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Behaviour of a common phthalate plasticizer (dioctyl phthalate) during the alkali- and/or acid-catalysed steps in an AOCS method for the preparation of methyl esters

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ABSTRACT

It is shown that the alkali-catalysed methanolysis step of an official method for determination of long-chain marine oil fatty acids produced dimethyl and mixed alcohol esters from the plasticizer dioctyl [actually di(2-ethylhexyl)] phthalate. An independent boron trifluoride-catalysed methanolysis step produced a lower level of artifacts but the official two-step process gave an even higher conversion figure than either catalyst independently. The retention times for the dimethyl, mixed alcohol, and dioctyl phthalates are discussed in relation to methyl esters of common fatty acids on polyglycol wall-coated open-tubular gas–liquid chromatographic columns.

INTRODUCTION

Di(2-ethylhexyl) phthalate (DEHP) is a widely used plasticizer and a widespread contaminant in an increasingly plastic world. Classed among the phthalic acid esters (PAEs) it has been found to be a ubiquitous environmental contaminant [1–3] and has also been classified as a priority pollutant [4]. Many papers pertaining to DEHP have appeared in recent years as part of a voluminous literature relating to the biological activity and toxic effects of PAE [2,5–9].

Equally of importance are the problems arising in analytical laboratories due to contamination of various commercial reagents and equipment by the phthalates which could lead to errors in analytical results. Background contamination by phthalates is commonly encountered in the chromatographic analysis of lipid samples [10–12] and has also been reported in prostaglandin analysis [13]. Middleditch [3], in a book on analytical artifacts, listed in detail the various contaminating sources of DEHP and other phthalate esters. While most of the literature lists dibutyl phthalate (DBP) and DEHP as the major contaminants in lipid analyses, only a few papers actually speak about their transesterification products. Pascaud [12] used hydrochloric acid in methanol for transesterification and noted that phthalates were strongly resistant to transmethylation. Later, Ishida *et al.* [14] analysed by gas–liquid chromatography (GLC) the different products arising from the use of different acid-catalysed transesterification reagents on the phthalates. Both results indicated that dialkyl phthalates were transesterified very slowly to give the methyl ester products. The amounts of these transesterified products formed would differ with the nature of the reagent used.

In this study, DEHP was subjected to both the base- and acid-catalysed transesterification steps of the new American Oil Chemists' Society (AOCS) Official Method Ce-1b-89 [15], with slight modifications. The amounts of different phthalate ester products obtained by subjecting the DEHP sepa-

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rately to the base-catalysed and the acid-catalysed reagents, and to both reagents in sequence as given in the AOCS procedure, were determined by GLC and by thin-layer chromatography with flame ionization detection (TLC–FID) (the Chromarod/Iatroscan system). Their interference with the GLC analysis of fatty acid methyl esters on a polyethylene glycol-based capillary column (Supelcowax-10) is also discussed.

EXPERIMENTAL

Dioctyl phthalate [bis(2-ethylhexyl) phthalate] was purchased from Aldrich (Milwaukee, WI, USA). The standard was tested for purity by GLC and with the TLC–FID Iatroscan system. Dimethyl phthalate for use as reference standard was prepared by methylation of phthalic acid, which was available in the laboratory. The dioctyl phthalate (>99% purity) (*ca.* 25 mg) was treated with alcoholic sodium hydroxide and boron trifluoride–methanol according to AOCS method Ce-1b-89 with a few modifications. Thus the sample was treated with 0.5 *M* alcoholic sodium hydroxide for 2 min instead of the official 7 min and for 20 min with 12% boron trifluoride–methanol instead of 5 min [16]. The sample was subjected to three different transesterification conditions: (A) base-catalysed with sodium hydroxide in methanol, (B) acid-catalysed with boron trifluoride–methanol and (C) both base- and acid-catalysed transesterification according to the modified AOCS conditions described earlier. The reaction products were extracted with isooctane and injected three times into the GLC system. The reaction products were also analysed with the TLC–FID Iatroscan system. All experiments were carried out in duplicate.

GLC analysis was performed on a Perkin-Elmer Model 8420 gas chromatograph equipped with a digital integrator, using a Supelcowax-10 column (30 m × 0.32 mm I.D., phase thickness 0.25 μm) (Supelco, Bellefonte, PA, USA). The oven temperature was programmed from 195 to 240°C at 3°C/min after an initial hold of 8 min at 195°C, and was maintained at 240°C for 10 min. The other parameters were splitting ratio 1:32, helium flow-rate 1.2 ml/min and injection port temperature 250°C. The peak-area output was recorded on a Perkin-Elmer GP-100 graphic printer.

