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Attogram-Level Detection and Relative Response of Strong Electrophores by Gas Chromatography with Electron Capture Detection

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The responses as peak areas of some divergent compounds, most of which are strong electron absorbers, are measured by gas chromatography with electron capture detection (GC-ECD). The most sensitive compounds are derivatized iodothyronines, which are essentially 20-fold more sensitive than lindane. *N,N*-Dipentafluorobenzoylpentafluoroaniline, a somewhat less sensitive but more volatile substance, was selected for determination of a detection limit. The value was 90 ag (1.6×10^{-19} mol), largely due to an anomalous increase in its response at the trace level. This increases the reported sensitivity of GC-ECD by 100-fold.

Electrophores in the vapor state are molecules or substituents which absorb thermal electrons (*1*, *2*). This type of electron uptake takes place in the electron capture detector in both gas chromatography (GC-ECD, e.g., ref 3) and liquid chromatography (*4*), and also negative chemical ionization mass spectrometry (*5*, *6*). Relatively few molecules are both volatile and intensely electrophoric.

The relative response of different electrophores has been studied previously, as has been reviewed (*3*, *7*, *8*), but this work can be extended in several respects. Only a limited variety of electrophoric structures were often examined in any particular study. Certain interesting molecules were not included, such as the derivatized iodothyronines (*9*), or were not compared with other electrophores, such as flophemesyl (*7*). Various types of EC detectors and GC-ECD conditions were involved, which can influence the response (*8*). Thus, it is useful to conduct a sensitivity study of strong electrophores under a set of constant and typical conditions. The conditions employed here are capillary GC with a fused silica column and a constant current, variable frequency, small volume ECD.

The purpose of this study is to define the types of structures which are most sensitive as well as volatile by GC-ECD, in order to guide the future development of improved derivatizing agents for GC-ECD and related techniques. The main conclusions reached here are that (1) the derivatized iodothyronines are the most sensitive but least volatile of the compounds examined, (2) the flophemesyl group, which has been employed as a derivatizing group for analyses by GC-ECD (*7*), is not highly sensitive, at least for the derivatization of alcohols, (3) the pentafluorobenzamide group (pentafluorobenzoylamide) offers a good combination of sensitivity, volatility, and chemical accessibility for incorporation into potential derivatizing agents, and (4) the response for *N,N*-dipentafluorobenzoylpentafluoroaniline increases at the trace level, yielding a detection limit for this compound of 90 ag.

EXPERIMENTAL SECTION

Materials and Methods. Methyl pentafluorobenzene, pentafluorostyrene, pentafluoroiodobenzene, pentafluoroacetophenone, decafluorobenzophenone, pentafluorobenzoyl chloride, and pentafluoroaniline were obtained from PCR Research Chemicals, Inc. 1,4-Dibromobenzene, 1-octanol, ethylamine, pentachlorobenzene, 1-butanol, 2,4,6-triiodophenol, glycine methyl ester hydrochloride, β-alanine, 2,5-diiodobenzoic acid, 3,5-dibromotyrosine, 3-iodotyrosine, 3,5-diiodotyrosine, 3,5-diiodothyronine, 3,3′,5-triiodothyronine, thyroxine, trifluoroacetic anhydride, pentafluoropropionic anhydride, and heptafluorobutyric anhydride were obtained from Aldrich Chemical Co. Flophemesyl chloride was purchased from Lancaster Synthesis Ltd., Lancaster, England, and used to derivatize 1-octanol according to the procedure of Poole et al. (*10*). A standard solution of lindane and aldrin in isooctane was obtained from Varian Associates.

***N*-Acetylpentafluoroaniline.** Pentafluoroaniline (0.4 g, 0.002 mol) and freshly distilled acetic anhydride (2 mL, 0.02 mol) were heated under reflux at 90 °C for 1 h. After the mixture was cooled to 4 °C, water (5 mL) was added followed by methylene chloride (10 mL) and shaking. The organic layer was separated, washed with 10 mL of water, and dried over anhydrous magnesium sulfate overnight. Nitrogen was used to evaporate the solvent, yielding white crystals. The product was recrystallized from toluene, and then acetonitrite, mp 144–145 °C. The structure was confirmed by mass spectrometry.

