Journal of Chromatography, 249 (1982) 183–186
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM 15,201

Note

High-performance liquid chromatographic analysis of short chain carboxylic acids as *p*-bromophenacyl esters: identification and separation of a decay product

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(Received July 16th, 1982)

It has been shown previously[1] that short chain carboxylic acids in aqueous systems can be determined by extraction with diethyl ether, esterification with *p*-bromophenacyl bromide[2] and analysis by reversed-phase high-performance liquid chromatography (HPLC). However, one problem encountered was the formation of a "decay" product from *p*-bromophenacyl bromide in the presence of chloride ion[1]. This non-reactive product co-eluted with the propanoate ester under the chromatographic conditions used As many aqueous samples —for example marine waters— contain significant amounts of chloride, this "decay" product could be mistaken for propanoate Thus a method was described which largely overcame this problem However, as *p*-bromophenacyl bromide itself appears to contain a small amount of this component, ultimately the best solution is to find chromatographic conditions — reported here— which allow separation of propanoate and the decay component In addition, the latter has been characterized from its mass spectrum, so that its suspected origin could be confirmed

EXPERIMENTAL

Reagents

Glassware, double distilled water and chemicals were prepared as before[1]

HPLC apparatus

The HPLC system consisted of 6000A and M45 pumps coupled to a M660 solvent programmer (all Waters Assoc.), a Rheodyne 7125 loop injector (20 μl) and μBondapak C_{18} column (30 cm × 3.9 mm I D , Waters Assoc.). Column effluent passed through an 8-μl flow-cell in a Cecil Instruments CE 2112 UV variable-wavelength spectrophotometric detector set at 254 nm, connected to a Bryans 28000 chart recorder. The mobile phase was acetonitrile and water, total flow-rate 2 ml min^{-1} Gradient and isocratic conditions are given below

Mass spectra

Mass spectra of *p*-bromophenacyl bromide and the decay product were obtained via a probe inlet on a Kratos MS 25 double focussing mass spectrometer.

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ionizing voltage 70 eV; filament emission current 100 μA; source temperature 200°C. Spectra were collected on a Kratos DS 55 data system.

Procedure

(a) Esterification of standard acids. Briefly, carboxylic acids (acetic, propanoic and butanoic) were converted into K^+ salts by addition of aqueous potassium hydrogen carbonate. Esterification occurred with addition of excess *p*-bromophenacyl bromide/18-crown-6 (ratio *ca.* 10:1) in acetonitrile and heating (80°C, 20 min)[2].

(b) Formation of the "decay" product. Hydrochloric acid (1 *M*) and potassium hydrogen carbonate (0.1 *M*) were added in equal mole amounts to form potassium chloride. *p*-Bromophenacyl bromide/18-crown-6 were added so that no bromide remained after heating. The decay product was the only organic component detected when the sample was analysed by HPLC. It was purified by thin-layer chromatography on silica gel G (eluent: hexane–diethyl ether, 7:3), before analysis by mass spectrometry.

(c) HPLC separation of propanoate ester and decay product. Aliquots of esteri-

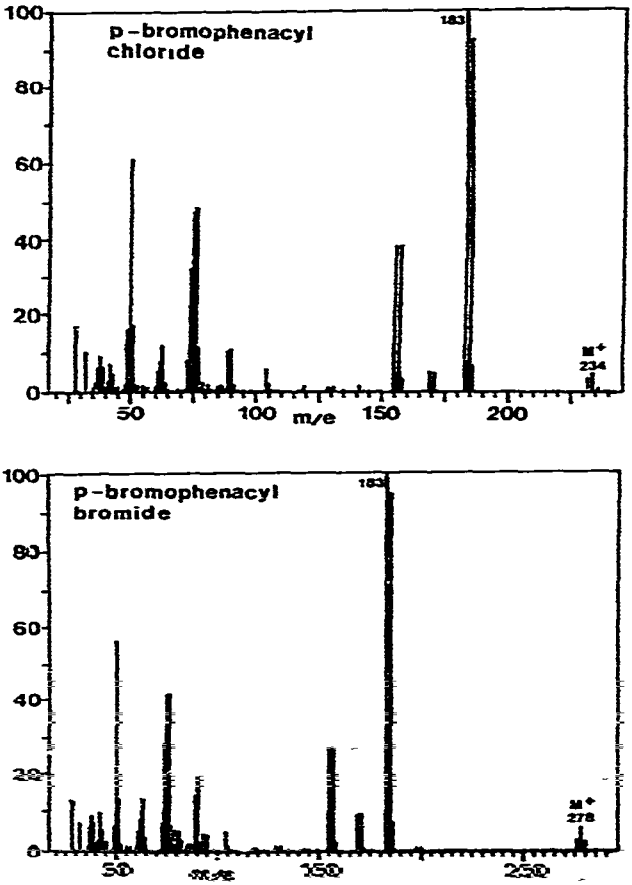

Fig. 1. Electron impact mass spectra of *p*-bromophenacyl chloride and *p*-bromophenacyl bromide.

