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Determination and identification of pyrazole derivatives by gas chromatography–mass spectrometry

Pyrazole and its derivatives are potent inhibitors of liver alcohol dehydrogenase (LADH, E.C. 1.1.1.1.) *in vitro*[1] and *in vivo*[2,3], of yeast ADH[3-5], and of some other enzymes[6,7]. However, only little is known about the analysis and biotransformation of pyrazole and its derivatives. Some data concerning a spectrophotometric method for the determination of pyrazole have been given[8].

The present paper describes suitable conditions for the analysis of some pyrazole derivatives by gas chromatography–mass spectrometry.

Materials and methods

Chemicals. Pyrazole was supplied by Schuchardt Ltd., Munich. 4-Methylpyrazole (in hydrochloride form) and 4-iodopyrazole were kindly made available to us by Prof. HUGO THEORELL, Dept. of Biochemistry, Karolinska Institutet. The syntheses of the 4-substituted derivatives were performed by Dr. BERNDT SJÖBERG, Astra Ltd., Södertälje, according to THEORELL *et al.*[9].

The gas chromatograph. The apparatus was a Pye chromatograph series 104, fitted with a flame ionization detector, column temperature 120° or 200° (see below), injection port temperature 250°, detector temperature 280°. Nitrogen carrier gas, flow rate 40 ml/min.

The gas chromatography column. Chromosorb W, acid-washed, 100–120 mesh, coated with 5 % Carbowax 20M, was packed in a 1.7-m coiled glass column, I.D. 4 mm. The column was conditioned at 230° before use.

Gas chromatography–mass spectrometry. An LKB 9000 instrument for com-

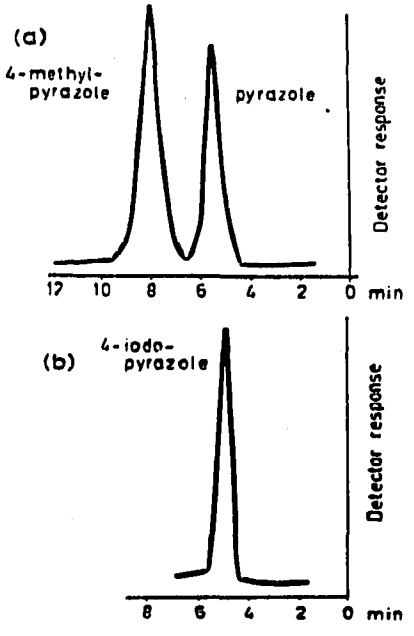

Fig. 1. (a) Gas chromatograms of pyrazole and 4-methylpyrazole; 120°. (b) Gas chromatogram of 4-iodopyrazole; 200°.

bined gas chromatography–mass spectrometry was used. The energy of the bombarding electrons was 22.5 eV and the temperature of the ion source was 290°.

The mass spectrometer data were stored on magnetic tape and were evaluated off-line in an IBM 1800 computer[10,11].

Results

Representative gas chromatograms of the different substances are shown in Fig. 1. Pyrazole and 4-methylpyrazole were separated on a column with 5 % Carbowax 20M on Chromosorb W at 120°, and 4-iodopyrazole was analyzed on the same column at 200°, isothermally. Methylene unit (MU) values[12] were determined for all the derivatives with aliphatic hydrocarbons as reference standards (Table I).

A solution of pyrazole in redistilled water gave a rectilinear detector response within the range of 10 ng–40 μg (n = 38) examined. The correlation coefficient of the rectilinear regression line in a log-log scale plot was 0.999 ± 0.007 (Fig. 2).

Structural confirmation of pyrazole and its derivatives was obtained by combined gas chromatography–mass spectrometry. The m/e values of significant mass peaks are shown in Table II. Loss of 27 mass units corresponds to loss of HCN or less probably, of C_2H_3. This may be compared with literature data for imidazole[13]. Spectra of pyrazole and 4-methylpyrazole are in agreement with published data[14], but no spectra of halogenated pyrazoles have been published earlier. The mass spectrum of 4-iodopyrazole is shown in Fig. 3. Loss of 128 mass units corresponds to loss of HI.