Perfluoroacyl Alkyl Ester Derivatives of the Amino Acids. Typically the dried amino acid (0.0003 mol) was heated at 60 °C with a 25% (w/w) methanolic solution of HCl (15 mL) for 1.5 h. Excess reagents were removed at 50 °C on a rotary evaporator under reduced pressure (water aspirator) and the product was dried in vacuo over sodium hydroxide in a desiccator overnight. The methyl ester amino acid hydrochloride was dissolved in dry acetonitrile (10 mL) and freshly distilled perfluoroacyl anhydride (0.01 mol) was added. The mixture was heated under reflux for 1.5 h. Excess reagents were removed at

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80 °C on a rotary evaporator under reduced pressure (water aspirator) and subsequently dried as above. The product was recrystallized twice from toluene and the purity was established by gas chromatography with both FID and ECD detectors.

Heptafluorobutyryl-2,4,6-triiodophenol. 2,4,6-Triiodophenol (0.1 g, 0.00022 mol) was dissolved in acetonitrile (5 mL) and freshly distilled heptafluorobutyric anhydride (0.01 mol) was added. The mixture was heated for 1.5 h at 80 °C after which excess reagent was removed on a rotary evaporator under reduced pressure (water aspirator). The residue was dried overnight in a desiccator over sodium hydroxide and recrystallized from toluene. A single peak was obtained by GC (FID and ECD), mp 51–52 °C.

2,5-Diiodobenzoic Acid Methyl Ester. 2,5-Diiodobenzoic acid (0.1 g, 0.000 25 mol) was heated with methanolic HCl (25% w/v, 15 mL) for 1 h at 60 °C. After the excess reagent was removed on a rotary evaporator at 60 °C under reduced pressure (water aspirator), the product was recrystallized twice from toluene–acetonitrile (3:1). The purity of this compound was determined by GC(FID and ECD), mp 75–76 °C.

***N*-Benzoylpentafluoroaniline.** Pentafluoroaniline (0.5 g, 0.002 mol) was dissolved in a solution made up of 5% aqueous sodium carbonate (5 mL) and 2 N NaOH (1 mL). The solution was cooled to 0–4 °C and benzoyl chloride (0.5 mL, 0.004 mol) was slowly added with stirring over a period of 15 min. The pH was adjusted to 7 with concentrated HCl, and the white precipitate was filtered, washed with cold water, and dried in vacuo at room temperature. The product was recrystallized twice from ethanol. The structure was confirmed by mass spectrometry, mp 113–114 °C.

***N*-Pentafluorobenzoylethylamine.** Ethylamine (1 mL, 0.016 mol) was combined with 1.4 mL of water and 5 mL of 2 N sodium hydroxide. After cooling in an ice bath, this solution was treated with 0.5 mL (0.003 mol) of pentafluorobenzoyl chloride in five aliquots over a period of 15 min. The solution was left to stir for another 15 min, after which it was neutralized with concentrated HCl. A white precipitate was filtered, washed with cold water, dried in vacuo at room temperature and recrystallized twice from ethanol. The structure was confirmed by mass spectrometry, mp 88–89 °C.

***N*-Pentafluorobenzoylpentafluoroaniline.** Pentafluorobenzoyl chloride (0.75 g, 0.0032 mol) was slowly added over a period of 15 min to an ice cold solution of pentafluoroaniline (0.5 g, 0.002 mol) in 5% aqueous sodium carbonate (5 mL) and 2 N sodium hydroxide (1 mL). After being stirred for 10 min at room temperature, the solution was neutralized with concentrated HCl and the resultant precipitate was filtered, washed with water, and dried in vacuo at room temperature. The product was recrystallized twice from toluene and confirmed by mass spectrometry, mp 179–180 °C.

***N,N*-Dipentafluorobenzoylpentafluoroaniline.** Pentafluoroaniline (0.5 g, 0.002 mol) was heated at 90 °C with pentafluorobenzoyl chloride (1.0 g, 0.004 mol) and triethylamine (0.5 g, 0.004 mol) in toluene (3 mL) for 1 h. A precipitate gradually formed and was filtered hot at the end of the reaction, mp 253–255 °C. (Reported melting point for triethylammonium chloride is 254–252 °C.) Cooling the filtrate to room temperature yielded another precipitate, mp 123–125 °C. This was recrystallized sequentially from acetonitrile and toluene. The structure was confirmed by mass spectrometry, mp 124–125 °C.

