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Note

Studies of pyrazines as their n–π charge-transfer complexes with some nitro aromatic compounds

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Pyrazines are flavour compounds formed in roasted foods as a result of the interaction of carbonyls with amino compounds. Different techniques such as gas chromatography (GC) and mass, infrared and nuclear magnetic resonance spectroscopy have been employed for their separation, identification and determination[1–5]. In GC it is difficult to resolve isomeric pyrazines using a single column. Dietrich and Mavier[6] employed paper chromatography for the separation of pyrazine carboxylic acids. Sizer[7] tried to separate some isomeric alkylpyrazines that are difficult to resolve by GC by employing thin-layer chromatography (TLC).

Even with TLC, in spite of employing different solvent systems, difficulties are encountered in the separation of pyrazines that have the same or very close R_F values.

Pyrazines are n–π donors and are known to form charge-transfer complexes with iodine[8]. Nitro aromatic compounds are powerful π-acceptors. Silica gel G impregnated with π-acceptors such as 2,4,6-trinitroanisole (TNA), 2,4,6-trinitrotoluene (TNT), 2,4,6-trinitrobenzene (TNB), picric acid (PA) and picramide (PM) has been used for the TLC separation of a variety of basic compounds[9–11].

In this work advantage was taken of n–π complexation of pyrazines with 2,4,6-trinitroresorcinol (TNR), picryl chloride (PC), 2,4,6-trinitrophenetole (TNP) and TNT for their separation on TLC plates. Pyrazines form complexes both in solution and on thin-layer plates impregnated with these nitro compounds. The complexation results in a distinct spectral shift in solution and a change in the R_F values. The resolution as n–π complexes of 25 pyrazines that are generally found in roasted foods has been readily effected. In particular, the separation of those pyrazines which have the same or close R_F values in different solvent systems has been achieved as a result of complexation. The complexes have distinct UV maxima.

Pyrazines could be detected on the TLC plates by spraying with Dragendorff's reagent[12] followed by 0.5% sulphuric acid with a detection limit of 2.5 μg, and determined after liberating them from the complexes with ethanol at levels down to 5 μg by using a UV spectrophotometer.

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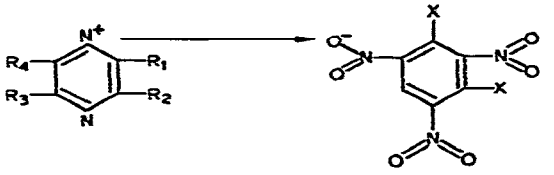

Pyrazine: Nitro aromatic compound
1:1 n–π complex
R_1, R_2, R_3, R_4 = H, alkyl, thioalkyl, alkoxy
X = H, OH, Cl, CH_3

EXPERIMENTAL

Authentic pyrazines (1–25, Table I) were received from Naarden International Research Centre (Naarden, The Netherlands) and Pyrazine Specialties (Atlanta, GA, U.S.A.). Styphnic acid, picryl chloride, trinitrotoluene and trinitrophenetole were of analytical-reagent grade.

Silica gel G (E. Merck, Darmstadt, G.F.R.) was used as the adsorbent. All solvents were dried and freshly distilled. The following eluting solvents were used with the ascending technique: I, chloroform–ethyl acetate–ethanol (60:38:2); II, benzene–acetone–ethanol (90:8:2).

Dragendorff's spray reagent was prepared as reported by Stahl[12].

A Perkin-Elmer Model 124 ultraviolet spectrophotometer was used for spectral measurements and a Chromatolite UV lamp (short-wave length radiation, 254 nm) for locating the compounds on the plates.

Preparation of plates, impregnation, spotting and elution

A fine slurry of silica gel G (50 g) in distilled water (100 ml) was poured on to glass plates (35 × 25 cm) and spread uniformly by tilting the plates from side to side. The plates were dried at room temperature overnight and activated at 110 °C in an oven for 1.5 h before use. The average coating thickness was 6.5 mg/cm^2. The plates were impregnated with a 0.01% solution of the nitro aromatic compound in carbon tetrachloride by the ascending technique. The plates were dried at room temperature and standard solutions of pyrazines (5 μg in 10 μl of carbon tetrachloride) were spotted. The compounds were also spotted on an untreated plate, which served as a control. The control plates were eluted with solvents I and II, but for the elution of the impregnated plates these solvents also contained 0.01% of the corresponding π-acceptor. After elution the plates were air dried. The compounds were located under UV light and by spraying with Dragendorff's reagent followed by 0.5% sulphuric acid, appearing as pink spots on both unimpregnated and impregnated plates. Fig. 1 shows two typical chromatograms obtained on an impregnated plate for some pyrazines with very close R_F values; on the picryl chloride-impregnated plate they were well resolved. Table I gives the R_F values of 25 pyrazines, and of n–π complexes with four acceptors in two solvent systems.

