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SYNTHESIS, GAS–LIQUID CHROMATOGRAPHIC ANALYSIS AND GAS CHROMATOGRAPHIC–MASS SPECTROMETRIC IDENTIFICATION OF NITROVANILLINS, CHLORONITROVANILLINS, NITROGUAIACOLS AND CHLORONITROGUAIACOLS

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SUMMARY

The synthesis of guaiacols and vanillins bearing nitro, or nitro and chloro, substituents is described. A procedure for analysis of these and other nitrophenolic compounds by gas–liquid chromatography after derivatization is given. Attention is directed to problems in the preparation and stability of the O-acetates and O-ethyl ethers due to their susceptibility to hydrolysis: the O-acetates were found to be the most suitable derivatives for quantitative analysis. Recoveries from aqueous solutions of >95% were achieved by extraction with *tert.*-butyl methyl ether or by adsorption to C_{18} Bond Elut columns. Mass spectra, electron impact and negative chemical ionization were examined and the diagnostic interpretation of the spectra is discussed.

INTRODUCTION

Kraft pulp produced by the sulphate process is generally brightened by removal of residual lignin. This is often accomplished by use of molecular chlorine, and results in the production of several hundred organochlorine compounds[1], including chloroguaiacols, chlorocatechols and chlorovanillins. On account of the potential hazard of discharging bleachery effluents into the aquatic environment[2], efforts have been directed to the use of delignification agents other than molecular chlorine.

The prenox process[3] is based upon the use of oxides of nitrogen to carry out delignification, and although the action of nitric acid on lignin was briefly noted as long ago as 1918[4], there have since been only sporadic studies on the nitration of lignin and lignin-related compounds[5,6]. By analogy with the reactions of molecular chlorine, it may be predicted that two series of reactions will occur: (i) nitration of the lignin residues with partial oxidation of the C_3 residues and formation of nitrovanillins and (ii) nitration with concomitant elimination of the C_3 residues and formation of nitroguaiacols. Subsequent chlorination of residual nitro compounds may then give rise to a range of guaiacols and vanillins containing both chloro and nitro groups. On the other hand, in contrast to reactions which take place during chlorination, dealkylation and formation of polynitrated products are probably of minor significance.

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We have developed experimental procedures for assessing the impact of industrial discharge on the aquatic environment[2]. As a prerequisite to the application of these procedures to effluents from the prenox process, we required analytical and identification procedures for all the compounds likely to be encountered, together with their potential microbial metabolites. In view of the complex range of organic compounds occurring in extracts of such samples, it is necessary either to introduce a purification step or to use derivatized samples: by choice of appropriate derivatives, purification is automatically and conveniently introduced.

In this communication, we describe: (i) the synthesis of the relevant vanillins and guaiacols containing nitro, and nitro and chloro substituents, (ii) analytical procedures for quantification which take into account certain inherent problems in extraction of these relatively water-soluble compounds from aqueous solutions and (iii) procedures for conclusive identification of all of these compounds based on mass spectrometry (MS) in electron impact (EI) and negative chemical ionization (NCI) modes.

To the best of our knowledge, none of these problems has been systematically investigated previously, though procedures for individual compounds[7,8] and mass spectra (NCI) of a restricted range of nitrophenols[9] have been published.

EXPERIMENTAL

Substrates

The chloronitrophenols were gifts from ICI, Agrochemicals Division (Jealott's Hill, U.K.); 2- and 4-nitrophenol and 4-nitrocatechol were obtained from Merck (Darmstadt, F.R.G.), 3-nitrophenol from Janssen (Beerse, Belgium) and 2,4-dinitrophenol from Serva (Heidelberg, F.R.G.). Other compounds were synthesized as described below.

Solvents and reagents

Fuming nitric acid (specific gravity 1.52), diethyl sulphate and acetic acid were obtained from Merck, *tert.*-butyl methyl ether, dimethylformamide and hexane from Burdick & Jackson (Muskegon, MI, U.S.A.), pyridine, acetyl chloride, tetramethyl- and tetrabutylammonium hydroxide, guaiacol, vanillin and veratrole from Janssen, acetic anhydride and ethyl iodide from Fluka (Buchs, Switzerland) and 2,6-dimethoxyphenol from EGA (Steinheim, F.R.G.).

Synthesis of substrates

The structures and ring position numbering of the unsubstituted compounds are given in Fig 1.

