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Effect of Solvent on Solid-Supported Reactions on Styrene/Divinylbenzene Copolymeric Macroreticular Resin

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Pentafluorobenzylation of organic acids on XAD-2 can be positively and negatively affected by organic solvent used as a diluent for the reagent, pentafluorobenzyl bromide (PFBBr). The increase or decrease in yield is determined by the nature of the solvent and the functional group undergoing derivatization. When 200 mg of XAD-2 is impregnated with 10 μL of PFBBr in 90 μL of certain organic solvents, rather than with the pure reagent, there is an increase in yield of pentafluorobenzyl (PFB) esters. Such increases are found with 1,1,2-trichloroethylene (TCE), saturated hydrocarbon (e.g., hexane), monochlorobenzene, and dichlorobenzene. In contrast, other aromatic hydrocarbons and hydroxylic solvents can decrease the yield by as much as 5- to 10-fold. Derivatization of phenols is also increased by diluting PFBBr in TCE as well as in other solvents, but most important the yield is also increased when the diluents are hydroxylic solvents. As a result, simply by changing the organic solvent used as a diluent, it is possible to achieve a 5- to 10-fold enrichment in the derivatization of phenolic analyte relative to the carboxylic acid background that is present in plasma.

Reactions on solid support are an approach to the automation of those methods that are predicated on analytical derivatization reactions. Such reactions can be used in both the on-line and off-line mode (*1-3*). The advantages and disadvantages of using these techniques have been recently discussed (*1, 3*). One intriguing possibility is the development of superior specificity for derivatization using solid-supported reagents (*3*). Such specificity can be particularly important as it would simplify subsequent chromatographic separations.

In developing the off-line reactor approach for automating analyses of organic analytes from biological matrix, we investigated the styrene/divinylbenzene copolymer, XAD-2, as a solid support for analytical derivatization reactions (*4-9*). Solid-supported reaction on XAD-2 has been used to determine carboxylic acid (*7, 8*) and phenolic analytes (*9*) from simple matrices such as incubates (*7, 8*) as well as more complex matrices such as plasma (*9*). This macroreticular resin is suited to biological applications in that such samples are predominantly aqueous and analytes are both basic and acidic. Thus, the support used for the reaction must be stable at both alkaline and acidic pH and XAD-2 meets these requirements. In addition, the resin is also compatible with all organic solvents which are required for eluting adsorbed compounds from the resin.

In these methods, organic solvent was used as a diluent for the reagent (PFBBr) during the impregnation step. It was suggested that this resulted in a more homogeneous distribution of the reagent throughout the beads and pores of the resin, which led to an increase in the yield of reaction (*6, 7, 9*). Subsequent investigation, however, demonstrated that the solvent used as a diluent for PFBBr exerted unexpected effects on the reaction yield of carboxylic acids and this in turn could be used to enhance the specificity for derivatization of phenolic analytes.

EXPERIMENTAL SECTION

Apparatus. The pentafluorobenzyl derivatives of pure analytes were determined on a Hewlett-Packard (H-P) 5710 gas chromatograph equipped with a pulse linearized electron capture detector. These determinations were carried out on a 2.8-m × 4.6-mm-i.d. glass column packed with 3% SE-30 on 100-120 mesh Supelcoport. The output of the detector was monitored on a H-P 3380A recording integrator. Plasma extracts were analyzed on an H-P 5790 gas chromatograph also equipped with a pulse linearized electron capture detector. In this case the output of the detector was monitored on a H-P 3390A recording integrator. The column was a J & W DB-17N with a thickness of 0.15 μm and a length of 15 m (0.23 mm i.d.). On the H-P 5710 gas chromatograph the carrier gas was 10% methane in argon maintained at a flow rate of 20 mL/min; on the H-P 5790 instrument, the carrier gas was hydrogen with a linear velocity of 40 cm/s at 200 °C. In the latter case, 10% methane in argon was also used as make-up gas at a flow rate of 15 mL/min.

