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Note

Gas chromatographic determination of 2-ethylhexanol and 2-ethylhexanoic acid as derivatives suitable for electron-capture and nitrogen–phosphorus detection after single reaction with heptafluorobutyrylimidazole

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2-Ethylhexanol (2-EH) enters our environment largely in the form of esters with various poly-functional acids, especially phthalic. Di(2-ethylhexyl)phthalate (DEHP), the most widely used plasticizer, can leach slowly from plastic products. Both DEHP and its hydrolysis product, 2-EH, contaminate the environment[1,2]. The National Toxicology Program is investigating the toxicological properties of 2-EH because of the wide exposure of the general population to its precursor the hepatocarcinogen DEHP[3].

During the analytical methods development to support the toxicology studies, the major metabolite of 2-EH, 2-ethylhexanoic acid (2-EHA)[4], was found to react in quantitative yield with the derivatization reagents N-trifluoroacetyl-, N-pentafluoropropionyl- and N-heptafluorobutyrylimidazole (HFBI), which were being used for acylation of 2-EH. The resultant derivative N-2-ethylhexanoylimidazole (EHI) was shown to have good chromatographic properties. Although N-substituted fluorinated acyl imidazole reagents are known to form esters with phenols, alcohols and amines[5], their reaction with carboxylic acids to form imidazole derivatives has not been reported.

Derivatization of the hydroxyl and carboxy groups by means of fluoroacylimidazoles was accomplished by a one-step reaction.

This finding raised the possibility of simultaneous quantitation of 2-EH and 2-EHA in biological samples by means of gas chromatography (GC) with highly sensitive and selective electron-capture and nitrogen–phosphorus detectors, respectively. This paper describes the simultaneous derivatization of 2-EH and 2-EHA and focuses on the evaluation of HFBI as a reagent for the determination of 2-EHA. The mass spectral properties of the 2-EHA derivative are also discussed.

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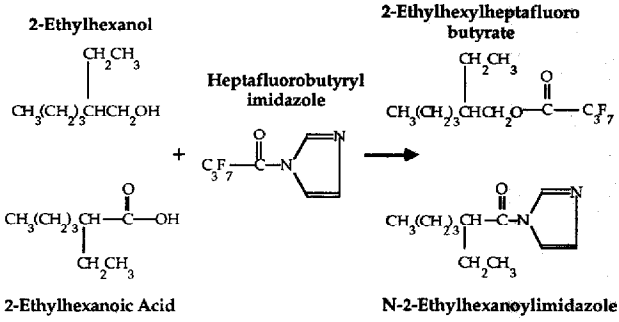

EXPERIMENTAL

Reagents

2-Ethylhexanol, 2-ethylhexanoic acid, octanol and 2-propylpentanoic acid (2-PPA) were obtained from Aldrich (Milwaukee, WI, U.S.A.), and were the highest purity available. HFBI as an imidazole kit (10 × 0.2 g) was obtained from Alltech/Applied Science, Deerfield, IL, U.S.A. All other chemicals were analytical-reagent grade. Solutions of NH_4OH were prepared in deionized water. Standard solutions of 2-EH and 2-EHA, as well as the internal standards, octanol and 2-PPA, were prepared by dissolving appropriate amount of each in *n*-hexane.

Instrumentation

For 2-EH analyses, capillary column GC was performed with a Perkin-Elmer Sigma 2000 gas chromatograph equipped with an ^{63}Ni electron capture detector, a split/splitless capillary inlet system, and a 1.0-μm DB-5 film, fused-silica capillary column (30 m × 0.32 mm; J and W Scientific, Rancho Cordova, CA, U.S.A.). The system was used in the split mode (*ca.* 1:45). The column was operated isothermally at 100°C with the injector and detector at 150 and 250°C, respectively. Helium, at a flow-rate of 1 ml/min, was used as the carrier gas, and argon–methane (90:10) was delivered at 30 ml/min as the detector makeup gas.

For 2-EHA, chromatographic analyses were performed on a Perkin-Elmer Sigma 2000 gas chromatograph equipped with both flame ionization and nitrogen–phosphorus detectors, and a 1.8 m × 2 mm glass column packed with Ultra-Bond FFAP 100–120 mesh (Alltech, Deerfield, IL, U.S.A.). Helium was used as the carrier gas at a flow-rate of 30 ml/min. The nitrogen–phosphorus detector was optimized for maximum selectivity and the bead current was kept below maximum sensitivity in order to increase the lifetime of the bead. The temperatures employed were as follows: oven, 130; injector, 200; detector, 250°C.

