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VOLATILE BENZOTRIAZOLE DERIVATIVES OF THE NITRITE ION

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SUMMARY

A method for determining low concentrations (< 1 μg/ml) of nitrite by gas chromatography, based on a derivatisation reaction with aryl 1,2-diamines is examined. The synthesis, chromatographic behaviour, retention data and detection limits for flame ionization detection, alkali flame ionization detection and electron-capture detection are reported for thirteen benzotriazole derivatives. Strong interaction of the derivatives with the column are minimised by suitable selection of deactivated support, stationary phase and reagent.

INTRODUCTION

Chromatographic techniques for determining nitrite in water include high-performance liquid chromatography (HPLC)[1,2], ion chromatography (IC)[3–6] and gas chromatography (GC)[7–18]. Of these, IC appears the most suitable when other anions, such as nitrate, chloride, sulphate and phosphate are to be determined simultaneously. However, as nitrite is unstable it is necessary that analyses be carried out quickly after sampling, while oxidation of this ion in the IC suppressor column[6] poses an additional problem. HPLC with UV detection has been applied, though not extensively, for determining nitrite both with[1] and without[2] derivatization. In GC, derivatization to a volatile form is essential but high selectivity and sensitivity can be achieved.

In the past, several independent approaches have converted nitrite to benzotriazole[16–18] for subsequent quantification by GC. Other methods investigated include conversion to nitrobenzene[7–11], nitrogen[12], aryl halides[13], a substituted benzene[14] and tetrazolophthalazine[15]. Akiba *et al.*[16] utilised derivatization to 1H-benzotriazole to determine relatively high concentrations of nitrite in water (20–160 μg/ml) using thermal conductivity detection (TCD). The calibration curves presented, however, indicated irreversible adsorption of the derivative below the 10 μg/ml level). The use of the more sensitive flame ionization detection (FID), alkali flame ionization detection (AFID), and electron-capture detection (ECD) for nitrite concentrations below

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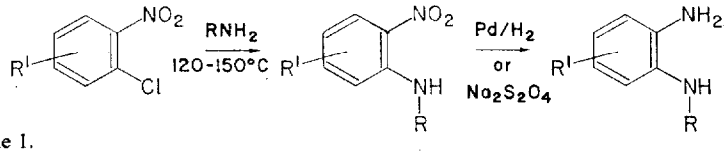
1 μg/ml becomes possible only if the benzotriazoles can be eluted satisfactorily at sub-nanogram levels. One method for overcoming the limitations of the polar 1H-benzotriazole employed a two-stage derivatization[17] procedure whereby the extracted derivative was silylated to form 1-trimethylsilylbenzotriazole prior to GC.

This paper is concerned with the derivatization reaction involving the conversion of nitrite to a benzotriazole. It is shown that with suitable choice of reagent, column conditions and detector, picogram amounts of nitrite can be detected.

EXPERIMENTAL

Synthesis

Aryl diamines (I, R = H) were obtained from commercial sources or prepared from the corresponding *o*-halogenated nitrobenzene[19] as shown in Scheme 1 (I, R = CH_3, C_2H_5, iso-C_3H_7, *tert.*-C_4H_9; R^1 = H, Cl, Br, CF_3) and stored as the stable, water-soluble hydrochloride salts. For analytical purposes, the salts were recrystallized from water (under nitrogen) before use. Benzotriazoles were prepared by condensation of I with nitrite in aqueous solution[20], extracted with dichloromethane and eluted from silica gel columns with ethyl acetate–dichloromethane mixtures. Solid products were purified by recrystallization from ethanol–water and liquids by vacuum distillation at 1–2 mmHg. The derivatives, listed below, were colourless and pure, single compounds as established by thin-layer chromatography (TLC), mass spectrometry (MS) and elemental analysis.



Scheme 1.

