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MACRORETICULAR RESIN XAD-2 AS A CATALYST FOR THE SIMULTANEOUS EXTRACTION AND DERIVATIZATION OF ORGANIC ACIDS FROM WATER*

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SUMMARY

The macroreticular resin XAD-2, when impregnated with benzyl or pentafluorobenzyl bromide, can effect the simultaneous extraction and derivatization of organic acids from water. Reaction takes place in an aqueous–solid mixture, the derivatized products being retained on the surface of the resin. The resin is isolated by filtration and the derivatized analytes are eluted with a volatile organic solvent in the usual way. The functionalities studied were carboxylic acids, phenols and barbituric acids. The reaction conditions were developed to obtain optimal yields of the pentafluorobenzyl and benzyl derivatives. With pentafluorobenzyl derivatives quantitative yields were obtained.

INTRODUCTION

Derivatization is a common and often obligatory step in many instrumental methods for the determination of organic analytes in biological and aqueous matrix[1,2]. Two frequently used reactions are phase-transfer catalysis (PTC) and extractive alkylation (EA), which combine extraction and derivatization into a single step[1,2]. Although PTC and EA have been widely applied in both analytical and synthetic organic chemistry, they are subject to some difficulties, arising in part from co-extraction of the phase-transfer catalyst used in these reactions. For purposes of analysis, such co-extraction results in interferences in detection at high sensitivity[3–6]. In addition, the large excess of co-extracted catalyst (frequently milligram amounts) could be deleterious to capillary columns, which are particularly sensitive to overloading. Thus analytical methods based on PTC or EA often require an extra step to remove the catalyst prior to analysis[3–5,7,8], adding to the technical complexity of a procedure initially designed to simplify sample preparation. The technical complexity also highlights a second type of problem. As with many other liquid–liquid partioning pro-

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cedures, it may be relatively difficult to automate methods based on PTC or EA, particularly those requiring removal of catalyst prior to analysis.

Two approaches have been developed to deal with these problems. One of these, triphasic catalysis (TC), is based on a phase-transfer catalyst covalently linked to a solid and insoluble support. The reaction takes place in a triphasic mixture of two immiscible liquids (such as toluene and water) and a solid support which can be separated from the liquid phases by filtration[9–12]. A second approach is based on systems consisting of solid macroporous supports impregnated with reagents (herein termed impregnated reagents)[13–20]. The use of solid, insoluble catalysts and supports for chemical syntheses has been well reviewed[13,14,21–24], stressing the operational simplicity[13,14] and application to automation[23,24], but such reagents and catalysts have not been exploited in *analytical* derivatization reactions of analytes in aqueous or biological samples.

In analytical organic chemistry, insoluble supports have been used in semi-automated[25,26] and automated[27] methods for the isolation of organic analytes from aqueous solution. A frequently utilized solid support is the macroreticular resin XAD-2, a thermostable, hydrophobic polystyrene–divinylbenzene cross-linked copolymer which acts as an adsorbent. In this paper it is demonstrated that XAD-2 is also effective as a support for an impregnated reagent to effect the simultaneous extraction and benzylation or pentafluorobenzylation of a number of organic acids from water. This approach alleviates the problem of co-extraction of the catalyst and may provide an approach to the automation of analytical derivatization reactions of organic analytes in aqueous matrices.

EXPERIMENTAL

Apparatus

In general, gas chromatographic (GC) analyses of the derivatives were carried out on a Hewlett-Packard 5710 gas chromatograph with flame-ionization detection (FID). However, with of Δ^9-tetrahydrocannabinol (Δ^9THC) electron-capture detection (ECD) was necessary owing to the very low solubility of this compound in aqueous media. Determination of the derivatized analytes was carried out on a 1.8 m × 2 mm I.D. glass column packed with 1.5% OV-17 on Chromsorb W (80–100 mesh). Determinations of benzyl bromide and pentafluorobenzyl bromide (PFBBr) were carried out on a column of Chromsorb 101 (1.8 m × 2 mm I.D.) but on a Varian 2100 gas chromatograph equipped with FID. For the analysis of PFBBr the column temperature was 220°C and for benzyl bromide 260°C.

