Journal of Chromatography, 358 (1986) 137–146
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 492

SOLID-SUPPORTED REAGENTS FOR THE SIMULTANEOUS EXTRACTION AND DERIVATIZATION OF CARBOXYLIC ACIDS FROM AQUEOUS MATRICES

STUDIES ON OPTIMIZATION OF REACTION CONDITIONS

J. ROSENFELD*, M. MUREIKA-RUSSELL and S. YEROUSHALMI
Department of Pathology, McMaster University Health Sciences Centre, 1200 Main Street West, Hamilton, Ontario L8N 3Z5 (Canada)
(First received July 22nd, 1985; revised manuscript received January 23rd, 1986)

SUMMARY

Solid-supported reagents for affecting a simultaneous extraction and derivatization of lipophilic and hydrophilic carboxylic acids from aqueous matrices were further investigated. The reagent consisted of pentafluorobenzyl bromide impregnated on the macroreticular resin XAD-2. New impregnation methods were developed which reduced the amount of pentafluorobenzyl bromide required and which were compatible with existing methods that utilize XAD-2 as an adsorbant. Factors affecting pentafluorobenzylation of these analytes were studied and the reaction conditions were optimized. Compatibility with simple biological samples was demonstrated using arachidonic acid as a model. Evidence was obtained suggesting that metal ions on the resin may be catalytic factors.

INTRODUCTION

Solid-supported reagents are used extensively in synthetic organic chemistry because of their ease of use, mild reaction conditions and adaptability to automation[1–4]. Simultaneous isolation and derivatization of reactive volatile analytes in air on solid-supported reagents has been reported as a means of enhancing sensitivity of analysis[5,6]. Post-column reactors based on solid supports have been described[7] in automated analysis of drugs in plasma by high-performance liquid chromatography and the use of solid-supported reactions in this area has been reviewed by Xie *et al.*[8]. Solid-supported reagents have also been utilized for esterification of carboxylic acids[9], including a reagent for the simultaneous extraction and derivatization of organic acids from water prior to gas chromatographic analysis[10]. In the latter case, a solid-supported reagent consisting of pentafluorobenzyl bromide (PFBBr) impregnated on the macroreticular rexin XAD-2 was used to affect reactions analogous to extractive alkylation. The reagent is used to make pentafluorobenzyl (PFB) derivatives of organic acids and bases which can be detected at high sensitivity using gas

0021-9673/86/$03.50

chromatography (GC) with electron-capture detection (ECD) or GC–mass spectrometry (MS) with negative ion chemical ionization (NICI) detection. The resin is a copolymer of styrene and divinylbenzene which has a highly adsorbent lipophilic surface. A number of automated and semi-automated methods for isolation of organic analytes from plasma are based upon macroreticular resins such as XAD-2[11,12]. In order to develop further the potential of solid-supported reagents, we have studied the effects of different impregnation methods, different fractional loadings, and different pretreatments of the resin on reaction yield. We have also utilized the solid-supported reaction to measure arachidonic acid in platelets.

MATERIALS AND METHODS

The macroreticular resin XAD-2 was bought from British Drug Houses (BDH, Toronto, Canada). The derivatizing reagent, PFBBr, was purchased from Chromatographic Specialties (Brockville, Canada). Tridecanoic acid, pentadecanoic, cosanoic and arachidonic acids were obtained from Sigma (St. Louis, MO, U.S.A.). Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) was obtained from Upjohn (Kalamazoo, MI, U.S.A.). Linoleic and linolenic acids were bought from Supelco (Mississauga, Canada).

Synthesis of the derivatives for use as standards

The PFB esters were synthesized by standard procedures: 25 mg of the parent compound of the analytes were dissolved in 1 ml of acetone and powdered potassium carbonate was added followed by 25 μl of PFBBr. The reaction mixture was stirred at room temperature for 2 h. At this point, thin-layer chromatographic (TLC) analysis showed only one spot. Methylene chloride and water were added and the ester was extracted into the methylene chloride. The methylene chloride phase was then washed three times with distilled water, dried with sodium sulphate and evaporated to dryness. The derivative was purified by preparative-scale TLC and isolated in 25–50% overall yield. This material was stored in solutions of 1 mg/ml in toluene and served as a standard for determination of recovery of yield.