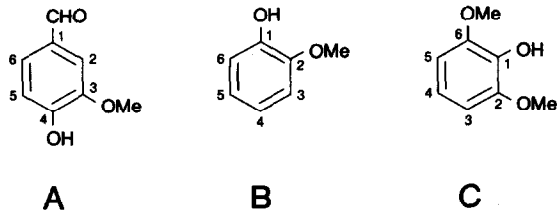

Fig. 1. Structure and ring numbering of vanillin (A), guaiacol (B) and syringol (C). Me = Methyl.

5-Nitrovanillin was prepared as follows. Vanillin (Fig. 1A) (10 g) was dissolved in acetic acid (*ca.* 30 ml), and a solution of fuming nitric acid (3 ml) in acetic acid (20 ml) slowly added with stirring. The mixture was poured into water, the product was removed by filtration, washed with water and recrystallized from acetic acid (*m/z* of the parent ion of the O-acetate $C_{10}H_9NO_6$: 239).

The compunds 2- and 6-nitrovanillin were prepared by the method of Raiford and Stoesser[10]. 2-Nitrovanillin was recrystallized from benzene, and 6-nitrovanillin from acetic acid (*m/z* of the O-acetates as for the 5-nitro isomer).

4,6-Dinitroguaiacol was prepared from guaiacol (Fig. 1B) by the procedure used for 5-nitrovanillin. The product, which was almost black, was purified by repeated recrystallization from acetic acid (*m/z* of parent ion of the O-acetate $C_9H_8N_2O_7$: 256).

3,5-Dinitroguaiacol was prepared as follows. Guaiacol was acetylated with acetic anhydride in refluxing acetic acid (1 h), the mixture poured into water and the product extracted with *tert.*-butyl methyl ether. Nitration was carried out by the procedure used for 2-nitrovanillin and, after hydrolysis with methanolic KOH, the product was recrystallized from benzene–cyclohexane (1:1) (*m/z* of the O-acetate as for the 4,6-dinitro isomer). The orientation of the nitro groups was unambiguously shown from the identity of the mass spectrum of the O-methyl ether with that of 3,5-dinitroveratrole prepared from 4,6-dinitroguaiacol (*m/z* for $C_8H_8N_2O_6$: 228).

4,5-Dinitroguaiacol was prepared from 4,5-dinitroveratrole by the method of Ehrlich and Bogert[11] and recrystallized from toluene. The parent ion of the O-acetate had *m/z* values identical to those of 3,5- and 4,6-dinitroguaiacol, whereas the mass spectrum of the O-methyl ether (veratrole) had a different gas–liquid chromatographic (GC) retention time and mass spectrum from that of 3,5-dinitroveratrole.

3,5-Dinitrosyringol was prepared from syringol (Fig. 1C) by the procedure used for 3,5-dinitroguaiacol and was recrystallized from benzene–cyclohexane (1:1) (*m/z* of the parent ion of the O-acetate $C_{10}H_{10}N_2O_8$: 286).

4-Chloro-3-nitrosyringol was prepared as follows. Syringol was chlorinated (*ca.* 1 h) in refluxing diethyl ether with sulphuryl chloride (1.1 mol), solvent removed and the product acetylated with acetic anhydride in refluxing acetic acid. Nitration and hydrolysis were carried out as for the synthesis of 3,5-dinitrosyringol to yield an oil. This was chromatographed on a column of SiO_2 (5 cm × 2 cm I.D., Merck Kieselgel 60, 70–230 mesh), the product eluted with dichloromethane and recrystallized from benzene–cyclohexane (1:1) (*m/z* of the parent ions of the O-acetate $C_{10}H_{10}ClNO_6$: 275, 277 in the ratio 3:1).

2-Nitro-5-chlorovanillin and 6-chloro-2-nitrovanillin were prepared by the method of Raiford and Lichty[12]. The former was recrystallized from acetic acid and the latter from water (*m/z* of the parent ions of the O-acetate $C_{10}H_8ClNO_6$: 273, 275 in the ratio 3:1).

4-Chloro-6-nitroguaiacol was prepared from 4-chloroguaiacol[13] by nitration in acetic acid as described for the synthesis of 5-nitrovanillin, and the product recrystallized from benzene (*m/z* of the parent ions of the O-acetate $C_9H_8ClNO_5$: 245, 247 in the ratio 3:1).

GC

Analyses were carried out with a Varian (Palo Alto, CA, U.S.A.) 3700 Model gas chromatograph equipped with a Model 8000 autosampler and an Hewlett-Packard

3390A integrator. The electron-capture detector was held at 300°C, the injector at 250°C and the following temperature programme was used: 1 min isothermal at 150°C followed by an increase at 2°C/min to 245°C. Helium was used as the carrier gas at a flow-rate of 20 cm/s with a splitting ratio of 1:25. A fused-silica capillary column, DB-5-30N, with an I.D. of 0.25 mm and a film thickness of 0.25 μm (J & W Scientific, Folsom, CA, U.S.A.) was used.

