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IDENTIFICATION OF GENOTOXIC COMPONENTS OF THE HEAVY ENDS OF ENERGY-RELATED MATERIALS

FINAL REPORT

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TABLE OF CONTENTS

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TABLE OF CONTENTS (CONTINUED)

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ABSTRACT

The objective of this work was to develop capillary supercritical fluid chromatographic methods for the analysis of the high-molecular-weight components in high boiling, heavy end materials that are responsible for genotoxic activity. Since these compounds were largely unknown, 62 standard reference compounds were synthesized for comparison with actual high boiling coal-derived products. These compounds were comprised of two- and three-ring aromatic moieties coupled together with carbon, oxygen, sulfur, and nitrogen bridging groups. Mass spectral evidence indicated that a number of these compound types could be present in various coal products at a concentration level of 0.01-0.2 percent, although none of them could be positively identified because of the complexities of the fractions. A number of capillary supercritical fluid chromatographic instrumental developments were made as a result of this work. These include a supercritical fluid fractionation system for group-type separation of complex samples and a direct coupled supercritical fluid chromatograph/double focusing mass spectrometer system. Finally, a number of supercritical fluid chromatographic mobile phase combinations were evaluated for the analysis of large polycyclic aromatic compounds.

I. INTRODUCTION

It has been found in recent studies that genotoxic activity, particularly initiation of tumorigenesis, resides mainly in high boiling, heavy end materials.[1] For instance, the 850°F+ distillate from a solvent-refined coal liquid (SRC II) was significantly more active for initiation of skin carcinogenesis in CD-1 mice than the 800-850°F or other lower-boiling distillates. At the present time, the identities of the compounds present in these high-boiling fractions and tars are unknown. This is primarily due to the lack of high resolution chromatographic methods which can be applied to complex tar samples. Direct spectroscopic analysis of these tars can provide general knowledge about their compositions, but a detailed knowledge (including specific health-related issues) can only come from the preliminary separation of mixture constituents prior to spectroscopic measurements. For the particular application to coal-derived heavy ends, capillary column gas chromatography with its high resolving power is limited by the low volatilities of these molecules, and high performance liquid chromatography is limited by its much lower column efficiency and oftentimes lower solvating ability for large compounds containing various functional groups.

In 1981, representatives of a number of laboratories were selected to participate in a symposium/workshop held in Seattle, WA, sponsored by the U.S. Department of Energy, Office of Health and Environmental Research.[2] The primary purposes of this meeting were to summarize and discuss the current state-of-the-art analytical approaches for measuring the biologically active constituents in the highly complex synthetic fuel-derived materials, and to identify research and methodology needs and future research directions. One of the major conclusions of this workshop was stated as: "Future increased efforts

should be directed toward developing fractionation and analysis methods for extremely polar compounds and compounds of high molecular weight". It was reported that 15-40% of the organic content of many of these materials remained to be identified. Unaccounted-for constituents were most common in high boiling point materials. It was also concluded that mass spectrometry techniques probably held the greatest potential for compound specific measurements of high-molecular-weight fractions.

The recent development of capillary supercritical fluid chromatography (SFC) provides a promising approach to the characterization of these high molecular weight materials. The unique feature of SFC is that the mobile phase is subjected to pressures and temperatures near its critical point. A supercritical fluid possesses solvating properties similar to a liquid, and solute diffusivities intermediate between a gas and a liquid. Therefore, relatively high efficiencies can be obtained for nonvolatile solutes which cannot be analyzed by gas chromatography. In addition, the low pressure drop across a capillary column allows higher efficiencies to be achieved than obtainable with a packed column. The small pressure drop also allows more sensitive control of the density of the supercritical fluid. Since the density of the supercritical fluid determines its solvating ability, density programming of the supercritical fluid mobile phase provides a powerful technique to extend the molecular weight range of compounds which SFC can analyze. The sensitive density control which is possible in open-tubular columns makes density programming in capillary SFC extremely useful for analysis of complex samples.

Supercritical fluid chromatography-mass spectrometry (SFC-MS) can provide the greatest amount of information about resolved components than any of the

detectors that are currently interfaced with SFC. Both electron impact (EI) and chemical ionization (CI) can be used with SFC-MS. With capillary column SFC, the entire effluent (often amounting to less than 1 mL min^{-1} gas) is generally handled by existing GC-MS pumping systems. By increasing the flow rate through the chromatographic column or by adding additional CI reagent, source pressures can be increased to allow for CI work. Molecular weight information and fragmentation patterns have become valuable aids in research in this area.

In this study, it was proposed to separate and identify the components of the heavy ends of coal-derived materials. Prefractionation using size exclusion chromatography and/or supercritical fluid fractionation was deemed to be a necessary first step. Final identifications would be done using capillary supercritical fluid chromatographic methods. Of particular importance was the interfacing of the supercritical fluid chromatograph to a high-resolution mass spectrometer.

II. WORK PERFORMED

A. Prediction of High-Molecular-Weight Compounds in Coal-Derived Materials

During the last ten years, numerous PAC have been identified and quantified by us in coal-derived materials. (see Appendix 1) Vital to this work was the synthesis of literally hundreds of standard reference compounds. Polycyclic aromatic hydrocarbons (PAH), nitrogen and sulfur heterocycles, and substituted (alkyl, amino, and hydroxy) PAC have been studied in detail. The structural conclusions of this work to date can be summarized by referring to Figures 1 and 2:

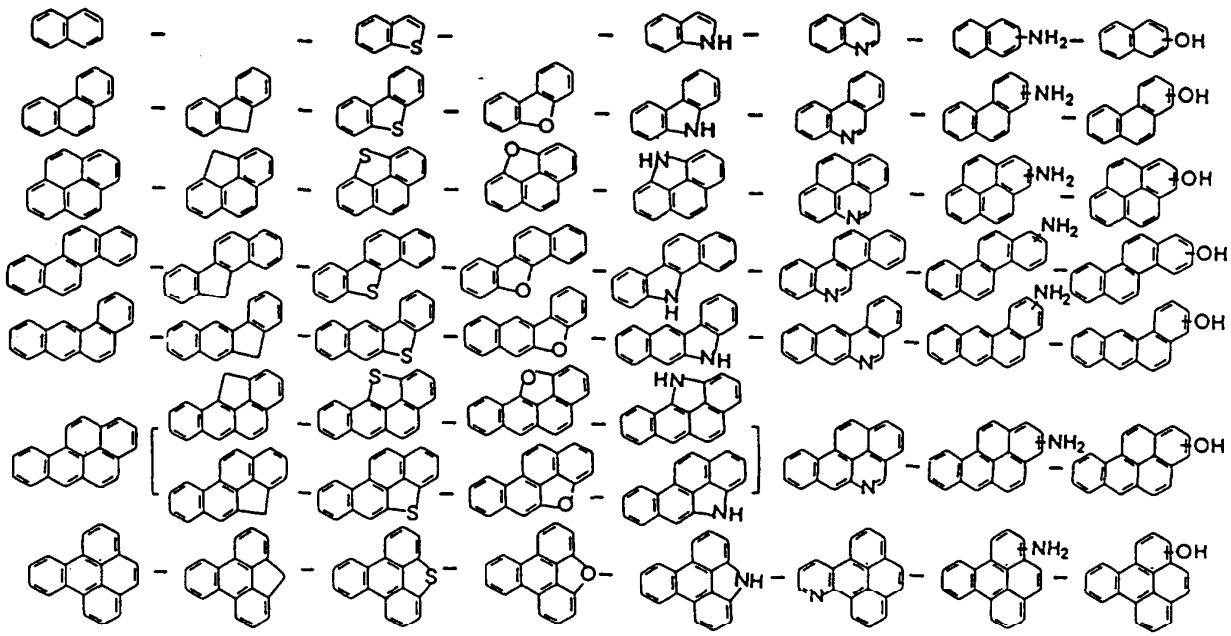

Figure 1. Structural similarities of major PAC identified in an SRC II HD coal liquid.

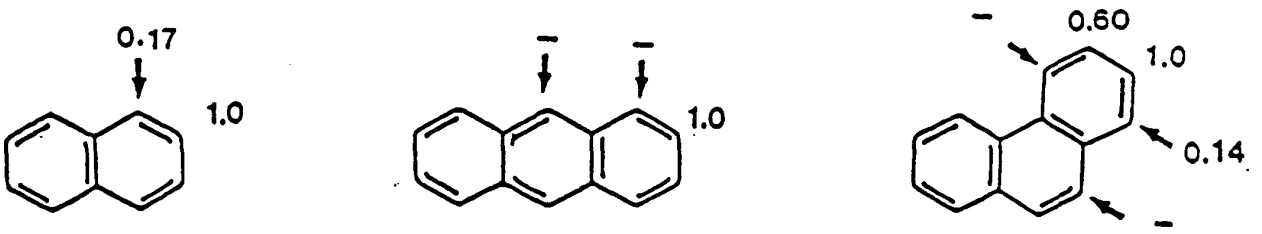

Figure 2. Relative abundances of methyl-substituted PAH in an SRC II HD coal liquid. Arrows indicate the sites of steric hindrance.

(a) Preferred structures exist for the PAC found in coal-derived materials. The major structures are based on naphthalene, phenanthrene, pyrene, chrysene, benz[*a*]anthracene, benzo[*a*]pyrene, benzo[*e*]pyrene, benzo[*ghi*]-perylene, dibenzo[*def*,*mno*]chrysene and coronene.

(b) The heterocyclic compound structures present are analogous to those of the major PAH. For example, by replacing one of the aromatic rings in the preferred PAH with a thiophene ring, the most abundant sulfur heterocycles are generally described. Major cyclopenta-containing PAH such as fluorene and benzofluorene can also be derived from replacing one of the aromatic rings in the most abundant PAH with a five-membered ring.

(c) Lower-molecular-weight (fewer aromatic rings) PAC are generally the most abundant components in coal-derived materials. Hence, naphthalene and phenanthrene are the major PAC in such materials, unless they have been removed by processes such as distillation. As the number of aromatic rings in the structures increases, the abundance of the compounds generally decreases.

(d) There are preferred positions of substitution on the aromatic rings (see Figure 2). Beta-substituted isomers are more abundant than α-substituted isomers; other substituted positions are generally very low or absent. It appears that steric hindrance is a major factor that dictates the preferred positions of substitution.

From these results, we predicted that the high-molecular-weight materials could contain significant concentrations of smaller polycyclic aromatic moieties coupled together with bridging groups. Therefore, preliminary experiments were performed to search for evidence of such linking groups. The first step in this effort was to synthesize a variety of representative compounds that contained

bridging groups to use as standards for methodology development. The next section describes these synthetic accomplishments.

B. Synthesis of Bridged Aromatic and Heteroaromatic Compounds as Reference Standards

General methods. Figure 3 shows the structures of the bridged standard reference compounds that were synthesized. Details of the synthetic methods are described in the following sections. Reprints of papers published are attached as Appendices 2-6.

The standard amino-linked compounds were prepared according to the general procedure of Lieber and Somasekhare.[3] In this method equal molar quantities of the aminonaphthalene (1 or 2), naphthol (3 or 4), ammonium chloride, and zinc chloride were heated to a melt followed by washing to remove unreacted starting materials, to afford 1,1'-dinaphthylamine (5) (21%), 1,2'-dinaphthylamine (6) (37%), and 2,2'-dinaphthylamine (7) (27%) after recrystallization.

Both acid and base catalyzed reactions provided the ether-bridged derivatives. Reaction of 1-naphthol (3) in the presence of sodium bisulfate furnished 1,1'-dinaphthyl ether (8) (15%). Following the Ullman ether synthesis,[4] 1,2'-dinaphthyl ether (10) (5%) was prepared from 2-naphthol (4) and 1-bromonaphthalene (9) by action of potassium hydroxide and copper powder. Refluxing 2-naphthol (4) in xylene with a catalytic amount of *p*-toluenesulfonic acid afforded 2,2'-dinaphthyl ether (11) (6.5%). When 1-naphthol (3) was allowed to react with 2-bromodibenzothiophene (13) (44%) (from the direct bromination of dibenzothiophene (12) under modified Ullmann conditions in the presence of pyridine, potassium carbonate, and copper iodide), only a trace amount of 2-naphthyl-2'-dibenzothienyl ether (14) (3%) was isolated.