Derivatives: 1H-benzotriazole (BT), m.p. 102°C; 1H-6-chlorobenzotriazole (ClBT), m.p. 149°C; 1H-6-nitrobenzotriazole (NO_2BT), m.p. 212°C; 1H-5,6-dichlorobenzotriazole (Cl_2BT), m.p. 264°C; 6-trifluoromethylbenzotriazole (CF_3BT), m.p. 134°C; 1-methylbenzotriazole (N(M)BT), b.p. 98°C (1 mmHg); 1-ethylbenzotriazole (N(Et)BT), b.p. 112°C (2 mmHg); 1-isopropylbenzotriazole (N(*i*-Pr)BT), b.p. 119°C (2 mmHg); 1-*tert.*-butylbenzotriazole (N(*t*-Bu)BT), b.p. 86°C (1 mmHg); 1-ethyl-6-chlorobenzotriazole (N(Et)ClBT), b.p. 98–102°C (1 mmHg); 1-ethyl-6-bromobenzotriazole (N(Et)BrBT), m.p. 86°C; 1-ethyl-6-trifluoromethylbenzotriazole (N(Et)CF_3BT), m.p. 92°C; 1-ethyl-5,6-dichlorobenzotriazole (N(Et)Cl_2BT), m.p. 106°C.

Instrumentation

A Varian 1400 gas chromatograph fitted with a flame ionization detector and Hewlett-Packard 3380 Integrator and a Packard Becker 427 instrument fitted with AFID and ECD instruments were used for the study. Columns employed were silanized borosilicate glass coils (5 ft. × 1/4 in. O.D.) packed with 10% OV-1 on Chromosorb W, Chromosorb 750, Gas Chrom Q or Ultrabond 20M (all 80–100 mesh). Carrier gas was high purity nitrogen (30 ml/min). Both injection and detector

temperatures were 200°C and column temperature was held at various settings between 120 to 200°C. Identification of chromatographic peaks was accomplished with a Varian MAT 112 GC–MS system employing a similar chromatographic column. Other columns evaluated were borosilicate glass (1 m × 4 mm I.D.) packed with Tenax GC (80–100 mesh), 3% OV-25, 3% OV-210, 3% OV-225, 3% Carbowax 20M, 3% OV-330, 3% OV-275 or 3% DEGS on Gas Chrom Q. All columns were silanized at 200°C by on-column injection of Silyl-8 (Pierce, 10 × 10 μl) and conditioned at suitable temperatures between 230 and 340°C for at least 12 h before use.

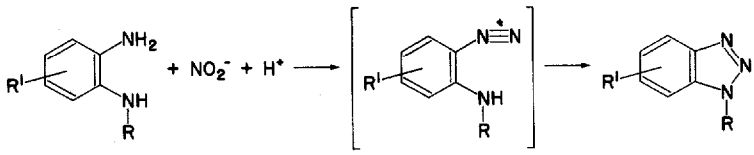
Derivatization and extraction procedure

The aqueous standards or samples of water were adjusted to pH 4–5 by addition of dilute acetic acid or sodium acetate solution and an aliquot (2 ml) treated with 2 ml of the diamine hydrochloride reagent (4%, w/v). The mixture were allowed to stand (15 min), extracted with carbon disulphide (2 ml) and the extracts (2 μl) examined by GC–FID. For quantitative work, *n*-pentadecane at an appropriate concentration was employed as internal standard.

For preliminary GC studies, pure benzotriazoles were prepared initially as solutions (0.1%, w/v) in carbon disulphide, dichloromethane or benzene and diluted as required.

