a are found (Fig. 6 for 3- and 4-substituted pyridines; Table IV). As the pK_a value increases, *i.e.*, as the pyridine becomes more basic, the difference in retention on the two columns becomes smaller. This observation is the result of weaker interactions between the more basic pyridine derivatives and the base-treated polar liquid phase.

Substituent effects on retention behavior can be further explained on the basis of electromerism using the Hammett free energy relationship. Fig. 7 correlates ΔI values with the substituent constant σ for the 3- and 4-substituted pyridines in which hydrogen bonding and chelation effects are absent (pyridine, alkylpyridines, pyridine esters and cyanopyridines). The linear correlations (Table IV) indicate that the change in retention index between the two columns is directly proportional to the change in free energy of ionization brought about by the introduction of substituents

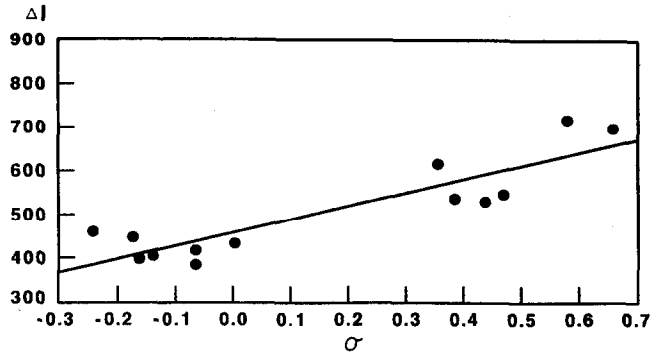

Fig. 7. Plot of ΔI as a function of substituent constant σ for pyridine and 3- and 4-substituted alkyl-, carbalkoxy- and cyanopyridines.

on the pyridine ring. ΔI increases as σ becomes more positive, *i.e.*, as the substituent becomes more electron-withdrawing. As electron density is increasingly removed from the ring, the π clouds above and below the ring become electron deficient, and the more acidic pyridines interact more strongly with the basic polar liquid phase. This enhanced acid–base interaction is reflected in larger ΔI values.

CONCLUSIONS

Capillary gas–liquid chromatographic methodology has been developed for analysis of pyridines on both polar (CAM) and non-polar (DB-5) columns. This method possesses the following advantages:

(1) High resolution of similarly substituted pyridines.
(2) High sensitivity to low pyridine concentration.
(3) Utility over a wide range of pyridine concentrations.
(4) Good peak shape and reproducibility of separations.

In general, the polar column provides greater resolution. Kováts retention indices have been determined for the 50 pyridines studied. Moreover, separations and relative elution orders of substituted pyridines have been related to physicochemical principles; linear relationships between I or ΔI and boiling point, dipole moment, acidity and Hammett σ values have been found. Since complex mixtures of pyridines have been successfully separated, this methodology should have wide utility, *e.g.*, for analyses in medicinal, agricultural and industrial chemistry.

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