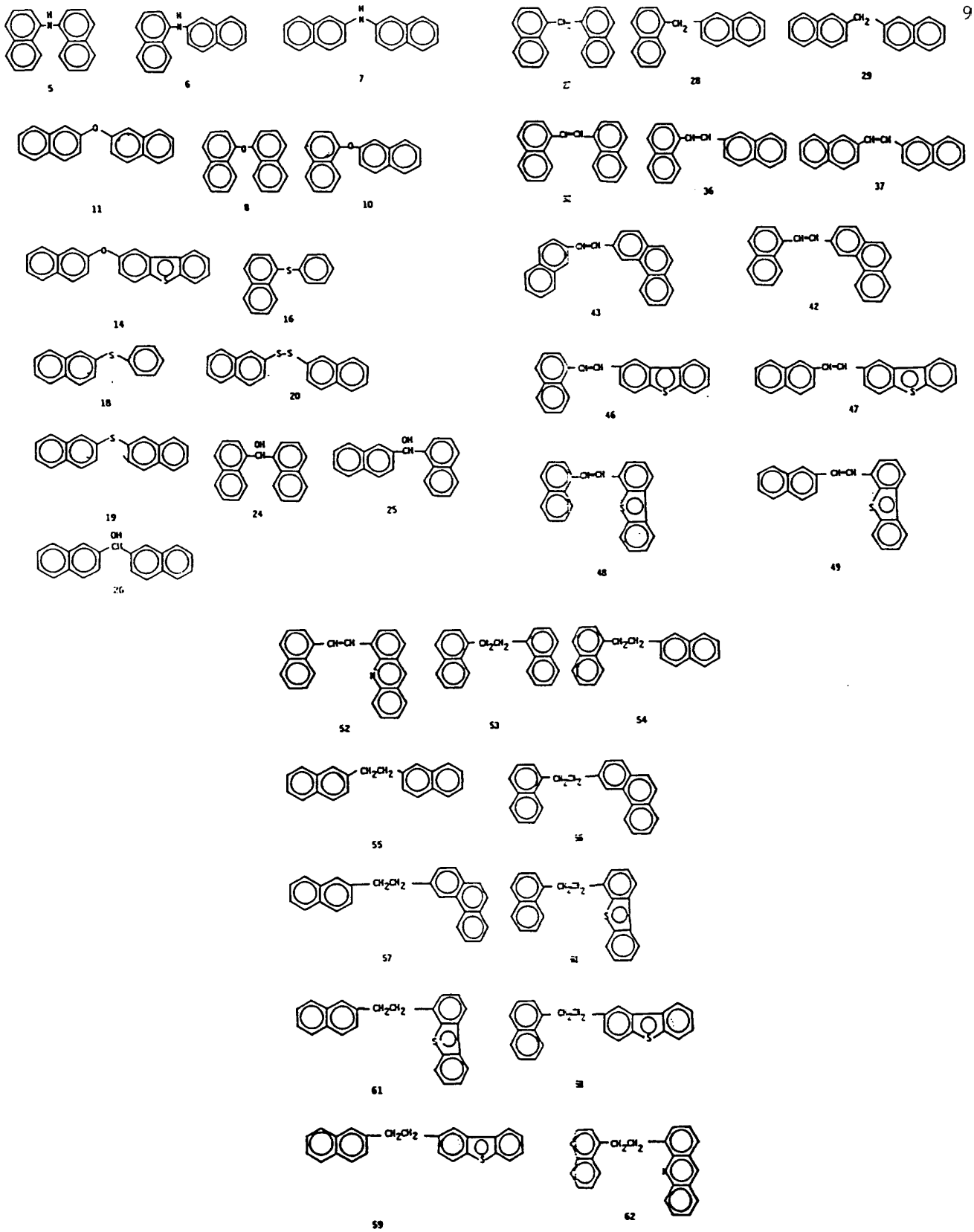

Figure 3. Final products synthesized.

The sulfur-linked reference compounds were synthesized following the acid catalyzed coupling of a thio with a naphthol as reported by Furman and coworkers.[5] Thus, 1-naphthylphenylsulfide (16) (7.3%) was prepared by refluxing thiophenol (15) with 1-naphthol (3) in the presence of *p*-toluenesulfonic acid in toluene. Both, thiophenol (15) and 2-naphtholenethiol (17) were reacted with 2-naphthol (4) under similar acid-catalyzed conditions to afford 2-naphthylphenylsulfide (18) (15%) and 2,2'-dinaphthyl sulfide (19) (17%), respectively. Utilizing the procedure of Weinstein and Pierson,[6] 2-naphthalenethiol (17), by action of iodine crystals, was converted into 2,2'-dinaphthyl disulfide (20) (48%).

The methyl bridged standard compounds have been previously reported.[7] However, instead of utilizing the classical Grignard approach, we chose to react the lithio derivative of 1-bromonaphthalene (9) or 2-bromonaphthalene (21), generated *via n*-butyllithium, with 1-naphthaldehyde (22) or 2-naphthaldehyde (23) to give 1,1'dinaphthylmethanol (24) (44%) 1,2'-dinaphthylmethanol (25) (48%), or 2,2'-dinaphthylmethanol (26) (45%). A 10% palladium on carbon catalyst under acidic conditions and a hydrogen atmosphere readily reduced (24) to furnish 1,1'-dinaphthylmethane (27) (63%). Reduction of (25) was achieved with either sodium borohydride[8] (Method A) or hydroiodic acid[9] (Method B) to afford 1,2'-dinaphthylmethane (28) in 49% or 87% yields, respectively. Lithium aluminum hydride in the presence of a Lewis acid[10] converted (26) into 2,2'-dinaphthylmethane (29) (45%).

The Wadsworth-Emmons reaction[11] was utilized in the preparation of the ethyl-linked compounds. 1-Naphthylmethylphosphonate (31), prepared in 64% yield from 1-chloromethylnaphthalene (30) according to the procedure of Kosolapoff,[12] was reacted with 1-naphthaldehyde (22) to give 1-(1-naphthyl)-

2-(1-naphthyl)ethylene (32) (69%). In a like manner, 2-naphthylmethylphosphonate (35) (59%), resulting from bromination of 2-methylnaphthalene (33) to give 2-bromomethylnaphthalene (34) (65%) followed by treatment with triethylphosphite as reported by Zimmerman *et al.*,[13] furnished 1-(1-naphthyl)-2-(2-naphthyl)ethylene (36) (72%) or 1-(2-naphthyl)-2-(2-naphthyl)ethylene (37) (54%) when reacted with 1-naphthaldehyde (22) or 2-naphthaldehyde (23). Reaction of 3-formylphenanthrene (41) (84%), from oxidation of 3-acetylphenanthrene (38) to 3-phenanthrylcarboxylic acid (39) (22%) following the method of Mosettig and Van De Kamp.[14] Then reduction to 3-hydroxymethylphenanthrene (40) (72%) and oxidation with chromium oxide, with the naphthylmethylphosphonates (31) or (35) produced 1-(1-naphthyl)-2-(3-phenanthryl)ethylene (42) (75%) and 1-(2-naphthyl)-2-(3-phenanthryl)ethylene (43) (76%). Preparation of 1-(1-naphthyl)-2-(2-dibenzothienyl)ethelene (46) (55%), 1-(2-naphthyl)-2-(2-dibenzothienyl)ethylene (47) (59%), 1-(1-naphthyl)-2-(4-dibenzothienyl)ethylene (48) (30%), and 1-(2-naphthyl-2-(4-dibenzothienyl)ethylene (49) (27%) was accomplished by treating the naphthylmethyl phosphonates (31) and (35) with either 2-formyldibenzothiophene (44) (86%), generated from the lithio intermediate of 2-bromodibenzothiophene (13) and N,N-dimethylforamide, or 4-formyldibenzothiophene (45) (63%), produced from direct lithiation of dibenzothiophene (12) followed by formylation with N,N-dimethylformamide. Finally, 1-(1-naphthyl)-2-(4-acridinyl)ethylene (52) (66%) was synthesized from 4-acridinylmethylphosphonate (51) (55%), by treating 4-bromomethylacridine (50)[15] with triethylphosphite and 1-naphthaldehyde (22). The ethylene spacer was reduced catalytically (10% Pd/C) under a hydrogen atmosphere to afford 1-(1-naphthyl)-2-(1-naphthyl)ethane (53) (60%), 1-(1-naphthyl)-2-(2-naphthyl)-ethane (54) (52%), 1-(2-naphthyl)-

2-(2-naphthyl)ethane (55) (84%), 1-(1-naphthyl)-2-(3-phenanthryl)ethane (56) (87%), 1-(2-naphthyl)-2-(3-phenanthryl)ethane (57) (74%), 1-(1-naphthyl)-2-(2-dibenzothienyl)ethane (58) (55%), 1-(2-naphthyl)-2-dibenzothienyl)ethane (59) (64%), 1-(1-naphthyl)-2-(4-dibenzothienyl)ethane (60) (80%), 1-(2-naphthyl)-2-(4-dibenzothienyl)ethane (61) (33%), and 1-(1-naphthyl)-2-(4-acridinyl)ethane (62) (4%), respectively.

Synthetic experimental details. M.p.s were determined on a Thomas Hoover melting point apparatus and are uncorrected. I.r. spectra were recorded on a Beckmann FT 1100 spectrophotometer as KBr pellets. Routine n.m.r. spectra were obtained on a JEOL FX-90Q spectrometer in the solvent indicated with tetramethylsilane as the internal standard. Chemical shifts are reported in p.p.m. (δ) and J values in Hz.

1,1'-Dinaphthylamine (5). - A stirred mixture of 1-aminonaphthalene (1) (20 g, 140 mmol), 1-naphthol (3) (20 g, 139 mmol), ammonium chloride (20 g, 374 mmol), and anhydrous zinc chloride (20 g, 147 mmol) was gently heated until all the reactants had melted. This resulted in a vigorous reaction that lasted for *ca.* 15 min. The mixture was maintained at this temperature an additional 30 min, then cooled to room temperature when the mixture was washed with boiling water, a 10% potassium hydroxide solution, and dilute hydrochloric acid. The resulting residue was recrystallized from ethanol accompanied by treatment with charcoal to give (5) (8 g, 21%), m.p. 111-112°C (lit.,[16] m.p. 111°C); $\lambda_{max.}$ (KBr) 3 394, 1 592, 1 584, 1 571, 1 279 cm^{-1}; δ_H ($CDCl_3$) 5.54 (1H, bs, exchangeable with D_2O), 6.94 (2H, dd, J 4.9, J' 0.9), 7.16-7.57 (8H, m), 7.63-8.04 (4H, m).

1,2'Dinaphthylamine (6). - The title compound was prepared from 1-aminonaphthalene (1) and 2-naphthol (4) exactly as that described for the

synthesis of 1,1-dinaphthylamine (5) to give (6) (13.8 g, 37%), m.p. 100-101°C (lit.,[3] m.p. 98-100°C); $\lambda_{max.}$ (KBr) 3 381, 1 630, 1 599, 1 576, 1 281, cm^{-1}; δ_H (CDCl3) 5.53 (1H, vbs, exchangeable with D_2O), 7.07-8.08 (14H, m).

2,2'-Dinaphthylamine (7). - The title compound was prepared from 2-aminonaphthalene (2) (2.4 g, 17 mmol), 2-naphthol (4) (2.4 g, 17 mmol), ammonium chloride (2.4 g, 45 mmol), and anhydrous zinc chloride (2.4 g, 18 mmol) in a manner similar to that described for the synthesis of 1,1'-dinaphthylamine (5). The resulting residue was recrystallized from ethanol accompanied by treatment with charcoal to give (7) (1.2 g, 27%), m.p. 169-170°C (lit.,[3] m.p. 172.5°C); $\lambda_{max.}$ (KBr) 3 412, 1 627, 1 602, 311 cm^{-1}; δ_H ($CDCl_3$) 4.96 (1H, v bs), exchangeable with D_2O), 7.22-7.86 (14H, m).