Instrumentation. The basic instrumentation and equipment were as follows: gas chromatograph, Varian Model 3740 equipped with a constant current, variable-frequency ^{63}Ni (8 mCi) ECD at 310 °C and direct injector at 250 °C; carrier gas, ultra-high-purity nitrogen (Matheson) filtered with 13X molecular sieves, charcoal, and an Oxyclear disposable purifier (Oxyclear Co.); column, fused silica 15 m × 0.25 mm nonbonded, SE52 (J&W Scientific Co.); injection solvent, toluene unless noted otherwise; septa, HT-X blue (Applied Sciences). A constant flow rate through the detector of 30 cm^3 min^{-1} was maintained by adjusting the makeup at the capillary insert base as required, unless noted otherwise. The flow rates (temperature corrected) of carrier gas through the column were 2.08, 6.07, 6.43, 6.81, and 4.00 cm^3 min^{-1} for groups A, B, C, D, and E, respectively, in Table I. Further details are provided elsewhere (*11*).

Calibration Curve for *N,N*-Dipentafluorobenzoylpentafluoroaniline (DPPA). For this experiment, the column, column

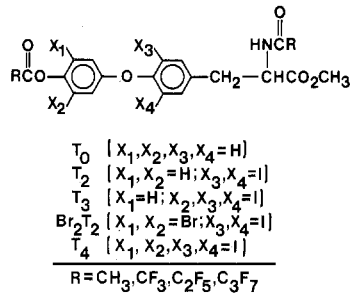

Figure 1. Structures of derivatized thyronines.

makeup, and ECD makeup rates of nitrogen flow (measured at room temperature and uncorrected) were 5.8, 4.0, and 5.2 mL/min, respectively. The direct injector was fitted with a silanized quartz insert. The syringes were kept clean between dilutions or injections by intermediate washing with acetonitrile at 75 °C. Blank solvent injections with the same syringe were made before and after every sample injection for concentrations of DPPA below 100 fg, and periodically for higher concentrations of DPPA. All analytical steps were performed either in duplicate or in triplicate. Final dilution into toluene instead of acetonitrile gave a broader solvent peak. The acetonitrile and toluene (Burdick and Jackson) were used as received.

RESULTS AND DISCUSSION

Conditions Used To Compare Responses. The compounds were analyzed as five groups based on volatility differences shown in Table I. Lindane was chosen as a reference solute because of its inertness, high electron-capturing characteristics, and moderate retention time. The responses were determined as peak areas relative to the peak area of lindane under constant conditions in the detector. In order to minimize the occurrence of artifacts such as O_2 effects, coulometric nonlinearity, or nonlinearity from excessive solute capture of electrons in the ECD, we adjusted the amounts of the solutes injected to give comparable peak heights in all cases. Further, the amounts (typically ~100 pg) of the strongest electron absorbers injected were always confirmed to be within the linear range for these compounds. For example, we have previously reported a linear range of 0.4–700 pg for the derivatized iodothyronines (*11*).

Although detection temperatures of 250, 280, and 310 °C were evaluated, only the responses at 310 °C are shown because the differences in all cases among the areas for each solute were never significantly greater than a factor of 2 throughout this temperature range. This is a small difference considering that the electron capture coefficient (K) covers a range of at least 10^7 (*2*). Significant changes in EC response due to detector temperature apparently tend to arise only when less sensitive solutes are analyzed especially at lower temperatures and over broader temperature ranges. For example, although the sensitivity of 1,2-dichloroethane, a weak electrophore, increases by a factor of 1000 as the temperature is changed from 80 to 350 °C, the response toward carbon tetrachloride, an intense electrophore, is constant throughout this entire range (*12*). Also, the variation in the electron capture coefficient for the most sensitive compounds ($K > 10^9$) among a series of strong electrophores, involving pentafluorobenzoate, pentafluorophenacetate, and pentafluorophenoxyacetate derivatives of some amines and alcohols, always varied by less than a factor of 2 over the temperature range of 250–350 °C (*13*). This limited effect of temperature on the response of strong electron absorbers has been noted previously (*14*).

