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2-CYANOETHYLDIMETHYL(DIETHYL)AMINOSILANE, A SILYLATING REAGENT FOR SELECTIVE GAS CHROMATOGRAPHIC ANALYSIS USING A NITROGEN–PHOSPHORUS DETECTOR

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SUMMARY

The synthesis of 2-cyanoethyldimethyl(diethyl)aminosilane (CEDMSDEA) as a reagent to form silyl derivatives detectable using nitrogen–phosphorus detection (NPD) in gas chromatographic (GC) analysis, is described. This new reagent was reacted with carboxyl, phenolic and secondary hydroxyl groups from fatty acids, phenols and cholesterol, respectively. The corresponding 2-cyanoethyldimethylsilyl (CEDMS) derivatives were analyzed by GC using NPD and their retention and detection characteristics compared with trimethylsilyl equivalents, analyzed by flame ionization detection. Results indicate that these new derivatives have chromatographic properties which are similar to other silyl derivatives with longer retention times then trimethylsilyl analogues. This new silylating reagent can thus be used selectively to analyze non-nitrogen containing compounds with high sensitivity using NPD. The mass spectral fragmentation of the CEDMS derivatives is briefly discussed with reference to their detection by selected ion monitoring GC–mass spectrometry.

INTRODUCTION

In the last two decades, considerable attention has been given to derivatization techniques and their use is widespread in the fields of chemical synthesis and chemical analysis[1,2]. Derivatives are used to protect reactive groups in synthesis and to enhance volatility and stability in instrumental techniques such as gas chromatography (GC) and mass spectrometry (MS). In the early developments in derivatization, the search was oriented towards universal reagents that would produce a one-step reaction with most functional groups. This led to a series of techniques of which acylation and silylation reactions are successful examples. As detection limits were lowered and the mixtures to be analyzed became more complex, chemically selective reactions were developed to discriminate against the background.

Numerous derivatization reagents presently exist and their applications have been presented in several reviews[1,3,4]. Within the existing reagents, silylating agents have received wide acceptability and are extensively used in the fields of chromato-

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graphy and MS because they offer excellent properties for chemical analysis, are easy to use and have universal applicability. The silylating reagents can be classified as universal or selective depending on the detection properties of the formed derivatives. For example, trialkylsilyl groups possess universal properties and their derivatives are generally analyzed using flame ionization detection (FID) and MS[5]. In order to make use of the advantages of selective detectors while retaining the basic chromatographic properties of alkylsilyl derivatives, halogenated silylating reagents have been introduced to form derivatives detectable by electron-capture detection (ECD)[6–8]. These derivatives have been used to analyze trace levels of biological materials and subnanogram detection limits have been reported[8].

In order to expand the applications of selective silylating reagents, which so far have been limited to the use of ECD, we have sought a reagent that would allow the use of an alternate selective detection method in situations where the use of ECD is not desirable. Such examples are the analysis of mixtures of halogenated compounds having a wide range of response factors for ECD or analysis of matrices that can seriously contaminate the electron-capture detector or cause strong interferences. A detection method that offers analytical potential for selective detection of organic compounds at trace levels is nitrogen–phosphorus detection (NPD). The nitrogen–phosphorus detector, of the Kolb type[9], has good sensitivity to nitrogen (10^{-13}g N), selectivity (10^4), a wide dynamic range (10^5), stability, is easy to operate and fairly resistant to contamination[10]. Because of this analytical potential, we have searched for a selective silylating reagent forming derivatives that would be detectable by NPD and also have mass spectral fragmentation suitable for detection by selected ion monitoring in GC–MS.

