Journal of Chromatography, 214 (1981) 131–134
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,034

Note

Alternate procedure for the preparation and separation of benzyl derivatives of organic acids

W. D. KORTE
Department of Chemistry, California State University at Chico, Chico, CA 95929 (U.S.A.)
(Received April 23rd, 1981)

The advantages of the benzylation of low-molecular-weight organic acids prior to gas chromatographic analysis have been described in several papers[1–6]; however, the reported derivatizing procedure using phenyldiazomethane (PDM) is not ideal for routine application. PDM must be prepared regularly and peaks from the degradation products of the derivatizing reaction are found as interferences in the chromatograms[6]. Alkylation with benzyl chloride and silver oxide, although quite rapid when heated in a sealed tube, gave by-product peaks in the chromatogram after the C_5 benzyl ester[7], and a similar alkylation procedure using the potassium salts of the acids required long heating periods[8].

While seeking an alternative simple procedure for the analysis of certain urinary acids, various rapid methods of esterification found in the literature[9–12] were evaluated. A modification of Greeley's general procedure for alkylation[9] appeared superior to PDM esterification for it required minimal sample and reagent handling and led to quantitative (>99%) esterification in less than 10 min. Benzyl esters were formed by converting the organic acids to benzyltrimethylammonium salts and then alkylating the salts with benzyl chloride. Gas chromatographic separation of the benzyl esters on the polar Silar-10C liquid phase led to well defined peaks for compounds ranging from benzyl formate to benzyl stearate and interfering peaks from the derivatizing process were not observed.

EXPERIMENTAL

Instrumentation

Separations were performed on a Varian 1400 gas chromatograph interfaced with a Varian-MAT CH-7 mass spectrometer (Varian Assoc., Palo Alto, CA, U.S.A.) and employed a 6 ft. × 2 mm I.D. glass column packed with either 2% OV-17 on 80–100 mesh Chromsorb W or 10% Silar-10C on 100 mesh Chromsorb Q (Applied Science Labs., State College, PA, U.S.A.).

Chemicals

Benzyl chloride, butyric acid, isovaleric acid, 4-methylvaleric acid, lauric acid, palmitic acid, margaric acid, and stearic acid were obtained from Eastman-Kodak (Rochester, NY, U.S.A.). Benzyl acrylate, benzyl butyrate, and benzyl isobutyrate

0021-9673/81/0000–0000/$02.50 © 1981 Elsevier Scientific Publishing Company

were obtained from Pfaltz & Bauer (Stamford, CT, U.S.A.). All other chemicals were obtained from Aldrich (Milwaukee, WI, U.S.A.).

Method

The samples analyzed in the chromatograms shown in Figs. 1 and 2 were prepared by mixing 10 μl dimethylformamide (DMF) solutions of the organic acids (approximately 1 μg/μl) and then adding a 40% solution of benzyltrimethylammonium hydroxide in methanol (5 μl). After shaking gently for a few seconds, benzyl