1,1' Dinaphthyl ether (8).[17] - A mixture of 1-naphthol 3 (3 g, 21 mmol) and sodium bisulfate (0.6 g, 5 mmol) was heated to 195°C overnight. After cooling, the dark mass was distilled under reduced pressure. The distillate, containing 1-naphthol 3, was treated with hot sodium hydroxide (2M) and the gummy solid that formed after cooling was collected. Recrystallization from ethanol afforded 8 (0.8 g, 15%) as yellowish prisms, m.p. 106-108°C (lit.,[18] m.p. 109-110°C); R_f = 0.65 ($CHCl_3$/pet. ether, 1:4); $\lambda_{max.}$ (KBr) 3 054, 1 234 cm^{-1}; δ_H ($CDCl_3$) 6.87 (2H, dd, J 7.6, J' 1.2), 7.21-7.64 (8H, m), 7.82-7.92 (2H, m), 8.25-8.36 (2H, m).

1,2'-Dinaphthyl ether (10). - Copper power (0.3 g) and 1-bromonaphthalene 9 (1.94 g, 9.4 mmol) was added to a mixture of 2-naphthol 4 (1.35 g, 9.4 mmol) and potassium hydroxide (0.62 g) which had been preheated to 130-140°C until all the alkali had dissolved. The mixture was then heated at 190-195°C for 58 h. After cooling, the mixture was distilled in a Kugelrohr under reduced pressure to give a mixture of naphthalene and crude product. After removal of naphthalene (0.42 g) by sublimation, the remaining material (0.22 g) was recrystallized from

ethanol to yield 10 (0.12 g, 5%), m.p. 80-81°C (lit.,[4] 81°C); R_f = 0.62 ($CHCl_3$/pet. ether, 1:4); λ_{max}. (KBr) 3 052, 1 250 cm^{-1}; δ_H ($CDCl_3$) 7.05 (1H, dd, J 7.3, J 1.2), 7.21-7.93 (12H, m), 8.18-8.24 (1H, m).

2,2'-Dinaphthyl ether (11).[19] - p-Toluenesulfonic acid (0.82 g, 4 mmol) was added to a solution of 2-naphthol 4 (1.75 g, 12 mmol) in xylene (80 mL) then heated at reflux for 6 days. The resulting brown solution was poured into a solution of sodium hydroxide (2 M, 100 mL) then extracted with ethyl acetate (2 x 80 mL), washed with water (80 mL), and dried ($MgSO_4$). Excess solvent was evaporated to dryness *in vacuo* and the residue was recrystallized from water to remove unreacted 2-naphthol 4. The mother liquid was then evaporated to dryness and the residue was distilled under reduced pressure to give 0.47 g of crude product along with some 2-naphthol 4. The yellowish distillate was recrystallized from ethanol to afford 11 (0.21 g, 6.5%) as colorless shiny leaflet crystals, m.p. 102-103°C (lit.,[18] 104-105°C); R_f = 0.58 ($CHCl_3$/pet. ether, 1:4); λ_{max}. (KBr) 3 052. 1 270 cm^{-1}; δ_H ($CDCl_3$) 7.20-7.88 (14H, m).

2-Bromodibenzothiophene (13). - A solution of bromine (5.6 mL, 109 mmol) in chloroform (20 mL) was added dropwise to a stirred solution of dibenzothiophene[12] (20 g, 109 mmol) in chloroform (200 mL) at room temperature. After the addition was complete, the orange solution was allowed to stir at room temperature for 12 h. The reaction solution was washed with a 5% sodium hydroxide solution (200 mL) then water (200 mL) and dried ($MgSO_4$). Excess solvent was removed under reduced pressure to give a white solid. This material was dissolved in hot ethanol (*ca.* 500 mL), treated with charcoal, then allowed to cool and stand at room temperature overnight. The precipitate was collected by filtration and treated as above to afford 13 (12.5 g, 44%) as a white solid,

m.p. 121-123°C (lit.,[20] m.p. 127°C); Beilstein test positive; λ_H ($CDCl_3$) 7.41-7.66 (4H, m) 7.75-7.89 (1H, m), 8.03-8.26 (2H, m).

2-Naphthyl-2'dibenzothienyl ether (14). - 2-Bromodibenzothiophene 13 (2.63 g, 10 mmol) was added to a stirred mixture of 2-naphthol 4 (1.44 g, 10 mmol) and powdered potassium carbonate (1.38 g, 10 mmol) in pyridine (10 mL) under a nitrogen atmosphere. When the mixture began to reflux, copper (I) iodide (1.5 g) was added and heating at reflux continued for 137 h. The mixture was evaporated to dryness *in vacuo* and the residue was distilled in a Kugelhohr to give 1.93 g of crude product containing 2-bromodibenzothiophene 13. The filtrate was evaporated to dryness and the residue was subjected to column chromatography with dichloromethane/pet. ether (1:10) as the eluent. The fractions containing product were pooled, evaporated to dryness, then recrystallized from ethanol to give 14 (0.1 g, 3%) as colorless crystals, m.p. 162-163°C; R_f = 0.54 ($CHCl_3$/pet. ether, 1:4) (found:C, 80.9; H, 4.4; S, 9.7. $C_{22}H_{14}OS$ requires C, 80.95; H, 4.3; S, 9.8%); $\lambda_{max.}$ (KBr) 3 050, 1 252, 1 213 cm^{-1}.

1-Naphthylphenylsulfide (16). - A mixture of 1-naphthol 3 (10 g, 69 mmol), thiophenol 15 (7.6 g, 69 mmol), p-toluene-sulfonic acid (3.8 g, 20 mmol), and toluene (20 mL) was stirred at 110°C for 20 h. The reaction was washed with waer (50 mL), 2% sodium hydroxide (50 mL), and water (100 mL), then dried ($MgSO_4$). The product was evaporated under reduced pressure and the remaining oil solidified upon standing. Recrystallization from ethanol afforded 16 (1.2 g, 7.3%) as white crystals, m.p. 40-41°C (lit.,[6] m.p. 39-40.5°C); δ_H ($CDCl_3$) 7.20-7.65 (10H, m), 7.83-7.91 (2H, m).

2-Naphthylphenylsulfide (18). - This compound was prepared by using a mixture of 2-naphthol 4 (10 g, 69 mmol), thiophenol 15 (7.6 g, 69 mmol), p-toluenesulfonic acid (3.8 g, 20 mmol), and toluene (20 mL) in a manner similar

to the preparation of 1-naphthylphenylsulfide 16. The title compound 18 (2.4 g, 15%) was obtained as white crystals (ethanol), m.p. 51-52°C (lit.,[5] m.p. 50-51°C); δ_H ($CDCl_3$) 7.23-7.55 (8H, m), 7.69-7.85 (4H, m).

2,2'-Dinaphthylsulfide (19). - A stirred suspension of 2-naphthalenethiol 17 (2 g, 12.5 mmol), 2-naphthol 4 (1.8 g, 12.5 mmol), and p-toluenesulfonic acid (0.24 g, 1.3 mmol) in toluene (25 mL) was heated at reflux for 36 h. After standing at room temperature for 12 h, the precipitate was collected by filtration. This solid was dissolved in hot benzene (*ca.* 30 mL), treated with charcoal, allowed to cool and stand at room temperature 3 h. The precipitate was collected by filtraiton and dried to give 19 (0.62 g, 17%) as white flakes (initially long needles formed, however, when stirred, flakes resulted), m.p. 146-147°C (lit.,[21] m.p. 150-151°C); δ_H ($CDCl_3$); 7.36-7.54 (6H, m), 7.68-7.86 (8H, m).

2,2'-Dinaphthyldisulfide (20). - A mixture of iodide crystals (1.6 g, 6.3 g/atoms) in methanol (25 mL) was added to a stirred suspension of 2-naphthalenethiol 17 (2 g, 12.5 mmol) in absolute ethanol (15 mL) which had been filtered hot into the reaction flask. The mixture was heated at reflux for 1 h then allowed to cool to room temperature when the precipitate was collected by filtration. This solid was dissolved in hot benzene (*ca.* 50 mL), treated with charcoal, allowed to cool and stand at room temperature overnight. The precipitate was collected by filtration and dried to give 20 (0.96 g, 48%) as faint yellow flakes, m.p. 140-141°C (lit.,[6] m.p. 141.8-142.6°C); δ_H ($CDCl_3$) 7.38-7.83 (12H, m), 7.97 (2H, d, J 1.1).

1,1'-Dinaphthylmethanol (24). - A solution of 1-bromonaphthalene 9 (6.6 g, 32 mmol) in anhydrous ether (50 mL) was added dropwise to a stirred solution of *n*-butyllithium (1.55 M, 20.6 mL, 32 mmol) in hexanes at ice bath temperature

under a nitrogen atmosphere. After the addition was complete, the mixture was allowed to warm to room temperature, and stirring was continued for 30 min. The mixture cooled to ice bath temperature then a solution of 1-naphthaldehyde 22 (5 g, 32 mmol) in anhydrous ether (50 mL) was added dropwise. After the addition was complete, the mixture was heated at reflux for 1 h then colled to ice bath temperature and diluted with water (100 mL). The organic layer was separated and the aqueous portion was extracted with ether (200 mL). The combined organic portions were dried ($MgSO_4$). Excess solvent was removed under reduced pressure to give a viscous light yellow paste which solidified upon standing, m.p. 86-110°C. This solid was dissolved in hot methanol (*ca.* 75 mL), treated with charcoal, allowed to cool to room temperature then placed at -15°C for 12 h. The precipitate was collected by filtration, washed with hexane (25 mL) and dried to give 24 (3.9 g, 44%) as a white solid, m.p. 143-145°C (lit.,[9] m.p. 144°C). The filtrate was concentrated to dryness *in vacuo* and upon standing overnight a solid resulted. This solid was suspended in hexane (10 mL) then the precipitate was collected by filtration and dried to afford 2,3 g of crude 24, m.p. 135-141°C; δ_{max}. (KBr) 3 466 cm^{-1}; δ_H ($CDCl_3$) 3.43 (1H, s, exchangeable with D_2O), 7.23-7.56 (9H, m), 7.76-8.07 (6H, m).

1,2'-Dinaphthylmethanol (25). - The title compound was prepared from 1-bromonaphthalene 9 (6.6 g, 32 mmol) and 2 naphthaldehyde 23 (5 g, 32 mmol) exactly as that described for the synthesis of 1,1'-dinaphthylmethanol 24. The crude isolated solid was dissolved in hot methanol (*ca.* 75 mL), treated with charcoal, then allowed to cool to room temperature followed by standing at -15°C for 48 h. The precipitate was collected by filtration, washed with hexane (25 mL) and dried to give 25 (4.4 g, 48%) as an off-white solid, m.p. 103-106°C (lit.,[7] m.p. 108-109°C). The filtrate was concentrated to dryness under reduced

pressure to give a viscous oil which solidifed upon standing at room temperature. This solid was suspended in hexane (25 mL) and the precipitate was collected by filtration to afford 2.7 g of crude 25, m.p. 103-109°C; $\lambda_{max.}$ (KBr) 3 350 cm^{-1}; δ_H ($CDCl_3$) 2.58 (1H, d, J 2.6, exchangeable with D_2O), 6.62 (1H, d, J 2.2, collapses to a singlet at 6.61 after treatment with D_2O), 7.35-7.57 (7H, m), 7.64-7.89 (6H, m), 8.01-8.12 (1H, m).

2,2'-Dinaphthylmethanol (26). - The title compound was prepared from 2-bromonaphthalene 21 (6.6 g, 32 mmol) and 2-naphthyldehyde 23 (5 g, 32 mmol) in a manner similar to that described for the synthesis of 1,1'-dinaphthylmethanol 24 except that the reaction was heated at reflux for 4 h. The crude isolated solid was dissolved in hot hexane (800 mL), treated with charcoal, then allowed to cool and stand at room temperature for 3 h. The precipitate was collected by filtration and dried to give 26 (4.1 g, 45%) as a white solid, m.p. 113-115°C (lit.,[22] m.p. 116.5°C); $\lambda_{max.}$ (KBr) 3 299 cm^{-1}; δ_H ($CDCl_3$) 2.48 (1H, bs, exchangeable with D_2O), 6.13 (1H, s), 7.40-7.54 (6H, m), 7.74-7.92 (8H, m).