GC–MS

GC–MS analyses were carried out with a TRIO 2 mass spectrometer (VG MassLab, Altrincham, U.K.) and an Hewlett-Packard 5890A gas chromatograph equipped with a DB-5-15N fused-silica column with an I.D. of 0.25 mm and a film thickness of 0.25 μm (J & W Scientific) and using helium as the carrier gas. The gas chromatograph was operated in the splitless mode under the following conditions: injector temperature 220°C; temperature programme, 50°C for 1 min isothermal, increasing at 15°C/min to 220°C which was held for 10 min. For EI mass spectra, the operating conditions were: source temperature 200°C, emission current 210 μA and an electron energy of 40 eV. The NCI mass spectra were obtained with methane as a reagent gas, a source temperature of 150°C, an emission current of 900 μA and a electron energy of 40 eV.

Preparation and stability of derivatives

O-Acetates were prepared as follows. The nitrophenols were dissolved in *tert.*-butyl methyl ether–hexane (1:1), pyridine (50 μl) and acetic anhydride or acetyl chloride (125 μl) added. The mixture was heated at 75°C for 30 min with occasional shaking, pyridine was removed with HCl (2 ml, 0.5 *M*) and the excess of acetylating reagent hydrolysed with 4 ml water for 15 min. The organic phase was used for analysis.

The yield of the products, the optimum derivatization conditions and the stability of the derivatives were determined from the results of experiments using pure samples of the O-acetates of 2,4-dinitrophenol and 6-chloro-2-nitrovanillin. These standards were prepared by acetylation of the phenolic compounds with acetic anhydride in refluxing acetic acid (1 h), the products isolated after destroying the excess of reagent with ice–water and recrystallization from cyclohexane. Based on the optimum derivatization procedure, the stability of all the O-acetates was examined.

O-Ethyl ethers were prepared by dissolving the compounds in 1 ml each of *tert.*-butyl methyl ether–hexane (1:1) and dimethyl formamide. A solution of tetrabutylammonium hydroxide in methanol (0.8 *M*, 15 μl) was added followed by ethyl iodide (10 μl) and the mixture shaken at room temperature for 15 min. The excess of ethyl iodide was removed by reaction with pyridine (50 μl), the sample shaken with HCl (2 ml, 0.5 *M*) to remove pyridine and finally with water (2 ml) to remove dimethylformamide. The organic phase was used for analysis. Due to the extreme sensitivity of the products to hydrolysis, this method was not uniformly suitable for preparation of the O-ethers of *ortho*-nitro-substituted phenolic compounds.

Recovery procedures for water samples and their efficiency

The extraction efficiency was determined using a synthetic mixture of the compounds dissolved in water (1 or 5 ml) at concentrations of 20 and 100 μg l^{-1}.

Solvent extraction with two solvents was examined: *tert.*-butyl methyl ether–hexane (1:1) and *tert.*-butyl methyl ether alone. The samples were acidified to a pH of < 1, ascorbic acid (100 mg) added and the solutions saturated with NaCl. Extraction was carried out with the two test solvents [1.5 ml, containing 2,4,6-tribromophenol (0.4 $\mu g\ ml^{-1}$) as the internal standard], and twice more with solvent (1.5 ml) lacking the internal standard. The organic phases were pooled, and the volume reduced to 2 ml in a stream of nitrogen at room temperature. In order to facilitate drying of the samples before acetylation, hexane (1 ml) was added, the volume again reduced to 2 ml and the extract dried (Na_2SO_4) for at least 15 min.

Use of C_{18} adsorption was also evaluated. The acidified solution was applied to an activated Bond Elut column (Analytichem, Harbor City, CA, U.S.A.), washed with HCl (1 ml, 0.1 *M*) and the nitrophenols eluted with *tert.*-butyl methyl ether [3.5 ml containing 2,4,6-tribromophenol (0.4 $\mu g\ ml^{-1}$) as the internal standard]. The eluate was treated as above for direct solvent extraction.

RESULTS AND DISCUSSION

Synthesis of substrates

Different procedures were used depending on the substitution pattern of the desired products. Synthesis of 2- and 4-nitro-substituted phenolic compounds was successfully carried out with fuming nitric acid in acetic acid at ambient temperature, whereas synthesis of the 3-substituted compounds required acetylation before nitration of the solid compound which was added portionwise to fuming nitric acid at 2–4°C. The purity of the compounds after recrystallization was >98% (GC–MS).