EXPERIMENTAL

Chemicals

2-Cyanoethyldimethyl(diethyl)aminosilane (CEDMSDEA) was prepared by the addition of 66 mmol dimethylchlorosilane (Pierce, silylation grade) to 65 mmol acrylonitrile (Aldrich) in the presence 65 μmol tris(triphenylphosphine)rhodium chloride (Aldrich) according to an existing proceddure[11]. The reactants were sealed in a test tube under an atmosphere of argon and heated to 50°C until the reaction mixture became dark (60–70 min). The reaction mixture was distilled under reduced pressure (5 Torr) and 2-cyanoethyldimethylchlorosilane (CEDMCS), a colorless liquid, was collected at 62°C. The chlorine radical was further displaced by diethylamine in tetrahydrofuran (THF)[12]. In 100 ml of THF containing 68 mmol of CEDMCS, 145 mmol of diethylamine were added over a period of 2 h. The mixture maintained under an atmosphere of argon was filtered and the desired compound isolated by distillation (80°C, 2 Torr). The reagent was treated with a 0.5% (w/v) solution of 3,4,5-trimethoxybenzoic acid (Aldrich) in THF to remove reactive side products. CEDMSDEA was kept and used as a 10% (v/v) solution in ethyl acetate. This solution may be kept for several months without degradation.

Derivatization reactions were carried in Reacti-Vials using ethyl acetate as a solvent. To a solution of ethyl acetate containing the compound of interest ($\approx$ 1 m*M*) CEDMSDEA was added in excess (80:1). The reaction time and temperature are given with the kinetic data.

All chemicals and solvents used in this study were purified by usual procedures prior to their use. Fatty acids (C_8–C_{18}) were obtained from Polyscience (IL, U.S.A.), cholesterol from Dr. J. C. Roy, Ste Justine Hospital (Montreal, Canada) and chlorophenols from Aldrich. In all cases, chlorophenols were purified by vacuum sublimation prior to their use.

Instrumentation

Mass spectral data were obtained on a Kratos MS-50TA mass spectrometer. The electron energy was 70 eV, the source temperature 220°C and the transfer line was kept at 260°C. The IR spectra were obtained on Digilab FT-15C/E Fourier Transform Michelson interferometer, the ^{1}H NMR spectra on a Bruker WH-90 spectrometer. All GC data were obtained on a Perkin Elmer (Norwalk, CT, U.S.A.) sigma 2B gas chromatograph equipped with a nitrogen–phosphorus detector and a flame ionization detector. The capillary column was 15 m × 0.23 mm I.D. coated with polydimethylsiloxane DB-1 (Chromatographic Specialties, Brockville, Ontario, Canada). The oven temperature was programmed from 120 to 280°C at 12°C/min for the analysis of fatty acids and chlorophenols derivatives. The derivative of cholesterol was analyzed at 280°C. The split injector ratio was 32:1 and injector and detector temperature was 300°C. Helium was used as carrier gas and in all cases aliquots (1 μl) of the reaction mixture were injected without prior separations of the products from the reactants. The nitrogen–phosphorus detector was operated with 30 ml/min of helium as make up gas and tuned for maximum selectivity according to a procedure use in this laboratory[13,14]. The hydrogen and air flow-rates at the detector were 2.6 and 100 ml/min, respectively. The bead current was kept below the optimum sensitivity (routine operation) to increase the lifetime of the bead.

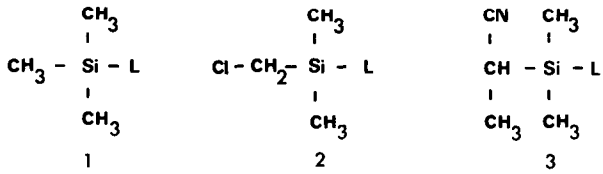
RESULTS AND DISCUSSION

Only a few derivatization techniques have been introduced to permit the selective analysis of non-nitrogen or non-phosphorus containing compounds using NPD. A reaction using dimethylthiophosphinic chloride to form esters has been reported[15]. This procedure introduces phosphorus in the derivatives, as the NPD selective element. Another approach made use of the silylating reagent N,N-diethylaminodimethylsilane[16] to form nitrogen-containing derivatives. The latter had limited application because of the chemical reactivity of the formed derivatives[16]. Following the example of chloromethyldimethylsilyl derivatives where the trialkyl group was modified to insert an ECD compatible group, we have synthetized CEDMSDEA in which a cyano group (NPD reactive) is attached to the dimethyl silicone moiety.