Sensitivity of Derivatized Thyronines. It is first interesting to observe that the most intense electrophores in Table I (largest peak areas) are the *N,O*-diperfluoracyl-derivatized (e.g., HFB) iodothyronine methyl esters, the struc-

Table I. GC-ECD Response and Retention Characteristics of Some Electrophores[a]

compound	rel molar response	retention time, min
A. 90 °C[b]		
methylpentafluorobenzene	0.000095	0.62
pentafluorostyrene	0.004	0.73
lindane	1.00	7.10
B. 140 °C		
pentafluoroiodobenzene	0.49	0.42
N-HFB-β-alanine-ME	0.076	0.44
pentafluoroacetophenone	0.12	0.67
1,4-dibromobenzene	0.074	0.71
N-acetylpentafluoroaniline	0.22	0.73
decafluorobenzophenone	1.5	0.81
N-PFB-ethylamine	0.86	0.87
O-HFB-*p*-methoxyphenol	0.13	1.38
pentachlorobenzene	0.53	1.65
1-bromonaphthalene	0.11	1.80
diphenyl ether	0.0000081	1.83
pentafluoroanisole	0.065	2.90
lindane	1.0	5.62
C. 165 °C		
N-PFB-glycine-ME	1.2	1.37
N-PFB-pentafluoroaniline	1.4	1.37
N-PFB-β-alanine-ME	1.1	1.43
flophemesyl *n*-octyl ether	0.035	1.48
N,N-di-PFB-pentafluoroaniline	5.00	1.95
N-PFB-aniline	0.78	2.25
lindane	1.00	2.39
N-benzoylpentafluoroaniline	0.14	2.46
O-HFB-2,4,6-triiodophenol	1.00	4.69
D. 190 °C		
lindane	1.00	2.05
2,5-diiodobenzoic acid-ME	0.005	2.53
N,O-di-HFB-3-iodotyrosine-ME	0.63	3.34
aldrin	1.30	3.80
N,O-di-HFB-3,5-dibromotyrosine-ME	1.90	4.47
N,O-di-HFB-3,5-diiodotyrosine-ME	0.90	9.40
E. 265 °C		
(lindane)[c]	(1.00)	0.32
N,O-di-HFB-3,5-diiodotyrosine-ME	1.28	0.81
N,O-di-HFB-T_0-ME	0.9	1.55
N,O-di-HFB-T_2-ME	19	2.45
N,O-di-AC-T_0-ME	0.01	2.60
N,O-di-HFB-T_3-ME	18	4.93
N,O-di-HFB-Br_2T_2-ME	22	6.23
N,O-di-AC-T_2-ME	5.7	9.87
N,O-di-PFP-T_4-ME	17	10.00
N,O-di-HFB-T_4-ME	19	10.00
N,O-di-TFA-T_4-ME	19	10.86
N,O-di-AC-T_3-ME	7.5	19.67
N,O-di-AC-T_4-ME	6.7	39.26

[a] The following abbreviations are used: HFB, heptafluorobutyryl; ME, methyl ester; PFB, pentafluorobenzoyl; PFP, pentafluoropropionyl; TFA, trifluoroacetyl; T_2, 3,5-diiodothyronine; T_3, 3,5,3′-triiodothyronine; Br_2T_2, 3,5-diiodo-3′,5′-dibromothyronine; T_4, 3,5,3′,5′-tetraiodothyronine; T_0, thyronine; flophemesyl, pentafluorophenyldimethylsilyl; and AC, acetyl. The relative molar responses are the peak areas of the compounds relative to the peak area of lindane, under constant flow rate conditions in the detector, and include any differences in the recovery of these compounds in the GC-ECD. [b] After initial hold at 90 °C for 1 min, the oven temperature for the column was programmed at 1 deg/s up to 150 °C. [c] The values in group E are expressed relative to lindane by carrying over the peak area for *N,O*-di-HFB-3,5-diiodothyronine-ME relative to lindane from group D, since lindane coeluted with the solvent tail at 265 °C.

tures of which are represented in Figure 1. The exact origin of this sensitivity is not well defined. Nevertheless, the results with several analogues suggest that (1) this sensitivity arises primarily from the substituted ring system (without much contribution from the perfluoroacylamide group), (2) this ring system can be rendered highly electrophoric in different ways, and (3) a considerable degree of "diminishing returns" can develop in this system, referring to the smaller proportion by which an electrophoric substituent increases the sensitivity when incorporated into a strong, as opposed to a weak electrophore.

The contribution of the perfluoracylamide group to the electron capture response probably is negligible not only because *N*-HFB-β-alanine-ME is not highly sensitive (its relative molar response is 0.076, which is 260-fold less than that of the *N,O*-diperfluoracyliodothyronine derivatives) but because the substitution of *N,O*-acetyl groups in place of *N,O*-perfluoracyl groups in these iodothyronine derivatives leads to only a 3-fold reduction in response (i.e., to a relative peak area value of about 7). In contrast, high sensitivity (relative peak area 1.0) is seen for *O*-HFB-2,4,6-triiodophenol, a model for the ring system under discussion.