RESULTS AND DISCUSSION

The derivatization reaction is depicted in Scheme 2 and involves formation of a diazonium ion (II) which then cyclizes to a benzotriazole (III) in acid medium[20]. In the absence of an ortho-amino group, II can undergo other reactions typical of



(I) (II) (III)

Scheme 2.

the diazonium ion. Indeed, several of these reactions have been utilised in GC derivatization and include pyrolysis to nitrogen[12], conversion to an aryl halide (by the Sandmeyer reaction)[13] and reduction to a substituted benzene[14]. On the other hand, benzotriazole formation has been applied to determining nitrite by other techniques including UV absorbance[21], spectrofluorimetry[21,22] and HPLC[1,2]. In GC, this derivatization reaction has several attractive features including high selectivity, ease of separating derivative from excess reagent and a potential for varying ring substituents to control reagent reactivity, extraction characteristics and GC retention, and detection sensitivity. The selectivity of the reaction shown in Scheme 2 is illustrated by the fact that selenite is the only other common inorganic anion which condenses with the diamine reagent, I[2,23–26]. Although some organic species, such as 1,2-dicarbonyls (*e.g.* dehydroascorbic acid) condense with these reagents[27], extraction and GC provide a high degree of selectivity.

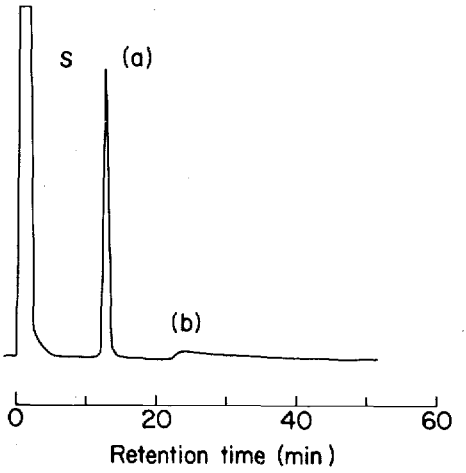


Fig. 1. Chromatogram of a mixture (0.1 μg each) of N(Et)BT (a) and BT (b) on the OV-1 column at 150°. Solvent: dichloromethane (S). Support: Gas Chrom Q. (Other conditions as in Experimental.)

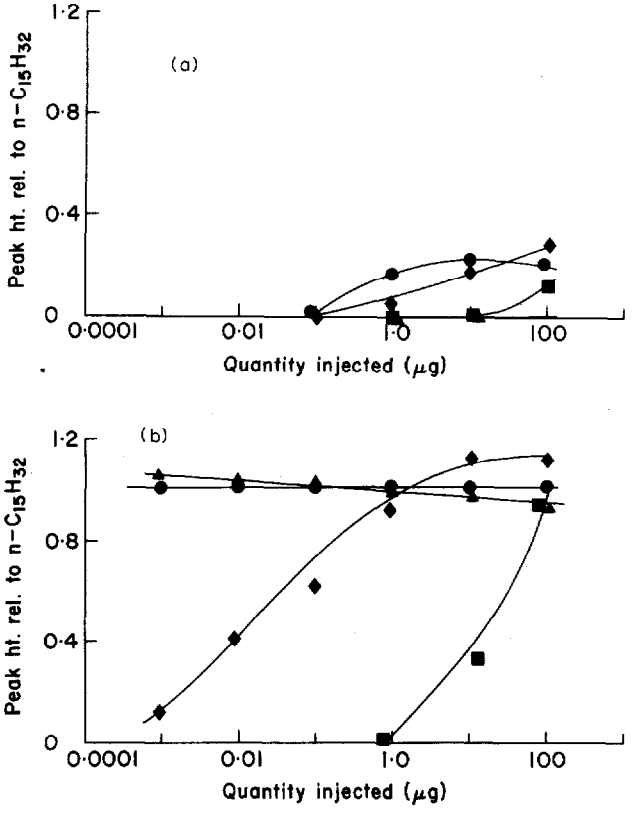
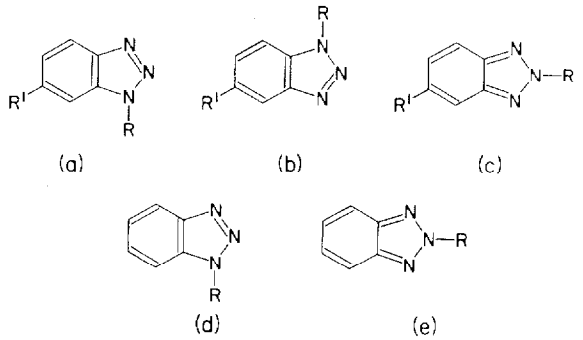