1,1'-Dinaphthylmethane (27). - A 10% Pd/C catalyst (0.1 g) was added portionwise to a stirred solution of 1,1'-dinaphthylmethanol 24 (3 g, 11 mmol) and sulfuric acid (d 1.84, 1 mL) in ethanol (200 mL) at room temperature. The mixture was stirred under a hydrogen atmosphere for 24 h then the catalyst was removed by filtration through a pad of Celite. Excess solvent was removed under reduced pressure and the resulting mixture was diluted with benzene (200 mL), stirred for 15 min, then the aqueous portion (*ca.* 3 mL) separated and the organic phase dried ($MgSO_4$). Excess benzene was removed *in vacuo* to give a viscous off-white oil. This oil was dissolved in hot ethanol, treated with charcoal, then allowed to cool and stand at room temperature overnight. The precipitate was collected by filtration and dried to afford 27 (1.8 g, 63%) as white crystals,

m.p. 105-107°C (lit.,[8] m.p. 108-109°C); δ_H ($CDCl_3$) 4.87 (2H, s), 7.03-7.11 (2H, m), 7.24-7.57 (6H, m), 7.71-8.89 (6H, m).

1,2'-Dinaphthylmethane (28). - Method A. Sodium borohydride (2.6 g, 70 mmol) was added portionwise to trifluoroacetic acid (50 mL) stirring over an ice bath under a nitrogen atmosphere. After the addition was complete, a solution of 1,2'-dinaphthylmethanol 25 (2 g, 7 mmol) in dichloromethane (25 mL) was added dropwise. After the addition was complete, the mixture was stirred at room temperature for 20 h then cooled to ice bath temperature when sodium hydroxide pellets were added portionwise until the mixture was basic. The mixture was extracted with ether (2 x 200 mL) and the combined extracts dried ($MgSO_4$). Excess solvent was removed under reduced pressure to give a viscous oil. This oil was dissolved in hot ethanol, treated with charcoal, then allowed to cool and stand at room temperature overnight. The precipitate was collected by filtration then recrystallized from ethanol to afford 28 (0.9 g, 49%) as white crystals, m.p. 90-92°C (lit.,[23] m.p. 94.5-95.5°C); δ_H ($CDCl_3$) 4.56 (2H, s), 7.16-7.50 (7H, m), 7.57-7.89 (6H, m), 7.96-8.07 (1H, m).

Method B. A mixture of 1,2'-dinaphthylmethanol 25 (1.14 g, 4.2 mmol) and acetic acid (25 mL) was heated on a hot plate, then to this solution was added hydroiodic acid (5.5 mL, d 1.701) dropwise and the mixture was boiled for a few minutes. After standing at room temperature for 5 h, 28 (0.94 g, 87%), which had separated in the form of colorless needles, was collected by filtration, m.p. 92-94°C. Spectral data were consistent with that observed in Method A.

2,2'-Dinaphthylmethane (29). - A solution of aluminum chloride (1.41 g, 10.6 mmol) in anhydrous ether (40 mL) was added portionwise to a stirred suspension of lithium aluminum hydride (0.4 g, 10.6 mmol) in anhydrous ether (50 mL) at ice bath temperature under a nitrogen atmosphere. After the addition

was complete, a solution of 2,2'-dinaphthylmethanol 26 (3.0 g, 10.6 mmol) in anhydrous ether (125 mL) was added dropwise. After the addition was complete, the mixture was heated at reflux for 2 h then excess lithium aluminum hydride and aluminum chloride were deactivated by the dropwise addition of water (4 mL) over *ca.* 1 h at ice bath temperature. The precipitate was removed by filtration, washed with anhydrous ether (25 mL), then the filtrate was dried ($MgSO_4$). Excess solvent was removed under reduced pressure to give a white solid, m.p. 78-82°C. This solid was dissolved in hot ethanol (*ca.* 125 mL), filtered, then allowed to cool and stand at room temperature for 2 h. The precipitate was collected by filtration and dried under high vacuum (1.2 mmHg at *ca.* 50°C for 2 h) to afford-- (1.3 g, 45%) as small white irregular-shaped crystals, m.p. 89-90°C (lit.,[7] m.p. 93°C). The recrystallization filtrate was concentrated to dryness *in vacuo* to yield 1.1 g of crude 29 as a white solid, m.p. 79-82°C; δ_H ($CDCl_3$) 4.29 (2H, s), 7.29-7.52 (6H, m), 7.67-7.85 (8H, m).

1-Naphthylmethylphosphonate (31). - A solution of 1-chloromethylnaphthalene 30 (200 g, 1.13 mmol) and triethyl phosphite (194 mL, 1.13 mmol) was heated at *ca.* 185°C for 12 h. The orange solution was subjected to a fractional distillation and a forerun was collected between 70-170°C at 0.15 mmHg followed by a fraction collected between 170-173°C at 0.16 mmHg to afford 31 (201 g, 64%) as a clear viscous liquid (lit.,[12] b.p. 205-206°C at 5 mmHg); δ_H ($CDCl_3$) 1.12 (6H, t, J 4.7), 3.60 (2H, d, J 1.47), 3.91 (4H, p, J 14.7), 7.36-7.53 (4H, m), 7.66-7.86 (2H, m), 8.04-8.14 (1H, m).

1-(1-Naphthyl)-2-(1-naphthyl)ethylene (32). - A solution of 1-naphthylmethylphosphonate 31 (8.9 g, 32 mmol) in 1,2-dimethoxyethane (35 mL) was added dropwise to a stirred suspension of a 60% oil dispersion of sodium hydride (1.5 g), prewashed with hexane (15 mL), in 1,2-dimethoxyethane (15 mL) at room

temperature under a nitrogen atmosphere. After the addition was complete, the suspension was stirred for 30 min then a solution of 1-naphthaldehyde 22 (5 g, 32 mmol) in 1,2-dimethoxyethane (50 mL) was added dropwise. After the addition was complete, the mixture was stirred for 12 h then diluted with water (100 mL) and stirring continued for 30 min. The precipitate was collected by filtration and dried. This solid was dissolved in hot ethanol, treated with charcoal, then allowed to cool and stand overnight at room temperature. The precipitate was collected by filtration to give 32 (6.2 g, 69%) as a light yellow solid, m.p. 161-163°C (lit.,[24] m.p. 164-165°C); δ_H ($CDCl_3$) 7.38-7.59 (6H, m), 7.78-7.93 (8H, m), 8.19-8.30 (2H, m).

2-Bromomethylnaphthalene (34). - A stirred mixture of 2-methylnaphthalene 33 (100 g, 703 mmol), N-bromosuccinimide (125.2 g, 703 mmol), and benzoyl peroxide (1 g, 4 mmol), in benzene (800 mL) was heated at reflux for 18 h. The following workup of the reaction mixture should be carried out in the hood since this compound was found to be an extreme lachrymator. The mixture was allowed to cool to room temperature, the solids were removed by filtration and the filtrate was washed with a 5% sodium hydroxide solution (500 mL), water (500 mL), then dried ($MgSO_4$). The dried organic phase was diluted with hexane (300 mL), cooled to ice bath temperature, then the precipitate collected by filtration, washed with cold (*ca.* 0°C) hexane (50 mL) to give 34 (100.3 g, 65%) as a white solid, m.p. 52-55°C (lit.,[25] m.p. 54°C); δ_H ($CDCl_3$) 4.55 (2H, s), 7.25-7.85 (7H, m). This material was used without further purification.

2-Naphthylmethylphosphonate (35). - The title compound was prepared from 2-bromomethylnaphthalene 34 (86 g, 389 mmol) and triethylphosphite (66.7 mL, 389 mmol) in a manner similar to that described for 1-naphthylmethylphosphonate 31 to afford 35 (63.5 g, 59%) as a yellow liquid collected between 181-183°C at 0.17

mmHg (lit.,[13] b.p. 148-153°C at 0.05 mmHg); δ_H ($CDCl_3$) 1.23 (6H, t, J 4.7), 3.30 (2H, d, J 14.7), 4.01 (4H, p. J 4.7), 7.38-7.49 (3H, m), 7.74-7.84 (4H, m).

1-(Naphthyl)-2-(2-naphthyl)ethylene (36). The title compound was prepared from 1-naphthylmethylphosphonate 31 (8.9 g, 32 mmol) and 2-naphthaldehyde 23 (5 g, 32 mmol) in the same manner as that described for the synthesis of 1-(1-naphthyl)-2-(1-naphthyl)ethylene 32. The isolated crude product was dissolved in hot benzene (*ca.* 400 mL), treated with charcoal, precipate collected by filtration and dried to give 36 (4.2 g, 47%) as a light lime-green powder, m.p. 186-187°C (lit.,[24] m.p. 191-192°C). The filtrate was concentrated to dryness under reduced pressure to afford 2.3 g of -- as a light yellow solid, m.p. 181-183°C; 72% yield based on 6.5 g recovered; δ_H ($CDCl_3$) 7.39-7.57 (6H, m), 7.77-8.10 (9H, m), 8.22-8.33 (1H, m).

1-(2-Naphthyl)-2-(2-naphthyl)ethylene (37). - The title compound was prepared from 2-naphthylmethylphosphonate 35 (8.9 g, 32 mmol) and 2-naphthaldehyde 23 (5 g, 32 mmol) in the same manner as that described for the synthesis of 1-(1-naphthyl)-2-(1-naphthyl)ethylene 32. The isolated crude product was suspended in hot ethanol (500 mL), heated at a slow boil for 30 min, filtered and the filtrate allowed to cool to room temperature. The precipitate was collected by filtraiton and dried to give 37 (4.8 g, 54%) as a white solid, m.p. 253-256°C (lit.,[26] m.p. 258°C); δ_H ($CDCl_3$) 7.40-7.51 (6H, m), 7.77-7.90 (10H, m).

3-Phenanthrylcarboxylic acid (39). - A stirred mixture of 3-acetyl-phenanthrene 38 (20 g, 90% m.p. 66-71°C from Aldrich Chemical Company) and sodium hypochlorite (300 mL, 5% minimum chlorine available) was heated to a boil for 3 h. During the course of the reaction 30 mL aliquot of sodium hypochlorite was added at 30 min intervals (3 x). The mixture was allowed to cool to room

temperature then it was diluted with water (2 ℓ) and the solids were removed by filtration. The filtrate was extracted with ether (3 x 150 mL) then made acidic with hydrochloric acid. The precipitate was collected by filtration and recrystallized from acetic acid to give 39 (4 g, 22%) as yellow plates, m.p. 262-267°C (decomp.) (lit.,[14] m.p. 270°C). This material was utilized without further purification.

3-Hydroxymethylphenanthrene (40). - 3-Phenanthylcarboxylic acid 39 (3.2 g, 14.4 mmol) was added portionwise to a stirred suspension of lithium aluminum hydride (2.2 g, 55.1 mmol) in anhydrous ether (150 mL) at ice bath temperatures. After the addition was complete, the mixture was stirred at room temperature for 20 min then heated at reflux for 1 h. The mixture was poured portionwise into an ice-cooled solution of hydrochloric acid (2N, 600 mL). The aqueous phase was extracted with ether (3 x 200 mL) and the combined ether portions evaporated to dryness under reduced pressure to give a yellow solid. Recrystallization from benzene afforded 40 (2.2 g, 72%) as pale yellow needles, m.p. 96-98°C (lit.,[27] m.p. 103-103.5°C); $\lambda_{max.}$ (KBr) 3 307, 1 355, 1 047 cm^{-1}; δ_H ($CDCl_3$) 4.95 (2H, s), 7.50-7.72 (4H, m), 7.83-7.95 (3H, m), 8.66-8.77 (2H, m); the OH proton was not observed.

3-Formylphenanthrene (41). - A mixture of 3-hydroxymethylphenanthrene 40 (1.5 g, 7.2 mmol) in pyridine (20 mL) was added in one portion to a stirred mixture of chromium oxide (2.2 g, 22 mmol) in pyridine (50 mL) at room temperature. After stirring for 1 h, solids were removed by filtration then washed with ether. The combined organic phases were washed with 5% sodium hydroxide, 5% hydrochloric acid, 5% sodium bicarbonate, and brine. Excess organic solvent was removed *in vacuo* to give a yellow solid which was recrystallized from hexane to afford 41 (1.25 g, 84%) as colorless prisms, m.p.

78-79°C (lit.,[28] m.p. 78-79°C); $\lambda_{max.}$ (KBr) 2 844, 2 828, 2 726, 1 694 cm^{-1}; δ_H ($CDCl_3$) 7.45-8.08 (7H, m), 8.68-8.85 (1H, m), 9.14 (1H, s), 10.25 (1H, s).