TABLE I

METHYLENE UNIT (MU) VALUES FOR PYRAZOLE AND ITS DERIVATIVES AS DETERMINED BY USING CARBOWAX 20M AS THE LIQUID PHASE

Substance	*MU values*
Pyrazole	17.98
4-Methylpyrazole	18.58
4-Iodopyrazole	25.02

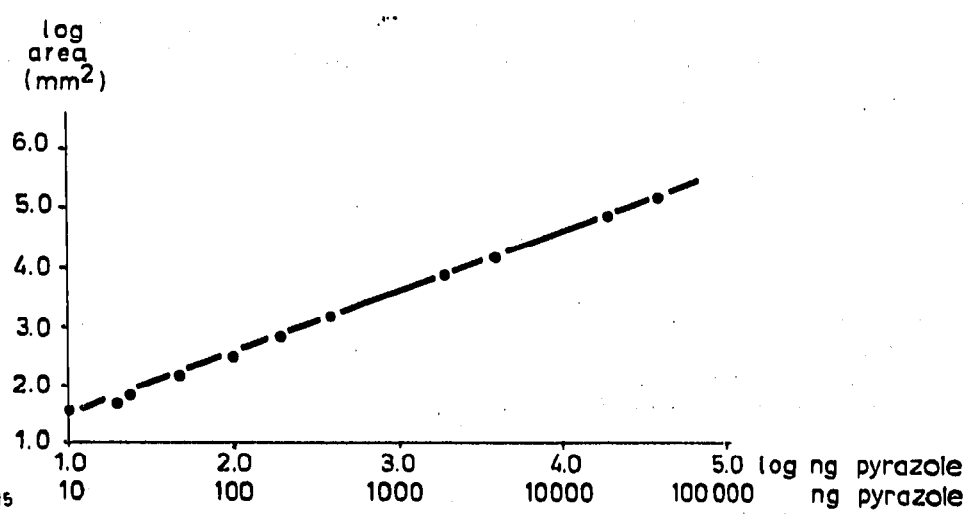

Fig. 2. Standard curve for the determination of pyrazole in a log-log scale.

Columns of Porapak Q or Polypak-1 50 % mixed with Chromosorb W have been tested for the same separation but gave less reproducible results and tailing[15].

TABLE II

MASS SPECTRA OF PYRAZOLE[a], 4-METHYLPYRAZOLE AND 4-IODOPYRAZOLE

Pyrazole							
17	(27)	18	(100)	39	(4)	40	(6)
41	(25)	42	(2)	68	(100)	69	(4)
4-Methylpyrazole							
17	(47)	18	(100)	51	(1)	52	(3)
53	(9)	54	(43)	55	(9)	80	(2)
81	(96)	82	(100)	83	(5)	85	(1)
4-Iodopyrazole							
39	(6)	40	(7)	41	(20)	47	(2)
66	(5)	67	(9)	68	(100)	69	(3)
194	(17)						

[a] Relative intensities are shown in parentheses (mean of 4–7 spectra).

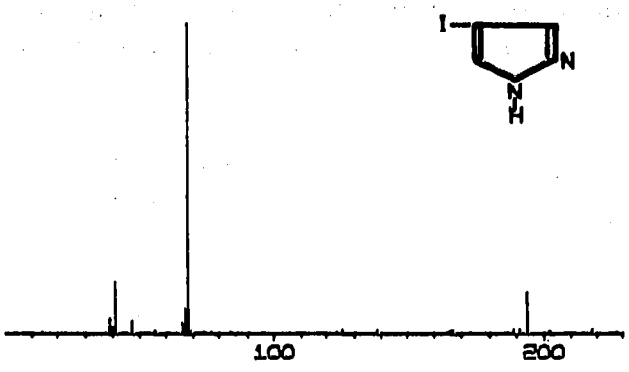

Fig. 3. Mass spectrum of 4-iodopyrazole.

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1 H. THEORELL AND T. YONETANI, *Biochem. Z.*, 338 (1963) 537.
2 D. LESTER, W. Z. KEOKOSKY AND F. FELZENBERG, *Quart. J. Stud. Alc.*, 29 (1968) 249.
3 L. GOLDBERG AND U. RYDBERG, *Biochem. Pharmacol.*, 18 (1969) 1749.
4 M. REYNIER, *Acta Chem. Scand.*, 23 (1969) 1119.
5 T. E. SINGLEVICH AND J. J. BARBORIAK, *Fed. Proc.*, 29 (1970) 275.
6 K.-H. KIESSLING AND U. RYDBERG, *Commun. Dept. Alcohol Res. Karolinska Inst.* 18.06/1969, 8 pp.
7 C. S. LIEBER, E. RUBIN, L. M. DE CARLI, P. MISRA AND H. GANG, *Lab. Invest.*, 22 (1970) 615.
8 D. LESTER AND G. D. BENSON, *Science*, 169 (1970) 282.
9 H. THEORELL, T. YONETANI AND B. SJÖBERG, *Acta Chem. Scand.*, 23 (1969) 255.

10 B. HEDFJÄLL, P.-Å. JANSSON, Y. MÅRDE, R. RYHAGE AND S. WIKSTRÖM, *J. Sci. Instr.*, 2/2 (1969) 1031.
11 R. REIMENDAL AND J. SJÖVALL, in *Proc. Int. Congr. Hormonal Steroids, 3rd, Hamburg, 7–12 Sept., 1970*, Excerpta Medica, Amsterdam, in press.
12 C. E. DALGLIESH, E. C. HORNING, M. G. HORNING, K. L. KNOX AND K. YARGER, *Biochem. J.*, 101 (1966) 792.
13 H. BUDZIKIEWICZ, C. DJERASSI AND D. H. WILLIAMS, *Mass Spectrometry of Organic Compounds*, Holden-Day, San Francisco, 1967.
14 A. COMM AND R. MASSOTT, *Compilation of Mass Spectral Data*, Heyden & Sons, London, 1966.
15 U. RYDBERG AND J. C. BUIJTEN, *Commun. Dept. Alcohol Res. Karolinska Inst.*, 25.07/1970, 5 pp.

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