GC analysis

Industrial effluents invariably contain a large and structurally heterogeneous range of organic compounds. Experience in the analysis both of bleachery effluents which contain several hundred structurally diverse chlorinated organic compounds[1], and samples from metabolic experiments with microorganisms which contain a variety of metabolites[2], has clearly shown the advantage, and in general the necessity, of preparing derivatives of the phenolic compounds before attempting GC analysis. Use of the O-acetates of chlorophenolic compounds has also provided good resolution of isomeric compounds[14], and in addition, a satisfactory degree of purification is thereby introduced. We were concerned with the development of comparable procedures for nitrophenolic compounds. Use of such relatively clean samples also considerably simplifies problems in GC–MS structural determination (see below).

Derivatization for GC analysis presented a number of problems, primarily due to the susceptibility of the derivatives to hydrolysis: for example, trifluoroacetylation was accomplished readily, but the derivatives were so susceptible to hydrolysis that during destruction of the excess of reagent with water they were completely hydrolysed. On the other hand, acetylation was not quantitatively accomplished with sodium acetate as the base: the use of pyridine was obligatory. Again, it was found that hydrolysis of the O-acetates presented a problem: to minimize this, pyridine must be removed from the reaction mixture immediately after derivatization. Hydrolysis of excess of acetic anhydride with sodium carbonate[15] could not be used since hydrolysis of the nitrophenol O-acetates occurred: it was found, however, that shaking with water

alone was satisfactory. Two of the more recalcitrant compounds, 2,4-dinitrophenol and 6-chloro-2-nitrovanillin, were chosen for detailed examination. The rates of acetylation of these are shown in Fig. 2. The yields of the acetylated products were virtually quantitative within 20 min, though in practice the reaction time was routinely extended to 30 min. Since the products from the acetylation of 2,4-dinitrophenol and 6-chloro-2-nitrovanillin, as well as the pure O-acetates from these, were stable in the extraction solvent maintained at room temperature for 20 h, it was judged that O-acetates could be used for quantitative analysis. All the O-acetates were stable at room temperature for at least 18 h, and, with the exception of those derived from 2-nitro-, 2-nitro-5-chloro- and 2-nitro-6-chlorovanillin, were stable for up to 66 h. At −20°C, all were stable for at least 4 days. It was therefore judged that the stability of the derivatives was sufficient for repeat GC analyses to be carried out. The relative retention times (Table I) showed clearly that adequate resolution of the substances examined can be achieved with the operating conditions used. Whereas use of the O-ethyl ethers may be a valuable complement for identification, the structural discrimination of these was significantly poorer (Table I). In addition, on account of the difficulty encountered in their preparation and their susceptibility to hydrolysis (discussed below), they were not suitable for quantitative studies.

Recovery of compounds from aqueous solutions

The solvent extractability of the compounds using *tert.*-butyl methyl ether–hexane (1:1) was low for the nitrovanillins, 6-chloro-2-nitrovanillin and 4-nitrocatechol: by use of *tert.*-butyl ether alone, however, the extractability exceeded 95% for all compounds. The C_{18} adsorption procedure provided equally quantitative recovery, and we therefore conclude that either procedure would be applicable to the recovery of a wide range of structurally diverse nitrophenolic compounds from waste-water samples.

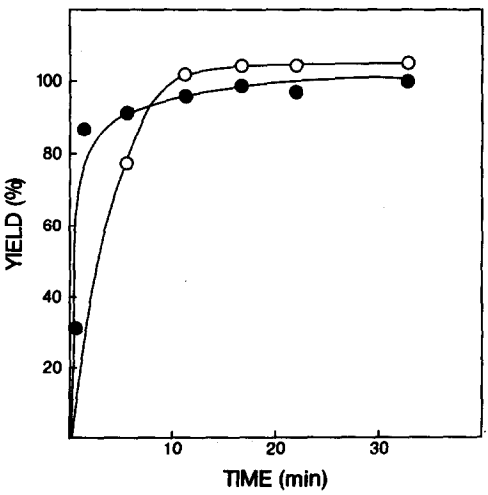

Fig. 2. Yield (%) of the O-acetates of 2,4-dinitrophenol (●) and 6-chloro-2-nitrovanillin (○) as a function of the reaction time (min) at 75°C. Yields were calculated on the basis of the pure O-acetates.