Reagents. Pentafluorobenzyl bromide was purchased from Caledon Laboratories, Georgetown, Ontario. The macroreticular resin, XAD-2, a cross-linked copolymer of styrene/divinylbenzene was obtained from BDH Laboratories, Toronto, Ontario. Solvents were bought from a variety of the usual commercial suppliers,

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such as Fisher and Aldrich Canada). Straight chain carboxylic acids were purchased from Sigma, St. Louis, MO, and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) from Upjohn. The cannabinoid, Δ^9-tetrahydrocannabinol, (Δ^9-THC), was provided by the National Institute on Drug Abuse of the USA under the auspices of the Food and Drug Directorate of Canada.

Derivatization of Pure Analyte. Pentafluorobenzylation of pure analyte was carried out by reported methods (*6*, *7*, *9*). Two hundred milligrams of resin prepared by methods previously described (*4*, *7*, *9*) were weighed into 16 × 100 mm screw-cap silanized glass vials. The carboxylic acid analytes were added to the resin in 4 mL of 0.1 M phosphate buffer solution at pH 7.4. The concentrations of straight chain carboxylic acids and $PGF_{2\alpha}$ were 0.25 μg/mL and 1.25 μg/mL, respectively. The reaction mixture was then shaken for 5 min at room temperature. These conditions are sufficient to adsorb the straight chain carboxylic acids at this pH (*7*). Prostaglandin $F_{2\alpha}$, however, remained in solution under these conditions.

For all carboxylic acids investigated, the reaction was initiated by the addition of 10 μL of PFBBr in 90 μL of diluent. Reaction time and temperature for straight chain carboxylic acids was 10 min at 30 °C and 1 h at 40 °C for $PGF_{2\alpha}$. The resin was then isolated by aspiration of the aqueous phase and washed with water, and the derivatized analytes were eluted with 36 mL of 10% EtOH in Et_2O. After evaporation of the solvent, $PGF_{2\alpha}$ was silylated with 100 μL of 10% TMCS (trimethylsilyl chloride) in BSTFA (*N*,*O*-bis(trimethylsilyl)trifluoroacetamide). The silylation mixture was dissolved in 1 mL of toluene containing 1 μg of pentafluorobenzyl tetracosanoate as an external standard. The residue from the reaction of the straight chain carboxylic acids was dissolved in 1 mL of toluene containing the PFB ester of a two carbon atom homologue of the analyte.

Derivatization of Δ^9-THC was initiated as described above for lipophilic acids by using preadsorption prior to the reaction (*6*, *9*). Thus, Δ^9-THC was adsorbed onto 200 mg of resin from 4 mL of aqueous solution at pH 3.5 containing 1.25 μg/mL of analyte. In this instance, however, after adsorption of analyte, the resin was isolated by aspiration and 4 mL of 0.1 M NaOH in H_2O/CH_3CN (4:1, v/v) was added followed by 10 μL of PFBBr in 90 μL of diluent (*6*, *9*). The reaction time and temperature were 10 min and ambient temperature. Heptadecanoic acid was similarly derivatized for purposes of comparing the effect of different diluents on yield of a carboxylic acid and a phenol. The procedure for the isolation of the pentafluorobenzyl derivatives was the same as described above.

Isolation and Derivatization of THC from Plasma. A solution of 100 ng of Δ^9-THC/mL of plasma was prepared by adding 100 μL of EtOH containing 1 μg of Δ^9-THC to 10 mL of plasma. One milliliter of this solution was transferred to a 16 × 100 mm screw-cap silanized glass vial containing 200 mg of resin. One hundred seventy microliters of acetonitrile was added to this mixture to enhance the adsorption of the analyte onto the resin, and the reaction mixture was shaken for 1 h (*9*). The resin was isolated by aspiration and washed with water until the aspirate was clear (this required approximately 40 mL). After the resin was washed off, 4 mL of 0.1 M NaOH in H_2O/CH_3CN (4:1, v/v) was added followed by 10 μL of PFBBr in 90 μL of diluent. In one set of samples, the diluent was TCE and in the second set the diluent was heptan-1-ol. The reaction time was 45 min at room temperature, and the PFB derivative was isolated by the procedure described above for other analytes. One microliter of the toluene solution of the residue was injected onto the capillary column by using the Grob splitless injector with a 30-s delay. The oven temperature was maintained at 220 °C for 1 min and then increased to 250 °C at 2 °C/min.