Standard preparation of resin

The resin beads were added to water, the mixture stirred and the beads were allowed to settle. The cloudy supernatant, containing a dispersion of fine particles of the resin (the fines) and inorganic salts used to preserve the resin against microbial growth, was removed by aspiration and this procedure was repeated until the supernatant was clear. The resin was then suspended in ethanol, the mixture was stirred and the beads allowed to settle. The supernatant was then removed by aspiration and the resin was washed with methanol. The methanol-saturated resin was transferred to a Soxhlet thimble and the resin underwent Soxhlet extraction in methanol for 5 h with a cycle time of 5 min. The resin sample was then Soxhlet extracted with diethyl ether for 5 h with a cycle time of 5 min. The resin was then transferred to a round-bottomed tube and the excess diethyl ether removed *in vacuo* overnight. The sample was stored at room temperature in the round-bottomed flask, sealed with a ground-glass joint.

Pretreatment of resin with reaction inhibitors

In one experiment, the resin was leeched with agents which solubilize metals. The resin was treated as before to remove the fines and inorganic preservatives. Following this, the batch was split into three portions. One portion was washed five times with 0.05 *M* aqueous EDTA, the second portion was washed five times with 0.1 *M* methanolic hydrochloric acid and the third portion was washed five times with distilled water. All batches were soaked overnight in the treatment fluid. The following day, the EDTA and water batches were washed successively with methanol–water (1:3), 50% methanol–water (1:1) and 100% methanol. Subsequent to the methanol wash, all batches of resin were tretaed as described above, *i.e.* Soxhlet extraction with methanol and diethyl ether. In the case of the acid-treated batch, sodium bicarbonate was added to the round-bottomed flask during Soxhlet extraction, thus any acid washed off the resin was neutralized.

In a second experiment, the resin was treated with a reagent which would complex the metals *in situ*. Triplicate 200-mg quantities of resin were treated with ammonium sulphide for 1 h and then washed to neutral pH with distilled water prior to use. Control batches of 200 mg of resin were treated with 0.1 *M* sodium hydroxide for 1 h and were also washed to neutral pH prior to use.

Reaction conditions

Procedure A. A 100-mg quantity of resin prepared as described above was transferred to a 16 × 100 mm silanized screw-capped tube. A 2-ml volume of trichlorofluoromethane (Freon-11, b.p. 24°C) was added to the 100-mg sample of resin and a volume of PFBBr was then added to this mixture. The volumes of PFBBr adsorbed onto the resin were 5, 10 and 15 μl. In one set of experiments, 10 μl of PFBBr were added to the Freon in 1,1,2-trichloroethylene (TCE) solution. The Freon-11 was then evaporated by shaking at 30°C for 15 min. At the end of this time, the vial was sealed with a PTFE-lined screw cap and set aside for further use.

A 4-ml volume of 0.1 *M* phosphate buffer (pH 7.4) containing 0.25 μg/ml of pentadecanoic acid was added to the solid-supported reagent which had been prepared by the above procedure. The reaction mixture was then shaken at 30°C and at a rate of 200 cycles/10 min. At the end of the time period, the resin was rapidly isolated by filtration and the PFB derivative was eluted with 20 ml of hexane. The residue was taken up in 1 ml of toluene containing 1 μg of PFB tridecanoate and injected into the gas chromatograph.

Procedure B. A 200-mg quantity of XAD-2 was weighed into a 16 × 100 mm tube, 4 ml of buffer containing 1 μg of pentadecanoic acid were added and the mixture was shaken for 5 min. A 100-μl volume of a 9:1 mixture of TCE–PFBBr was added and the reaction mixture was shaken for 10 min and worked up as described above. In the case of the $PGF_{2\alpha}$, the buffer contained 5 μg of analyte and the reaction mixture was shaken for 1 h at 40°C. The PFB ester of $PFG_{2\alpha}$ was eluted with 20 ml of diethyl ether–ethanol (9:1, v/v) and the residue was trimethylsilyated in 50 μl of bis(trimethylsilyl)trifluoroacetamide–trimethylchlorosilane (9:1, v/v) for 1 h at 60°C. The silylating solution was evaporated and the residue taken up in 500 μl of toluene containing PFB docosanoate as an external standard.