A laboratory data system, Hewlett-Packard Model 3359A, was used for data handling. The 0.8-ml autosampler vials capped with viton septa (Sunbrokers, Wilmington, NC, U.S.A.) were used for samples and standard preparation.

GC–mass spectrometry experiments were conducted on a Finnigan-MAT 4500 gas chromatograph–mass spectrometer–data system. The DB-5 capillary column was introduced directly into the ion source. The source temperature was maintained at

200°C and the electron energy was 70 eV. The gas chromatograph was programmed from 35 to 250°C at a rate of 4°C/min, with the injector at 200°C. Helium was used as the carrier gas at 1 ml/min in the splitless/split mode. The scanning speed was 1 sec over a mass range of 35–650 mass units. Data were acquired on an INCOS data system using a Nova 3C computer.

Derivatization

To 200 μl of a solution of 2-EH, 2-EHA and internal standard 2-PPA in a 0.8-ml autosampler vial were added 10 μl of the HFBI and the vial was capped. The solution was shaken briefly and after 5 min, 400 μl water was injected into the vial and vortexed until the organic phase was clear (*ca.* 20 s). The water was removed by syringe and 400 μl of 5% NH_4OH was injected into the vial. After vortexing, the NH_4OH phase was removed and the organic phase was washed twice with 400 μl water. A 150-μl aliquot of the organic phase was removed by syringe and transferred into a new vial. Aliquots of 2.5 μl of the hexane phase were injected into the gas chromatograph.

RESULTS AND DISCUSSION

A major problem in the development of GC methods for biological studies

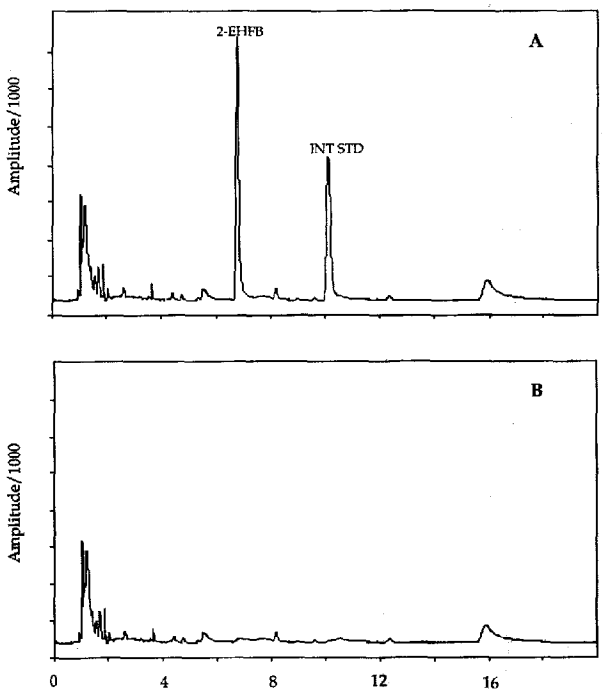

Fig. 1. GC–electron-capture detection of (A) derivatized 2-EH and octanol (internal standard, INT STD) (4 μg/ml) and (B) control. Chromatographic conditions: column, DB-5 FSCC 30 m × 0.32 mm. Temperature program: 100°C isothermal. Injector: split 1:45. Carrier gas: helium at 1.0 ml/min.

