Selective Complexation of Impurities in the Electron Capture Gas Chromatographic Determination of Some Chlorinated Polycyclodiene Pesticide Metabolites and Derivatives

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The quantitative conversion (1-3) of organic compounds to stable, easily separated (4) and identified derivatives (5-6) is a perpetual problem in organic and biological chemistry. Substitution of active hydrogen by a trimethylsilyl (TMS) group has been employed with increasing frequency to render such compounds more volatile and thus better suited for distillation or gas chromatography. TMS derivatives are also finding use as intermediates in organic syntheses and in some cases may provide useful information on conformational preferences (7) of the molecule. A number of different methods have been used for the preparation of these derivatives including those attending the gas-liquid chromatographic analysis of hydroxylated compounds. In the course of metabolic studies of the chlorinated polycyclodiene pesticides, a method was sought which would permit rapid characterization of low levels ($\leq$ 1 ppm) of hydroxylated and related metabolites. Since gas chromatography with electron-capture detection (EC-GC) had been employed (8) previously for determination of major urinary metabolites of these systems, it was the method of choice. However, it was immediately evident that analysis of low levels of metabolites via their TMS derivatives was complicated by impurities from the silylation procedures themselves or from those extractable from urine. In our laboratory europium nitrate, $Eu(NO_3)_3$, has been employed as a selective complexing agent which enabled the determination of low levels of metabolites which are normally masked by procedural impurities.

Experimental

Authentic standards were either commercially available (I and V), synthesized according to published procedures (II-IV, VI, VIII), or obtained by batch metabolic studies (9) (VII). The silylating reagent was a premixed formulation (Tri-Sil Z) obtained from Pierce Chemical Company and the europium nitrate, $Eu(NO_3)_3$, was used as the solid (99.9%) obtainable from Research Organic/Inorganic Chemical Corporation. The gas chromatography was done on a Varian Aerograph Model 1868-40 chromatograph equipped with a tritium foil electron capture detector and connected to a 1-mv recorder. The gas chromatographic column conditions are described in Table 1.

Typical spiking, extraction, and silylation procedures were as follows. One ml aliquots of control rat urine were spiked with 0.1 or 1 µg (0.1 and 1 ppm) of material introduced in 1 µl

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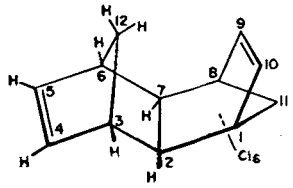

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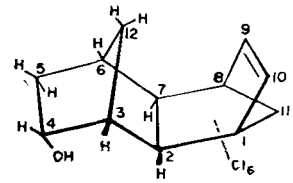

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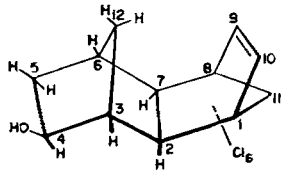

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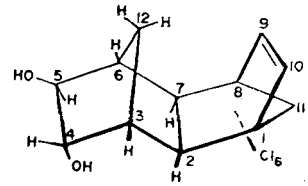

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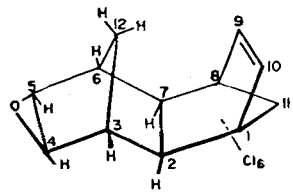

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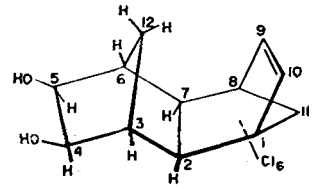

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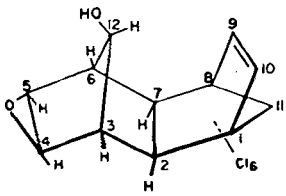

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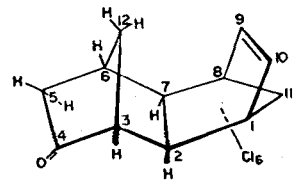

VIII

of acetone followed by thorough mixing on a Mini-shaker. To this solution was added 0.1 ml of conc. HCl, and the solution was again shaken. The resulting aqueous solution was extracted with three 2-ml portions of anhydrous ether which were aided in separation by centrifugation. The combined ethers were evaporated to dryness under a nitrogen stream, and the residue was treated directly with 0.2 ml of Tri-Sil Z. The resulting solution was heated at 80°C for 10 minutes. After cooling, the solution was diluted to 1 ml with pesticide quality benzene followed by the addition of solid ($Eu(NO_3)_3$ ca. 50 mg). The mixture was thoroughly shaken on a Mini-shaker for 5 minutes and centrifuged. The centrifugate (1 µl) was injected directly into the gas chromatograph.