Analysis of carboxylic acids in platelets

Human platelets were obtained in a calcium-free Tyrode Buffer suspension. The concentration was 500 000 platelets/μl, which is within the normal range. In one experiment, duplicate aliquots of 250 μl were treated with 1 ml of 0.1 *M* sodium hydroxyde for 5 min at room temperature to hydrolyse fatty acid esters and lyse the membrane. This solution was then transferred to 200 mg of XAD-2 in 4 ml of phosphate buffer and the reaction was carried out using procedure B. In a second experiment, duplicate aliquots of 250 and 50 μl were added directly to a tube containing 200 mg of XAD-2 and 4 ml of phosphate buffer (pH 7.4) and left at room temperature for 1 h. The reaction was then carried out using procedure B.

Gas chromatographic analysis

Gas chromatographic analyses of model compounds were carried out on a Hewlett-Packard 5710 gas chromatograph–electron-capture detector. The output of the detector was monitored on a Hewlett-Packard 3380 recording integrator. The chromatographic phase was 3% SE-30 on Chromsorb-W (80–100 mesh) and the carrier gas was 15% methane in argon at a flow-rate of 15 ml/min. For the analysis of pentafluorobenzyl pentadecanoate, the temperature was programmed from 220 to 250°C at 4°C/min. Prostaglandin $F_{2\alpha}$ PFB ester was analysed on the same instrumentation but with a temperature program from 250 to 300°C at 8°C/min.

Gas chromatographic analyses of the platelet extracts were carried out on a Hewlett-Packard 5790 gas chromatograph using a DB-17 capillary column, 15 m × 0.25 mm I.D., 0.25 μm thick. The output of the electron-capture detector was recorded on a Hewlett-Packard 3390 recording integrator.

Calculation of yield

The yields were calculated by external calibration using weights of synthetic PFB esters of the analytes equimolar to the weight of analyte. In the case of arachidonic acid, however, the yield was calculated relative to extractive alkylation. This was done because of the relatively small amounts of analyte available. Calculations were based on peak areas as reported by the HP-3380 recording integrator.

RESULTS AND DISCUSSION

In the initial investigations, PFBBr was added dropwise onto the resin[10]. While adequate to test the paradigm, it is unlikely that this was the optimum impregnation technique. It is possible that not all beads were uniformly impregnated with this procedure and that some beads of the resin adsorbed more than others. This would be particularly true when relatively small volumes of PFBBr were added to large amounts of resin, and in such instances reduced yield was observed[10]. Furthermore, manual impregnation could be expected to be subject to considerable interindividual variation. Finally, 50 μl of PFBBr were required to affect quantitative yield in 1 h. This relatively large volume of PFBBr increased both the amount of interfering compounds obtained and the expense of the procedure. Thus, reduction of the amount of PFBBr and of the reaction time was considered a priority.

Optimization studies were carried out with pentadecanoic acid for a number of reasons: the analyte is inexpensive and synthetic scale work is reasonable; the

compound is moderately soluble in buffer at pH 7.4; the PFB derivative is not subject to loss during the evaporation step; and, finally, the analyte is not a contaminant of glassware and reagents. Subsequently, the reaction was tested on acids found in mammalian systems. As a model of lipophilic acids we investigated arachidonic acid, and the model for hydrophilic acids was $PGF_{2\alpha}$. These biosynthetically linked carboxylic acids, as well as other acids arising from arachidonic acid, are involved in a variety of physiological and pharmacological phenomena[13].