RESULTS AND DISCUSSION

Solvent effects provide insight into reaction mechanisms and a means to control the rate of reaction (*10*). In the case of solid-supported reactions on XAD-2, diluting the reagent PFBBr in TCE increased the reaction yield (*6*, *7*, *9*). For pentafluorobenzylation of carboxylic acids, the reaction yield increased approximately 2-fold upon dilution of 10 μL of PFBBr in 90 μL of TCE: in the case of pentadecanoic acid the reaction time was 10 min and the yield increased from 32 ± 6 to 67 ± 12% (average ± RSD, n = 6); for prostaglandin $F_{2\alpha}$ (a hydrophilic, trihydroxycarboxylic acid) a 2-h reaction time was required, but the yield also increased from 48 ± 17 to 90 ± 10% (average ± RSD, n = 6) upon dilution of PFBBr in TCE (*7*). In a similar fashion the yield for pentafluorobenzylation of Δ^9-THC increased from 58 ± 6 to 80 ± 3% (average ± RSD, n = 5) upon dilution of 10 μL of PFBBr in 90 μL of TCE (*9*). Moreover, prewetting of the resin with EtOH was found to be essential for pentafluorobenzylation of the phenolic and basic indoleamines *N*-acetylserotonin and melatonin at the oxygen and nitrogen positions, respectively (*11*). In this case, the increase in yield was attributed to improved wetting of the hydrophobic resin, thus enhancing adsorption (*12*). Regardless of the mechanism, use of solvents at different stages of sample preparation appeared to be a facile way of increasing reaction yield.

Studies on pentafluorobenzylation of the prostaglandins on XAD-2, however, suggested that the effect of solvents on these reactions was complex. These investigations were undertaken because pentafluorobenzylation of such polar analytes is much slower than that of lipophilic carboxylic acids (*7*). For instance, a straight chain carboxylic acid was completely derivatized in 10 min at 30 °C but *quantitative* derivatization of $PGF_{2\alpha}$ required 2 h at 40 °C. While this reaction time was not prohibitive with respect to analysis, clearly an increase in reaction rate for derivatization of these important analytes would be useful.

Given the polarity of the analytes, it was reasonable to propose that a more hydrophilic diluent would improve the reaction yield by (a) dispersing the PFBBr throughout the beads and pores of the resin in a more homogeneous manner as in the case of lipophilic analytes (*6*, *7*, *9*) and (b) wetting the resin to enhance permeation of the aqueous phase into the pores (*12*). Consequently, MeOH and EtOH were used in lieu of TCE as diluent. In addition to being hydrophilic, these solvents are quite close to TCE in polarity (*13*, *14*). Although both these solvents are highly water soluble and thus poorly adsorbed on the resin, it was still feasible to utilize them to distribute the reagent and prewet the resin because the beads floated on the surface of the 0.1 M buffer; thus, the solvent and reagent passed over the resin as they came in contact with the water.

For both MeOH and EtOH the yield of the PFB derivative was lower relative to that obtained for TCE. In addition, the yield for EtOH was lower than that for MeOH. This trend continued for the homologous series of alcohols containing one to eight carbon atoms (Figure 1). No effect of geometric isomerism was observed; thus, all geometric isomers of propanol, butanol, and pentanol (in the last case, those that were liquids at 40 °C) produced the same result.