Reagents and chemicals

The macroreticular resin XAD-2 was purchased from Rohm and Haas (Philadelphia, PA, U.S.A.) in the form of 20–60-mesh beads which had a surface area of 300 m^2/g, a pore volume of 50 μL per 100 mg and, when cleaned, a specific gravity of 1. Benzyl bromide and PFBBr were purchased from Aldrich (Milwaukee, WI, U.S.A.) and Pierce (Rockford, IL, U.S.A.) respectively. Benzyl alcohol was purchased from Fisher Scientific (Montreal, Canada).

Pentafluorobenzyl alcohol (PFBOH) was synthesized in-house by reacting 50 μl PFBBr in 5 ml of ethanol and 2 ml of 0.5 *N* potassium hydroxide at 25°C for 2 h. The PFBOH so prepared was free from starting material. Silver oxide was purchased from

Canlab (Canada). Straight-chain carboxylic acids, phenobarbitol (PB), 5-ethyl-5-toluylbarbituric acid (ETB) and estradiol (E_2) were purchased from Sigma (St. Louis, MO, U.S.A.). Δ^9-Tetrahydrocannabinol was obtained by courtesy of R. A. Graham, Chief, Scientific Services Food and Drug Directorate, Department of National Health and Welfare, Canada. Gas chromatographic supplies were obtained from Chromatographic Specialties (Brockville, Ont., Canada).

Synthesis of standards

The pentafluorobenzyl (PFB) and/or benzyl (Bz) derivatives of palmitic (C_{16}), heptadecanoic (C_{17}) and stearic (C_{18}) acids were synthesized by reaction with the appropriate reagent in dimethylformamide (DMF) using potassium carbonate as a base[8]. The PFB ether of Δ^9THC (Δ^9THC-PFB) and the Bz ether of E_2 (E_2 (E_2Bz) were similarly synthesized. For the synthesis of the diPFB and diBz derivatives of PB it was necessary to use 2 ml of DMF–methylene chloride (1:1, v/v).

In all instances work-up was as follows: the reaction mixture was shaken for 1 h, an equal volume of water was added and the product extracted into 5 ml of methylene chloride. The organic layer was washed an additional five times with 5 ml of water, dried with sodium sulphate and evaporated. The Δ^9THC-PFB was purified by thin-layer chromatography (TLC) on silica gel C, the plates being developed in diethyl ether–hexane (1:8, v/v). The PFB and Bz derivatives of C_{16} and C_{18} acids and E_2 and the diPFB derivative of PB were found to be homogeneous by GC and TLC analysis. In the benzylation of PB, approximately 10% of the monobenzyl derivative had formed. The dibenzylated derivative was isolated by preparative high-performance liquid chromatography. Stock solutions of the derivatives at concentrations of 1 mg/ml were prepared in acetonitrile for those products analysed by FID and toluene for the products analysed by ECD. Pure compounds were characterized by gas chromatography–mass spectrometry.

Preparation of stock solutions of analytes

Stock solutions of C_{16}, C_{17} and C_{18} acids, Δ^9THC and E_2 were prepared in ethanol at concentrations of 1 mg/ml. Phenobarbital stock solution was prepared in acetonitrile at a concentration of 1 mg/ml. All solutions were kept refrigerated until used to prepare dilute working solutions.

Preparation of macroreticular resin

The commercially available resin was initially cleaned using standard procedures recommended by the manufacturer to prepare an active adsorbant surface. Fines were removed by suspension of the commercial product in distilled water, aspiration of the supernatant and repeating this procedure until the supernatant was clear. The water-washed resin was then isolated by filtration, washed with methanol to remove the water and stored under methanol until used in the subsequent step. Sufficient resin to fill a 43×123 mm Soxhlet thimble was transferred to a Soxhlet apparatus and extracted for 3 h with diethyl ether and with a cycle time of 10–12 min. The Soxhlet-extracted resin was transferred into a round-bottomed flask and the ether was evaporated *in vacuo* at 50°C.