TABLE I

RETENTION TIMES AND RESPONSE FACTORS OF THE O-ACETATES OF THE NITROPHENOLS RELATIVE TO THAT OF 2,4-DINITROPHENOL, AND RELATIVE RETENTION TIMES OF THE O-ETHYL ETHERS RELATIVE TO 2,4-DINITROPHENETOLE

Substance	*O-Acetates*		*O-Ethyl ethers*
	Relative retention time	*Relative response*	*Relative retention time*
2-Nitrophenol	0.445	1.05	0.361
3-Nitrophenol	0.521	1.05	0.365
4-Nitrophenol	0.550	1.43	0.447
4-Chloro-2-nitrophenol	0.616	4.79	0.528
2-Chloro-4-nitrophenol	0.706	4.64	0.659
2,4-Dichloro-6-nitrophenol	0.759	4.30	0.534
2,4-Dinitrophenol	1.000	1.00	1.000
4-Chloro-6-nitroguaiacol	1.034	2.75	0.723
2-Nitrovanillin	1.084	2.02	0.953
4-Nitrocatechol	1.113	2.56	0.809
5-Chloro-2-nitrovanillin	1.247	4.73	0.934
5-Nitrovanillin	1.260	1.79	0.895
4-Chloro-3-nitrosyringol	1.332	2.74	0.872
6-Nitrovanillin	1.351	1.01	1.090
3,5-Dinitroguaiacol	1.400	4.87	1.141
4,6-Dinitroguaiacol	1.466	2.01	1.142
6-Chloro-2-nitrovanillin	1.636	3.87	1.455
3,5-Dinitrosyringol	1.730	8.27	1.218
4,5-Dinitroguaiacol	1.782	2.07	1.822

Recommended analytical procedure

On the basis of the above investigations, we suggest the following steps for analysis of nitrophenolic compounds in aqueous samples: (i) extraction with *tert.*-butyl methyl ether, or adsorption onto a C_{18} Bond Elut column followed by elution with *tert.*-butyl methyl ether, (ii) derivatization of the nitrophenolic compounds by pyridine-catalysed acetylation with acetic anhydride following the detailed procedures provided and (iii) using known standard compounds, quantitative GC analysis of the organic extracts using a fused-silica DB-5-30N capillary column.

GC–MS analysis

Previous mass spectrometric studies have used underivatized samples[8]. For analysis and identification of environmental samples, however, this is not an ideal procedure. Such samples are generally contaminated with a large number of compounds other than those to be analysed so that inclusion of a separation stage before analysis is highly advantageous. This is conveniently combined with derivatization of specific functional groups, and considerable effort was therefore expended in finding suitable derivatives. They should fulfil the following criteria: (i) be produced in quantitative yield, (ii) be chemically stable for at least 20 h and (iii) yield structurally informative mass spectra.

It had been hoped that alkylation would provide stable derivatives: these would

be attractive for GC–MS analysis in the EI mode since the intensity of the parent ions would be greater than from the corresponding acetates. Alkylation, however, introduced a number of serious problems to which no satisfactory general solutions were found. Use of diazoalkanes, which has been successfully used by other workers for analysis of Dinoseb[7], was suitable only for nitrophenols themselves since a complex reaction with the aldehyde group of the nitrovanillins precluded its general use[16]. Among other alkylation reagents, diethyl sulphate in aqueous solution was unsuitable since it was found that, before GC–MS analysis, the excess of reagent must be removed and this could not readily be accomplished using ammonia or alkali without hydrolysis of the alkali-sensitive O-ethyl ethers. In order to solve simultaneously the problems of extraction and derivatization, attempts were made to use extractive alkylation procedures. Use of ethyl iodide and tetrabutylammonium hydroxide in dichloromethane resulted in rapid alkylation, but simultaneous hydrolysis of the O-ethyl ether occurred so that this was an unsuitable procedure for quantitative work. On the other hand, use of tetramethylammonium hydroxide was precluded due to ineffective transfer of the ion pair to the organic phase. We therefore reluctantly concluded that the compounds for analysis must first be extracted with an organic solvent, and then alkylated in dimethylformamide using the procedure detailed in the Experimental section. Even by this procedure, however, hydrolysis sometimes occurred although usually to a minor extent: for example, ethylation of 4,5-dinitroguaiacol produced small amounts of the catechol di-O-ethyl ether and this method was generally not effective for 2- and 4-nitro-substituted phenolic substances. The rapid rate of hydrolysis of the O-ethyl ethers clearly precluded this as a quantitative method. Other potentially suitable solvents such as dimethyl sulphoxide clearly cannot be used for the nitrovanillins due to reaction between the active methylene group of the solvent and the carbonyl group.