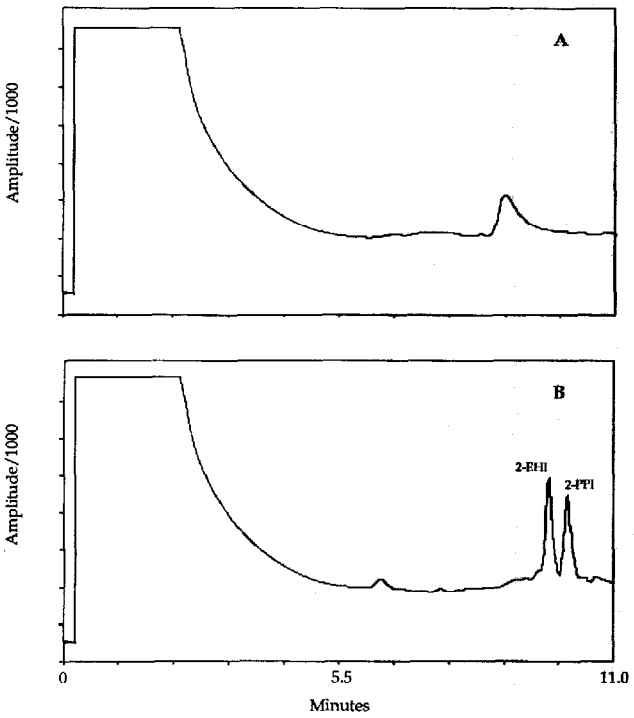

Fig. 2. GC–flame ionization detection of 2-EHA and 2-PPA (0.4 μg/ml) before (A) and after (B) reaction with HFBI. Chromatographic conditions: column, glass, 1.8 m × 2 mm packed with Ultra-Bond FFAP 100–120 mesh. Temperature program: 100°C for 5 min, then to 150°C at 10°C/min hold for 1 min. Carrier gas: nitrogen at 30 ml/min.

involves achieving highly sensitive and selective detection. From literature data[1], as well as preliminary studies conducted in our laboratory, it was apparent that expected levels of 2-EH in blood would be very low. For this reason the procedure described here, involving the formation of the heptafluorobutyryl ester of 2-EH, was chosen for investigation. It is known that HFB esters of alcohols may be formed readily, that these compounds have good GC properties and that the HFB group is detectable with high sensitivity by electron-capture detection[6].

A capillary column was chosen for use in the studies with 2-EH because low levels were expected and because of its potential for high resolution (Fig. 1). A packed column was used for the 2-EHA studies because much higher levels of the metabolite were anticipated (Fig. 2).

During the development work with 2-EH it was observed that the chromatographic properties of 2-EHA also changed. On further evaluation by mass spectrometry the derivative of 2-EHA was shown to be N-2-ethylhexanoylimidazole (EHI). This derivative exhibited good GC properties with sharp peaks, an absence of tailing, no evidence of decomposition and a wide linear response.

In order to optimize the analytical procedure, the kinetic parameters of the exchange reaction were studied. In these experiments 100 μg of 2-EHA and 2-PPA

were reacted with 10 μl HFBI at room temperature, and the reaction yield was measured as a function of time. The yield was calculated by measuring the disappearance of the 2-EHA and 2-PPA with a flame ionization detector. The results obtained indicate that the reaction reached equilibrium (91.0 ± 3.0% yield) in less than 6 min and that the derivatives of 2-EHA and 2-PPA are stable in *n*-hexane for at least 24 h. The effect of temperature on the reaction yield was also studied. No noticeable effect was observed when the reaction temperature was raised to 60°C, a further indication that the reaction is quantitative. The effect of concentration on the reaction kinetics was studied for 2-EHA and 2-PPA by using solutions from 8 to 500 ng/μl. In all cases the yields reached a plateau rapidly and remained constant for hours, indicating no effect of concentration on the total yield over the stated concentration range.

The results obtained from the reaction kinetic studies indicate that HFBI is a good analytical reagent for 2-EHA as well as for the well characterized reaction with alcohols such as 2-EH, since the reaction is rapid, quantitative, and can be easily performed under very mild conditions. Comparison between nitrogen–phosphorus

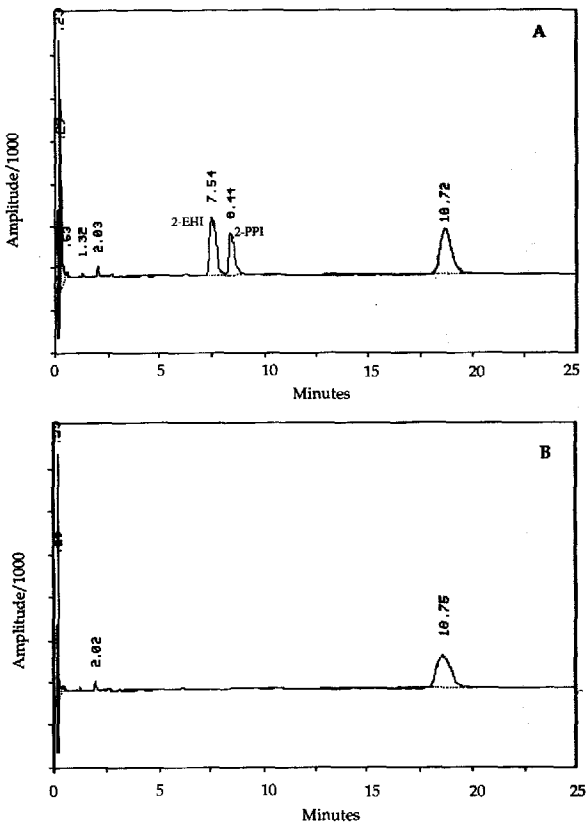

Fig. 3. GC-nitrogen–phosphorus detection of (A) derivatized 2-EHA (0.8 μg/ml) and 2-PPA (0.8 μg/ml) extracted and (B) control. Chromatographic conditions: column, glass, 1.8 m × 2 mm packed with Ultra-Bond FFAP 100–120 mesh. Temperature program: 130°C isothermal. Carrier gas: helium at 30 ml/min.