In order to prevent microbiological contamination during storage, the cleaned and dry resin was kept in a glass-stoppered round-bottomed flask at –20°C until used.

Under these conditions reproducible yields were obtained over a period of 3 weeks with the same batch of resin.

Impregnation

The dried resin was treated with a volume of ethanol prior to impregnation with derivatizing agent. This was effected by the dropwise addition of ethanol followed by vortexing until the beads were again free flowing. Initially, studies were conducted using volume/weight (v/w) ratios of 0, 10, 25 and 40 μl of ethanol per 100 mg of dried resin prior to the addition of derivatizing agent. The standard conditions so developed used a v/w ratio of 25 μl of ethanol to 100 mg of resin for optimal yield. All subsequent experiments were conducted using resin pre-wetted with ethanol at a v/w ratio of 25 μl per 100 mg of resin.

The derivatizing agent (either BzBr or PFBBr) was similarly impregnated on the resin. The liquid reagent was added dropwise to resin that had been pre-wetted with ethanol at a v/w ratio of 25 μl per 100 mg and the resin was vortexed until it was free flowing. The ratio of the volume of derivatizing agent to the weight of resin used is reported below in the discussion of individual experiments.

Reaction conditions

The general reaction conditions were as follows. Resin was weighed into a 16×100 mm siliconized screw-capped glass tube and impregnated *in situ* by the described procedure. A 4-ml volume of aqueous phase was added to the impregnated beads, which sank to the bottom of the aqueous phase. A 25-μl aliquot containing the analyte in solution was added to the mixture. The aqueous phase used for the various analytes and the amounts of analyte used are shown in Table I. The screw-capped tubes were sealed with PTFE-lined screw-caps and the mixture was shaken at room temperature for 1 h at a speed of 175 cycles per minute (cpm).

After shaking, the beads were isolated by filtration through a plug of silanized glass-wool. The aqueous phase was removed by suction and the resin washed with approximately 5 × 10 ml of distilled water, which was also removed by suction. The derivatized analytes were eluted with 10 ml of diethyl ether and this solution was dried with sodium sulphate. the external standards were added to the dry diethyl ether solution. For the C_{16} and C_{18} carboxylic acids the external standard was the corresponding ester of the C_{17} acid. For benzylation of E_2 the external standard was the benzyl tetracosanoate. For the study of the pentafluorobenzylation of Δ^9THC the standard was PFB tetracosanoate. For both the benzylation and pentafluorobenzylation of PB the

TABLE I

ANALYTE, AMOUNT OF ANALYTE DERIVATIZED AND COMPOSITION OF THE AQUEOUS PHASE

Analyte	*Amount (μg)*	*Composition of aqueous phase*
C_{16} and C_{18} acids	10	0.05 *M* phosphate buffer (pH 7.4)
PB	25	0.05 *M* carbonate buffer (pH 11)
E_2	25	0.1 *N* NaOH
Δ^9THC	0.5	0.1 *N* NaOH

external standard was the dibenzyl ETB. (It was not possible to separate the diPFB derivatives of ETB and PB). The anhydrous ether solutions were transferred into siliconized glass tubes and evaporated to dryness under a stream of nitrogen with warming in a sand-bath. The derivatives were taken up in 25 μl of acetonitrile for GC with FID analysis and 150 μl of toluene for GC with ECD analysis. The yield was calculated from the ratio of the peak heights of the derivatized analyte to the amount of the external standard. This ratio was compared with the corresponding ratio obtained for mixtures containing amounts of authentic material equimolar to that of analyte used in the derivatization and containing the same amount of external standard.

