CHROM. 6226

The separation and identification of imidazoles by gas–liquid and thin-layer chromatography

One of the problems in the experimental chemistry of imidazoles has been the difficulty encountered in the separation and identification of mixtures of these compounds. Although paper and thin-layer chromatography (TLC) have been widely used (the field has been surveyed recently[1]) there have been few references to the successful use of gas–liquid chromatography (GLC)[2-7], and certainly no systematic study. The relatively low volatilities and high polarities of the compounds have made the use of GLC a somewhat unrewarding technique. During an investigation of the rearrangement of some 1-substituted imidazoles it became necessary to analyse complex mixtures of isomeric imidazoles, both substituted and unsubstituted on ring nitrogen. Considerable effort has been expended to accomplish this, and has resulted in the development of a versatile GLC separation procedure. In conjunction with this, a TLC survey of imidazoles has been carried out.

Experimental

Gas–liquid chromatography. The apparatus used was a Loenco Model 160-PM with flame ionization detector and nitrogen as the carrier gas. The column used was 2 m × 3 mm I.D., glass with coiled diameter 195 mm. The support was Chromosorb W, AW-DMCS (H.P.), 80–100 mesh (Varian Aerograph). This support was coated (using the "pan coating" method described by McNAIR AND BONELLI[8]) with 5 % OV-17 (Applied Sciences Laboratories). After the coated support had been vacuum dried and then further dried at 80° for 5 h, the column was packed using a vacuum pump and some external tapping.

The operating conditions were, nitrogen flow-rate of 20 ml/min, the temperatures were: initial 70°, programmed at 2°/min, injection oven 200°, detector 250°. By means of a syringe 1-μl samples were injected.

For imidazoles in which there was no substitutent on the nitrogen, the samples were prepared by adding the imidazoles (10 mg) to dry tetrahydrofuran (0.8 ml) and acetic anhydride (0.2 ml). For 1-substituted imidazoles, the compounds (10 mg) were dissolved in dry tetrahydrofuran (1 ml).

Thin-layer chromatography. The adsorbents used were Aluminium Oxide G and Aluminium Oxide PF_{254} (Type E) (E. Merck AG, Darmstadt, G.F.R.). The plates were spread to a thickness of 250 μ in the normal way and left to air-dry without activation. In order to reduce tailing 1-substituted imidazoles were separated on chromatoplates prepared from slurries in 0.1 *N* NaOH.

The solvents used were (1) chloroform–methanol (6:0.2) for imidazoles unsubstituted on the ring nitrogens and (2) benzene–acetone–methanol (6:1:0.1) for 1-substituted imidazoles. The compounds were visualized with iodine vapour.

Materials. With the exception of the imidazoles: 2-methylimidazole, 2-ethylimidazole, 2-ethyl-4-methylimidazole, 2-phenylimidazole, 4,5-diphenylimidazole, imidazole-4-carboxylic acid, histamine dihydrochloride, imidazole-4-acetic acid, 2-mercaptoimidazole, urocanic acid, imidazole pyruvic acid, 1-butylimidazole, 1-vinylimidazole, 1-*trans*-cinnamoylimidazole, 1,2-dimethylimidazole, 1-methyl-2-mercapto-

imidazole, 1-vinyl-2-methylimidazole (Koch-Light Laboratories Ltd.), all other compounds were synthesized by standard methods and will be reported elsewhere. Their purity and identities were determined by examination of NMR and mass spectra, and other physical properties.

Results and discussion

Gas–liquid chromatography. Excellent separations have been achieved using the OV-17 column. 1-Substituted imidazoles can be injected directly and give little tailing. Imidazoles unsubstituted on ring nitrogens are first converted to the acetyl derivatives using acetic anhydride in dry tetrahydrofuran. This convenient modification of a method reported earlier[9,10] gives yields of the order of 100% (except with 2,4,5-trimethylimidazole) and has the advantage of often forming only one of the two possible isomers[11]. The formation of acetyl derivatives is almost instantaneous and they are stable for several days, besides giving rise to symmetrical, triangular-shaped peaks (see Fig. 1) when chromatographed.