The general structure of silylating reagents incorporates two parts that gives them specific properties. These are the silicone moiety and the leaving group L (see structures 1–3). The silicone moiety caracterizes the formed derivatives and the leaving group determines the reactivity of the reagent. Since the objective of this study was to develop a reagent for selective detection, attention was given to the silicone moiety. However, since the electronic effects of the cyano group on the silicon were not known, diethylamine was chosen as the leaving group because its reactivity is intermediate both in the trialkylsilyl and flophemesyl reagents[8].

In order to modify the character of the silicone moiety so that the derivatives

could be detected by NPD while retaining the basic properties of trimethylsilyl (TMS) derivatives (structure 1), a cyano group was introduced on one of the alkyl groups substituting the silicone atom. A cyano group was chosen because it is easily detected by NPD and it is believed to play an important role in the detection mecanism of NDP[17]. The cyano radical is used as a chemical tag in a fashion similar to the halogen in the chloromethyldimethylsilyl radical (structure 2) for ECD. Since the cyano radical could not be introduced directly on the silicone atom because silico-cyanides readily isomerize to their isocyanide form[18], it was introduced on the silicone moiety using the ethyl group as a carrier (structure 3).



The use of the ethyl group instead of the methyl, confers additional chemical stability to the methine proton which can be acidic in the cyanomethyl group. Furthermore, the presence of the cyanoethyl group, while keeping the mass of the added

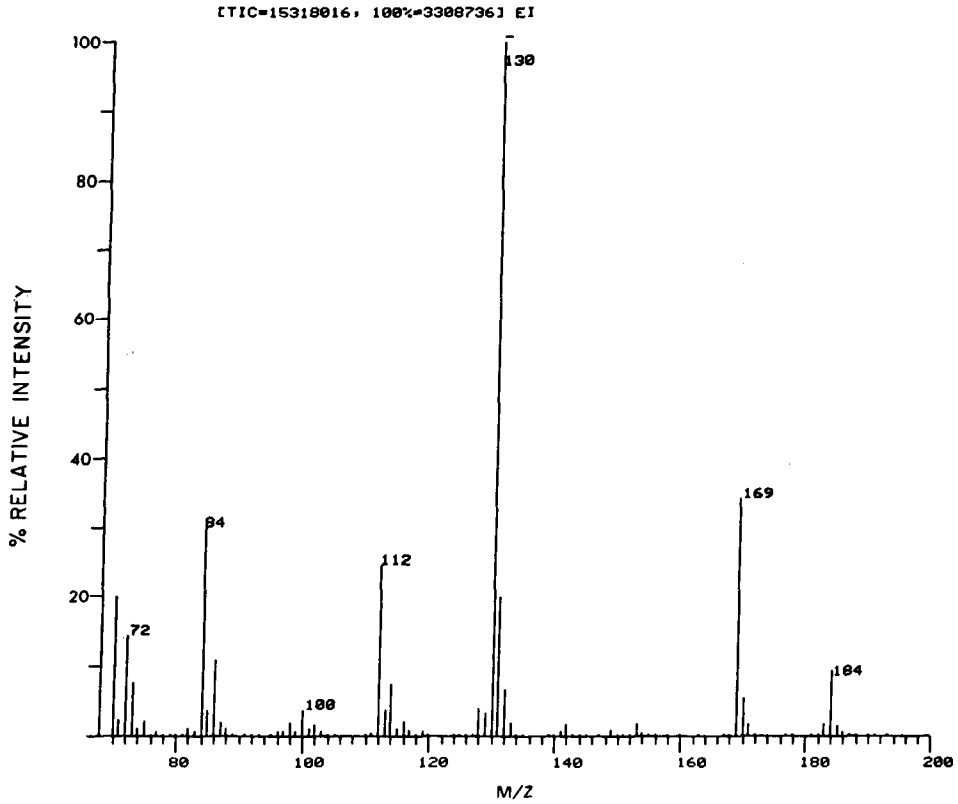


Fig. 1. Mass spectrum of 2-cyanoethyldimethyl(diethyl)aminosilane.

radical to a minimum, should favor the elimination of the cyanoethyl radical under electron impact providing an intense and specific ion at [M − 54].