These results conformed with the initial hypothesis that a more hydrophilic diluent would enhance the reaction rate for hydrophilic analytes. The reduced yield with MeOH relative to TCE may have reflected hydrogen bonding between the hydroxyl group and the carboxylate anion rather than an effect of polarity. Such hydrogen bonding effects were suggested as the cause for reduced yield for derivatization of carboxylic acids with phase-transfer catalysts in the presence of ethyl alcohol (*15*). The reduced yield with higher alcohols may have been due to a higher adsorption of these lipophilic cosolvents on the lipophilic resin, thus increasing the mole fraction of the hydroxyl functionality adsorbed.

Experiments with other solvents and analytes indicated that these simple models of the effect of solvent may not be correct. First, the most lipophilic diluents available (pentane, hexane, and octane) produced the same yield of PFB ester of $PGF_{2\alpha}$ as TCE, which is considerably more polar (*13*, *14*) than the hydrocarbon solvents (Table I). The corresponding alcohols,

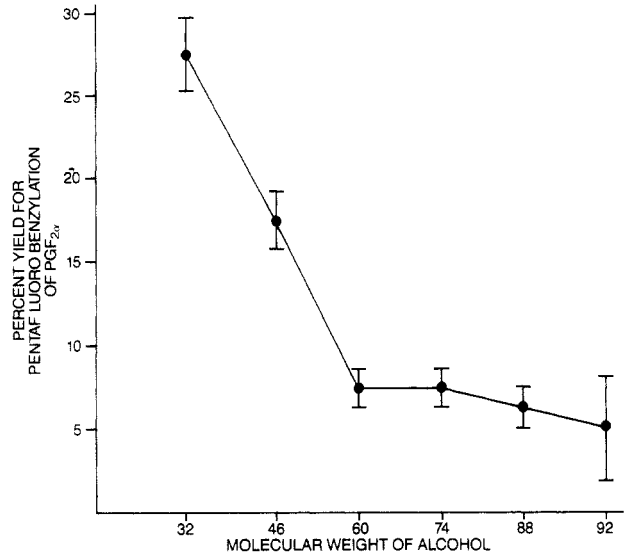

Figure 1. The effect of increasing molecular weight of the diluent alcohol on yield for pentafluorobenzylation of $PGF_{2\alpha}$.

Table I. Effect of Hydrocarbons on the Yield for Pentafluorobenzylation of $PGF_{2\alpha}$

solvent class	solvent	normalized yield[a] (average ± RSD, n = 5)
straight chain hydrocarbons	pentane	102 ± 12 (95 ± 7)[b]
	hexane	124 ± 5 (94 ± 14)[b]
	octane	109 ± 9 (100 ± 14)[b]
aromatic hydrocarbons	benzene	62 ± 15
(i) methylated	toluene	63 ± 13 (58 ± 10)[b]
	xylene	60 ± 9 (65 ± 4)[b]
	mesitylene	56 ± 5 (61 ± 3)[b]
(ii) nitrated	nitrobenzene	62 ± 6
(iii) chlorinated	monochlorobenzene	96 ± 11
	dichlorobenzene	100 ± 3

[a] As a result of day-to-day and between-batch variability, the yield for this reaction at 1 h varied from 65% to 75%; consequently the results are normalized to derivatization with TCE as a diluent, using the same batch of resin and run on the same day as the solvent class. [b] Results in parentheses were obtained with a different batch of resin and were obtained 6 months previously.

which are much closer in polarity to the parent hydrocarbon, however, reduced the yield by 5-fold. In addition, diols reduced the yield of pentafluorobenzylation of $PGF_{2\alpha}$ from 70 ± 8% for TCE to 29 ± 10% for ethylene glycol and 23 ± 15% for propylene glycol (average ± RSD, n = 6). Thus, wetting of the resin with a polar cosolvent, while required for adsorption (*12*), was, in fact, detrimental to optimizing reaction conditions even for polar, hydrophilic analytes such as $PGF_{2\alpha}$.