In the derivatized iodothyronines, the perfluoracylamide group is separated, as seen in Figure 1, from the strongly electrophoric ring system by two saturated carbon atoms. This is similar to the separation of the two dinitrophenyl electrophores in the didinitrophenyl derivatives of the amino acids lysine, ornithine, and cystine (*15*). Consistent with the apparent failure of the isolated perfluoroacylamide electrophore to add significantly to the sensitivity of the ring system in the derivatized iodothyronines, the preceding didinitrophenyl derivatives were found to be no more sensitive than the monodinitrophenyl derivatives of the other common amino acids (*15*).

Different, Highly Sensitive Diphenyl Ether Ring Systems. Apparently a diphenyl ether ring system is quite susceptible to electrophoric enhancement by the incorporation of appropriate substituents, even though innately it is a very weak electrophore. As seen in Table I, it is 1.2×10^5 times less sensitive than lindane. The addition of acetoxy (–OCO-CH_3) and $-CH_2CH(NHCOCH_3)(CO_2CH_3)$ groups at opposite ends of this ring system, affording *N,O*-di-AC-T_0-ME, increases the electrophoricity by a factor of 1200, up to a peak area of 0.01 relative to lindane. This is in spite of the fact that the $-CH_2CH(NHCOCH_3)(CO_2CH_3)$ group apparently contributes negligibly to this change, as just discussed, and the acetoxy group is inherently only a weak electrophore. Replacing the acetoxy group with an $-OCOC_3F_7$ group, giving *N,O*-di-HFB-T_0-ME, further increases the sensitivity up to a relative peak area value of 0.9. (This also involves a change of *N*-acetyl to *N*-HFB, but this substitution, based on the above arguments, is assumed not to contribute significantly to the observed increase in sensitivity.)

Alternatively, the further attachment of two iodine atoms to *N,O*-di-AC-T_0-ME, giving *N,O*-di-AC-T_2-ME, yields a peak area relative to lindane of 5.7. Thus, even different electrophoric modifications of diphenyl ether (*O*-HFB at one site vs. two iodine atoms at other sites, i.e., *N,O*-di-HFB-T_0-ME vs. *N,O*-di-AC-T_2-ME) result in structures which readily capture electrons. Combining these *O*-HFB and iodine electrophoric groups, as in *N,O*-di-HFB-T_2-ME, raises the response even further, to the relative peak area value of 19.

Diminishing Returns. The continued addition of equivalent or similar electrophoric groups to a given molecular structure sooner or later adds proportionately less and less to the electron capture response (*3*, *14*, *16*). We shall refer to this as "diminishing returns". For example, the relative sensitivities of the trifluoroacetyl (TFA), pentafluoropropionyl

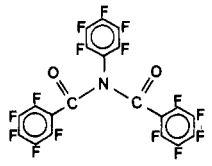

Figure 2. Structure of *N,N*-dipentafluorobenzoylpentafluoroaniline (DPPA).

(PFP), and heptafluorobutyryl (HFB) mono-*N*-acyl derivatives of benzylamine were 0.8, 299, and 715, respectively (*16*). Thus, the first extra CF_2 group in PFP (over TFA) gives a 286-fold enhancement, whereas another such group (in HFB) only gives a further 3.1-fold increase.

Diminishing returns are observed here for the derivatives of the thyronines. The addition of two iodine atoms to *N,O*-di-AC-T_0-ME, a weak electrophore, gives *N,O*-di-AC-T_2-ME, and this involves an increase in sensitivity of 570-fold (from 0.01 to 5.7). However, the same addition of two iodine atoms to *N,O*-di-HFB-T_0-ME, a stronger initial electrophore, affording *N,O*-di-HFB-T_2-ME, only increases the sensitivity by 21-fold (from 0.9 to 19). Further, for both the acetyl and HFB derivatives of the iodothyronines, which are all very strong electron absorbers, the electron absorption is essentially constant at relative peak areas of about 7 and 20, respectively, irrespective of the presence of two, three, or four iodine atoms; or, alternatively, two bromine and two iodine atoms in the case of the HFB derivative. Also, for the methyl ester of T_4, the strong response is constant within experimental error whether TFA, PFP, or HFB groups are present.