The identity of CEDMSDEA has been ascertained by IR, ^{1}H NMR and MS. The IR spectrum shows an intense absorption band at 2220 cm^{-1} (C≡N). The ^{1}H NMR signals at 0.25 ppm (CH_3–S_i) (s), 1.01 ppm (CH_3–CH_2) (t), 1.26 ppm (CH_3–CH) (d), 1.74 ppm (CH) (q) and 2.86 ppm (CH_2–CH_3) (q) are supportive of the structure. The mass spectrum (Fig. 1) shows an M^+ ion at *m/z* 184 and a base peak at *m/z* 130 due to the loss of the cyanoethyl radical. Peaks at *m/z* 169 and 112 are explained by the elimination of the CH_3 and $N(C_2H_5)_2$ radicals.

Model compounds were selected to study the reactivity of the reagent and the analytical characteristics of the formed derivatives. The general reactivity was studied using carboxyl, phenolic and secondary hydroxyl groups from fatty acids, chlorophenols and cholesterol, respectively. Fatty acids (C_8–C_{18}) are of biological interest and represent a good system to study, in the same series, the reactivity of the carboxylic group and retention properties of the derivatives. Mono- and pentachlorophenols permit the study of two halogenated phenolic compounds with different pK_a values (9.2 and 4.8) and different halogen content. Cholesterol, which is often used as a reference compound, was chosen to examine the application of these derivatives to steroid analysis both in chromatography and mass spectrometry.

The carboxyl group of fatty acids reacts readily with CEDMSDEA in ethyl acetate. Fig. 2A shows kinetic data for the C_8 and the C_{18} homologues and it can be seen that quantitative reaction is achieved within a few minutes in ethyl acetate at room temperature. The use of higher temperatures does not affect the overall yield and the derivatives are stable in the reaction mixture for hours indicating that no secondary reactions or thermal degradation are occurring. Fig. 2B shows the kinetic

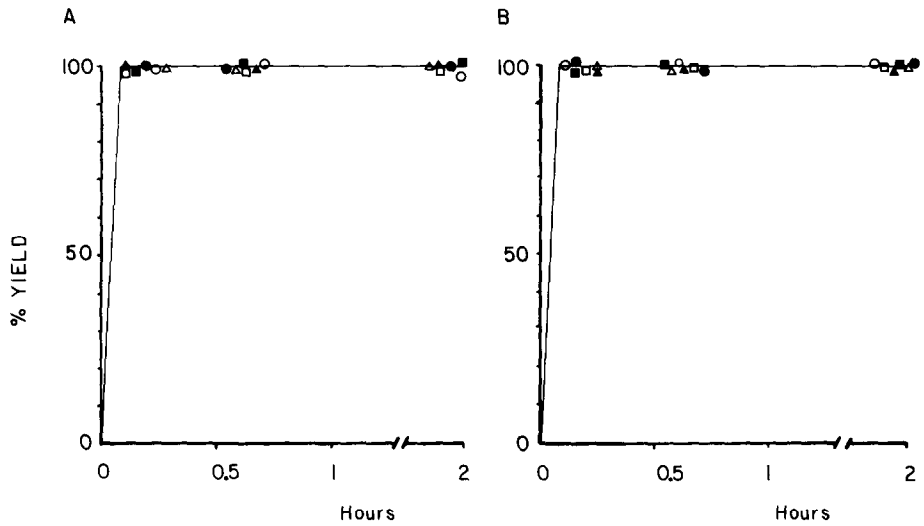


Fig. 2. (A) Reaction kinetics of octanoic (C_8) and octadecanoic (C_{18}) acids with CEDMSDEA in ethyl acetate at different temperatures. C_8: (●) 25°C, (■) 55°C and (▲) 75°C. C_{18}: (○) 25°C, (□) 55°C and (△) 75°C. (B) Reaction kinetics of mono- and pentachlorophenols with CEDMSDEA in ethyl acetate at different temperatures. 2-Chlorophenol: (●) 25°C, (■) 55°C and (▲) 75°C. Pentachlorophenol: (○) 25°C, (□) 55°C and (△) 75°C.