Fig. 2. Response of (a) BT and (b) N(Et)BT relative to an equal quantity of pentadecane on OV-1 on various supports. Identities are Chromosorb 750 (◆); Gas Chrom Q (●); Ultrabond (▲) and Chromosorb W (■). Other conditions as in Fig. 1.

The benzotriazoles examined were all stable and volatile at 200–300°C as established by thermal analysis, and were expected to chromatograph readily at these temperatures. Because of their polar nature, however, the derivatives were difficult to elute at submicrogram levels on conventional packed columns. For example, on deactivated non-polar columns, together with several prepared from Ultrabond 20M[28,29], certain derivatives (III, R = H) tailed severely and were irreversibly adsorbed at levels of 0.5–1 μg. This is illustrated in Figs. 1 and 2 for the parent compound BT. However, improved peaks were obtained with highly polar stationary phases such as OV-225, OV-275, OV-330, DEGS and Carbowax 20M.

Although tailing persists on the polar columns, BT, ClBT, NO_2BT, Cl_2BT and CF_3BT were readily detected at the nanogram level. Poor peaks were obtained with other stationary phases such as Porapak Q and Tenax GC, phases of medium polarity (OV-25 and OV-210) and with OV-225 containing added phosphoric acid (1%, w/w). Of the polar phases, Carbowax 20M produced least tailing, but unacceptable bleeding and decomposition of the stationary phase were observed at 230°C. The most suitable polar phase for this application was OV-330. Retention data and FID detection limits for the acidic benzotriazoles are given in Table I.

As previously stated, the poor peaks obtained for BT have been overcome by its conversion to the N-silyl derivative[17]. Our attempts at alkylating BT and its analogues with ethyl iodide–potassium carbonate or dimethyl sulphate–potassium carbonate to produce N-alkyl derivatives (III, R = C_2H_5 or CH_3, respectively), improved peak shapes also but produced two or three N-alkyl derivatives readily separated by GC* as shown in Fig. 3. Generally, three isomers (a), (b) and (c) are obtainable, but where positions 1 and 3 are equivalent only two isomers, (d) and (e) form.



Clearly, a two-stage derivatisation is not satisfactory. However, by incorporating an N-alkyl substituent in I it becomes possible to carry out the derivatisation in a single step. With one exception**, reagents of this type are not currently available commercially but can be prepared as shown in Scheme 1. As expected derivatives

* These isomers result from the alkylation of any one of the three nitrogens in the triazole ring, as was verified for N(Et)BT and N(Et)Cl_2BT by separating the two forms on silica gel columns and characterising them by elemental analysis, and ^1H nuclear magnetic resonance (NMR).

** The reagent N-methyl-*o*-phenylenediamine dihydrochloride is available from Eastman, Rochester, NY, U.S.A.

TABLE I

RETENTION DATA AT 220°C AND FID DETECTION LIMITS FOR THE ACIDIC BENZOTRIAZOLES ON OV-330

Derivative	*Retention time (min)*	*Detection limit (ng)*
BT	3.8	1
ClBT	8.3	4
Cl_2BT	21.5	50
CF_3BT	3.4	1.5
NO_2BT	30.3	50