1-(1-Naphthyl)-2-(3-phenanthryl)ethylene (42). - A 60% oil dispersion of sodium hydride (0.1 g, prewashed with hexane) was added portionwise to a stirred solution of diethyl 1-naphthylmethylphosphonate 31 (0.75 g, 2.5 mmol) and 3 formylphenanthrene 41 (0.5 g, 2.4 mmol) in dimethylsulfoxide (10 mL) at ice bath temperature. After the addition was complete, the mixture was stirred for 20 min then allowed to warm to room temperature when stirring was continued for 2 h. The reaction was poured onto a mixture of crushed ice and water and the precipitate collected by filtration. Recrystallization from toluene afforded 42 (0.6 g, 75%) as pale green prisms, m.p. 168-170°C (found: C, 94.4; H, 5.4. $C_{26}H_{18}$ requires C, 94.5; H, 5.5%); $\lambda_{max.}$ (KBr) 3 070, 3 055, 3 015, 1 602, 1 509, 1 453, 1 422, 1 399, 1 383, 954, 849, 797, 771, 753 cm^{-1}.

1-(2-Naphthyl)-2-(3-phenanthryl)ethylene (43). - The title compound was prepared from 2-naphthylmethylphosphonate (35) (0.8 g, 2.6 mmol), 3-formylphenanthrene (41) (0.55 g, 2.7 mmol), and sodium hydride (0.2 g, 60% oil dispersion) in a manner similar to that described for the synthesis of 1-(1-naphthyl)-2-(3-phenanthryl)ethylene (42). The crude isolated product was recrystallized from benzene to give (43) (0.67 g, 76%), m.p. 205-211°C (found: C, 94.3; H, 5.4. $C^{26}H_{18}$ requires C, 94.5; h, 5.5%); $\lambda_{max.}$ (KBr) 3 083, 3 055, 3 016, 1 602, 1 594, 1 509, 1 455, 1 422, 1 401, 1 386, 954, 849, 833, 820, 748 cm^{-1}.

2-Formyldibenzothiophene (44. - A solution of *n*-butyl lithium in hexanes (1.55 N, 41.3 mL, 64 mmol) was added dropwise to a stirred solution of 2-bromodibenzothiophene (13) (16.8 g, 64 mmol) in anhydrous ether (650 mL) under nitrogen atmosphere at ice bath temperature. After the addition was complete,

the mixture was allowed to warm to room temperature and stirring continued for 30 min; then the reaction was cooled to ice bath temperature and a solution of N,N-dimethylformamide (9.9 mL, 128 mmol) in anhydrous ether (50 mL) was added dropwise. After the addition was complete, the mixture was allowed to warm and stir at room temperature for 12 h. A 10% hydrochloric acid solution (200 mL) was added to the mixture, it was stirred for 30 min, the aqueous layer was separated, then the ether portion was washed with water (200 mL) and dried ($MgSO_4$). Excess ether was removed *in vacuo* and the resulting was solid dissolved in hot ethanol (*ca.* 150 mL), treated with charcoal, then cooled and allowed to stand at room temperature overnight. The precipitate was collected by filtration and dried to give (44) (11.6 g, 86%) as slightly off-white crystals, m.p. 100-102°C (lit.,[29] m.p. 107.5-108.5°C); Beilstein test negtive; $\lambda_{max.}$ (KBr) 1 700 cm^{-1}; δ_H ($CDCl_3$) 7.42-7.57 (2H, m), 7.77-7.89 (3H, m), 8.10-8.21 (1H, m), 8.52 (1H, t), 10.08 (1H, s).

4-Formyldibenzothiophene (45). - A solution of *n*-butyl lithium in hexanes (1.55 M, 53 mL, 81 mmol) was added dropwise to a stirred solution of dibenzothiophene (12) (10 g, 54 mmol) in anhydrous ether (500 mL) at room temperature under a nitrogen atmosphere. After the addition was complete, the yellow solution was heated at reflux for 12 h, then cooled to room temperature whereupon a solution of N,N-dimethylformamide (8.4 mL, 108 mmol) in anhydrous ether (50 mL) was added dropwise. Heating at relflux continued for 6 h. The reaction was cooled to room temperature, then diluted with a 10% hydrochloric acid solution (200 mL) and allowed to stir for 30 min. The organic portion was separated, washed with water (200 mL), then dried ($MgSO_4$). Excess solvent was removed under reduced pressure to afford a light yellow solid which was further dried under high vacuum (0.12 mm at 50°C) for 2 h to give crude (45), m.p. 72-

98°C. This solid was recrystallized from benzene (*ca.* 75 mL) to give (45) (7.4 g, 63%) as faint yellow irregular crystals, m.p. 123-125°C (lit.,[30] m.p. 124-125°C); $\lambda_{max.}$ (KBr) 1 671 cm^{-1}; δ_H ($CDCl_3$) 7.39-7.69 (3H, m), 7.85-8.02 (2H, m), 8.11-8.22 (2H, m), 10.25 (1H, s).

1-(1-Naphthyl)-2-(2-dibenzothienyl)ethylene (46). - The title compound was prepared from 1-naphthylmethylphosphonate (31) (3.9 g, 14 mmol) and 2-formyldibenzothiophene (44) (3 g, 14 mmol) in a manner similar to that described for the synthesis of 1-(1-naphthyl)-2-(1-naphthyl)ethylene (32). The crude isolated product was suspended in hot hexane (*ca.* 900 mL), filtered, then allowed to cool and stand at room temperature overnight. The precipitate was collected by filtration then recrystallized (2 x) from hexane to give (46) (2.6 g, 55%) as short fine lime-green needles, m.p. 159-160°C, softens *ca.* 138°C (lit.,[31] m.p. 155-156°C) (found: C, 85.9; H, 4.9; S, 9.3. $C_{24}H_{16}S$ requires C, 85.7; H, 4.8; S, 9.5%); δ_H ($CDCl_3$) 7.18-7.72 (5H, m), 7.74-7.93 (6H, m), 8.07-8.32 (3H, m).

1-(2-Naphthyl)-2-(2-dibenzothienyl)ethylene (47). - The title compound was prepared from 2-naphthylmethylphosphonate (35) (3.9 g, 14 mmol) and 2-formyldibenzothiophene (44) (3 g, 14 mmol) in a manner similar to that described for the synthesis of 1-(1-naphthyl)-2-(1-naphthyl)ethylene (32). The crude isolated product was recrystallized (3 x) from benzene to give (47) (2.8 g, 59%) as a white solid, m.p. 229-231°C (lit.,[31] m.p. 208-209°C) (found: C, 85.9; H, 4.5; S, 9.4. $C_{24}H_{16}S$ requires C, 85.7; H, 4.8; S, 9.5%); δ_H (DMSO-d_6) at 100°C) 7.34-7.58 (5H, m), 7.76-8.05 (7H, m), 8.35-8.46 (1H, m), 8.59 (1H, d, J 0.7).

1-(1-Naphthyl)-2-(4-dibenzothienyl)ethylene (48). - The title compound was prepared from 1-naphthylmethylphosphonate (31) (3.9 g, 14 mmol) and 4-formyldibenzothiophene (45) (3 g, 14 mmol) in a manner similar to that described for the synthesis of 1-(1-naphthyl)-2-(1-naphthyl)ethylene (32). The

crude isolated product was suspended in hot hexane (*ca.* 900 mL), stirred for 15 min, filtered and the filtrate allowed to cool and stand at room temperature overnight. The precipitate was collected by filtraiton and dried to give (48) (1.4 g, 30%) as a light lime-green solid, m.p. 171-173°C (lit.,[31] m.p. 181-182°C); δ_H ($CDCl_3$) 7.32-7.61 (7H, m), 7.71-7.97 (5H, m), 8.08-8.25 (4H, m).

1-(2-Naphthyl)-2-(4-dibenzothienyl)ethylene (49). - The title compound was prepared from 2-naphthylmethylphosphonate (35) (3.9 g, 14 mmol) and 4-formyl-dibenzothiophene (45 (3 g, 14 mmol) in a manner similar to that described for the synthesis of 1-(1-naphthyl)-2-(1-naphthyl)ethylene (32). The crude isolated product was dissolved in hot hexane (*ca.* 800 mL), treated with charcoal then allowed to cool and stand at room temperature overnight. The precipitate was collected by filtration and dried to give (49) (1.3 g, 27%) as an off-white solid, m.p. 146-148°C (lit.,[31] m.p. 163-164°C); δ_H ($CDCl_3$) 7.39-7.54 (7H, m), 7.68-8.21 (9H, m).

Diethyl 4-acridinylmethylphosphonate (51). - The title compound was prepared from 4-bromoethylacridine (50)[15] (3 g, 11 mmol) and triethyl phosphite (6 mL, 37 mmol) in a manner similar to that described for 1-naphthylmethyl-phosphonte (31) except that the reaction was heated at reflux for 4 h. Excess triethyl phosphite was removed under reduced pressure and the remaining material was subjected to a vacuum distillation whereupon (51) (2 g, 55%) was collected between 216-219°C at 2 mmHg. This material was utilized without further purification.

1-(1-Naphthyl)-2-(4-acridinyl)ethylene (52). - The title compound was prepared from 4-acridinylmethylphosphonate (51) (1.8 g, 5.5 mmol), 1-naphthal-dehyde (22) (1 g, 6.4 mmol), sodium hydride (0.2 g, 60% oil dispersion), and dimethylsulfoxide (30 mL) in a manner similar to that described for the synthesis

of 1-(naphtyl)-2-(3-phenanthryl)ethylene (42) except that the reaction was carried out under a nitrogen atmosphere. The resulting mixture was poured onto a mixture of crushed ice and water. The precipitate was collected by filtration and dried to give (52) (1.2 g, 66%) as a yellow solid, m.p. 186-187°C (found: C, 90.9; H, 5.3; N, 4.0. $C_{25}H_{17}N$ requires C, 90.6; H, 5.2; N, 4.2%); δ_H ($CDCl_3$) 7.20-8.90 (17H, m).

1-(1-Naphthyl)-2-(1-naphthyl)ethane (53). - A 10% Pd/C catalyst (0.1 g) was added portionwise to a stirred solution of 1-(1-naphthyl)-2-(1-nahthyl)ethylene (32) (2 g, 7 mmol) in benzene (150 mL) at room temperature. The mixture was stirred at room temperature under a hydrogen atmosphere for 48 h. The catalyst was removed by filtration through a pad of celite then excess solvent was removed under reduced pressure to give a solid. This solid was dissolved in hot ethanol (*ca.* 400 mL), filtered, then cooled and left standing overnight at room temperature. The precipitate was collected by filtration and dried to afford (53) (1.2 g, 60%) as small white branches, m.p. 161-162°C (lit.,[32] m.p. 161-162°C); δ_H ($CDCl_3$) 3.50 (4H, s), 7.31-7.54 (8H, m), 7.67-7.92 (4H, m), 8.05-8.16 (2H, m).

1-(1-Naphthyl)-2-(2-naphthyl)ethane (54). - The title compound was prepared from 1-(1-naphthyl)-2-(2-naphthyl)ethylene (36) (3 g, 11 mmol) in a mixture of benzene (800 mL) and a 10% Pd/C catalyst (0.2 g) in a manner similar to that described for 1-(1-naphthyl)-2-(1-naphthyl)ethane (53) except that the mixture was stirred for 36 h. Removal of excess solvent under reduced pressure resulted in a clear viscous oil which partially solidifed upon standing overnight at room temperature. This material was dissolved in hot hexane (*ca.* 50 mL), filtered, then allowed to cool to room temperature and after standing 2 h, cooled to *ca.* -15°C. The precipitate was collected by filtration, then treated as above to give

(54) (1.6 g, 52%) as a white solid, m.p. 71-73°C (lit.,[33] no melting point was given) (found: C, 93.7; H, 6.45. $C_{22}H_{18}$ requires C, 93.6; H, 6.4%); δ_H ($CDCl_3$) 3.06-3.27 (2H, m), 3.36-3.55 (2H, m), 7.17-7.49 (7H, m), 7.53-7.89 (6H, m), 7.91-8.17 (1H, m).