Sensitivity of Other Groups. As seen in Table I, a pentafluorophenyl group can be quite insensitive, e.g., methylpentafluorobenzene is 10^4 times less sensitive than lindane. Neither the attachment of a vinyl group (giving pentafluorostyrene, sensitivity of 0.004 relative to lindane) nor of a dimethylsilyl ether moiety (giving flophemesyl *n*-octyl ether, for example, which has a sensitivity relative to lindane of 0.035) yields structures with high sensitivity. However, the pentafluorobenzyl derivatives of some organic acids are reported to have a sensitivity similar to that of aldrin (*17*), suggesting that the response of a pentafluorophenyl group can be enhanced significantly even by a suitable substituent attached through a methylene group.

The pentafluorobenzamide group is a strong electron absorber, as seen by the high sensitivities for the *N*-PFB derivatives of ethylamine, glycine-ME, and β-alanine-ME. The high sensitivity of the pentafluorobenzamide group has been reported several times previously (e.g., ref 5 and 13), and analogous Schiff bases of pentafluorobenzaldehyde are similarly sensitive (*18, 19*). The additional sensitivity of the imide, *N,N*-di-PFB-pentafluoroaniline, is in agreement with previous reports that imides involving electrophoric acyl groups are strong electron absorbers (*20, 21*). For example, the *N,N*-di-HFB derivative of dodecylamine is 17-fold more sensitive than the corresponding mono-HFB derivative (*21*).

Attogram Detection Limit and Anomalous Response. *N,N*-Dipentafluorobenzoylpentafluoroaniline (DPPA), the structure of which is shown in Figure 2, was selected for determination of a detection limit due to the combination of high sensitivity and moderate volatility observed for this compound. For this value, first a solvent blank of toluene is injected into the gas chromatograph giving chromatogram A in the insert of Figure 3. A 12.5 pg/μL toluene solution of DPPA then is diluted by 0.5 μL per 25 mL into acetonitrile, giving a 0.25 fg/μL solution, and 1 μL is injected immediately into the instrument. The resulting chromatogram, labeled B in the insert of Figure 3, reveals a peak which corresponds to an extrapolated detection limit of 90 ag (1.6×10^{19} mol) of DPPA at a signal-to-noise ratio of 2.

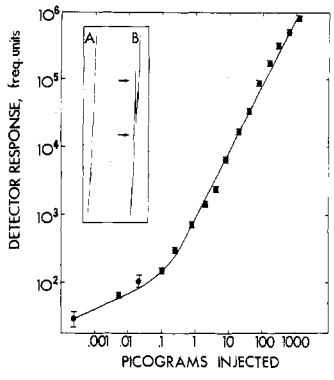

Figure 3. Calibration curve and (insert) detection limit for DPPA by GC-ECD. In the insert, chromatogram (A) is a solvent blank, and chromatogram (B) is an injection of 0.25 fg of DPPA in 1 μL of acetonitrile at an attenuation × range of 1 × 1. The peak height in (B) is defined by the arrows, and the signal-to-noise ratio for this peak is 6:1, giving an extrapolated detection limit of 90 ag at a signal-to-noise ratio of 2. The severe base line slope is due to the solvent peak. The retention time for DPPA is 4.4 min.

Essentially the same chromatogram is obtained whether the syringe is filled once or pumped several times with the 0.25 fg/μL solution of DPPA prior to filling, demonstrating the absence of any significant trace enrichment in the syringe. Reinjection of this solution after 20 min of storage in the dark shows almost complete loss of the solute peak. The extent to which this loss results from adsorption onto the vessel wall vs. decomposition of DPPA in this most dilute solution is not defined. At a higher concentration (e.g., 29 pg/μL) a solution of DPPA is significantly more stable, e.g., a loss of only 35–40% of DPPA is observed by GC-ECD after storage of this solution, in either acetonitrile or toluene, for 72 h at room temperature. DPPA is stable for at least 1 month at a concentration of 600 μg/mL in toluene.