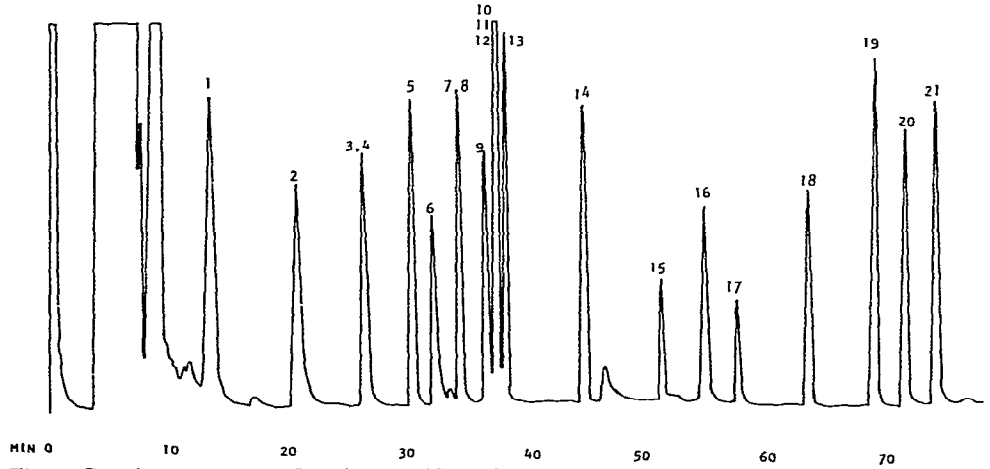

Fig. 1. Gas chromatogram of a mixture of benzyl esters analyzed on OV-17 (3%) programmed from 65°C at 3°C/min with a 10-min delay. The peaks shown are benzyl esters of the following acids: 1 = formic; 2 = acetic; 3 = propionic; 4 = acrylic; 5 = isobutyric; 6 = butyric; 7 = isovaleric; 8 = lactic; 9 = valeric; 10 = tiglic; 11 = 3,3-dimethylacrylic; 12 = 4-methylvaleric; 13 = hexanoic; 14 = octanoic; 15 = decanoic; 16 = undecanoic; 17 = lauric; 18 = myristic; 19 = palmitic; 20 = margaric; 21 = stearic.

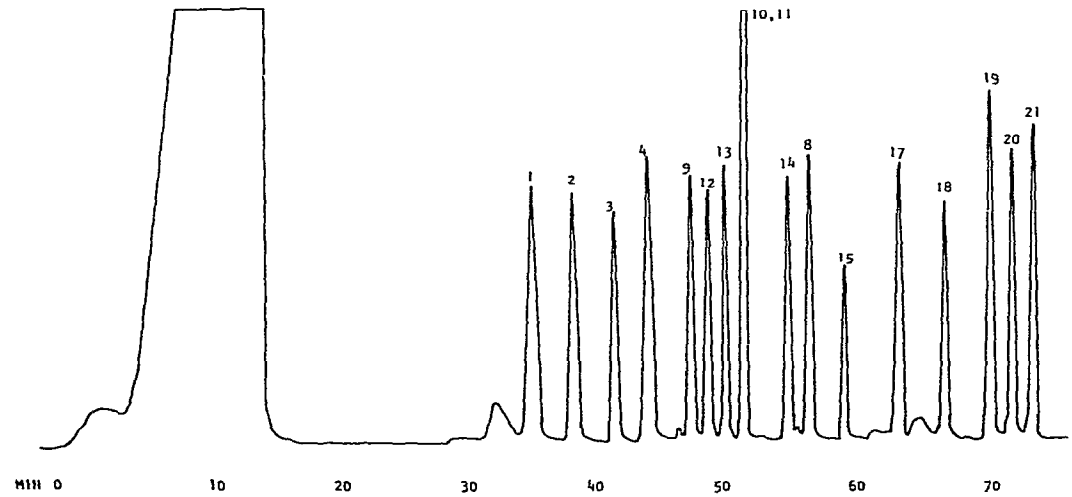

Fig. 2. Gas chromatogram of a mixture of benzyl esters analyzed on Silar-10C (10%) programmed from 100°C at 3°C/min with a 30-min delay. Peaks as in Fig. 1.

chloride (5 μl) was added and the mixture heated for 10 min at 60°C. A 10-μl aliquot was then injected into the chromatograph with a Hamilton syringe. Chromatographic conditions are described under the corresponding figures. Subsequent study found that the lachrymator, benzyl chloride, was more conveniently handled as a dilute solution in cyclohexane or benzene.

Quantitative measurements were obtained by comparing the areas under the peaks of the derivatized acids with the peaks obtained from the injection of freshly distilled benzyl esters. Benzyl acetate, benzyl acrylate, benzyl butyrate, and benzyl isobutyrate were used for these comparisons. The structures of the benzyl derivatives were confirmed by mass spectroscopic analysis during the separation process[3,13].

RESULTS AND DISCUSSION

A broad range of organic acids including many volatile low-molecular-weight species were converted to benzyl esters for chromtographic analysis by first forming benzyltrimethylammonium salts of the acids and then alkylating the salts with benzyl chloride. In the cases of the four acids, acetic, acrylic, butyric, and isobutyric, which were studied thoroughly, the yields of alkylation were quantitative (>99%). The benzylation reaction was completed in less than 10 min at 60°C in DMF solvent and the process can be accelerated at higher temperatures although significant quantities of benzyl formate are obtained by the decomposition of DMF at sustained temperatures over 100°C. Other solvents such as ketones and chlorinated hydrocarbons can be used[12]; however, the rate appeared optimized and there was minimal solvent loss on heating when DMF was used. Aqueous or alcoholic samples of several of the organic acids, formic, acetic, and lactic, were used with no significant effect on either the yield or the rate of the process if the total solvent mixture was at least 95% DMF, in contrast to observations of great reaction rate retardation with small quantities of water or alcohol[9,12].