Ultraviolet spectra of n–π complexes in solution

Solutions of $1 \cdot 10^{-6}$ *M* of each of the pyrazines and nitro aromatic compounds were prepared in carbon tetrachloride. The solutions were mixed in various pro-

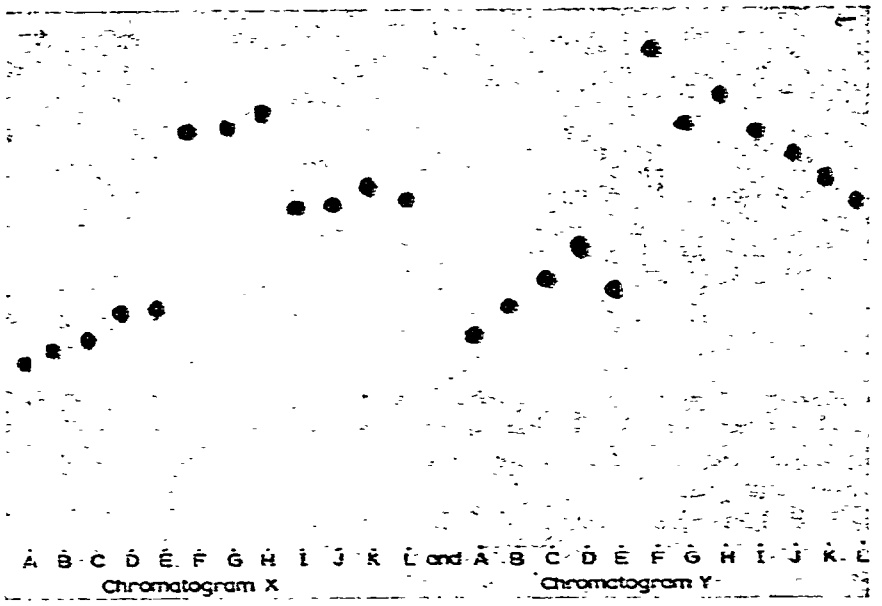

Fig. 1. Thin-layer chromatograms showing the separation of pyrazines having very close R_F values. Chromatogram X, without impregnation with picryl chloride; chromatogram Y, impregnated with picryl chloride. A = 2,3-Dimethylpyrazine; B = 2,5-dimethylpyrazine; C = 2,6-dimethylpyrazine; D = 2-ethyl-5-methylpyrazine; E = pyrazine; F = 2-methoxy-3-isopropylpyrazine; G = 2-methoxy-3-sec.-butylpyrazine; H = 2-methylthio-3-ethylpyrazine; I = 2-ethoxy-3-methylpyrazine; J = 2-acetyl-3-methylpyrazine; K = 2-propyl-3,6-dimethylpyrazine; L = 2-ethyl-6-methylpyrazine. Adsorbent: Silica gel G (E. Merck). Solvent system: Chloroform–ethyl acetate–ethanol (60:38:2) for chromatogram X; for Y the system also contained 0.01 % of picryl chloride.

portions and the UV spectrum of each mixture was recorded together with those of the individual compounds. Table I gives the $\lambda_{max.}$ valves of the n–π complexes and of the individual compounds. Figs. 2–5 show the absorption patterns of some typical n–π complexes.

Micro-determination of pyrazines

Amounts of 5 μg of each pyrazine were doubly spotted on two half-sides of a picryl chloride-impregnated plate and eluted with solvent I containing 0.01 % of this nitro compound. After elution the first half was sprayed with Dragendorff's reagent followed by 0.5% sulphuric acid. The spots corresponding to the first half of the plate were marked on the second half of the plate. The spots on the second half (unsprayed) portion were also visible under UV light. These were scraped with a micro-spatula into a sintered-glass funnel (I.D. 0.5 cm). Pyrazines were liberated from their complexes by treatment with ethanol, the pyrazines being found in the filtrate whereas the nitro compound remained adsorbed on the silica gel. The solvent was carefully removed from the filtrate under a partial vacuum, the residue was dissolved in carbon tetrachloride and the volume made up to 10 ml. The absorbance at the $\lambda_{max.}$ values were measured. The amounts were calculated from previously drawn standard absorption graphs for each pyrazine. The recovery of the pyrazines was 97–98% and the results were reproducible.