fied acids formed in *a* and the product from *b* were combined to form one sample, which was used for separation of the two co-eluting components

RESULTS AND DISCUSSION

It was suggested previously that the decay product, formed from *p*-bromophenacyl bromide in the presence of chloride ion, might be *p*-bromophenacyl chloride This is indeed the case, as the mass spectrum in Fig 1 shows (the mass spectrum of the bromide is included for comparison). This is important for three reasons: (i) environmental samples which contain short chain carboxylic acids often contain significant amounts of chloride as well. Extraction techniques which do not exclude chloride will result in formation of *p*-bromophenacyl chloride at the derivatization stage (ii) *p*-Bromophenacyl chloride is non-reactive for esterification purposes and appears to

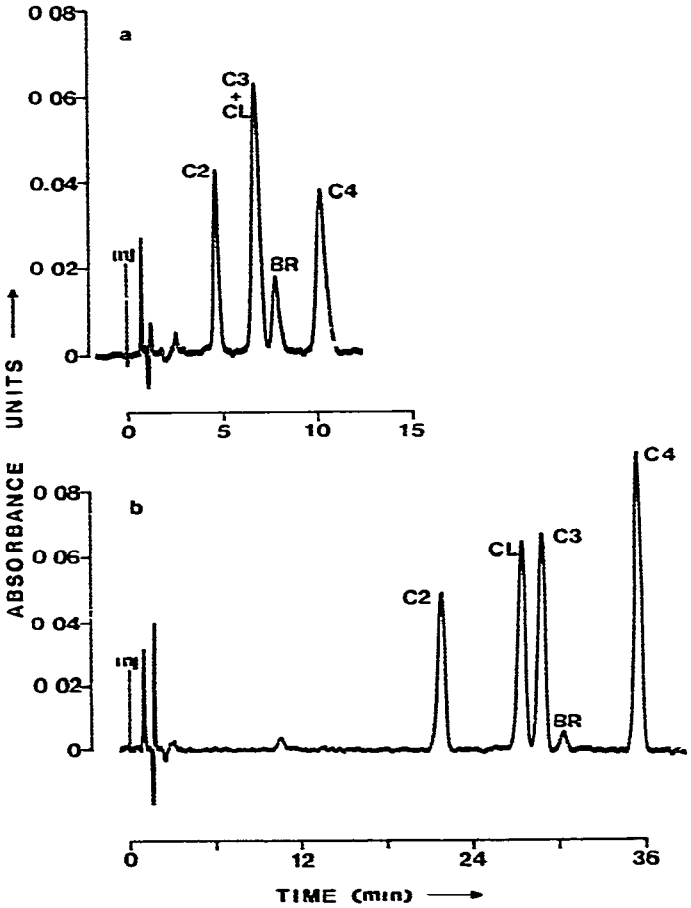

Fig. 2. HPLC separations of standard acids (C_2–C_4) (*p*-bromophenacyl esters) and *p*-bromophenacyl chloride Conditions 20-μl loop injection on μBondapak C_{18} column, flow-rate 2 ml min^{-1}, UV detection 254 nm (a) Acetonitrile–water (50 50) isocratic elution (b) Acetonitrile (20–52%) and water, linear gradient elution in 40 min C2 = acetate; C3 = propanoate, C4 = butanoate, CL = chloride, BR = bromide (starting material)

form more readily than the acid esters (iii) The chloride derivative and propanoate ester co-elute on reversed phase HPLC under isocratic conditions used previously (acetonitrile–water, 50:50) (Fig. 2a) As it is difficult to prevent completely formation of the chloride derivative, separation of the co-eluting components was attempted. This can be done under isocratic conditions, and baseline separation was achieved using 35% acetonitrile However, this leads to significant peak broadening and very long retention times for butanoate and higher acid esters (>60 min). Instead, a linear gradient programme of 20–52% acetonitrile in 40 min was found to be the best set of conditions (Fig. 2b). Each component is eluted as a sharp peak, and propanoate and the chloride are separated to baseline. Retention times are still longer than under the initial isocratic conditions, but this is felt to be a worthwhile sacrific since propanoate can be assigned with greater confidence.

ACKNOWLEDGEMENTS

We thank UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (grant T16/181/B2/2(B)) and SERC (grant GRB 51239) for financial support, and the Royal Society for purchase of the HPLC equipment. We are also grateful to Mr. A M Greenway (School of Molecular Sciences, University of Sussex) for the mass spectra, and to Professor R. Andrew for providing laboratory facilities

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