Gas chromatography–mass spectrometry (GC–MS) with electron impact ionization was performed on a Finnigan MAT (San Jose, CA, USA) Model 700 ion-trap detector (ITD) system interfaced with a Perkin-Elmer Model 990 gas chromatograph. The fused-silica capillary GLC column was fed through a heated transfer line directly into the ITD gas inlet. For GC–MS, the separations were performed on a methylsilicone DB-1 column (60 m × 0.25 mm I.D., phase thickness 0.25 μm) (J & W Scientific, Folsom, CA, USA). The oven temperature was programmed from 165 to 240°C at 3°C/min after an initial hold of 8 min and with a final hold of 48 min at 240°C. The helium pressure was 15 p.s.i. The data system consisted of an IBM 286 compatible DTK TECHDATA-1230C computer (Technida, Dartmouth, Canada). All studies were conducted with version 4.0 of the ITD software supplied by Finnigan. The ITD was tuned by using the procedure supplied by the manufacturer. The ITD was operated in the full-scan mode and scanned with a 1-s cycle time.

TLC–FID analysis was performed as described elsewhere [17]. Hexane–diethyl ether–formic acid (97:3:1, v/v/v) was used as the developing solvent system.

RESULTS AND DISCUSSION

Table I gives the composition of the different products formed when the dioctyl phthalate was subjected to the different transesterification conditions. Three peaks were observed in the GLC analysis of the reaction products. The peak due to 2-ethylhexanol eluting just after the solvent peak has been ignored. The earliest eluting phthalate peak had a retention time similar to that of methyl heptadecanoate (17:0) or methyl hexadecadienoate (16:2$n-4$) under the conditions of analysis [18], and was identified as dimethyl phthalate based on the mass spectra and comparison with the retention time of dimethyl phthalate standard.

The mass fragment corresponding to the acylium ion [$M^+ - 31$] at m/z 163 formed the base peak (Fig. 1), in keeping with the data published by Middleditch [3] and others [19]. However, we obtained a protonated parent ion at m/z 195 (Fig. 1) and not a parent ion at m/z 194 as published [3]. It is not unusual to obtain the M + 1 ion under electron impact

TABLE I

GLC ANALYSES OF THE DIFFERENT PHTHALATE ESTERS RESULTING FROM THREE TRANSESTERIFICATION CONDITIONS

Type of transesterification	Relative concentration of phthalates[a]		
	Dimethyl	Methyloctyl	Dioctyl
Base-catalysed	0.3 ± 0.001	14.2 ± 1.04	85.5 ± 1.18
Acid-catalysed	n.d.[b]	3.6 ± 0.29	96.4 ± 0.29
AOCS (modified)	1.8 ± 0.02	28.3 ± 1.55	69.9 ± 2.00

[a] Expressed as weight percent + S.D. (n = 6); theoretical response factor of the phthalates calculated with respect to methyl octadecanoate (18:0), based on ionizable carbon content.
[b] Not detected.

in the ITD system [20,21]. The second-eluting peak, which fitted between the peaks for the methyl ester of the important eicosatetraenoic acid (arachidonic or 20:4$n-6$) and a later eluting (on polyglycols) eicosatrienoic acid (20:3$n-3$) was identified as methyloctyl phthalate based on the mass spectrum. Possibly it is a small component eluting at 13.34 min in a prior publication from this laboratory [22], with 20:4$n-6$ eluting at 13.21 min and 20:3$n-3$ at 13.56 min, and a similarly located but very large unknown component in Fig. 3 of a publication from another laboratory of the methyl esters of fatty acids from human plasma [23]. The protonated parent peak at m/z 293 was the base peak. The peak m/z 163 was, however, also prominent. The published mass spectral data for methyloctyl phthalate [3] show a very low-intensity parent peak at m/z 292.

These variations in the intensity of the peaks and the appearance of the protonated ions in this study from those published by Middleditch could be due to the different operating conditions used for spectra acquisition. A comparison of ion-trap mass spectra with quadrupole mass spectra has been presented in detail elsewhere [21].