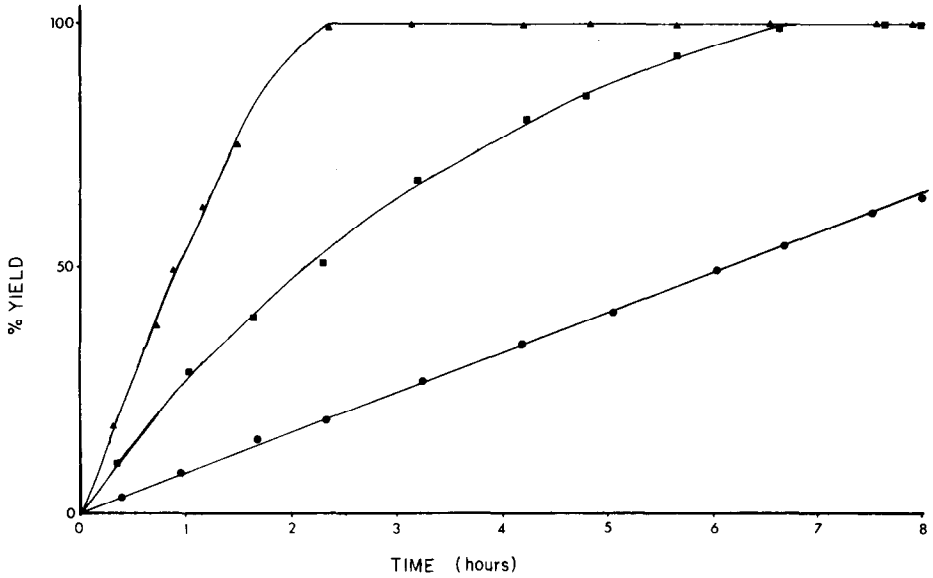


Fig. 3. Reaction of cholesterol with CEDMSDEA in ethyl acetate at (●) 25°C, (■) 55°C and (▲) 75°C.

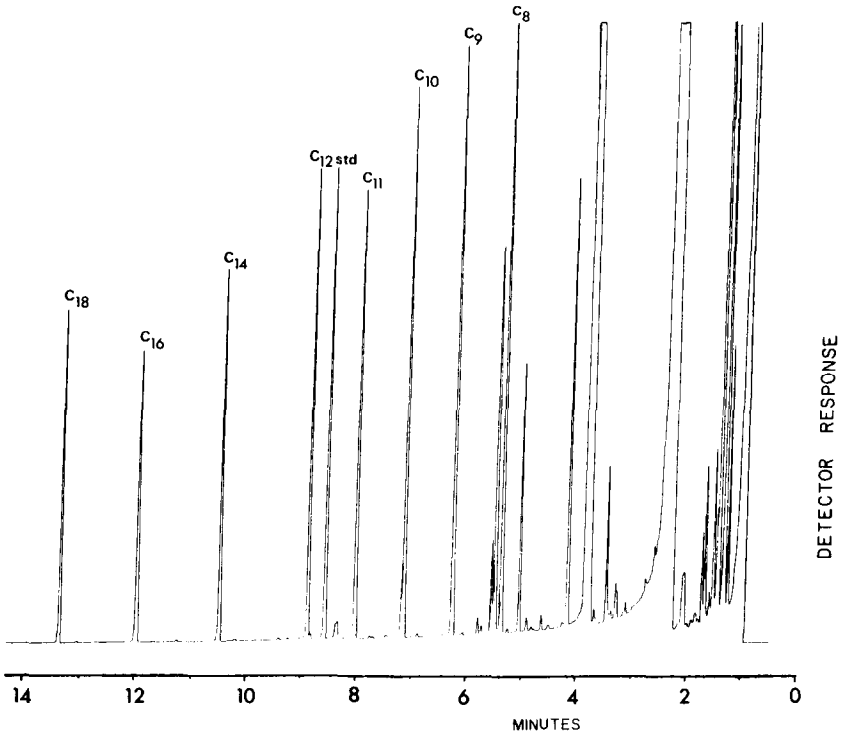


Fig. 4. Chromatogram of a mixture containing CEDMS derivatives of C_8–C_{18} fatty acids 70 ng analyzed with NPD. STD = triphenylamine (external standard).