Results and Discussion

Recent studies in our laboratory (10) of the proton magnetic resonance spectra of some chlorinated polycyclodiene pesticide metabolites and derivatives have demonstrated the use of the new NMR shift reagent, tris(dipivalomethanato)europium [$Eu(DPM)_3$] to induce paramagnetic shifts in protons via association with lone-pair electron bearing functional groups, and have thereby demonstrated its utility as a confirmatory technique of signal assignments and as a selective shift reagent enabling superimposed signals in these systems to be separated from one another. In addition, it was also shown that the equilibrium association is strongest with hydroxyl groups and is weakest with carbonyl and ether type oxygens. The weaker association can be argued on electronic grounds but is likewise affected by steric hindrance such as might be expected for the large TMS group in silyl ethers.

Therefore, it was thought that such europium compounds might be used prior to GC analysis to selectively complex with undesirable oxygen and nitrogen containing compounds after the desired compound has been protected by TMS derivatization. Although some TMS derivatization of impurities was certain to occur, it was suspected that impurities would be in much larger relative concentrations and would be preferentially complexed via equilibrium association. Such selective complexation of impurities in biological extracts would permit rapid detection of much lower concentrations of metabolites without laborious prior clean-up procedures. Aqueous work-up procedures have been shown (11) to effectively remove material which normally interferes in the GC analysis of TMS derivatizations; however, this is usually at the expense of some hydrolysis of the desired silyl ether and therefore is not routinely done by most workers. The NMR shift reagent, $Eu(DPM)_3$, possessed the desired selectivity, but had the disadvantages of being soluble in organic solvents and somewhat expensive. Europium nitrate [$Eu(NO_3)_3$] was chosen since it could be added as a solid only slightly soluble in benzene and can be removed by centrifugation after complexation has occurred. The complexes themselves were evidently sufficiently insoluble themselves since the yellow solutions of urinary extracts became essentially colorless on treatment with $Eu(NO_3)_3$ while the solid

material acquired a yellow color. Furthermore, the $Eu(NO_3)_3$ serves as a dehydrating agent and is considerably less expensive than $Eu(DPM)_3$.

A representative series of compounds (I-VIII) were available from our metabolic studies of dieldrin (V) and aldrin (I). All of these compounds were differentiated on one column substrate (5% QF-1) by employing silylation. The retention times and relative retention times (to dieldrin) are given in Table 1 along with the gas chromtographic conditions. The values listed are the average values from three to six determinations. The compounds I, V, and VIII can be determined directly before derivatization while compounds II-IV and VI-VII are determinable after silylation. The silylating procedure does not alter the properties of compounds I, V, and VIII permitting their detection in the presence of hydroxylic compounds and providing a confirmatory technique for their gas chromatographic identification.

TABLE 1

Gas Chromatography of Compounds I-VIII on 5% QF-1 on 100/120 Varaport 30 with Oven Temperature at 182°, Inlet at 190°, Detector at 200°C and a N_2 Flow Rate of 36 cc/min; 5'x1/8" stainless steel.

Compound	Retention Time (min.)	Relative Retention Time
I	1.42	0.36
II as TMS derivative	2.65	0.68
III as TMS derivative	2.76	0.71
IV as TMS derivative	3.36	0.86
V	3.90	1.00
VI as TMS derivative	4.39	1.12
VII as TMS derivative	5.52	1.41
VIII	5.56	1.43

Since all of the compounds listed in Table 1 have similar responses to the electron capture detector, the chromatograms (Figure 1) of the trans-aldrindiol (IV) are typical and illustrative of the selective and preferential binding of impurities with $Eu(NO_3)_3$ and hence the increased capability of ascertaining the presence of low concentrations of hydroxylated as well as other oxygenated metabolites of these systems on a given GC column.