1-(2-Naphthyl)-2-(2-naphthyl)ethane (55). - The title compound was prepared from 1-(2-naphthyl)-2-(2-naphthyl)ethylene (37) (2 g, 7 mmol) in a mixture of benzene (1,000 mL) and a 10% Pd/C catalyst (0.1 g) in a manner similar to that described for the synthesis of 1-(1-naphthyl)-2-(1-naphthyl)ethane (53). The crude isolated product was dissolved in a hot solution of ethanol and ethyl acetate (1:1, 800 mL) then boiled down to *ca.* 500 mL and allowed to cool and stand at room temperature overnight. The precipitate was collected by filtration and dried to afford (55) (1.7 g, 84%) as fine white branches, m.p. 183-184°C (lit.,[34] m.p. 185-186°C); δ_H ($CDCl_3$) 3.18 (4H, *s*), 7.28-7.47 (6H, m), 7.63-7.85 (8H, m).

1-(1-Naphthyl)-2-(3-phenanthryl)ethane (56). - The title compound was prepared from 1-(1-naphthyl)-2-(3-phenanthryl)ethylene (42) (0.6 g, 1.8 mmol) in a mixture of ethylacetate (200 mL) and a 10% Pd/C catalyst (0.2 g) in a manner similar to that described for the synthesis of 1-(1-naphthyl)-2-(1-naphthyl)-ethane (53, except that the mixture was stirred overnight. Removal of excess solvent *in vacuo* resulted in a colorless oil which afforded (56) (0.52 g, 87%) as colorless crystals when crystallized from hexane, m.p. 100-102°C (found: C, 94.0; H, 6.2. $C_{26}H_{20}$ requires C, 93.9; H, 6.1%); δ_H ($CDCl_3$) 3.91-3.61 (4H, m), 7.32-7.99 (13H, m), 8.07-8.24 (1H, m), 8.49-8.70 (2H, m).

1-(2-Naphthyl)-2-(3-phenanthryl)ethane (57). - The title compound was prepared from 1-(2-naphthyl-2-(3-phenanthryl)ethylene (43) (0.65 g, 2 mmol) in a mixture of ethylacetate (200 mL) and a 10% Pd/C catalyst (0.3 g) in a manner

similar to that described for the synthesis of 1-(1-naphthyl)-2-(1-naphthyl)-ethane (53) except that the mixture was stirred overnight. Removal of excess solvent under reduced pressure resulted in a crude solid. This material was recrystallized from hexane to give (57) (0.43 g, 74%) as colorless crystals, m.p. 116-118°C. (Found: C, 94.1; H, 5.9. $C_{26}H_{20}$ requires C, 93.9; H, 6.1%); δ_H ($CDCl_3$) 3.24 (4H, s), 7.31-8.06 (14H, m), 8.48-8.67 (2H, m).

1-(1-Naphthyl)-2-(2-dibenzothienyl)ethane (58). - The title compound was prepared from 1-(1-napthyl)-2-(2-dibenzothienyl)ethylene (46) (2 g, 5.9 mmol) in a mixture of benzene (250 mL) and a 10% Pd/C catalyst (0.1 g) in a manner similar to that described for the synthesis fo 1-(1-naphthyl)-2-(1-naphthyl)ethane (53) except that the mixture was stirred for 36 h. Removal of excess solvent under reduced pressure resulted in a light yellow oil which solidified upon standing overnight at room temperature. The solid was dissolved in hot ethanol (*ca.* 100 mL), treated with charcoal, then allowed to cool to room temperature whereupon the precipitate was collected by filtration. This material was recrystallized from ethanol to afford (58) (1.1 g, 55%) as white branches, m.p. 99-100°C. (Found: C, 85.1; H, 5.45; S, 9.5 $C_{24}H_{18}S$ requires C, 85.2; H, 5.4; S, 9.5%; δ_H ($CDCl_3$) 3.17-3.29 (2H, m), 3.38-3.47 (2H, m), 7.22-8.16 (14H, m).

1-(2-Naphthyl)-2-(2-dibenzothienyl)ethane (59). - The title compound was prepared from 1-(2-naphthyl)-2-(2-dibenzothienyl)ethylene (47) (1.5 g, 4.5 mmol) in a mixture of benzene (300 mL) and a 10% Pd/C catalyst (0.1 g) in a manner similar to that described for the synthesis of 1-(1-naphthyl)-2-(1-naphthyl)-ethane (53). The crude isolated product was dissolved in hot ethanol (*ca.* 200 mL), treated with charcoal, allowed to cool to room temperature whereupon the precipitate was collected by filtration. This material was recrystallized (2 x) from ethanol to afford 59 (1 g, 64%) as fine white clusters, m.p. 131-133°C.

(Found: C, 85.4; H, 5.15; S, 9.7. $C_{24}H_{18}S$ requires C, 85.2; H, 5.4; S, 9.5%); δ_H ($CDCl_3$) 3.19 (4H, s), 7.23-7.52 (6H, m), 7.65-7.89 (6H, m), 7.98-8.15 (2H, m).

1-(1-Naphthyl)-2-(4-dibenzothienyl)ethane (60). - The title compound was prepared from 1-(1-naphtyl)-2-(4-dibenzothienyl)ethylene (48) (1 g, 3 mmol) in a mixture of benzene (100 mL) and a 10% Pd/C catalyst (0.1 g) in a manner similar to that described for the synthesis of 1-(1-naphthyl)-2-(1-naphthyl)ethane (53) except that the mixture was stirred for 36 h. Removal of excess solvent *in vacuo* resulted in a viscous liquid which solidified upon standing at room temperature. This solid was dissolved in hot ethanol (100 mL), treated with charcoal, then allowed to cool to room temperature. The precipitate was collected by filtration and dried to give (60) (0.8 g, 80%) as short white branches, m.p. 115-116°C. (Found: C, 85.4; H, 5.6; S, 9.4. $C_{24}H_{18}S$ requires C, 85.2; H, 5.5; S, 9.5%); δ_H ($CDCl_3$) 3.27-3.40 (2H, m), 3.48-3.62 (2H, m), 7.29-7.60 (8H, m), 7.65-8.01 (4H, m), 8.07-8.26 (2H, m).

1-(2'-Naphthyl)-2-(4"-dibenzothienyl)ethane (61). - This compound was prepared from 1-(2'-napthyl)-2-(4"-dibenzothienyl)ethylene (49) (1 g, 3 mmoles) in a mixture of benzene (100 mL) and a 10% Pd/C catalyst (0.1 g) in a manner similar to that described for the synthesis of 1-(1'-naphthyl)-2-(1"-naphthyl)-ethane 53 except that the mixture was stirred for 72 h. Excess solvent was removed under reduced pressure to afford an off-white oil. This oil was dissolved in hot benzene:hexane (1:1, *ca.* 50 mL) to give, after standing 72 h at room temperature, 61 (0.34 g, 33%) as thick irregular plates having a faint purple tint, m.p. 71-72°; 1_H nmr (deuteriochloroform); δ 3.25 (4H, s), 7.17-7.51 (7H, m), 7.64-8.16 (7H, m). Anal. Calcd for: C, 85.2; H, 5.4; S, 9.5. Found: C, 85.0; H, 5.3; S, 9.2.

1-(1'-Naphthyl)-2-(4"-acridinyl)ethane (62). - This compound was prepared from 1-(1'-naphthyl)-2-(4"-acridinyl)ethylene (52) (0.3 g, 0.9 mmoles) in a mixture of ethyl acetate (200 mL) and a 10% Pd/C catalyst (1 g) in a manner similar to that described for the synthesis of 1-(1'-naphthyl)-2-(1"-naphthyl)ethane 53 except that the mixture was stirred for 4 days. Excess solvent was removed under reduced pressure and the residue chromatographed on an alumina column with hexane as the eluent to give 62 (11.8 mg, 4%) as pale green needles, m.p. 105-106°C; 1_H nmr (deuteriochloroform); δ 3.54-3.88 (4H, m), 7.28-8.03 (12H, m), 8.40 (1H, d, J 5.8), 8.70 (1H, s), 8.91 (1H, d, J 5.8). Anal. Calcd. for $C_{25}H_{19}N$: C, 90.1; H, 5.7; N, 4.2. Found: C, 89.9; H, 5.8; N, 4.2.

C. Screening of an SRC II Heavy Distillate for Compounds Containing Aromatic Moieties with Bridging Groups

An SRC II heavy distillate (HD, 260-450°C boiling point range, collected during the processing of a West Virginia coal from the Pittsburgh Seam, and obtained from the Fort Lewis, Washington, pilot plant which was operated by the Pittsburgh & Midway Coal Mining Company) was analyzed for the presence of compounds containing aromatic moieties with bridging groups. Representative standard reference compounds were selected from those that were synthesized during this period as well as from several others that were obtained from Dr. K.D. Bartle (University of Leeds, Leeds, England). The compounds selected for screening purposes were the most volatile linked compounds available (generally containing bridged two-ring moieties) so that high-resolution GC and GC-MS techniques could be used for this preliminary work. It was rationalized that if these compounds were detected by GC, the larger more nonvolatile linked compounds

would be sought for in the higher molecular weight fractions using supercritical fluid chromatography.

Fractions of the SRC II HD were analyzed by GC and GC-MS using a 10 m x 200 μm i.d. capillary column coated with a liquid crystalline polysiloxane stationary phase (0.15 μm film thickness). The gas chromatograph was a Hewlett-Packard Model 5890 equipped with a flame ionization detector and split-splitless injector, and the GC-MS system was a Hewlett-Packard Model 5970 GC-MSD which was operated at 70 eV ionization energy. Selected-ion-monitoring for the molecular ions and $(M-1)^+$ ions of the reference compounds was performed.

Figure 4 gives the compound types that were tentatively detected in the SRC II HD. The qualification for detection was based on approximate chromatographic retention as compared to the standard reference compounds and matching M^+ and $(M-1)^+$ ion abundance ratios with the representative standard compounds.

It was almost always possible to find peaks in the desired regions, because of the complexity of the sample. also, the compounds of interest coeluted with polycyclic aromatic hydrocarbons that were present in the sample in significant concentrations. Those compounds of interest, if they were present, were present in small amounts (<0.05%), such that they could be totally "buried" under large peaks of dominant compounds. Even if the separation of standard compounds was accomplished nicely under certain conditions, the same conditions would give a rather complicated picture of the sample. When peaks were found with retention times in the desired region, that region would be monitored for specific ions in the SIM mode.

For further investigation, new sample solutions were made with three different concentrations to allow monitoring of the changes in abundances of selected ions important for identification of the compounds of interest.

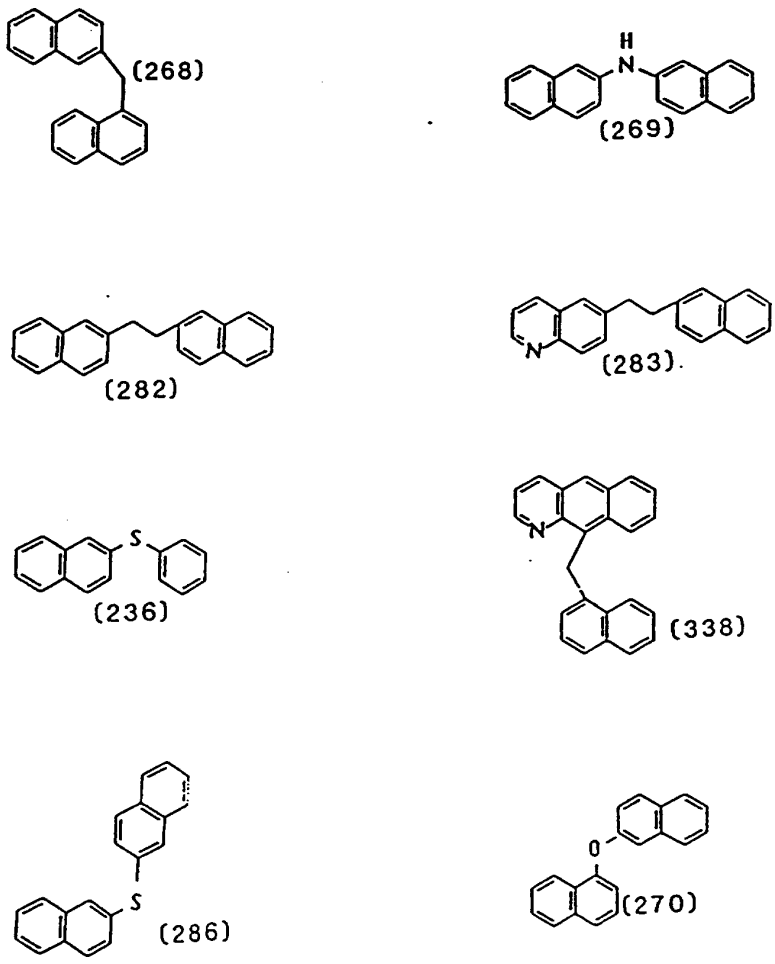

Figure 4. Representative linked compounds detected in an SRC II heavy distillate.