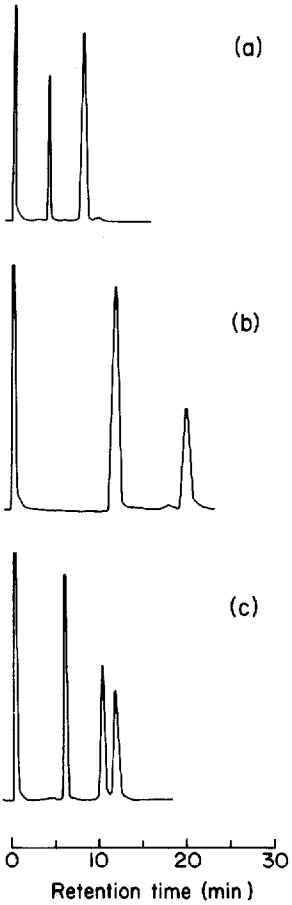


Fig. 3. Chromatograms, showing the separation of N-ethyl isomers of BT (a), Cl_2BT (b) and ClBT (c) obtained in the reaction with ethyl iodide–potassium carbonate. Column temperatures were 150, 160 and 160°C, respectively. Other conditions as in Fig. 1. Prior separation of N(Et)BT isomers and examination by NMR identified the first peak as 2-ethylbenzotriazole and the second peak as 1-ethylbenzotriazole.

with N-alkyl groups ($R = CH_3$, C_2H_5, iso-C_3H_7 or *tert.*-C_4H_9) gave sharp, symmetrical peaks (see Fig. 1 for N(Et)BT) and are the preferred derivatives. Although improved peaks are obtained for these derivatives, highly deactivated columns are required since adsorption effects persist on some columns as illustrated in Fig. 2. In our hands, columns prepared from Gas Chrom Q support repeatedly gave the sharpest, most symmetrical peaks and were used in all subsequent studies reported herein.

Retention data for the N-alkyl derivatives on the non-polar OV-1 phase are given in Table II and show the effect of different substituents on retention. For the N-ethyl series, relative retentions were consistent with the volatilities found by thermogravimetric analysis in that greater volatility resulted in shorter retention. On the other hand, for the series N(Me)BT, N(Et)BT, N(*i*-Pr)BT and N(*t*-Bu)BT the relative retention and volatility were:

$$\text{N}(t\text{-Bu})\text{BT} > \text{N}(i\text{-Pr})\text{BT} > \text{N(Et)BT} > \text{N(Me)BT}$$

that is, relative retention is the reverse of that predicted from the volatility data.

TABLE II

RETENTION DATA AT 150° FOR ALKYLATED BENZOTRIAZOLES ON OV-1

Derivative	*Retention time (min)*	*Retention index*
N(Me)BT	10.6	1340
N(Et)BT	13.6	1390
N(*i*-Pr)BT	16.5	1430
N(*t*-Bu)BT	22.0	1490
N(Et)ClBT	26.0	1530
N(Et)CF_3BT	11.8	1360
N(Et)BrBT	45.4	1650
N(Et)Cl_2BT	60.6	1720

Detection limits (for three detectors) are given in Table III and show, with one exception, that all values are similar and in the nanogram to picogram range. Several features of these data, however, require comment. Firstly, AFID and ECD, although not generally more sensitive to benzotriazoles than FID, are more selective and potentially more useful for analytical application. Another feature is that benzotriazoles, even those without halogen substituents, are readily detected by ECD. The triazole ring, therefore, seems an efficient electron-capturing moiety and this property can be advantageous in the GC determination of benzotriazoles.

In applying the derivatisation and extraction procedure described in the Experimental section, linear calibration curves were obtained for the range 0 to 1.0 μg/ml nitrite in water, using FID and the derivatives N(Me)BT, N(Et)BT and N(Et)CF_3BT (see Fig. 4). The analyte peaks were identified by GC–MS using electron impact ionisation and produced abundant molecular ions at *m/z* 133, 147 and 215, respectively.

Peaks additional to those of the analyte were frequently observed in the chromatograms and originate from the reagent and by-products of the derivatization. Similar, extraneous peaks have also been observed in the related GC methods for

TABLE III

FID, AFID AND ECD DETECTION LIMITS FOR THE N-ALKYLATED BENZOTRIAZOLES ON OV-1

Column temperature was adjusted between 150–200°C for each compound to give a retention in the range 8–12 min. ECD temperature was 300°C.