The last-eluting peak, with a retention time greater than that of methyl tetracosenoate (24:1), was the unreacted dioctyl phthalate. This is also probably a major and late-eluting peak in the already mentioned Fig. 3 published elsewhere [23] for human plasma fatty acid methyl esters, with dioctyl partially converted to a methyloctyl phthalate. The mass spectra of authentic dioctyl phthalate, with

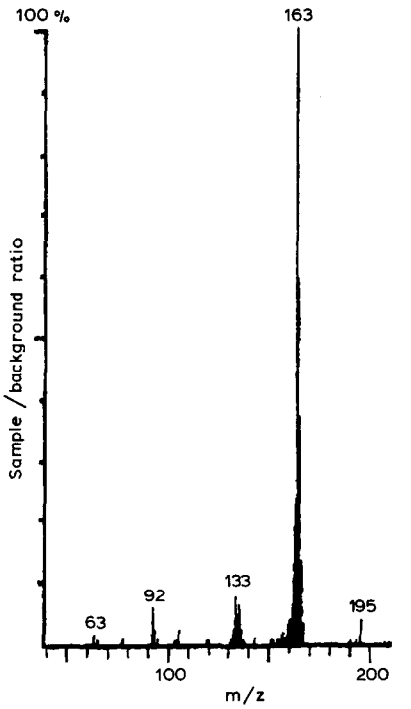

Fig. 1. Part of electron impact ionization mass spectrum of dimethyl phthalate obtained with a Finnigan MAT 700 ion-trap detector after passage through a Supelcowax-10 GC column as described.

the fragments at m/z 149, 167 and 279, was comparable to those published by Middleditch [3] and the protonated parent ion was observed at m/z 391.

The percentage composition of these products obtained by GLC (Table I) compares fairly well with those obtained from TLC–FID analysis (not shown). The three peaks resolved well on the Chromarods-SIII. The mobility decreased with decreasing chain length of the alcohol moiety, the dimethyl phthalate being the least and the dioctyl phthalate the most mobile.

This agrees with the elution order of a variety of phthalate esters in planar TLC on silica gel. The longer chain alcohol phthalates migrate close to methyl esters of common fatty acids [19]. This is in fact the key to a technique for eliminating phthalate esters from contaminated fatty acid methyl esters. Re-methanolysis with sodium methoxide converts all phthalate esters to dimethyl phthalate, which is much less mobile than the fatty acid methyl esters in TLC. The latter are not affected by the re-methan-

olysis and may be recovered from the TLC plate free from phthalate esters [19]. A more recent chromatographic separation of the phthalate esters [24] using a bonded methylphenylsilicone capillary column–quartz tube reactor–electron capture detection (ECD) system seems to be an interesting method for confirming alkyl phthalates. The quartz tube reactor maintained at a temperature of 900°C converts the phthalate esters to anhydrides and increases the sensitivity of the method for aliphatic phthalates.

Alkaline-catalysed transesterification, which is usually rapid [16,19,25], seemed to produce a relatively greater yield of the transesterified product when compared with acid-catalysed transesterification with boron trifluoride–methanol. Dimethyl phthalate was not observed in any detectable amounts following treatment of the dioctyl phthalate with boron trifluoride–methanol alone, and methyloctyl phthalate was also formed in low yield. The relatively higher yield of the transesterified products resulting from following the two-step modified AOCS procedure with contributions from both the acid- and the base catalysed reagents is not readily explained.

The modifications to the original published AOCS procedure [15] were not directed to reducing the yield of DEHP phthalate ester reaction products and the reasons for modifiying the reaction times [26] have been published in detail elsewhere [16].

All of the aliphatic fatty acids commonly encountered in food, plant or animal tissues are readily and almost totally transesterified by both base- and acid-catalysed reaction conditions. Although the 2-ethyl substituent could be involved in the slower reaction of these phthalate esters, the presence of the aromatic ring could also account for the resistance of DEHP to transesterification. Versino *et al.* [27] also observed that the presence of the aromatic ring in the dioctyl phthalate made it less biodegradable.

The proportion of the actual transesterified product of the dioctyl phthalate formed during the transesterification of oil samples contaminated with a small amount of the phthalate could differ, depending on the relative ratio of the dioctyl phthalate present and the reagent used. While the dioctyl phthalate eluting with a relatively greater retention time than the fatty acids of most interest (*e.g.*, docosahexaenoic acid or 22:6$n-3$) can probably be ignored in the GLC analysis of the fatty acid methyl esters on polygycol liquid phases, the dimethyl and the methyloctyl phthalate which elute along with the fatty acid methyl esters should be considered more carefully to avoid erroneous qualitative or quantitative results. The large number of liquid phases in common use in GLC [18] precludes the listing of definitive retention times, particularly as even changing the temperature on any one column could cause a shift of the aromatic esters relative to the common methyl esters of aliphatic acids. As methyl heptadecanoate (17:0) is often used as an internal standard in quantitative GLC [18,23], it is fortunate that only a small amount of dimethyl phthalate is formed with the popular boron trifluoride–methanol reagent. In high-performance liquid chromatographic analyses of fatty acids, as such or in the form of any aromatic derivative, similar problems could arise and the magnitude of the problem would depend on the type of detector in use.