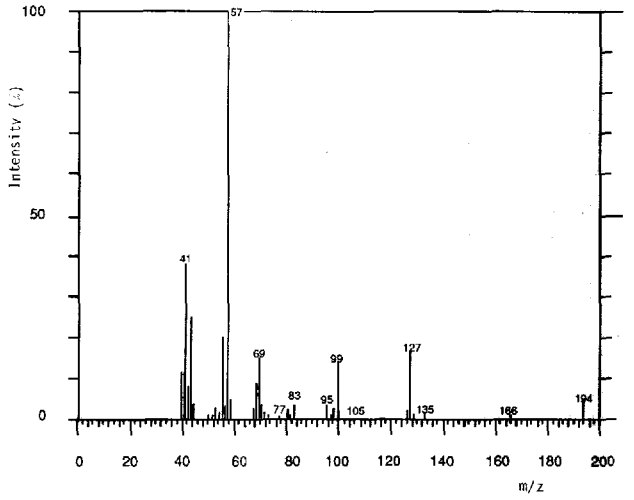

Fig. 4. Electron-impact (70 eV) mass spectrum of EHI.

and flame ionization detectors showed that sensitivity for EHI is *ca.* 10 times greater with nitrogen–phosphorus detection than with flame ionization detection. An example of GC–nitrogen–phosphorus detection of a 2-EHA and 2-PPA after derivatization and of a reagent blank are shown in Fig. 3. Under the routine detection conditions used, the lowest quantity detected was found to be 50 pg.

The mass spectral behavior of the imidazolo derivative of 2-EHA was also studied. The mass spectrum of EHI is shown in Fig. 4. Examination of this spectrum revealed that the spectrum contains sufficient information for structure confirmation and that relatively intense diagnostic ions were observed. The molecular ion of the

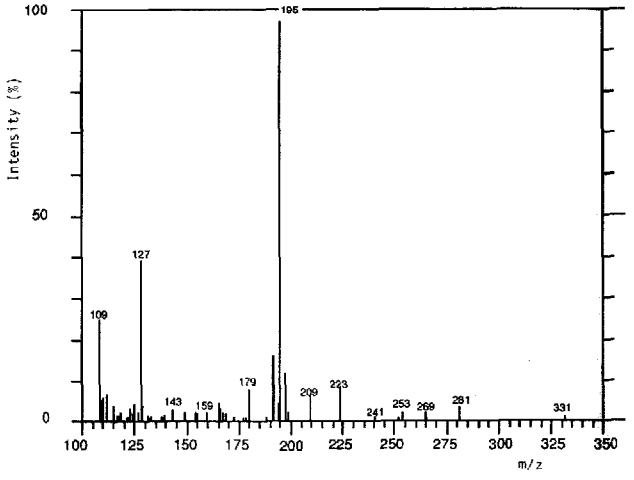

Fig. 5. Methane chemical ionization mass spectrum of EHI.

imidazolo derivative of 2-ethylhexanoic acid was observed at mass 194. A minor loss of 28 mass units observed at mass 166. The ion of mass 127 arose from an α-cleavage of the imidazolo group, with a subsequent cleavage of the carbonyl group to generate the ion of mass 99. Charge retention with transfer of two hydrogen atoms to the imidazolo group generated the ion of mass 69. McLafferty rearrangement, with charge retention on the hydrocarbon fragment of mass 57, was responsible for the generation of the base peak of the mass spectrum. Reinforcement for the assignment of mass 194 as the molecular weight of the imidazolo ester derivative was obtained from the methane chemical ionization mass spectrum (Fig. 5), which was dominated by the $(M + 1)^+$ species at mass 195.

The potential for simultaneous derivatization and analysis of 2-EH and 2-EHA has permitted the development of an analytical method for determining 2-EH and 2-EHA in biological samples which has facilitated our pharmacokinetic studies of 2-EH. Work with this derivatization reagent for other branched chain carboxylic acids is continuing in our laboratory.

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