Stability of reagents

The reagent stability under the described reaction conditions was determined by analysis of th diethyl ether eluate (described above). Thus a 0.5–1.0-μl aliquot of the 10-ml eluate was injected directly on to the Chromosorb 101 column maintained at the appropriate temperature. The hydrolysis products benzyl alcohol and PFBOH, were well resolved from the parent compound. Under these conditions, less than 1% of hydrolysis product could be detected in the presence of the parent compound.

Yield as a function of the ratio of derivatizing agent to resin

The yield of derivative formed was determined at various ratios of derivatizing agent to resin (D/R ratio) using a constant volume of derivatizing agent with different weights of resin, for instance, 50 μl of PFBBr impregnated on 50, 100, 200 and 400 mg of resin (dry weight) that had been pre-wetted with ethanol at a ratio of 25 μl per 100 mg of resin.

Yield as a function of the weight of resin used

Various amounts of resin were impregnated with a volume of derivatizing agent necessary to give the D/R ratio for optimal yield. For instance, in the benzylation of PB, 100 mg of resin were pre-wetted with 25 μl of ethanol and impregnated with 25 μl of benzyl bromide; 200 mg of resin were pre-wetted with 50 μl of ethanol and impregnated with 50 μl benzyl bromide, etc. The different weights of optimally impregnated resin were then used in the derivatization reaction.

RESULTS AND DISCUSSION

The standard method for the preparation of XAD-2 resin as an adsorbent requires a surface free from manufacturing by-products such as monomers and oligomers and the inorganic salts which act as preservatives. The resin so prepared must first be wetted with a suspension in a hydrophilic organic solvent such as methanol or ethanol in order to permit premeation of water into the pores[28]. Up to a point, this procedure for the preparation of adsorbent XAD-2 was adequate for the preparation of a resin suitable for a simultaneous extraction and derivatization. If the resin was suspended in ethanol prior to impregnation of derivatizing agent, the beads aggregated on addition of that agent and the yields of derivatives were markedly reduced and variable. When the resin was pre-wetted with less than 40 μl of ethanol per 100 mg of resin prior to impregnation with derivatizing agent the resin was free-flowing after the addition of the derivatizing agent and there were no visible droplets of derivatizing agent, sug-

TABLE II

YIELD OF C_{16} PFB ESTER AS A FUNCTION OF PRE-WETTING WITH ETHANOL

Ethanol per 100 mg of resin (μl)	*Yield of PFB ester (%)*
0	69, 71
10	78, 82
25	92, 100
40	85, 86

gesting that the resin had adsorbed the regent. If the v/w ratio of ethanol to resin was equal to or greater than 40 μl per 100 mg, the resin aggregated on the addition of the derivatizing agent. The optimal ratio of ethanol to resin was 25 μl per 100 mg (Table II).

Using impregnated XAD-2 and conditions compatible with analysis, a number of functionalities can be simultaneously extracted and derivatized from an aqueous matrix. Although the mechanism (or mechanisms) requires further study, two possible functions of the resin can be suggested at this point. The first is that the resin acts simply as a support for the derivatizing agent and that product formation is due to the inherent reactivity of benzylbromide and PFBBr[29,30]. This function is suggested by the data in Figs. 1 and 2, showing that optimal yields are obtained when the pores are at least half filled (as with PB) or almost completely filled (as with carboxylic acids). The second possible function of the resin is that of a catalyst[13,14]. A function other than a support for the reagents is suggested by the mild reaction conditions required to achieve high yields of certain derivatives and by the different organic acids that could be derivatized.