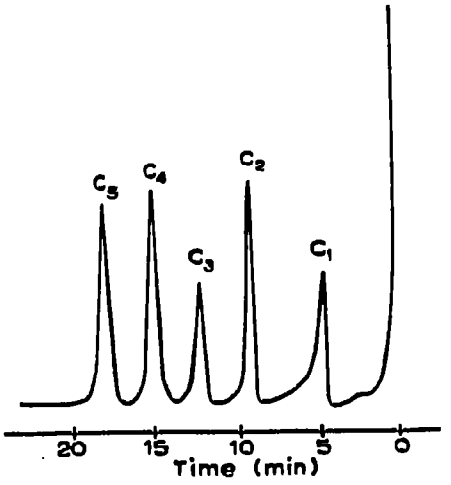

Fig. 1. Gas chromatogram showing the separation of C_1 = 1-methylimidazole; C_2 = imidazole; C_3 = 4-methylimidazole; C_4 = 2-ethylimidazole; C_5 = 2-ethyl-4-methylimidazole. The operating conditions are given in the text.

R_{ImAc}* and temperature-programmed KOVÁTS retention indices (I_p) have been recorded for a range of imidazoles (see Tables I and II), and good separations have been achieved for isomeric imidazoles.

Thin-layer chromatography. As other workers have found, it is not possible to obtain adequate separations of 1-substituted imidazoles and the more polar imidazoles with no N-substituent using any simple combination of solvent system and ad-

* For imidazoles substituted on ring nitrogens

$$R_{ImAc} = \frac{\text{adjusted retention time of the imidazole}}{\text{adjusted retention time of 1-acetylimidazole}}.$$

For imidazoles unsubstituted on ring nitrogens

$$R_{ImAc} = \frac{\text{adjusted retention time of the acetylated imidazole}}{\text{adjusted retention time of 1-acetylimidazole}}.$$

TABLE I

R_{ImAc} AND I_p VALUES OF IMIDAZOLES NOT SUBSTITUTED ON RING NITROGEN

Compound	R_{ImAc}	I_p
Imidazole	1	1282
2-Methylimidazole	1.26	1340
4-Methylimidazole	1.32	1350
2,4-Dimethylimidazole	1.53	1400
2-Ethylimidazole	1.61	1410
4-Ethylimidazole	1.78	1447
2-Ethyl-4-methylimidazole	1.90	1464
4,5-Dimethylimidazole	1.93	1474
2,4,5-Trimethylimidazole	2.47	1560
4-Methyl-5-carbethoxyimidazole	3.36	1719
2-Mercaptoimidazole	3.56	1765
2-Hydroxymethylimidazole	3.75	1800
2-Bromo-4-methyl-5-carbethoxyimidazole	3.96	1824
4-Hydroxymethylimidazole	4.00	1839
2-Phenylimidazole	4.50	1943
4-Carbethoxyimidazole	4.70	1991
Histamine	6.75	2452
4,5-Diphenylimidazole	7.97	2720

TABLE II

R_{ImAc} AND I_p VALUES OF 1-SUBSTITUTED IMIDAZOLES

Compound	R_{ImAc}	I_p
1-Vinylimidazole	0.50	1160
1-Methylimidazole	0.56	1200
1-Vinyl-2-methylimidazole	0.56	1200
1,4-Dimethylimidazole	0.74	1223
1-Ethylimidazole	0.80	1231
1,2-Dimethylimidazole	0.86	1249
1-Acetylimidazole	1.00	1282
1-Allylimidazole	1.00	1282
1,2,4-Trimethylimidazole	1.08	1297
1-Propylimidazole	1.12	1306
1,5-Dimethylimidazole	1.32	1344
1,2,5-Trimethylimidazole	1.60	1410
1-Butylimidazole	1.62	1416
1,2,4,5-Tetramethylimidazole	1.85	1456
1-Methyl-2-mercaptoimidazole[a]	3.56	1765
1-Benzylimidazole	4.04	1854
1-Benzyl-2-hydroxymethylimidazole	5.88	2241

[a] Acetylated.

TABLE III

R_{Im} VALUES OF IMIDAZOLES NOT SUBSTITUTED ON RING NITROGEN

Solvent (1): chloroform–methanol (6:0.2) was used.

Compound	*Alumina G-layer*	*Alumina (type E)-layer*
Histamine dihydrochloride	0.00	0.00
Imidazole-4-acetic acid	0.00	0.00
Imidazole-4-carboxylic acid	0.00	0.00
Imidazole pyruvic acid	0.00	0.00
Urocanic acid [(α-4-Imidazolyl)acrylic acid]	0.00	0.00
4-Hydroxymethylimidazole	0.12	0.10
2-Hydroxymethylimidazole	0.22	0.20
Imidazole-4-carboxamide	0.24	0.14
2-Mercaptoimidazole	0.93	0.71
Imidazole	1.00	1.00
4-Carbethoxyimidazole	1.19	1.88
4,5-Dimethylimidazole	1.22	1.84
4-Methylimidazole	1.23	1.58
2,4,5-Trimethylimidazole	1.30	1.96
4-Methyl-5-carbethoxyimidazole	1.43	2.48
2,4-Dimethylimidazole	1.79	2.10
2-Methylimidazole	1.86	1.88
4,5-Diphenylimidazole	2.03	3.80
2-Ethylimidazole	2.24	3.30
2-Ethyl-4-methylimidazole	2.30	3.45
2-Phenylimidazole	2.68	4.02
2-Acetyl-4-methylimidazole	2.72	4.54

TABLE IV

R_{Im} VALUES OF 1-SUBSTITUTED IMIDAZOLES

Solvent (2): benzene–acetone–methanol (6:1:0.1) was used.