Detailed discussion of the mass spectra is not justified but we draw attention to points of diagnostic significance. The following conclusions may be drawn from examination of the EI spectra:

(i) The parent ions of the O-acetates were weak, in contrast to the relatively strong ones produced from the O-ethyl ethers.

(ii) *ortho*-Nitrophenols produced no M − 30 ion (loss of NO) from the O-acetates and no M − 16 (loss of O) ion from the O-ethyl ethers.

(iii) For the O-acetates, the base peak was generally that corresponding to M − 42 (loss of $COCH_2$) and for the O-ethyl ethers to M − 28 (loss of C_2H_4).

(iv) Ions at M − 30 (loss of NO) were observed only from the vanillin O-ethyl ethers, and an M − 46 ion (loss of NO_2) only from 3-nitrophenol O-ethyl ether.

(v) Base peaks of low m/z (63–155) were observed for a number of O-acetates, and for some of the vanillin O-ethers.

Our decision to examine NCI mass spectra was motivated by the fact that this procedure has been recommended for analysis of nitroaromatic hydrocarbons on account of its enhanced sensitivity[17], and was used in a comprehensive study of a variety of aromatic nitro compounds including some nitrophenols[9]. Our spectra for the O-acetates and the O-ethers of 2-nitro- and 2,4-dinitrophenol correspond closely to those published in the latter study. We therefore concluded that the two studies were compatible and that the interpretations given by these authors were valid for the compounds investigated by us.

As expected, the NCI spectra were less amenable to formulation of fragmentation patterns than the EI spectra, and those of the O-acetates and O-ethyl ethers were, in general, significantly different with two important exceptions: (i) M − 15 ions (loss of CH_3) were formed both from both the O-acetates and O-ethyl ethers of 3,5-dinitroguaiacol, 4,5-dinitroguaiacol, 3,5-dinitrosyringol, and 4-chloro-3-nitrosyringol, all of which have two strongly electron-attracting groups on the ring; (ii) 2-nitrovanillins had a base peak at M − 30. On the basis of the data presented, however, we are unable to distinguish between formation of this ion by loss of NO or by reduction of the nitro to an amino group[9,18]. The spectra of the O-acetates showed the following characteristics:

(i) *ortho*-nitrophenols had no parent ions and had base peaks at M − 59 (loss of OCO–CH_3) or 59 (OCO–CH_3),

(ii) M − 46 ions (loss of NO_2) were observed only from 5-chloro-2-nitro- and 6-chloro-2-nitrovanillin, and

(iii) M − 43 ions (loss of CO–CH_3) were characterististic of 4-nitrophenols (except 4,6-dinitroguaiacol).

A simpler pattern was found for the O-ethyl ethers: with the exception of the 2-nitrovanillins and the substituted syringols noted above, all compounds had base peaks either at M or M − 29 (loss of C_2H_5).

Both the EI and NCI spectra of 4,5-dinitroguaiacol O-acetate and O-ethyl ether were unique in having ions corresponding to M − 16, M − 32 and M − 48: these can be attributed to successive loss of oxygen atoms from the *ortho* nitro groups with ultimate formation of an isoxadiazole ring (M − 48). A similar fragmentation pattern has been reported for the EI spectrum of 2,3-dinitrofluoranthene[19].

Examples of spectra illustrating these points, and the specific influence of molecular structure on the fragmentation patterns, are given in Figs. 3–6. We