data obtained for the two phenolic compounds studied and again the reaction is very rapid. Results indicate that the acidity of the hydroxyl group has no noticeable effect on the reaction rate and the reaction is complete in minutes at room temperature in ethyl acetate. The overall reaction yield does not vary when the temperature is raised to 55 or 75°C and the products are stable in the reaction mixture. The results obtained for the silylation of the secondary hydroxyl group of cholesterol are presented in Fig. 3. For this compound it can be seen that the reaction proceeds at a much slower rate taking 17 h to be completed at room temperature. In this case, quantitative reaction can be achieved in about 2 h if the reaction temperature is raised to 75°C.

The 2-cyanoethyldimethylsilyl (CEDMS) derivatives of the model compounds were analyzed on a polydimethylsiloxane stationary phase using NPD in order to study their retention and detection characteristics. Fig. 4 shows a typical chromatogram obtained from a mixture containing the C_8–C_{18} fatty acids after reaction with CEDMSDEA. As can be seen from the figure, the elution profile of the peaks is symmetrical and no tailing can be seen. The GC behavior of the CEDMS derivatives is similar to other silyl derivatives. Under our experimental conditions the retention times of the CEDMS derivatives are 1.4–2 times longer than the corresponding TMS

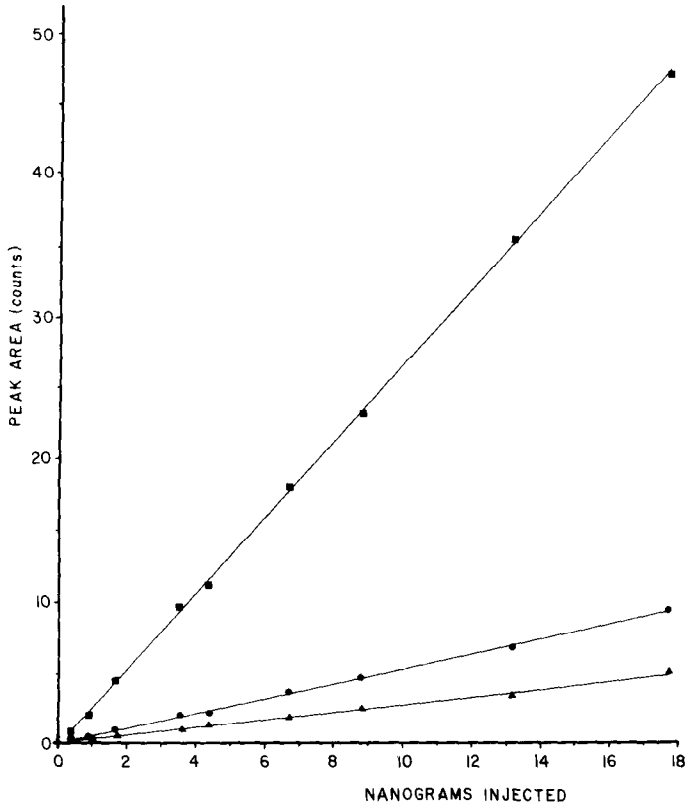


Fig. 5. Concentration–response plots of CEDMS and TMS derivatives of octadecanoic acid: (■) CEDMS derivatives with NPD, (●) TMS derivatives with FID and (▲) CEDMS derivatives with FID.