Investigation of the pentafluorobenzylation of lipophilic carboxylic acids confirmed these observations as the reaction yields for these compounds are also reduced by alcohols according to the molecular weight of the alcohol. However, because of the faster reaction rate of these analytes and given the reaction time used, the reduction is not evident until the alcohol has more that three carbon atoms. For instance, the yield for pentafluorobenzylation of pentadecanoic acid was 90 ± 5% (average ± RSD, n = 6) in 10 min at 30 °C with both TCE and EtOH as a diluent. If, however, the diluent was *tert*-butyl alcohol or benzyl alcohol, the yield was 20 ± 11% or 9 ± 20% (average ± RSD, n = 6), respectively. Thus the effect is not limited to polar analytes and may be a general phenomenon.

Studies with aromatic solvents further demonstrated the complexity of the effect of solvent on XAD-2 supported reactions. Benzene, methyl substituted benzenes, and nitrobenzene (Table I), all reduced the yield of reaction relative to TCE. In contrast, mono- and dichlorobenzene produced the same yield as TCE. The polarities of these methyl- and chloro-substituted benzenes are similar to each other and also similar to that of pentanol, hexanol, heptanol, and octanol (*13*, *14*). This demonstrated that the relative polarity of the diluent and analyte, while important for solubility, may not be a critical factor in the reaction.

Table II. Effect of Alcohols on the Yield of a Model Carboxylic Acid and a Phenol

	yield of PFB derivative[a]				
solvent	TCE	propan-3-ol	butan-1-ol	pentan-1-ol	hexan-1-ol
analyte					
HDC acid[b]	90 ± 10	40 ± 3	33 ± 6	23 ± 6	23 ± 3
THC	95 ± 9	78 ± 8	83 ± 6	92 ± 6	95 ± 8

[a] Yield calculated relative to extractive alkylation ± SD (n = 5). [b] HDC = heptadecanoic acid.

As these investigations showed that solvent has a significant effect on the reaction of carboxylate groups, the effect of solvent on other functionalities was investigated. There was reason to suspect that there might be differences in effect of solvents based on the nature of the functional group being derivatized. In heterogeneous liquid systems (CH_2Cl_2/alkaline H_2O) and *in the absence* of phase-transfer catalyst, phenols, but not carboxylic acids, undergo pentafluorobenzylation or benzylation (*16*, *17*). Under basic conditions, however, both functionalities are ionized and derivatized in catalyzed heterogeneous (e.g., phase-transfer catalysis) or homogeneous conditions (*18*).

We therefore investigated the possibility that solid-supported reactions on XAD-2 might also exhibit similar specificity. If carboxylate derivatizations could be selectively reduced, this would improve the specificity of analyses of phenols from biological samples where carboxylic acids constitute the major reactive element in the matrix.

These studies demonstrated that solvents can indeed be used to develop selectivity for phenols in the presence of carboxylic acids (Table II). In 0.1 N NaOH, the pentafluorobenzylation of phenols was found to be unaffected by alcohols regardless of molecular weight. In contrast, the derivatization of a model carboxylic acid (heptadecanoic acid) was markedly suppressed.

The consequence of this selectivity is demonstrated in Figure 2 by comparing chromatograms of plasma preparations for GC/ECD (electron capture detector) or GC/NICI-MS (NICI = negative ion chemical ionization) analysis of Δ^9-THC as the PFB derivative. The analyte was first adsorbed onto XAD-2 and then pentafluorobenzylated. There was a significant decrease in the carboxylic acid background when heptan-1-ol was used as solvent, as opposed to TCE. The enrichment in analyte, upon changing from TCE to heptan-1-ol, varied from 5- to 10-fold when compared to different plasma carboxylic acids.