Strictly, "dioctyl" phthalate should be based on *n*-octanol. However, the common phthalate ester commercially available is based on 2-ethyl-1-hexanol as the alkyl moiety. Similarly, "dibutyl" phthalate could refer to either esters of *n*-butanol or isobutanol (2-methyl-1-propanol), themselves sources of identification problems in the GLC of fatty acid methyl esters [10].

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REFERENCES

1 I. Tomita, Y. Nakamura and Y. Yagi, *Ecotoxicol. Environ. Saf.*, 1 (1977) 275.
2 Y. Nakamura and I. Tomita, *Eisei Kagaku*, 33 (1987) 71; *C.A.*, 107 (1987) 53536.
3 B. S. Middleditch, *Analytical Artifacts —GC, MS, HPLC, TLC and PC* (*Journal of Chromatography Library*, Vol. 44), Elsevier, Amsterdam, 1989, pp. 78, 279 and 350.
4 A. D. Sauter, L. D. Betowski, T. R. Smith, V. A. Strickler, R. G. Beimer, B. N. Colby and J. E. Wilkinson, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 4 (1981) 366.
5 R. W. R. Baker, *Toxicology*, 9 (1978) 319.

6 J. A. Thomas, T. D. Darby, R. F. Wallin, P. J. Garvin and L. Martis, *Toxicol. Appl. Pharmacol.*, 45 (1978) 1.
7 T. Gray and M. Gray, *Food Chem. Toxicol.*, 26 (1988) 811.
8 C. J. A. Doelman, P. J. A. Borm and A. Bast, *Lancet*, 335 (1990) 725.
9 K. Bergman and L. Albanus, *Food Chem. Toxicol.*, 25 (1987) 309.
10 J. C. Pascal and R. G. Ackman, *Comp. Biochem. Physiol. B.*, 53 (1976) 111.
11 M. Ishida, K. Suyama and S. Adachi, *J. Chromatogr.*, 189 (1980) 421.
12 M. Pascaud, *Anal. Biochem.*, 18 (1967) 571.
13 J. Mai, J. E. Kinsella and J. S. Chou, *Lipids*, 15 (1980) 968.
14 M. Ishida, K. Suyama and S. Adachi, *J. Chromatogr.*, 294 (1984) 339.
15 *Offical Methods and Recommended Practices of the American Oil Chemists' Society*, AOCS, Champaign, IL, 4th ed., 1989, Official Method Ce 1b-89.
16 R. G. Ackman, A. M. Timmins and N. C. Shantha, *INFORM*, 1 (1990) 987.
17 N. C. Shantha and R. G. Ackman, *Lipids*, 25 (1990) 570.
18 R. G. Ackman, in R. J. Hamilton and J. B. Rossell (Editors), *Analysis of Oils and Fats*, Elsevier Applied Science Publ., London, 1986, Ch. 4, p. 137.
19 A. Shibahara, M. Nakanishi and G. Kajimoto, *Yukagaku*, 32 (1983) 18.
20 W. M. N. Ratnayake, A. Timmins, T. Ohshima and R. G. Ackman, *Lipids*, 21 (1986) 518.
21 R. P. Adams, *Identification of Essential Oils by Ion Trap Mass Spectroscopy*, Academic Press, San Diego, CA, 1989.
22 R. G. Ackman, *Acta Med. Scand.*, 222 (1987) 99.
23 W. Welz, W. Sattler, H.-J. Leis and E. Malle, *J. Chromatogr.*, 526 (1990) 319.
24 Atlas of Chromatograms, *J. Chromatogr. Sci.*, 27 (1989) 685.
25 C. D. Bannon, G. J. Breen, J. D. Craske, N. T. Hai, N. L. Harper and K. L. O'Rourke, *J. Chromatogr.*, 247 (1982) 71.
26 R. G. Einig and R. G. Ackman, *J. Am. Oil Chem. Soc.*, 65 (1988) 510.
27 C. Versino and M. Novaria, *J. Synth. Lubr.*, 4 (1987) 3.