derivatives. Furthermore, Fig. 4 clearly demonstrates that these derivatives can be detected by NPD. Since the contribution of the discrimination effect on the signal-to-noise (S/N) ratio of the detector depends on the matrix to be analyzed, the increase in the sensibility obtained using this procedure was studied by comparison with FID. In these experiments, concentration–response plots were obtained by analyzing CEDMS and TMS derivatives of the fatty acids using FID and compared with the plots obtained for CEDMS derivatives when analyzed by NPD. The resulsts are shown on Fig. 5 and the response is linear with concentration in all cases. However,

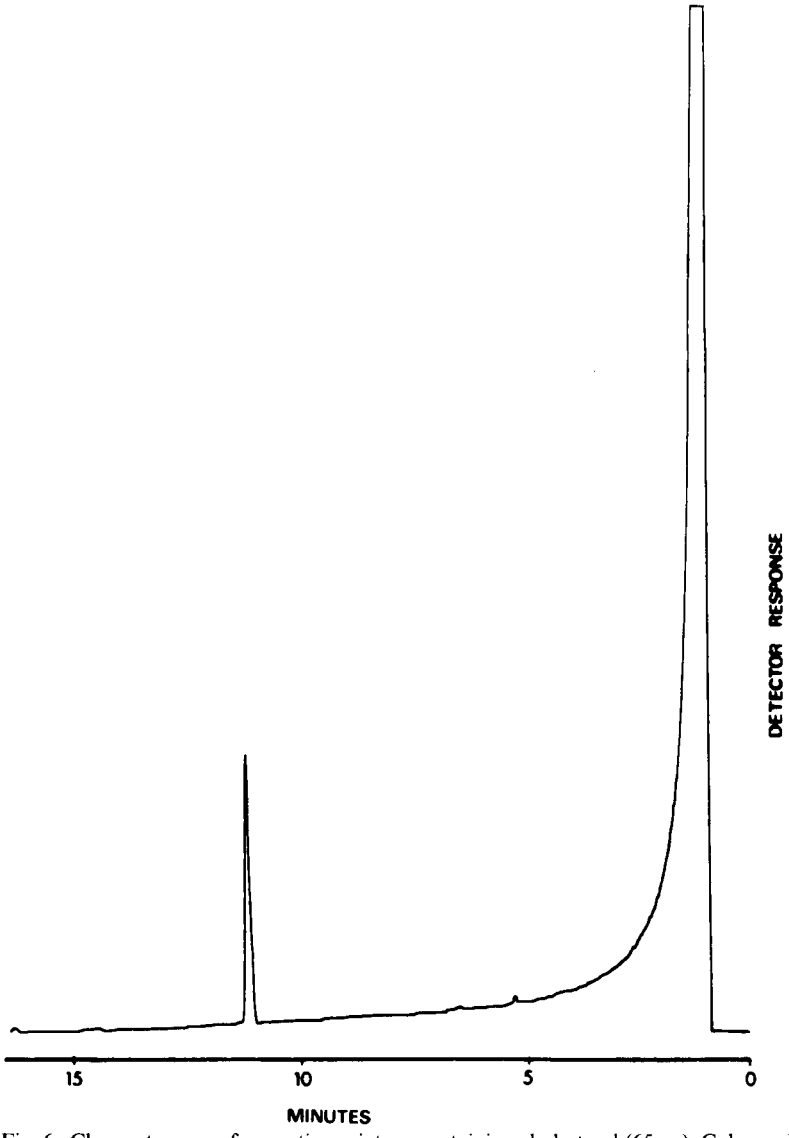


Fig. 6. Chromatogram of a reaction mixture containing cholesterol (65 ng). Column, DB1; isothermal at 300°C.