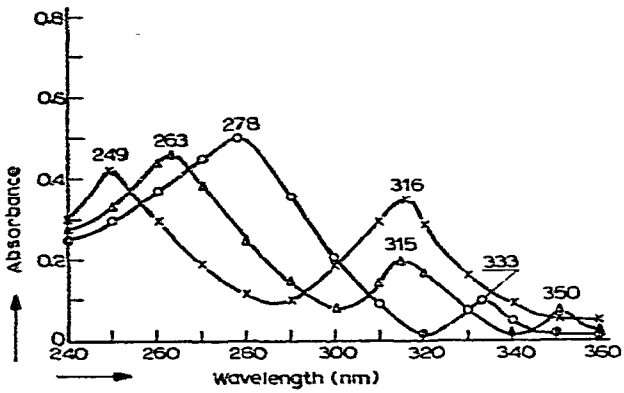

Fig. 2. UV spectra of 2-methylthio-3-isopropylpyrazine (×), styphnic acid (○) and their n–π complex (△).

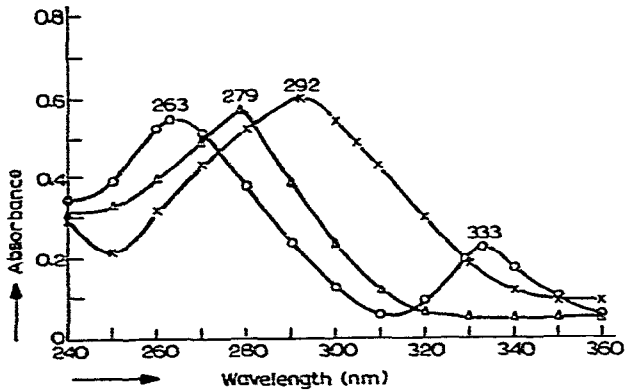

Fig. 3. UV spectra of 2-methoxy-3-ethylpyrazine (×), picryl chloride (○) and their n–π complex (△).

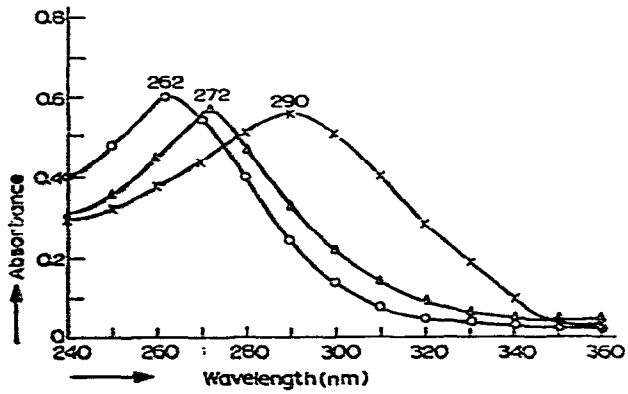

Fig. 4. UV spectra of 2-ethyl-5-methylpyrazine (×), TNT (○) and their n–π complex (△).

TABLE I

R_F VALUES OF PYRAZINES AND THEIR n–π COMPLEXES WITH STYPHNIC ACID, PICRYL CHLORIDE, TRINITROTOLUENE AND TRINITROPHENETOLE, AND ABSORPTION MAXIMA IN CARBON TETRACHLORIDE

Solvent systems: I = chloroform-ethyl acetate-ethanol (60:38:2); II = benzene-acetone-ethanol (90:8:2). Adsorbent: silica gel G (E. Merck). When the nitro compound-impregnated plates were eluted, the solvent also contained 0.01% of the respective nitro compound. $\lambda_{max.}$ values in carbon tetrachloride: styphnic acid, 278 and 333 nm; picryl chloride, 263 and 333 nm; trinitrotoluene, 262 nm; trinitrophenetole, 269 nm.