Some indication of the mechanism(s) can be obtained from comparisons of XAD-2-mediated reactions and biphasic derivatization reactions (BDR) previously described[29,30]. Under conditions of biphasic derivatization reactions, a solution of benzyl bromide or PFBBr in methylene chloride can effect a simultaneous extraction

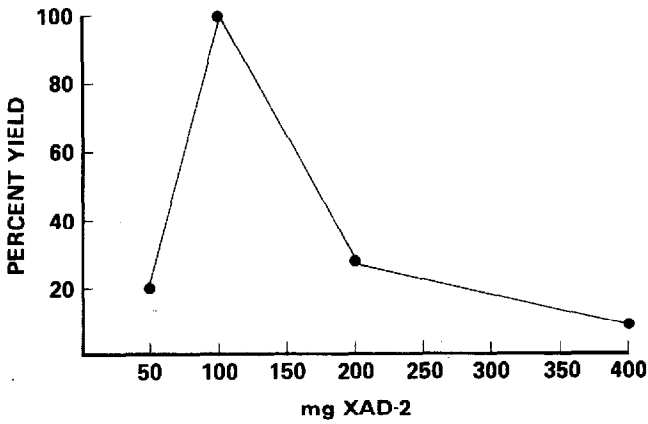

Fig. 1. Pentafluorobenzylation of palmitic acid; 50 μl of PFBBr were used to impregnate various weights of XAD-2.

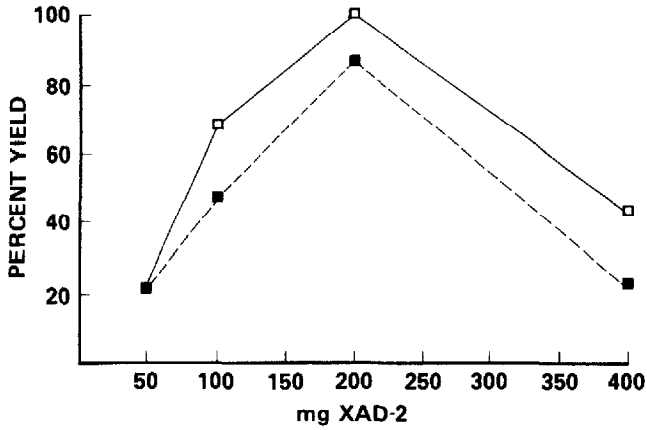

Fig. 2. Pentafluorobenzylation of phenobarbital (□) and benzylation of phenobarbital (■); 50 μl of PFBBr or benzylbromide were used to impregnate various weights of XAD-2.

and derivatization of phenols from alkali in the absence of any catalyst[29,30]. Those studies demonstrated that in the absence of benzyl bromide or PFBBr, sodium salts of E_2 and palmitic acid partition between the alkaline phase and methylene chloride[30], but in the presence of these reagents also undergo derivatization. Thus, it is possible that in the case of XAD-2-mediated reactions the volume of benzylbromide or PFBBr that either fills or partially fills the pores, acts both as an extraction solvent (similar to methylene chloride) and as a reagent. If this is so, the characteristics of the reaction should then be analogous to those of the BDR.

There are, however, differences in reactivity between the BDR and XAD-2-mediated reactions. Biphasic derivatization reactions require 8 *N* sodium hydroxide to achieve 70% yield for the benzylation of E_2 and 5 *N* sodium hydroxide for quantitative pentafluorobenzylation of Δ^9THC in a reaction time of 1 h[29,30]. Further, when 0.1 *N* sodium hydroxide was used, derivatives of Δ^9THC or E_2 did not form. In contrast, XAD-2-mediated derivatization requires much lower concentrations of base used to achieve comparable yields in the same time. For instance, with XAD-2-mediated processes 0.1 *N* sodium hydroxide can give an 80% yield of the E_2-Bz and a quantitative yield of the Δ^9THC-PFB in 1 h. Even more important is the fact that carboxylic acids and PB are benzylated and pentafluorobenzylated in high, and in the latter instance quantitative, yield using XAD-2-mediated processes. Under conditions of BDR no derivatives of these analytes were formed. This suggests some qualitative differences between the mechanism of XAD-2-mediated reactions and BDR. It is therefore reasonable to argue that not only does the resin serve as a support for the derivatizing agent, but that XAD-2 mediated processes act by mechanisms different from those involved in BDR.