In order to reduce the amount of PFBBr required, an impregnation procedure was developed which was expected to give a more homogeneous distribution of PFBBr on the resin. In this procedure, PFBBr (b.p. 174°C) was added to the resin in Freon-11 (b.p. 24°C) and the mixture was shaken for 30 min at 30°C. Thus, PFBBr was deposited on the surfaces as the Freon-11 was evaporated. With this impregnation procedure, as little as 5 μl of PFBBr deposited on 100 mg of resin were sufficient to give quantitative yield in 1 h. With the Freon-11 method, increasing the volume of PFBBr resulted in increased product yield (Table I) in a given reaction time, but even with 10 μl the reaction time required for quantitative reaction was markedly reduced.

TABLE I

THE EFFECT OF VOLUME OF REAGENT ON YIELD FOR THE DERIVATIZATION OF PENTADECANOIC ACID AT 30°C

Average ± S.D., $n = 6$.

*Volume PFBBr**	*Reaction time (min)*	
	10	*20*
5	34 ± 6	51 ± 4
10	51 ± 5	64 ± 5
15	59 ± 6	76 ± 5

* 100 mg of resin.

Further optimization of reaction conditions was investigated with respect to the weight of resin used. The effect of increased resin weight was investigated both at constant volume of PFBBr used and constant ratio of PFBBr to weight of resin. As shown in Table II, the weight of resin is an important determinant of the yield, regardless of the reagent/resin ratio.

The Freon-11 impregnation procedure was useful in developing a solid supported reagent, but this reagent might not be compatible with existing methods using XAD-2 as an adsorbant. In these methods, the analytes would be adsorbed prior to impregnation. An impregnation procedure compatible with this situation might allow the use of automated instrumentation already available. Consequently, the effect of order of impregnation was investigated.

In this study, pentadecanoic acid was adsorbed from buffer at pH 7.4 and was thus adsorbed as an anionic species. The adsorption was efficient and complete within 5 min. Pentafluorobenzyl bromide was then impregnated using procedure B. Using

TABLE II

THE EFFECT OF REAGENT/RESIN RATIO ON YIELD FOR DERIVATIZATION OF PENTADECANOIC ACID WITH VARYING AMOUNTS OF RESIN AT 30°C IN A REACTION TIME OF 10 MIN

Weight of resin (mg)	*Volume of PFBBr (μl)*	*Ratio of reagent/resin*	*Percentage yield**
100	10	0.1	47 ± 3
200	10	0.05	67 ± 5
300	15	0.05	80 ± 4

* Average ± S.D., $n = 6$.

this very simple impregnation procedure the solid-supported reaction was still affected, but relative to the Freon-11 impregnation method the yield was reduced (Table III). This reduction may have been due to non-uniform impregnation of the beads.

TABLE III

EFFECT ON YIELD (%) OF CO-IMPREGNATION PROCEDURE WITH 10 μl OF PFBBr ON 200 mg OF RESIN

Volume of TCE (μl)	*Analyte*		
	Pentadecanoic acid		*$PGF_{2\alpha}$*
	*Reaction procedure A**	*Reaction procedure B***	*Reaction procedure B****
0	64 ± 4	32 ± 2	48 ± 8
45	66 ± 4	57 ± 2	
90	57 ± 3	67 ± 8	90 ± 9

* Average ± S.D., $n = 4$.
** Reaction time of 10 min at 30°C; average of six determinations ± S.D.
*** Reaction time of 2 h at 40°C; average of six determinations ± S.D.

In order to improve the uniformity of impregnation, the 10 μl of PFBBr were impregnated in solution with TCE on 200 mg of resin in order to increase the volume of liquid being added. In the limit studied, the total volume of halogenated hydrophobic organic solvent (10 μl of PFBBr plus 90 μl of TCE) was thus equal to the pore volume. Assuming that a pore can only hold its own volume of fluid, this approach, in principle, ensured that each pore would contain a solution of PFBBr–TCE at a ratio of 1:9 (v/v). Given the large excess of PFBBr, we reasoned that such dilution might not adversely affect the yield. This was suggested by the fact that with the Freon-11 impregnation method, dilution of 10 μl of PFBBr in 45 or 90 μl of TCE did not strongly affect the reaction yield (Table III). In contrast, using procedure B

there was an increase in reaction yield with increasing volume of TCE, and at 90 μl it was equal to that obtained with the Freon-11 technique. Thus, it is possible to use a simple method of impregnation which can be utilized when the analytes are already adsorbed on the resin and which may be compatible with existing instrumentation.