Derivative	*Detection limits (ng)*		
	FID	*AFID*	*ECD*
N(Me)BT	0.05	0.2	0.02
N(Et)BT	0.05	0.2	0.02
N(*i*-Pr)BT	0.03	0.1	0.01
N(*t*-Bu)BT	0.03	0.1	
N(Et)ClBT	0.08	0.1	0.01
N(Et)CF_3BT	0.03	0.2	0.02
N(Et)BrBT	0.1	0.4	0.0001
N(Et)Cl_2BT	0.1	0.4	0.02

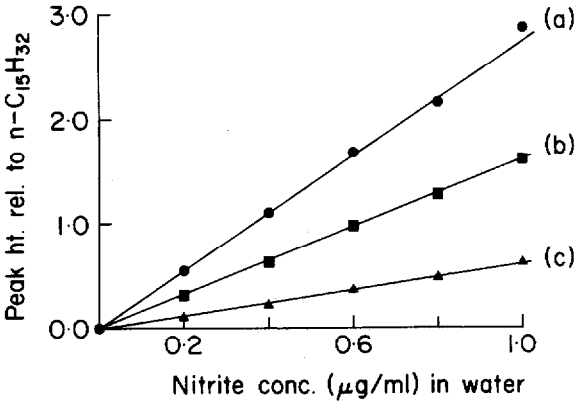
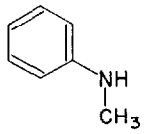


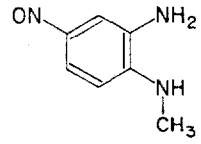
Fig. 4. Calibration curves for nitrite ion as N(Et)CF_3BT (a), N(Et)BT (b) and N(Me)BT (c). Derivatization and extraction procedure as given in Experimental section and GC conditions as in Fig. 1.

selenium[30–32] and require that steps be taken to reduce their number and intensity. Suitable precautions involve purifying the reagents and storing them away from air and light. Peaks due to the diamine reagent and by-products[33] can be minimised by extracting the derivative from an acid medium. A pH of 1–3 was satisfactory for the more basic diamines but higher acidity was found necessary for less basic reagents. For example, pH 3 was adequate for extracting derivative from excess reagent in the case of N(Me)BT and N(Et)BT but was inadequate for N(Et)CF_3BT. In moderately strong (2 *M*) hydrochloric acid, extraction of N(Me)BT is also incomplete so a compromise in the acidity is required. A detailed study of the extraction properties of suitable reagents and their derivatives is therefore a necessary aspect of further method development.

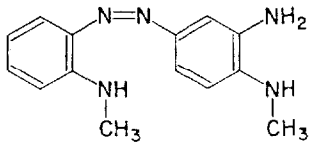
By-products obtained in the formation of the derivative, N(Me)BT, were identified by GC–MS. The main compounds obtained were IV and V which contributed about 5% of the total FID response. IV is thought to form by reduction of diazonium



(IV)



(V)



(VI)

ion II[14] by the diamine reagent, while V is a C-nitroso compound or the isomeric N-nitroso intermediate of II. Another minor component VI, probably formed by the normal coupling reaction of II with diamine reagent I, was also identified by MS following its separation from the extract by TLC. A further source of extraneous peaks can originate from the sample itself. This can occur because nitrite lability precludes prior treatment of samples (*e.g.*, with ammonium persulphate, or nitric and perchloric acids) to remove organic substances which may otherwise be co-extracted with the benzotriazole derivative and interfere with its determination.

In summary, aryl 1,2-diamines appear promising as reagent for the selective determination of trace levels of nitrite by GC. Highly deactivated columns are required in order to produce sharp chromatographic peaks where the sensitive and selective ECD can be used to advantage. The use of fused-silica capillary columns[34] may be expected to improve the GC of these compounds.

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