It is possible that the mechanism(s) shares some characteristics with other impregnated reagents[13,14]. Posner[13] and McKillop and Young[14] proposed that some of the driving force of the reaction may be attributable to adsorbtion of both reactants proximal to each other, thus lowering the entropy of activation of the reaction. McKillop and Young[14] also suggested that the nucleophilicity of the nucleophile is

TABLE III

YIELD AS A FUNCTION OF WEIGHT OF RESIN

Analyte	*Derivatizing agent*	*Weight of dry resin in (mg)**	Yield (%)
C_{16} acid	PFBBr	50	67, 73
PB	Benzyl bromide	25	45, 49
		100	69, 71
		200	82, 88
E_2	Benzyl bromide	25	29, 35
		50	43, 45
		100	50, 54
		200	70, 72

* Pre-wetted with ethanol at a 25 μl per 100 mg ratio and impregnated at optimal D/R ratio.

enhanced. An analogous situation may exist for the simultaneous extraction and derivatization of organic acids from water using reagent-impregnated XAD-2. It is argued that on a bead of impregnated resin a small percentage of the surface consists of regions that are only partially coated. The uncoated sections of these regions serve to adsorb the ionized analyte in sufficient proximity to those sections of the region that are coated with the impregnated derivatizing agent, thus permitting reaction between analyte and the reagent.

Given this model, the variation in reaction yield of the carboxylic acids (Fig. 1) and PB (Fig. 2) with changes in the D/R ratio can be rationalized as follows. At the optimal D/R ratio the number of partially coated regions is at a maximum. However, as the D/R ratio decreases there is an increase in the amount of uncoated surface area relative to the partially coated regions as well as an increase in unfilled or insufficiently filled pores. This increases the probability of adsorption of the analyte in a region of the surface remote from the derivatizing agent. The decrease in yield as the ratio increases beyond the optimal value can also be understood in terms of this model. As the D/R ratio increases there is a decrease in the surface area available for adsorbtion of the analyte. With decreased probability of adsorbtion there is a concomitant decrease in the reaction yield per unit time.

The increase in yield with increase in the amount of optimally impregnated resin can also be rationalized using this model. Whereas altering the D/R maximizes the amount of optimally coated regions per unit weight of resin, increasing the total weight of resin increases the absolute number of such sites in the reaction mixture. This in turn increases the yield (Table III).

Whereas the yield was dependent on the weight of derivatizing agent and resin, it was independent of concentration of the analyte, an essential characteristic of an analytical derivatization reaction. The benzylation of several analytes was studied over a concentration range to determine if the yield varies linearly with concentration. The range for the C_{16} acids was 0.5–2.5 μg/ml in the aqueous phase, that PB was 2.5–12.5 μg/ml and that for E_2 was 2.5–25 μ/ml. In all instances the yield was constant over the range of amounts of analyte studied.

Finally, under the described conditions PFBBr remained stable during the reaction. Thus, in our hands and using 0.1 *N* sodium hydroxide the hydrolysis of PFBBr

was less than 1% in a 1-h reaction time (in fact, no PFBOH could be detected). In contrast, under conditions of extractive alkylation at pH 10, hydrolysis of PFBBr was of the order of 25%[6].

CONCLUSIONS

The use of reagent-impregnated XAD-2 has potential as a possible alternative to PTC and EA in analytical organic chemistry. While these three reactions permit simultaneous extraction and derivatization directly from aqueous media, XAD-2-mediated derivatization has advantages common to impregnated reagents. The first of these is that the catalyst can be readily separated from the derivatized analyte without any additional steps in the procedure. Second, this is a solid-state synthesis utilizing a resin currently used in semi-automated[25,26] and automated extraction procedures[27]. As it can also affect derivatizations, reagent-impregnated XAD-2 may be suitable for the automation of analytical methods that require both extraction from an aqueous matrix and derivatization of the analyte.

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