It can be seen from Figure 1 (A) that without the addition of $Eu(NO_3)_3$ the silyl ether of IV is discernible as a shoulder of the large tailing peak of the impurities which largely consists of pyridine from the silylating reagent. Compounds I-III are completely masked by such a peak while compounds IV-VI are limited in detectability by such a peak, e.g., 0.1 ppm of IV is not detectable. However, on treatment (Figure 1, B) of the same solution with $Eu(NO_3)_3$, the broad tailing peak is essentially eliminated facilitating the detection of IV as well as the other compounds. Control experiments indicated that the other small peaks present are the result of the combination of the silylating reagent with $Eu(NO_3)_3$ and do not interfere in the analysis of any of these compounds. In addition, prior treatment with $Eu(NO_3)_3$ enabled the detection of IV in concentrations as low as 0.1 ppm (Figure 1, C) in rat urine with a ten-fold decrease in attenuation.

Figure 1, A and B, also indicates that the silyl ether of IV is not effectively complexed nor otherwise removed from solution at its relatively low concentrations. Furthermore, triplicate determinations and comparisons with standards showed that all of these compounds are recoverable in good yields (≥95%) from rat urine by simple ether extraction. In addition, it was demonstrated via mixed standards that the epoxide group in dieldrin (V) and the carbonyl group in oxodihydroaldrin (VIII), which have been shown from NMR spectroscopy to complex with $Eu(DPM)_3$, likewise do not appreciably complex with $Eu(NO_3)_3$ at the concentrations studied here. These compounds were not studied as a total mixture since this situation would never occur in reality and would only serve to complicate the picture.

In conclusion, it appears that the complexing properties of compounds of the rare earth metals such as europium may play a role in the analytical chemistry encompassing the detection of oxygenated metabolites of a variety of biologically important compounds. Although the scope of the method was not investigated, it is possible that even more specifically selective complexing agents will be found which can be applied to various body fluids and tissues (the method has also been applied successfully to extracts of rat liver microsomes and certain plant roots) to facilitate isolation and characterization of metabolites. It certainly seems desirable to apply such a convenient rapid-assay method to the low level determination of chlorinated phenolic systems which are becoming of increasing importance as possible metabolites and degradation products of diverse environmental

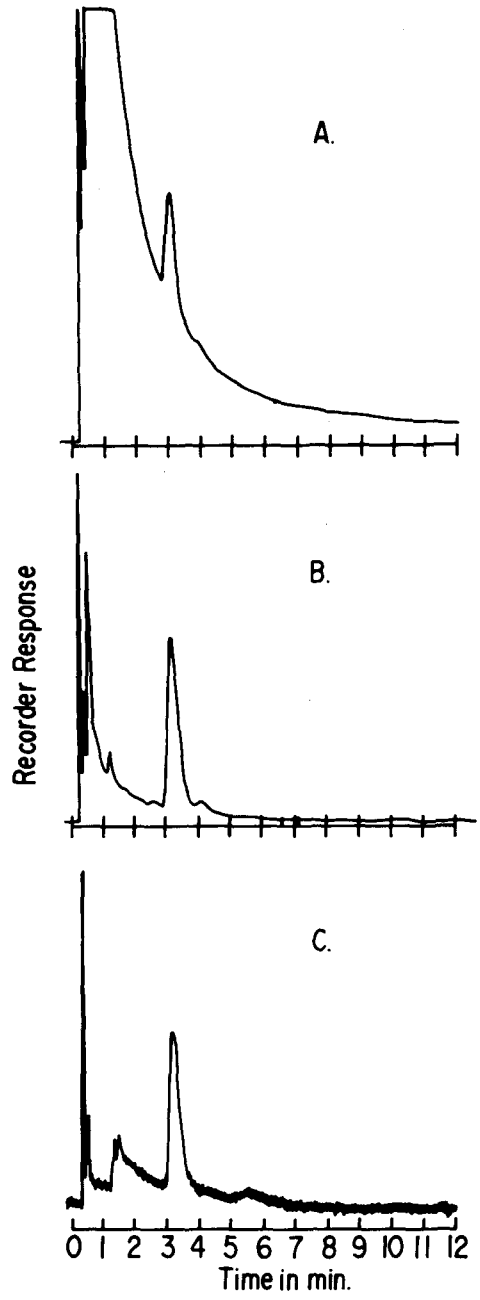

Figure I. EC-GC of TMS derivative of trans-aldrin diol (IV) from spiked rat urine.
A) I ng of IV without $Eu(NO_3)_3$
B) I ng of IV with $Eu(NO_3)_3$
C) 0.I ng of IV with $Eu(NO_3)_3$

agents including hexachlorophene and related systems, chlorobenzenes, and polychlorinated biphenyls.

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