Different sample concentrations would enable us to distinguish more clearly those compounds from the background noise and sample background. For each compound of interest, at least three representative ions were monitored: the molecular ion, the most abundant ion if different from the molecular ion, and one more specific for the compound in question. The SIM mode of operation was used as before.

The results were disappointing because they didn't give additional information needed about the compounds of interest. As the concentration increased, baseline noise increased and signal-to-noise stayed about the same. Also, ambiguity in the ratio of M^+ and $(M-1)^+$ ions prevented drawing any conclusions regarding the existence of compounds with bridging groups in the SRC II HD.

From previous work done in our lab, where specific compounds with bridging groups were found among the major compounds in certain coals, and from results in this study, it could be concluded that the existence of compounds containing aromatic moieties with bridging groups is very probable in small concentrations (<0.05%) in the SRC II HD, but this could not be firmly proved with the results at hand.

To determine the existence of these proposed compounds further, much more specific fractionation of sample is needed. Even though preliminary work was promising, this probing led us to the conclusion that further investigation in this direction would be very time-consuming with no certainty that successful identification of bridging compounds in the SRC II HD could be accomplished.

D. Prefractionation Using Size Exclusion Chromatography and Supercritical Fluid Fractionation

The most widely used method for prefractionation of the heavy ends of energy related materials has been fractional distillation. In this work, we wanted to avoid the high temperatures required in distillation methods, and wanted to rely on a chromatographic method to give us narrow molecular weight cuts. Therefore, size exclusion chromatography (SEC) and supercritical fluid fractionation (SFF) were evaluated.

The complexity of coal-derived materials makes necessary some initial separations based on polarity, functionality, or molecular weight prior to characterization. For high-molecular-weight materials, separations based on polarity or functionality are less effective because there may be several different polar substituents in a compound, and they may be buried in the compound structure. The most useful method of effecting separations of these materials is high performance size exclusion chromatography in which molecules of different size are separated according to their degree of penetration into the pores of a gel packed in a column as small-diameter spheres. SEC can be performed on a preparative scale or on an analytical-scale, depending on whether fraction quantity or resolution is most important.

For SEC, a solvent-refined coal vacuum residue (SRC VR) was fractionated into five fractions by preparative SEC on a 60 cm x 25 mm i.d., 50 nm porosity PL gel (Polymer Laboratories, Ltd) column with tetrahydrofuran (THF) mobile phase. The molecular weight distributions of these five fractions were determined by analytical high-performance SEC. Analytical SEC was carried out on a 60 cm x 7.7 mm i.d. PL gel column with 10 μm particle size and 50 nm

porosity; THF was the mobile phase. Figure 5 shows the relative distributions of the five fractions, and Figure 6 shows the molecular weight histograms of these fractions. The molecular weight (MW) calibration was according to the retention volumes (V_R) of polystyrene standards using UV detection at 254 nm. The linear portion of the calibration curve can be described by the equation $V_R = C_1 - C_2\log(MW)$. The slope (C_1) of the polystyrene standards is 3.6×10^{-3}, and the intercept (C_2) is 4.09. While there was observed a definite size separation, there was also significant overlap and broadening of the molecular weight distribution, particularly for the higher molecular weight fractions.

For the evaluation of SFF, the instrumental set-up diagrammed in Figure 7 was used. The system included a 375-mL syringe pump (Isco, Lincoln, NE), a 250-mL syringe pump (Varian 8500, Walnut Creek, CA), each modified for pressure control at flow rates of up to 8 mL min^{-1} (liquid), a chromatographic oven (Varian Series 2100, Walnut Creek, CA), and four 125-mL stainless steel fraction collection vessels which were fitted with cooling jackets. During fraction collection, the vessels were cooled to 3 ± 2°C *via* a circulating cooling bath (Grant Science/Electronics, Dayton, OH). A six-port switching valve (Valco Instrument Co., Houston, TX) was used to effect collection of successive fractions in different collection vessels. The collection vessels were pressurized with nitrogen gas from a high pressure tank with appropriate valving. A micrometering valve (Autoclave Engineering, Erie, PA) was used to control the flow when the effluent was vented directly to atmospheric pressure. A more detailed description is given in Appendix 7.

The fractions containing the more volatile compounds were collected by either bubbling the effluent through methylene chloride or by venting the effluent through a 60-cm length of 1/16 inch i.d. Teflon tubing which was

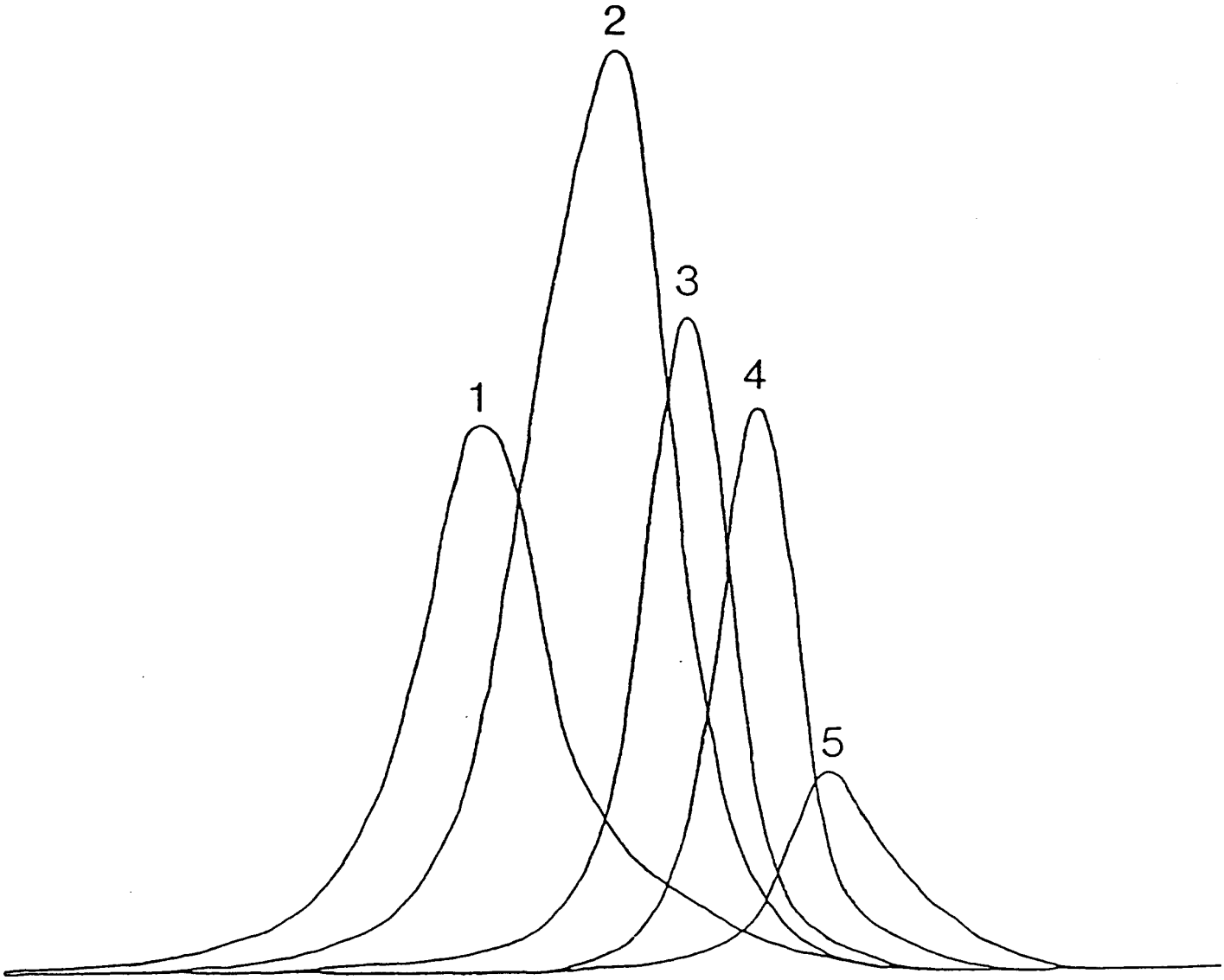

Figure 5. Relative molecular weight distributions of the SEC fractions from an SRC VR.

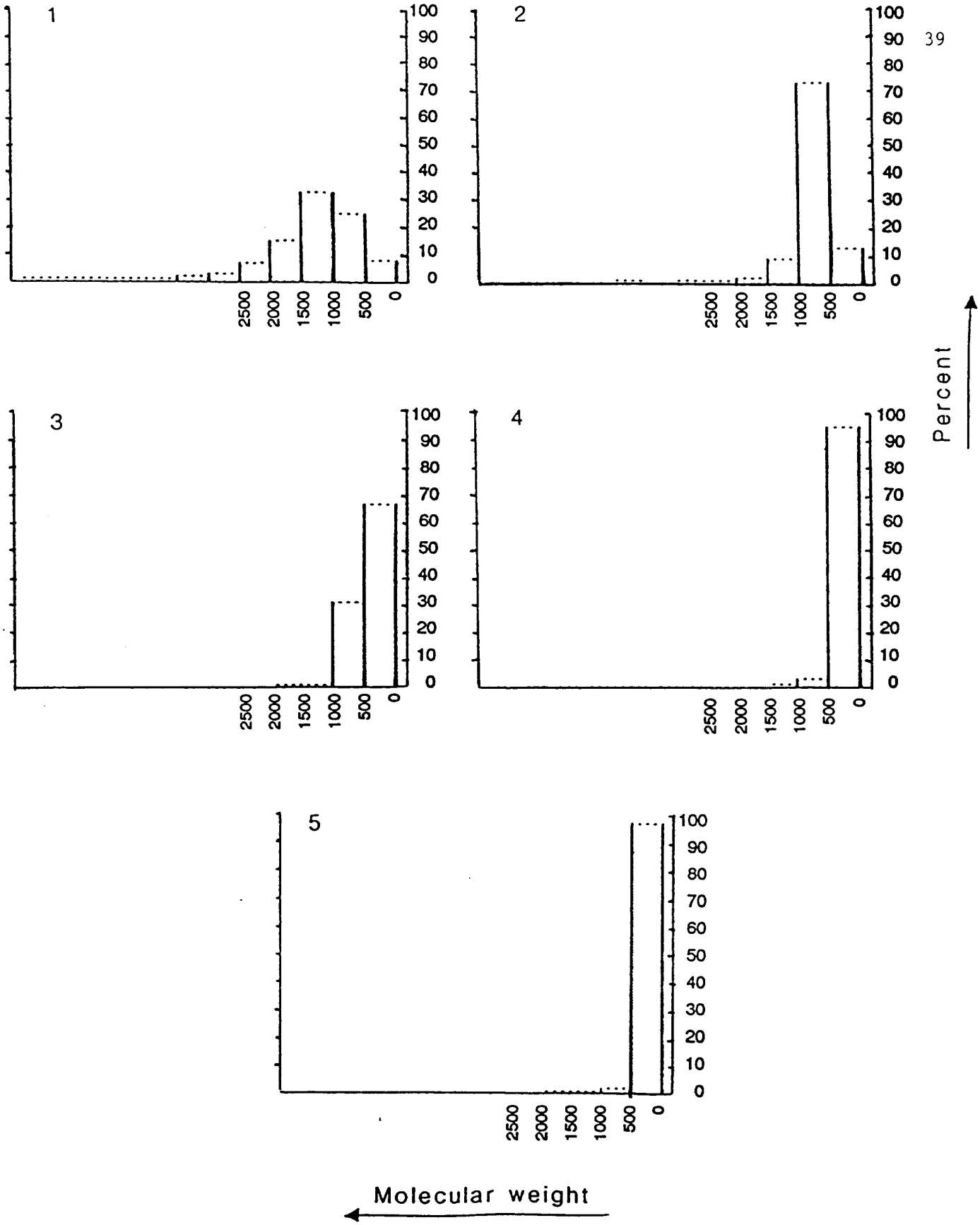

Figure 6. Molecular weight histograms of the SEC fractions from an SRC VR.