Compound	*Alumina G-layer*	*Alumina (type E)-layer*
1-Methylimidazole-4-acetic acid	0.00	0.00
1-Methylimidazole-5-acetic acid	0.00	0.00
1-*trans*-Cinnamoylimidazole	0.00	0.00
1-Methyl-5(2′-aminoethyl)imidazole	0.55	0.46
1-Methyl-4(2′-aminoethyl)imidazole	1.00	0.83
1-Benzyl-2-hydroxymethylimidazole	2.05	1.05
1-Methyl-2-mercaptoimidazole	3.20	2.27
1-Methylimidazole	4.45	3.26
1,5-Dimethylimidazole	4.92	2.97
1,2,5-Trimethylimidazole	5.25	3.25
1,2-Dimethylimidazole	5.65	3.85
1,2,4,5-Tetramethylimidazole	5.75	3.50
1,2,4-Trimethylimidazole	5.78	3.72
1-Benzyl-2-mercaptoimidazole	6.20	2.87
1-Ethylimidazole	6.20	3.72
1,4-Dimethylimidazole	6.30	3.94
1-Isopropylimidazole	7.65	4.14
1-*n*-propylimidazole	8.00	4.30
1-Allylimidazole	8.35	4.32
1-Vinylimidazole	9.20	5.07
1-Butylimidazole	9.42	4.80
1-Benzylimidazole	9.55	4.80
1-Vinyl-2-methylimidazole	9.80	5.32

sorbent. The parallel use of two sets of chromedia has proved a useful analytical and preparative tool.

For analytical studies imidazoles with no N-substituent have been separated on Aluminium Oxide G using solvent 1 (see Table III), while 1-substituted imidazoles are most conveniently examined using a basic layer and solvent 2 (see Table IV).

Preparative separations have been carried out with most success using Aluminium Oxide PF_{254} (type E).

As previously reported[12] the use of R_{Im}* values has given more reproducible results than R_F values.

One of us (C.G.B.) thanks the University Grants Committee of New Zealand for a Post Graduate Scholarship.

Department of Chemistry, University of Otago, Dunedin (New Zealand)

C. G. BEGG
M. R. GRIMMETT

1 M. R. GRIMMETT, *Advanc. Heterocycl. Chem.*, 12 (1970) 103.
2 R. THAM AND B. HOLMSTEDT, *J. Chromatogr.*, 19 (1965) 286.
3 D. ROACH, C. W. GEHRKE AND R. W. ZUMWALT, *J. Chromatogr.*, 43 (1969) 311.
4 D. H. FRAM AND J. P. GREEN, *J. Biol. Chem.*, 240 (1965) 2036.
5 P. BEAK AND W. MESSER, *Tetrahedron*, 25 (1969) 3287.
6 H. TIEFENTHALER, W. DÖRSCHELN, H. GÖTH AND H. SCHMID, *Helv. Chim. Acta*, 50 (1967) 2244.
7 H. J. M. DOU AND B. M. LYNCH, *Bull. Soc. Chim. Fr.*, (1966) 3815.
8 H. M. MCNAIR AND E. J. BONELLI, *Basic Gas Chromatography*, Consolidated Printers, Oakland, Calif., 1967, p. 223.
9 G. S. REDDY, L. MANDELL AND J. H. GOLDSTEIN, *J. Chem. Soc.*, (1963) 1414.
10 H. A. STAAB AND W. ROHR, in W. FOERST (Editor), *Newer Methods of Preparative Organic Chemistry*, Vol. 5, Academic Press, New York, 1968, p. 100.
11 R. A. OLOFSON AND R. V. KENDALL, *J. Org. Chem.*, 35 (1970) 2246.
12 M. R. GRIMMETT AND E. L. RICHARDS, *J. Chromatogr.*, 18 (1965) 605.

Received May 5th, 1972

---

* $R_{Im} = \frac{\text{distance travelled by compound}}{\text{distance travelled by imidazole}}$.