High yields of benzyl esters can be obtained for synthetic purposes by heating the benzyltrimethylammonium or benzyldimethylanilinium salts directly without the presence of benzyl chloride[14], however low yields (<5%) of methyl esters were also observed which limits the procedure for quantitative analysis and led to noticeable interference peaks in the chromatogram. Tetramethylammonium hydroxide can also be used as a catalyst in the alkylation reaction[9], but again observable traces of methyl esters appeared in the chromatograms. Additional crown catalyst[10] did not accelerate the alkylation reaction when the tetraalkylammonium salts were first formed, but they did catalyze the reaction when potassium or sodium salts of the acids were alkylated. PDM esterification may still be the method of choice for those organic acids which decompose in the presence of tetraalkylammonium hydroxides.

Figs. 1 and 2 compare the separation of various benzyl esters after derivatization on the liquid phases, OV-17 and Silar-10C. The latter polar liquid was tested because excellent separations of saturated and unsaturated fatty acid esters were reported[15]. The temperature was deliberately held at 100°C for the separation with Silar-10C in Fig. 2 before initiating the temperature program to exaggerate how all excess reagents and solvents, particularly the DMF, were separated from the lowest-molecular-weight ester, benzyl formate. Retention times for the benzyl esters of the unsaturated acids were significantly greater on Silar-10C than on OV-17 compared to

esters of saturated acids which led to the easy separation of benzyl propanoate from benzyl acrylate and benzyl tiglate from benzyl 4-methylvalerate on Silar-10C. The retention time of benzyl lactate, a derivatized hydroxy acid, was also considerably greater on Silar-10C than OV-17 compared to the saturated organic acids.

The broad small peak immediately before benzyl formate was unidentified, but appeared to be a by-product of the derivatization process. The other small unidentified peaks in Figs. 1 and 2 were from impurities in several practical grade chemicals used in the study.

ACKNOWLEDGEMENTS

The generous support of the Department of Clinical Pathology, University of California at San Francisco, Dr. Hans Loken, Director, and the NIH during my Sabbatical Leave is greatly appreciated. I would also like to thank Mr. Robert Stanfield and Ms. Lai Ping Wong for their assistance in the operation of the gas chromatograph–mass spectrometer.

REFERENCES

1 H.-P. Klemm, U. Hintze and G. Gercken, *J. Chromatogr.*, 75 (1973) 19.
2 E. K. Doms, *J. Chromatogr.*, 105 (1975) 79.
3 J. Oehlenschläger, U. Hintze and G. Gercken, *J. Chromatogr.*, 110 (1975) 53.
4 D. Karashima, A. Shigematsu, T. Furukawa, T. Nagayoshi and I. Matsumoto, *J. Chromatogr.*, 130 (1977) 77.
5 E. K. Doms, *J. Chromatogr.*, 140 (1977) 29.
6 R. Liardon and U. Kuhn, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 1 (1978) 47.
7 C. B. Johnson, *Anal. Biochem.*, 71 (1976) 594.
8 J. R. Watson and P. Crescuolo, *J. Chromatogr.*, 52 (1970) 63.
9 R. H. Greeley, *J. Chromatogr.*, 88 (1974) 229.
10 H. D. Durst, M. Milano, E. J. Kikta, Jr., S. A. Connelly and E. Grushka, *Anal. Chem.*, 47 (1975) 1797.
11 A. Arbin, H. Brink and J. Vessman, *J. Chromatogr.*, 170 (1979) 25.
12 A. Arbin, H. Brink and J. Vessman, *J. Chromatogr.*, 196 (1980) 255.
13 U. Hintze, H. Röper and G. Gercken, *J. Chromatogr.*, 87 (1973) 481.
14 K. Williams and B. Halpern, *Synthesis*, 10 (1975) 727.
15 R. G. Ackman and S. N. Hooper, *J. Chromatogr.*, 86 (1973) 83.