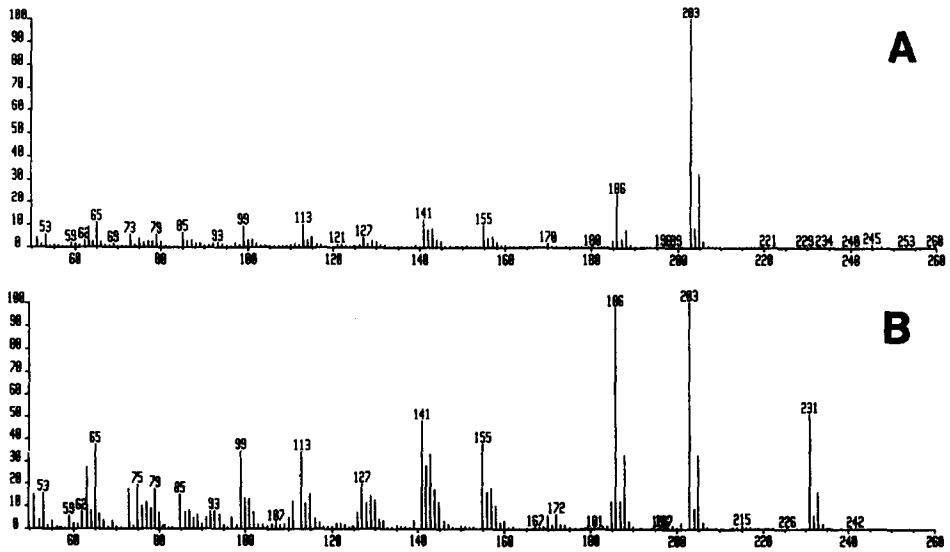

Fig. 3. Mass spectra (EI) of (A) 4-chloro-6-nitroguaiacol O-acetate and (B) 4-chloro-6-nitroguaiacol O-ethyl ether.

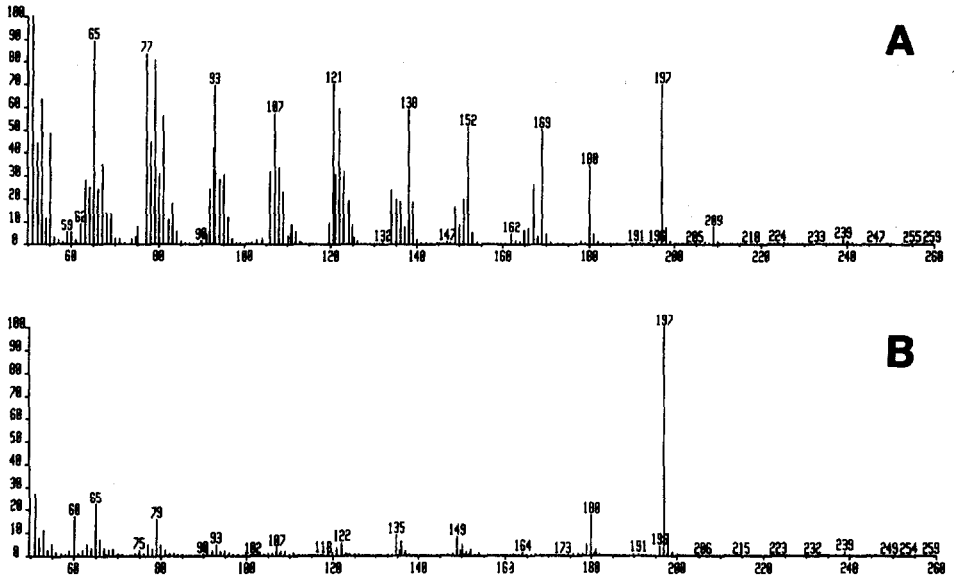

Fig. 4. Mass spectra (EI) of the O-acetates of (A) 2-nitrovanillin and (B) 5-nitrovanillin.

concluded that mass spectra provided a valuable basis for identification of the compounds examined in this study. The discrimination between isomers was clear-cut and contrasts with the situation in O-acetates of chlorophenols and chloroguaiacols. The strongly electron-withdrawing nitro group clearly played a significant rôle. Since, however, a complex array of fragmentation patterns was generally observed, this study emphasizes the need for access to reference compounds of known structure.