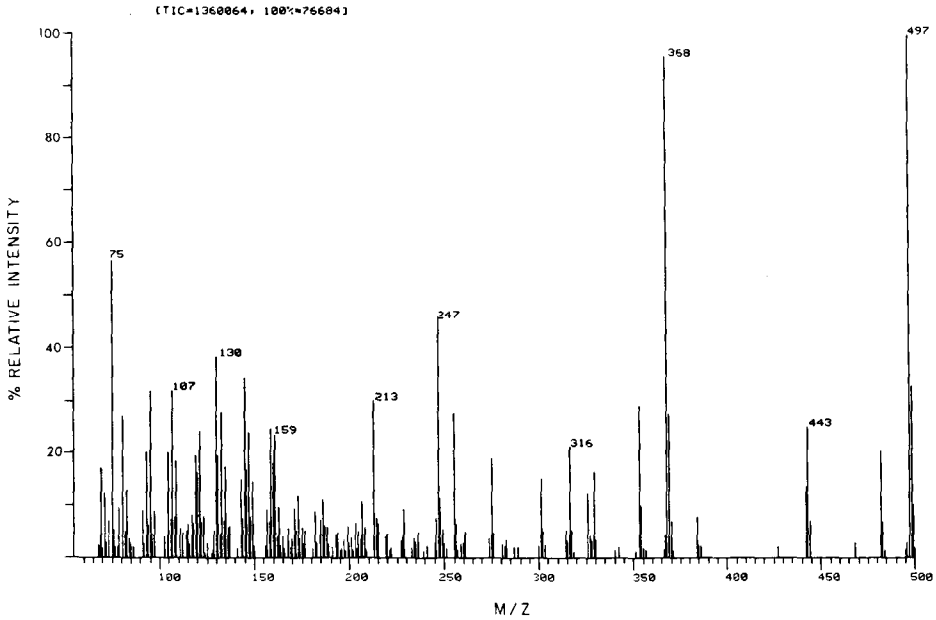


Fig. 7. Mass spectrum of the CEDMS derivative of cholesterol.

when the sensitivities obtained with both detectors are compared (slope ratio) it is found that the gain in sensitivity is at least fivefold when NPD is used. The combination of the increase in sensitivity and the selectivity is reflected in the detection limit for these compounds which was found to be $1 \cdot 10^{-13}$–$3 \cdot 10^{-13}$ g N (S/N = 3) under our experimental conditions. Similar results have been obtained for the derivatives of cholesterol and the two chlorophenols studied. The chromatogram of the derivative of cholesterol is shown on Fig. 6 and it can be seen that under our experimental condition the peak is unique and free from interferences from the reaction mixture. The chromatographic behaviour of chlorophenols is adequate for their analysis and this is reported elsewhere[21].

Since selected ion monitoring MS is often used as a specific detection method in conjunction with GC the behavior of the CEDMS derivatives under electron impact was briefly studied. The presence of specific ions at high mass is of particular interest since these ions are often used for quantitation or confirmation in selected ion monitoring GC–MS. For fatty acids derivatives the moleccular ions are present but their relative intensities are below 5% as in *tert.*-butyldimethyl derivatives[19]. However, the mass spectra are characterized by the presence of intense ions at m/z 75 and 129, [M – 54] and [M – 15] which are characteristic of the particular acid. The [M – 15] and [M – 54] ions are due to the losses of the CH_3 and C_2H_4CN radicals, the latter being more intense and occuring at an odd mass since the "nitrogen rule" is applicable. The mass spectrum of the cholesterol CEDMS derivative is shown on Fig. 7. The general features of the spectrum are like those of the equivalent TMS derivative[20] but some additional features can be seen. The molecular ion at m/z 497 is of odd mass and also the base peak in the spectrum. Other intense high mass peaks are seen at [M – 15], [M – 54] and [M – 129] corresponding to the losses of CH_3,

C_2H_4CN and the silanol. Again these four peaks are highly characteristic of the structure and may be used for selected ion monitoring detection. These preliminary results indicate that CEDMS may have fragmentation features under electron impact which are of analytical interest.

CONCLUSION

The results presented in this study indicate that CEDMSDEA is a silylating reagent that can be used to form derivatives for specific detection. The CEDMS derivatives have the general chromatographic properties of other silyl derivatives and offer new features that can be of analytical interest. They can be used in conjunction with NPD and as such can expand the field of application of silylating reagent. Furthermore, it appears from the mass spectra of the compounds studied that they offer mass spectral features which are complementary to existing TMS and *tert.*-butyldimethylsilyl derivatives for MS identification or analysis by selected ion monitoring GC–MS.

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