Whilst the changes in the impregnation procedures can moderately affect the yield, the structure of the carboxylic acid appears to have a much larger effect. Quantitative derivatization of $PGF_{2\alpha}$, a trihydroxy carboxylic acid, required 2 h at 40°C. This yield is only 25% in excess of the yield found for pentadecanoic acid in 10 min at 30°C (Table III). The slower reaction for the polar compound may be related to the polarity and higher water solubility of the $PGF_{2\alpha}$ anion, which, unlike straight-chain carboxylic acids, cannot be efficiently adsorbed onto the resin from buffer at pH 7.4. Interestingly, the effect of co-impregnation of PFBBr and TCE gave a similar increase in yield to that of the lipophilic analytes, indicating that addition of co-solvent is an effective approach to optimizing the yield for hydrophilic as well as hydrophobic analytes.

The solid-supported reagent appears to be an effective analogue of phase-transfer catalysis, but the nature of the reaction, particularly the role of the resin, is little understood. The resin can act simply as a support for the reagent. In this case, the reaction would be due to the highly reactive nature of the PFBBr. In fact, reactions of phenolates in an aqueous/organic heterogeneous system have been reported[14,15] but such reactions were not observed for carboxylic acids. Thus, it is unlikely that in the case of carboxylic acids, the resin acts only as a support for PFBBr. Moreover, if the resin was solely a support then, in analogy to homogeneous and heterogeneous reactions, a large solvent effect should be observed in changing from halogenated to alkyl hydrocarbons[16]. However, when cyclohexane was used instead of TCE, the yield remained unchanged at 67 ± 5% in a reaction time of 10 min at 30°C. These data indicate that the resin is an obligatory component of the reaction and may act as a catalyst as well as an adsorbant.

In contrast to a lack of effect with different co-solvents, different resin pre-treatments substantially reduced the reaction rate. Thus, treatment with acid during resin preparation produced a decrease in reaction yield (Table IV). This could have resulted from alteration of the hydrophobic surface of the resin or from alteration of other features on the surface. Since it has been reported that XAD-2 is a cation-exchange resin capable of removing metals[17] from water, one such feature might be

TABLE IV

EFFECT OF LEECHING WITH HYDROCHLORIC ACID AND EDTA DURING PREPARATION OF RESIN

Analyte	*Treatment*		
	Control	*HCl*	*EDTA*
Pentadecanoic acid*	48 (2)**	18 (3)**	22 (3)**
$PGF_{2\alpha}$	51 ± 4***	37 ± 6***	31 ± 2***

* Freon impregnation.
** Average of three determinations (range).
*** Average ± S.D., $n = 4$.

metals adsorbed or otherwise attached on the surface of the resin. Support for this was provided by the effect of pretreatment of the resin with EDTA, which suppressed the reaction, and the fact that both EDTA and hydrochloric acid partially leeched metals from the resin (Table V). Furthermore treatment with ammonium sulphide results in a decrease in yield of >90% for the pentafluorobenzylation of $PGF_{2\alpha}$. This reagent reacts with Fe^{3+} and Zn^{2+} to form sulphides that are insoluble at neutral pH[18,19]. Formation of such sulphides *in situ* may have resulted in the marked reduction of reaction yield. It is unlikely that the alkaline conditions encountered during treatment with ammonium sulphide are responsible, since treatment of the resin with alkaline buffer had no effect on yield. The data on pretreatment of the resin with hydrochloric acid and EDTA during resin preparation, and with ammonium sulphide *in situ*, suggest that metals may be catalytic factors in this reaction.

TABLE V

EFFECT OF PRETREATMENT ON TRACE METAL

Metal	*Treatment of resin*		
	Control	*HCl*	*EDTA*
Fe	62*	16	20
Cr	1.6	0.53	1.4
Zn	4.0	2.0	2.2

* Concentrations quoted in ppm determined by neutron activation analysis.