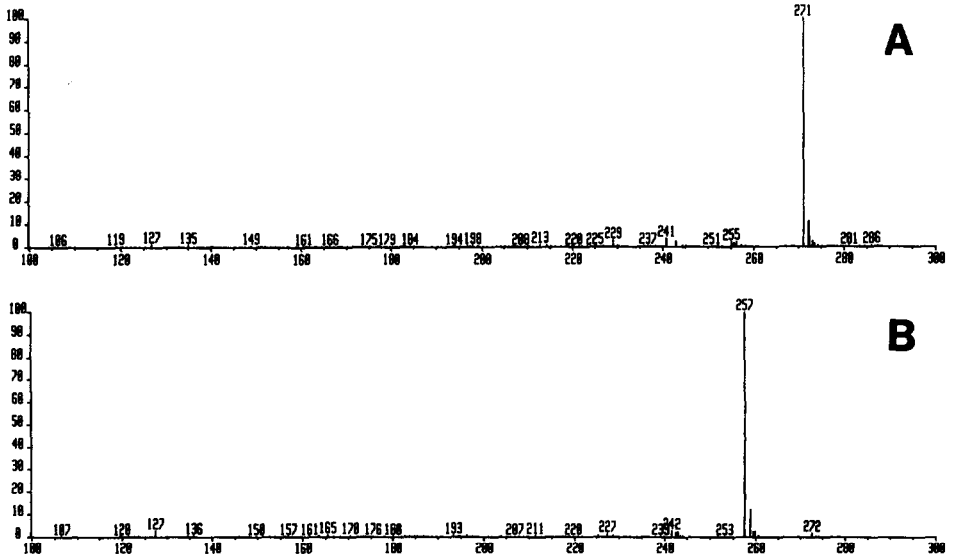

Fig. 5. Mass spectra (NCI) of (A) 3,5-dinitrosyringol O-acetate and (B) 3,5-dinitrosyringol O-ethyl ether.

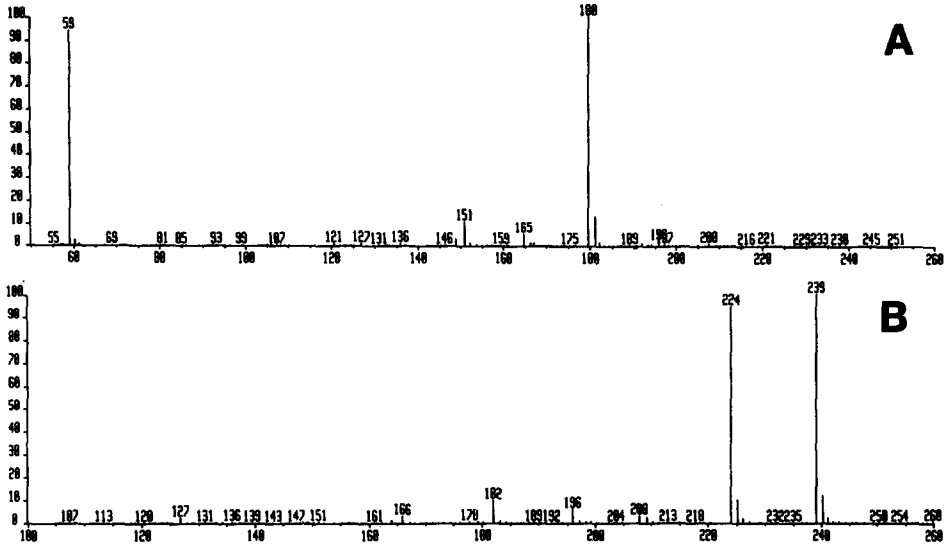

Fig. 6. Mass spectra (NCI) of the O-acetates of (A) 5-nitrovanillin and (B) 6-nitrovanillin.

General environmental significance

Nitrophenolic compounds are also of environmental significance in a wider context[20]. For example, some are employed as biocides or are used as starting material for their synthesis[21], while others may be formed by atmospheric nitration of phenolic compounds produced from aromatic hydrocarbons by reaction with hydroxyl radicals[22]: such compounds have been identified in rainwater[23] as well as in particulates collected from the atmosphere[24]. The methods developed in this study, modified as necessary, would clearly be applicable to the study of the environmental fate of these compounds also.

CONCLUSIONS

This study has provided experimental procedures for the quantitative analysis and identification of a structurally diverse range of nitrophenolic compounds in aqueous solutions. Attention is drawn to the following salient results.

(i) The susceptibility to hydrolysis of derivatives of some of the nitrophenolic compounds presented difficulties in their quantitative preparation. An optimum procedure for preparation of the O-acetates was developed, and it was shown that these derivatives were stable for at least 20 h at room temperature. Diazoethane cannot be used for preparation of the O-ethers of vanillins, and use of tetrabutylammonium hydroxide and ethyl iodide was not uniformly successful: these derivatives are not therefore suitable for quantitative analysis.

(ii) All test compounds were recovered from aqueous solutions either by extraction with *tert.*-butyl methyl ether, or by adsorption on C_{18} Bond Elut columns followed by elution with *tert.*-butyl methyl ether or, less desirably, acetone. Methanol cannot be used due to the ready formation of acetals from the nitrovanillins.

(iii) EI spectra of the O-acetates and O-ethyl ethers gave spectra displaying the expected fragmentation patterns although the parent ions of the former were usually weak, and the peak intensities of all ions were strongly dependent on the molecular structure: in particular, the EI spectra of compounds with nitro groups *ortho* to the O-acetyl or O-ethyl group were unique in failing to show M − 30 and M − 16 ions respectively. NCI spectra were structurally valuable even though a complex array of fragmentation patterns was found particularly for the O-acetates.

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