The effect of various solvents on reaction yield does not correlate in a simple manner to any physical property of the solvent. Thus, it is not possible, based on solvent effect, to draw many conclusions regarding the mechanism. The lack of solvent effect on reaction of phenols coupled with the significant effects on derivatization of carboxylic acids, however, implies a different reaction pathway for these two functionalities. Alternatively, it is possible that Δ^9-THC reacts much faster than the carboxylic acid and, while alcohols may reduce the yield for pentafluorobenzylation of this analyte as

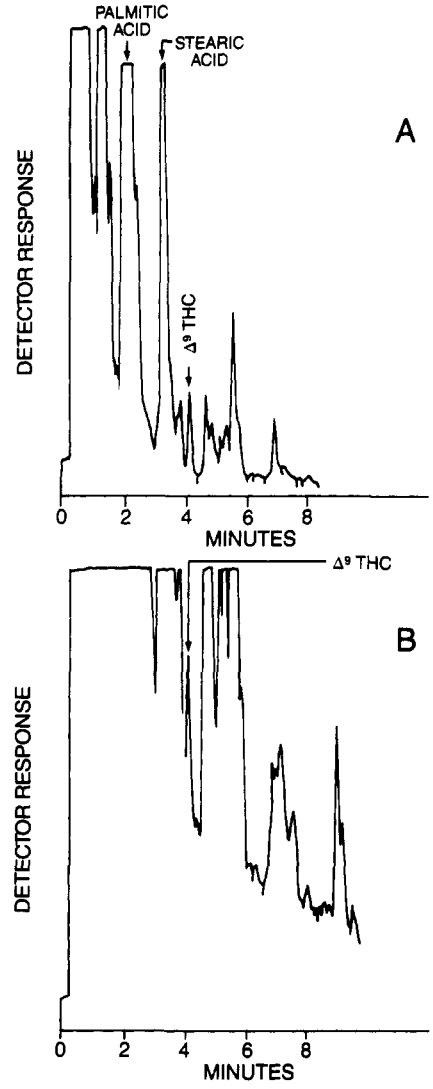

Figure 2. Gas chromatography/electron capture detector traces for preparations of analysis of Δ^9-THC from plasma at a concentration of 100 ng/mL: (A) heptan-1-ol used as a diluent for PFBBr; (B) TCE used as a diluent for PFBBr.

well, the reduction in yield is not evident in a 10-min reaction time.

While data from the present study are difficult to interpret, the information already available provides empirical guidelines for optimizing conditions of solid-supported reactions on XAD-2. First, use of a diluent for PFBBr has a major effect on the reaction yield for pentafluorobenzylation on XAD-2 supported reactions. Second, unlike the case for reactions in homogeneous medium or for extraction, polarity of solvent is not an appropriate guide for selection of diluent for optimizing conditions for XAD-2 supported reactions. It is possible, however, to empirically optimize the conditions for pentafluorobenzylation of carboxylic acids and phenols on the basis of the diluent. Furthermore, this selection can be used effectively to enhance the specificity of sample preparation. Further investigation could develop a theoretical framework for improving this selectivity of reaction.

Registry No. $PGF_{2\alpha}$, 551-11-1; $PGF_{2\alpha}$(PFB deriv.), 82205-49-0; XAD-2, 9060-05-3; PFBBr, 1765-40-8; Δ^9-THC, 1972-08-3; Δ^9-THC(PFB deriv.), 104335-68-4; TCE, 79-01-6; BuEt, 110-54-3; BuBu, 111-65-9; C_6H_6, 71-43-2; MePh, 108-88-3; NO_2Ph, 98-95-3; ClPh, 108-90-7; BuMe, 109-66-0; PrOH, 71-23-8; BuOH, 71-36-3; $BuCH_2OH$, 71-41-0; $Bu(CH_2)_2OH$, 111-27-3; $Me(CH_2)_{15}CO_2H$, 506-12-7; $Bu(CH_2)_3OH$, 111-70-6; $Me(CH_2)_{15}CO_2PFB$, 104324-30-3; xylene, 1330-20-7; mesitylene, 108-67-8; dichlorobenzene, 25321-22-6.

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