No.	*Compound*	*Pure pyrazines*			*$\lambda_{max.}$ of styphnic acid complexes in solution (nm)*	*R_F values on styphnic acid impregnated plate*		*$\lambda_{max.}$ of picryl chloride complexes in solution (nm)*	*R_F values on picryl chloride-impregnated plate*		*$\lambda_{max.}$ of trinitrotoluene complexes in solution (nm)*	*R_F values on trinitrotoluene-impregnated plate*		*$\lambda_{max.}$ of trinitrophenetole complexes in solution (nm)*	*R_F values on trinitrophenetole impregnated plate*	
		$\lambda_{max.}$ (nm)	*R_F values*													
			Solvent I	*Solvent II*		*Solvent I*	*Solvent II*		*Solvent I*	*Solvent II*		*Solvent I*	*Solvent II*		*Solvent I*	*Solvent II*
1	2-Methylpyrazine	266	0.30	0.35	274, 335	0.26	0.32	275	0.40	0.39	269, 315	0.41	0.43	260, 315	0.34	0.36
2	2,3-Dimethylpyrazine	274	0.36	0.37	271	0.38	0.35	270, 310	0.41	0.40	271, 309	0.43	0.40	271, 310	0.39	0.40
3	2,5-Dimethylpyrazine	275	0.38	0.39	276	0.40	0.37	270, 310	0.45	0.42	272, 314	0.46	0.45	272, 314	0.43	0.42
4	2,6-Dimethylpyrazine	275	0.40	0.39	276, 334	0.42	0.39	280	0.49	0.44	271, 314	0.49	0.48	270, 313	0.44	0.43
5	2-Ethyl-5-methylpyrazine	290	0.44	0.52	272, 335	0.53	0.57	275	0.53	0.58	272	0.52	0.59	273	0.48	0.49
6	2,3,5-Trimethylpyrazine	278	0.36	0.55	273, 335	0.40	0.61	274	0.44	0.63	271	0.40	0.50	282	0.32	0.59
7	2,3,5,6-Tetramethylpyrazine	279	0.41	0.37	281, 337	0.54	0.32	282	0.46	0.42	282	0.45	0.41	284	0.37	0.38

8	2-Methoxy-3-methylpyrazine	291	0.82	0.56	280, 340	0.91	0.50	293	0.86	0.66	279	0.71	0.51	286	0.89	0.69
9	2-Methoxy-3-isobutylpyrazine	290	0.87	0.70	280, 335	0.92	0.74	279	0.83	0.95	280	0.79	0.60	281	0.84	0.67
10	Pyrazine	261	0.44	0.51	274	0.40	0.55	270	0.46	0.56	263, 321	0.37	0.44	258, 316	0.40	0.46
11	2-Ethylpyrazine	266	0.58	0.50	270, 335	0.61	0.62	269	0.61	0.64	267, 315	0.53	0.42	268, 315	0.65	0.60
12	2-Methoxy-3-ethylpyrazine	292	0.90	0.81	276, 335	0.93	0.75	279	0.80	0.93	280	0.84	0.73	278	0.85	0.77
13	2-Methoxy-3-isopropylpyrazine	280	0.80	0.64	276, 331	0.84	0.84	275	0.93	0.58	275	0.75	0.56	274	0.77	0.59
14	2-Ethyl-3-methylpyrazine	274	0.48	0.37	270, 333	0.43	0.47	270	0.53	0.34	270	0.34	0.40	270, 312	0.52	0.41
15	2-Acetylpyrazine	271	0.75	0.34	268	0.78	0.40	266, 310	0.79	0.37	264	0.70	0.38	270, 310	0.70	0.38
16	2-Ethoxy-3-ethylpyrazine	285	0.86	0.60	280, 335	0.80	0.72	280	0.90	0.55	280	0.59	0.63	280	0.82	0.55
17	2-Ethoxy-3-methylpyrazine	290	0.66	0.53	277, 336	0.56	0.64	276	0.78	0.50	280	0.51	0.56	278	0.60	0.50
18	2-Methoxy-3-*sec.*-butyl pyrazine	280	0.80	0.65	284, 335	0.76	0.81	290	0.81	0.60	274	0.76	0.55	276	0.74	0.61
19	2-Acetyl-3-methylpyrazine	274, 222	0.67	0.53	277, 331	0.50	0.63	270	0.74	0.49	259, 312	0.59	0.45	271	0.61	0.59
20	2-Methylthio-3-ethylpyrazine	243, 316	0.81	0.66	275, 333	0.67	0.84	256, 317	0.85	0.56	259, 316	0.77	0.54	260, 315	0.78	0.61
21	2-Methylthio-3-isopropyl-pyrazine	249, 316	0.92	0.72	263, 315, 350	0.84	0.96	260, 315	0.88	0.62	260	0.88	0.58	317	0.86	0.76
22	2-Butyl-3-methylpyrazine	270	0.66	0.47	276, 310	0.76	0.63	266, 310	0.73	0.43	280	0.59	0.36	270, 312	0.61	0.40
23	2-Propyl-3,6-dimethylpyrazine	279	0.69	0.51	274, 331	0.63	0.66	284	0.70	0.46	284	0.61	0.42	284	0.60	0.44
24	2-Ethoxy-3-isopropylpyrazine	220, 278	0.96	0.81	271, 333	0.90	0.88	280	0.88	0.74	280	0.93	0.66	280	0.92	0.85
25	2-Ethyl-6-methylpyrazine	276	0.68	0.51	270, 333	0.52	0.63	282	0.66	0.52	272, 310	0.58	0.37	272, 310	0.64	0.53