The resin-supported reaction was tested for the determination of arachidonic acid, linoleic and linolenic acids. The latter two compounds are di- and tri-unsaturated C_{18} carboxylic acids found in platelets (Fig. 1), but whose function in platelet physiology is undefined. Relative to extractive alkylation, the yields for solid-supported pentafluorobenzylation were greater than 88% and the calibration curves were linear for arachidonic acid ($r = 0.996$) from 150 to 4 μg, and for linolenic and linoleic acid ($r = 0.993$ and $r = 0.998$, respectively) over the range 125–500 ng. In the case of linolenic acid, there was an interference corresponding to 40 ng; this limits the detection of the analyte in smaller biological samples. The presence of both C_{16} and C_{18} carboxylic acids on glassware and reagents has been previously documented[20]. No such interferences were found for linolenic and arachidonic acids.

Human platelets in amounts corresponding to 250 μl of whole blood ($12.5 \cdot 10^7$) were treated in two ways: hydrolysis with 0.1 *M* sodium hydroxide; and adding the platelets to the 200 mg of resin in 0.1 *M* phosphate buffer at pH 7.4. Treatment with 0.1 *M* sodium hydroxide resulted in the recovery of 2.42 ± 0.23 μg of arachidonic acid, whereas just adding the platelets to the resin and buffer resulted in recovery of 2.4 ± 0.06 μg. These data suggest that platelets adsorbed on the resin surface undergo a release reaction and the released unsaturated lipophilic acids are adsorbed directly onto the resin surface upon which they can be subsequently derivatized. Thus the resin, in addition to serving as the basis for solid-supported reagent, also permits mild conditions for the isolation of carboxylic acids from platelets. It also appears that the constituents of the platelets (*e.g.* proteins, neutral lipids, etc.)