This detection limit of 90 ag can be compared to a theoretical detection limit of 1 fg which has been estimated for this equipment (*22*). In this latter calculation, it was assumed that chlorinated pesticides such as lindane are maximally sensitive relative to all other types of compounds, as has been proposed (*14*) prior to the availability of the current data. In terms of observed detection limits by GC-ECD, values in the range of 10 fg at a signal-to-noise ratio of 2 can be extrapolated from previous reports on the analysis of hexachloroethane (*23*) and lindane (*24*) by capillary GC-ECD. Thus, the detection limit here of 90 ag for DPPA extends the reported sensitivity of GC-ECD by 100-fold.

The calibration curve for DPPA, the lowest point of which corresponds to the detection limit just discussed, also is shown in Figure 3. As seen, this curve is linear with a slope of 1.0 in terms of log units for injected amounts of this compound ranging from 1 to 1000 pg, revealing a constant sensitivity in this range. However, when lower amounts of DPPA are injected, nonlinearity is observed corresponding to an increased sensitivity. Normally one expects a decreased response at lower solute levels when a strong electron absorber is analyzed by gas chromatography with a constant current, variable frequency ECD (*25, 26*). This is the first time that a positive change in response at a trace solute level has been reported with this type of detector. We have no explanation for this phenomenon other than the speculation that a polyelectrophoric response (*3*) may develop or increase for this solute under these conditions of high electron density in the ECD.

Future Derivatizing Agents. Although the derivatized iodothyronines are highly sensitive by GC-ECD, their potential as ECD-active groups in derivatizing agents is limited severely by their low volatility. Potentially, however, the diphenyl ether component of these molecules can be converted by

appropriate substitution into a volatile, strong electrophore. Certain other structures, such as decafluorobenzophenone and the compounds involving the pentafluorobenzamide group (*N*-PFB), are both highly sensitive and volatile, and therefore also are worthy of further attention as potential electrophoric groups in future derivatizing agents for GC-ECD. The *N*-PFB group is immediately attractive for this purpose, because it is directly accessible in chemical terms, is reasonably volatile, and is as sensitive as lindane.

Strong electron absorbers at the trace level can encounter the problem of coulometric nonlinearity with this type of detector (*25–27*), or nonlinearity of an undefined nature as seen here for DPPA. However, a derivatization approach to GC-ECD analysis potentially allows internal standards to be developed which can significantly overcome this problem. This approach should be broadly applicable to the analysis of many trace biomolecules, due to their content of derivatizable groups.

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Determination of Trichloroacetic Acid at the Part-per-Billion Level in Water by Precolumn Trap Enrichment Gas Chromatography with Microwave Plasma Emission Detection

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Trichloroacetic acid was determined in water at the part-per-billion level by gas chromatography after extraction with diethyl ether and derivatization to methyl trichloroacetate with diazomethane. A 200-μL portion of derivatized extract volume was introduced into the gas chromatographic column by means of precolumn trap enrichment. Chlorine-containing compounds were detected with an element-selective atmospheric pressure microwave plasma emission detector. Recovery studies of trichloroacetic acid at various concentrations are described. The procedure was used to determine the amount of trichloroacetic acid formed during chlorination of water containing fulvic acid.

In characterizing the chlorinated products formed from the chlorination or organic material in natural waters, an analytical procedure to determine trichloroacetic acid (TCAA) at the microgram per liter level in water was needed. Interest in this compound arose when Quimby et al. (*1*) showed that relatively large amounts of TCAA appeared to be formed when water containing fulvic acid was chlorinated. Studies on the chlorination of model compounds for humic materials have given similar results (*2*). To understand the chlorination processes occurring in such systems, one requires quantitative methods for determining the major products formed during chlorination, including trichloroacetic acid.

The available methods for the determination of TCAA include both spectrophotometric and chromatographic procedures. The former methods are based on the Fujiwara reaction (*3, 4*) and are both simple and sensitive; however, interferences include chloroform and other chlorinated organics (*5*). Although several gas chromatographic procedures for the determination of TCAA and similar compounds have been reported (*6–8*), they have the disadvantage of needing extremely toxic derivatization agents or they lack reproducibility at trace levels. They also involve lengthy multiple liquid–liquid extractions of large sample volumes (500 mL) to separate the analyte from the sample matrix. By comparison, the method for the analysis of TCAA presented here has several advantages. First, high-resolution capillary column gas chromatography with a selective element microwave plasma emission detector (MED) eliminates the need for multiple extractions to separate analyte from the matrix. This decreases analysis time and increases sample throughput. Secondly, since this procedure employs precolumn trap enrichment of the ether extract instead of direct injection onto the column, smaller sample volumes (50 mL) may give de-

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