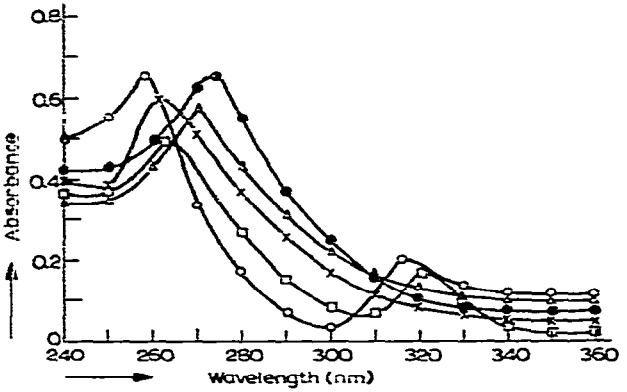

Fig. 5. UV spectra of pyrazine (×) and n–π complexes of pyrazine with styphnic acid (●), picryl chloride (△), TNT (□) and trinitrophenetole (○).

RESULTS AND DISCUSSION

As is evident from Table I, pyrazines form charge-transfer complexes with nitro aromatic compounds both in solution and on TLC plates, which is reflected in the spectral shifts and in the R_F values. For most of the compounds a large spectral shift is accompanied by a significant change in their R_F values.

Pyrazines are n donors. The nitrogen atoms in the ring deactivate all of the ring carbon atoms towards electrophilic substituents and activate them towards nucleophilic substituents. Hence nucleophilic substituents such as alkyl, alkoxy and thioalkyl groups on the pyrazine ring further modify the donor capacity of the molecule.

Of the four nitro aromatic compounds tried as acceptors, *viz.*, styphnic acid, PC, TNT and TNP, PC gave the best separation of the complexes. The chlorine atom attached to the nitro-aromatic ring further enhanced the acceptor properties.

Theoretically, pyrazines could form 1:1, 1:2 and 2:1 complexes with nitro aromatic compounds. From a Job's plot in solution it was observed that all of the pyrazines formed only 1:1 complexes. In some instances the complexes showed more than one absorption band in solution. This could be due to multiple charge transfer occurring between the closely related filled orbitals of the donor molecule and closely related unfilled orbitals of the acceptor molecule.

Silica gel G was suitable as an adsorbent. Of the two solvent systems examined, solvent I gave better resolutions of the complexes. The compounds could be detected with Dragendorff's reagent both on unimpregnated plates and on plates impregnated with nitro aromatic compounds.

This property of n–π complexation on TLC plates was found to be of great utility in the resolution of those pyrazines which had either identical or very close R_F values. For example, the following groups of pyrazines had very close R_F values in both solvent systems (Table I). Group I —2,3-dimethylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine; Group II —2-ethyl-5-methylpyrazine, pyrazine; Group III —2-methoxy-3-isopropylpyrazine, 2-methoxy-3-*sec.*-butylpyrazine; 2-methylthio-3-ethylpyrazine; Group IV —2-ethoxy-3-methylpyrazine, 2-acetyl-3-meth-

ylpyrazine, 2-propyl-3,6-dimethylpyrazine, 2-ethyl-6-methylpyrazine. However, as can be seen from Fig. 1, as the n–π complexes they gave very distinct separations.

The procedure described is very useful for the separation of pyrazines that are difficult to resolve. It is possible to detect and determine them with limits of 2.5 and 5 μg, respectively.

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