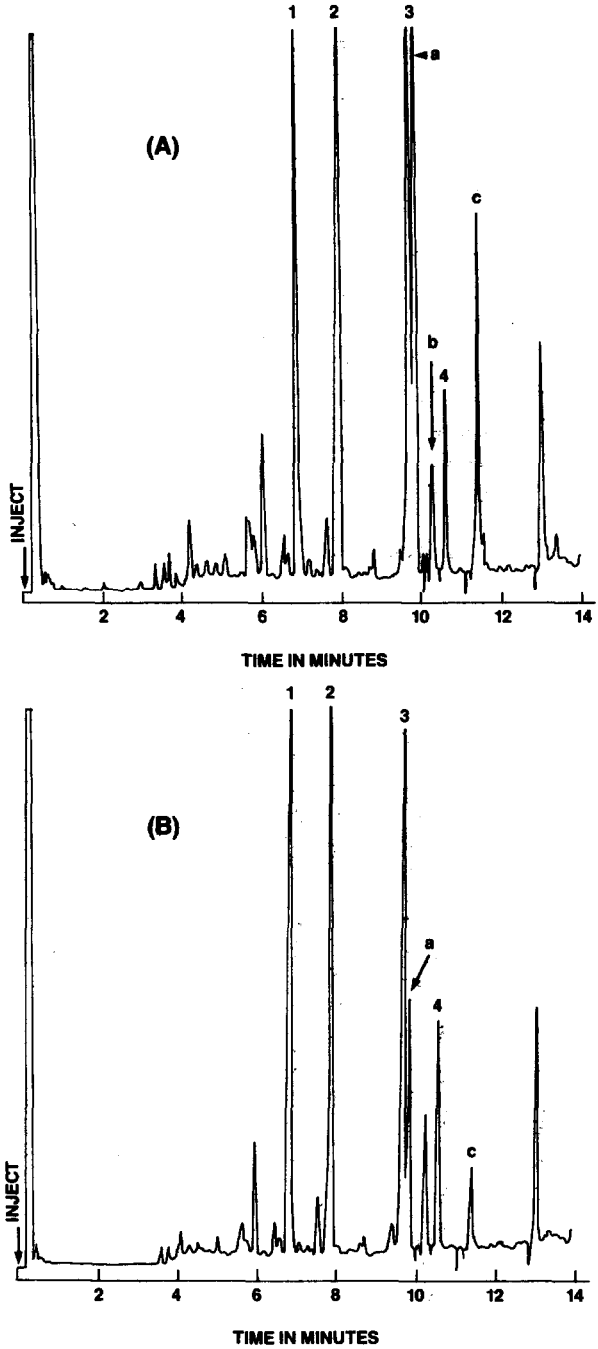

Fig. 1. Gas chromatographic analysis of fatty acids derived from human platelets by pentafluorobenzylation on XAD-2: (A) platelets derived from 250 μl of whole blood; (B) platelets derived from 50 μl of whole blood. Peaks labelled 1, 2, 3 and 4 are PFB esters of carboxylic acids found in the blank; peaks labelled a, b and c are PFB esters of linoleic, linolenic and arachidonic acid, respectively.

do not interfere in the reaction. The sensitivity of the method was tested by carrying out the procedure on a second batch of human platelets and using only $3 \cdot 10^7$ platelets; 176 ng of arachidonic acid were found (Fig. 1B). As this would be equivalent to the number of platelets found in 50 μl of blood, such sensitivity would permit experimental investigation in small animals or clinical study in the pediatric population.

CONCLUSION

Establishing the reaction mechanism of this catalytic process will require further investigation. The present data, however, indicate the applicability of solid-supported reactions on XAD-2 to biological problems. In addition, the lack of solvent effects argues that the reaction is distinct from phase-transfer catalysis and, finally, that the decreases of reaction yhield resulting from leeching with hydrochloric acid and EDTA or treating with ammonium sulphide suggests the involvement of metals in the reaction. This solid-supported reaction will be investigated as one approach[8] to the automation of methods in analytical organic chemistry.

REFERENCES

1 C. Leznoff, *Acc. Chem. Res.*, 11 (1978) 327.
2 G. H. Posner, *Angew. Chem. Int. Ed. Engl.*, 17 (1978) 487.
3 A. McKillop and D. Young, *Synthesis*, (1979) 401.
4 A. Akellah and D. C. Sherrington, *Chem. Rev.*, 81 (1981) 557.
5 E. R. Kennedy and R. Hill, *Anal. Chem.*, 54 (1982) 1739.
6 E. Lipari, *Anal. Chem.*, 56 (1984) 1820.
7 L. Nondek, R. W. Frei and U. A. Th. Brinkman, *J. Chromatogr.*, 282 (1983) 141.
8 K.-H. Xie, S. Colgan and I. S. Krull, *J. Liq. Chromatogr.*, 6 (1983) 125.
9 An Zhu and Gui-Yun Xu, *J. Chromatogr.*, 314 (1984) 421.
10 J. M. Rosenfeld, M. Mureika-Russell and A. Phatak, *J. Chromatogr.*, 283 (1984) 127.
11 L. M. St. Onge, E. Dolar, M. A. Anglim and C. J. Least, *Clin. Chem.*, 25 (1979) 1373.
12 J. Balkon, B. Donnelly and D. Prendes, *J. Forensic Sci.*, 27 (1982) 23.
13 H. Kalant, W. H. E. Roschau and E. M. Sellen (Editors), W. H. E. Roschlau (Executive Editor), *Principles of Medical Pharmacology*, University of Toronto Press, Toronto, 1985, Ch. 31, p. 361.
14 J. M. Rosenfeld and J. L. Crocco, *Anal. Chem.*, 50 (1978) 701.
15 J. M. Rosenfeld, T. L. Ting and J. L. Crocco, *Anal. Lett.*, 13 (1980) 283.
16 A. W. Herriot and D. Picker, *J. Am. Chem. Soc.*, 97 (1975) 2345.
17 D. J. Mackey, *J. Chromatogr.*, 242 (1982) 275.
18 *Analytical Chemistry Principles*, J. H. Kennedy Harcourt, Bruce, Jovanovich, 1984, p. 155.
19 *Macro and Semimicro Qualitative Inorganic Analysis*, A. I. Vogel Songmans, London, 4th ed., 1953, p. 260.
20 J. E. Greving, J. H. G. Jonkman and R. A. de Zeeuw, *J. Chromatogr.*, 148 (1978) 398.