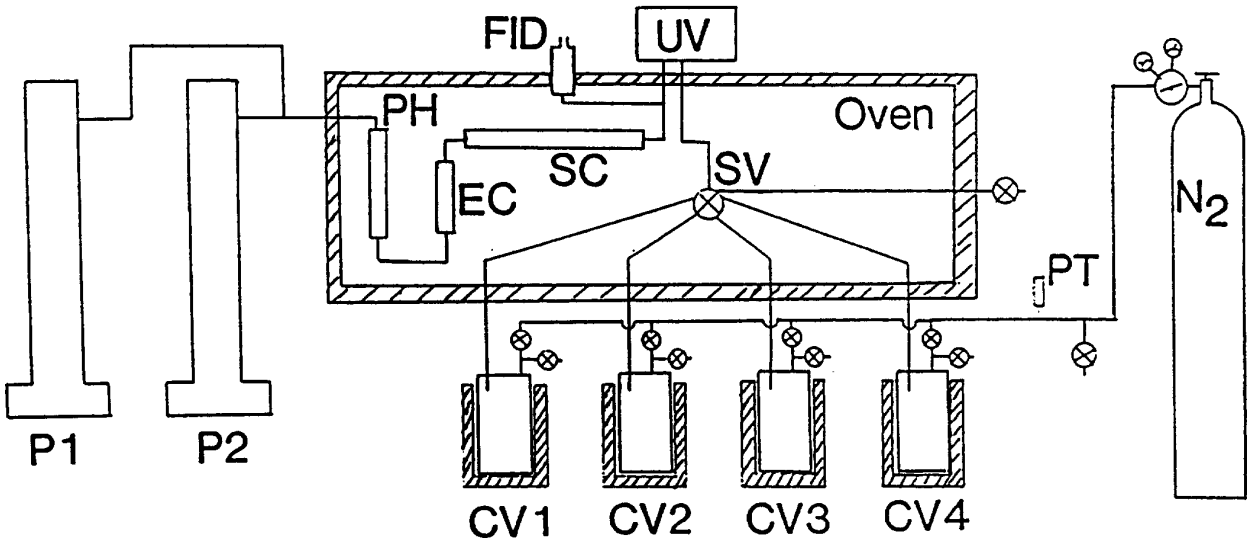

Figure 7. Schematic diagram of the supercritical fluid extraction/fractionation system. P1 and P2, syringe pumps 1 and 2; PH, solvent preheater/equilibrator; EC, extraction column; SC, separation column; SV, 6-port switching valve; FID, flame ionization detector; PT, pressure transducer; N_2, high pressure cylinder of nitrogen gas; CV1-4, pressurized fraction collection vessels equipped with cooling jackets.

immersed in a dry ice/isopropanol bath (-78°C). To recover the collected materials, the Teflon tubing was removed from the bath and rinsed with a small amount of an appropriate solvent. An extraction column (10 cm x 4.6 mm i.d.) and a separation column (25 cm x 4.6 mm i.d.) were placed in the oven, and effluents were monitored with a UV-absorbance detector (Hitachi Model 100-10, Tokyo, Japan) equipped with a high pressure flow cell (Hewlett-Packard, Avondale, PA). A small fraction of the column effluent was split into a flame ionization detector (FID) through a 15-cm length of tapered 25-μm i.d. fused silica tubing. The FID block was shortened by half and heated to 400°C with heat tape. All parts of the extraction/fractionation apparatus were constructed of stainless steel.

It was decided that a thorough study of the potential of CO_2 for the fractionation of high-molecular-weight polycyclic aromatic compounds (PAC) be undertaken. Theoretical solubility parameter data suggested that CO_2 might be a better solvent than n-pentane for high-molecular-weight PAC. There were also some indications that coal extracts and high-molecular-weight coal-derived fluids could undergo structural changes when heated. Aromatization, condensation, rearrangements, and other types of chemical reactions are expected to occur in coal mixtures at temperatures above 100°C. Therefore, a low temperature fractionation procedure would be much more desirable than a high temperature one. Both standard compounds and a carbon black extract were used to determine the upper molecular weight limit of the CO_2 fractionation system. Coronene was eluted in 8 min at 400 atm and 60°C on a 25 cm x 4.6 mm i.d. NH_2-Adsorbosil column. The only other high-molecular-weight polycyclic aromatic compound (PAC) that was available in sufficient quantity for study was ovalene. Elution of this compound could not be detected by either the UV-absorbance or flame ionization detectors at the maximum conditions of the system (400 atm and 60°C). Ovalene

is probably extracted in small amounts at these conditions and is not sufficiently soluble to give a distinct chromatographic peak.

A fractionation of a methylene chloride extract of a carbon black at 400 atm and 60°C with the UV-absorbance detector set at 300 nm was attempted. A clear ring-number fractionation occurred up to coronene, but after this, no compounds were detected. When this fractionation was repeated at 340 nm, the same result was obtained.

While a better separation was obtained using SFF than SEC, the technique could not be applied to the high molecular weight materials present in the heavy ends when CO_2 was used. It was decided to concentrate our efforts on improving the SEC separation instead of evaluating additional supercritical fluids.

E. Evaluation of SFC Mobile and Stationary Phases for Coal Molecules

Unfortunately, typical chromatographic methods, including supercritical fluid chromatography (SFC) with CO_2 mobile phase, have been inadequate for the separation and identification of the heavy end constituents. To this end, we have initiated a study to evaluate the addition of organic modifiers (entrainers) to CO_2 in SFC to improve chromatographic efficiency and to enhance the solubility of large polycyclic aromatic compounds (PAC) in the mobile phase. Additionally, a smectic biphenylcarboxylate ester liquid crystalline polysiloxane stationary phase has been studied to gain selectivity.

Initially, a comparison of the solubility of PAC in CO_2 and *n*-pentane, the two most common SFC mobile phases studied for coal-derived materials, under identical conditions was made using a 100% methylpolysiloxane column. The results are presented in Table 1. As expected, a dramatic decrease in capacity factor (increase in solubility), k', occurred with increasing density of

Table 1. Comparison of the Solubility of Selected PAC in Carbon Dioxide and *n*-Pentane at 210°C.

Solute	Carbon Dioxide		n-Pentane	
	k' at 0.182 g mL^{-1}	k'at 0.396 g mL^{-1}	k' at 0.182 g mL^{-1}	k' at 0.396 g mL^{-1}
9,9-Bianthryl	10.64	0.34	1.59	0.11
Coronene	16.36	0.66	1.62	0.11
Ovalene	>50	>50	5.49	0.28
Decacyclene	>50	>50	7.31	0.66

n-pentane, rather than CO_2, as the mobile phase. CO_2 was unable to elute PAC larger than coronene (MW = 300) due to their insolubilities in CO_2. Although supercritical *n*-pentane shows greater solvating power toward PAC, its high critical temperature (T_c = 196°C), as well as safety considerations, preclude it as a desirable mobile phase for SFC.

A device, shown schematically in Figure 8, has been constructed to produce homogeneous CO_2 + modifier mixtures of known composition. The mixing system consists of a commercially available sample cylinder, appropriate valves and fittings, and a CO_2 cylinder with eductor tube. All system components were constructed of 316 stainless steel. To prepare mixtures, liquid organic modifier is first injected by syringe into the evacuated mixing vessel. CO_2 is then metered into the vessel, the contents are thoroughly mixed, and then transferred to the SFC syringe pump. The mixture composition is determined by measuring the mass of each component as it is added.

The effects of three solvents (*i*-propanol (IPA), acetonitrile (ACN), and methylene chloride (MCl)), as CO_2 mobile phase modifiers with varying concentration, on chromatographic separations were investigated. The range of modifier mole percent studied was from 0 to 9%, and the capillary SFC system used a deactivated, nonpolar 100% methylpolysiloxane column and UV-absorbance detector. Critical parameters of the mixed mobile phase were estimated from mixing rules associated with a frequently cited equation of state. Test solutes included polar and nonpolar PAC.

The addition of any of the modifiers, under conditions of constant temperature and pressure (100°C and 150 atm), decreased the retention of all solute types as the modifier mole fraction (X) increased. At the highest modifier concentration studied (X = 8.9% IPA), the two largest PAC, picene and

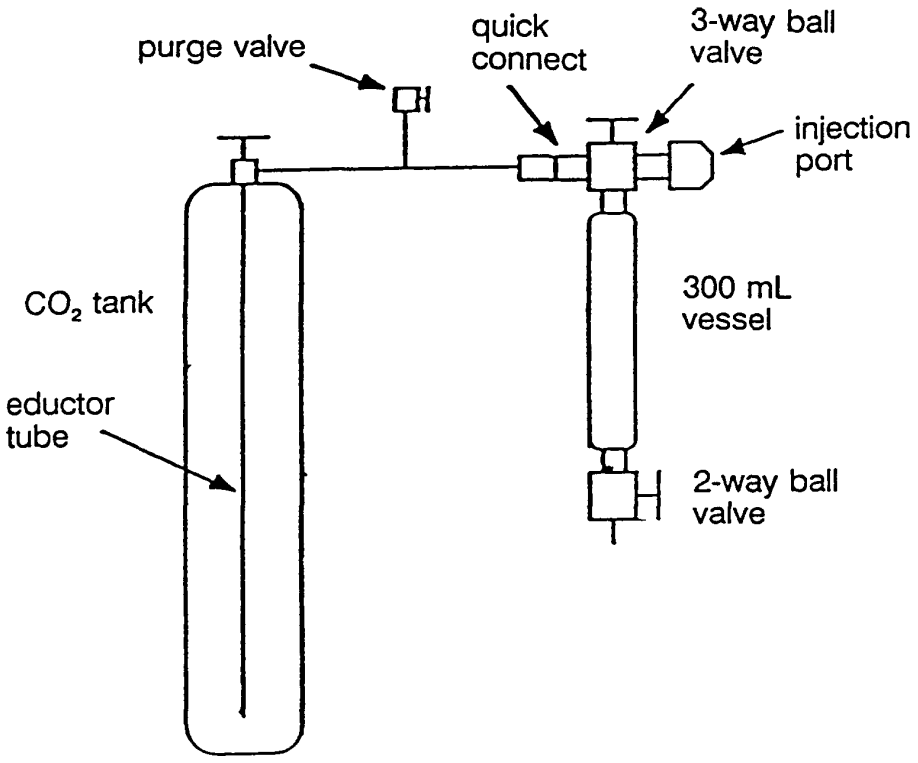

Figure 8. Schematic diagram of fluid mixing device.

coronene, exhibited an order of magnitude decrease in retention relative to pure CO_2 mobile phase (Figure 9). The other test solutes also showed dramatic differences in retention. Pyrene, chrysene, and polar pyrene derivatives decreased by about 80%, while phenanthrene and polar naphthalene derivatives decreased by 60 to 70%. The elution order of all solutes was constant with all modifiers over all concentrations studied, except for phenanthrene and naphthalene acetamide with IPA or MCl modifier (Figure 10). However, at high X the initial elution order was restored for IPA.

Differences were observed between the modifiers in their ability to increase the solvating power of the CO_2 mobile phase. In general, for all solutes, the magnitudes of decreases in k' with modifier was in the order IPA > ACN > MCl (Figures 9-11). This is a very interesting result considering that ACN is a much more polar liquid solvent than IPA.

A mixture of 8.9 mole% IPA in CO_2 was used to study the elution behavior of large PAC in SFC with mixed mobile phases. As previously discussed, pure CO_2 is capable of eluting compounds only up to coronene. In the experiments with CO_2 + IPA, ovalene, decacyclene, and rubrene (MW = 398, 450, and 533, respectively) eluted at constant conditions of 120°C and 205 atm. (Figure 12). Rubrene eluted before either of the planar PAC, with significantly higher efficiency. Use of a higher temperature and density programming would produce better peak shapes for the planar PAC.

Using the above results as guidelines, the evaluation of CO_2 modifiers was undertaken. Initially, a wide range of organic solvents (propylene carbonate, dimethyl formamide, dimethyl sulfoxide, *o*-dichlorobenzene, toluene, and pyridine) with different physicochemical properties were chosen as modifiers. The physical properties of these solvents are listed in Table 2. Various chromatographic

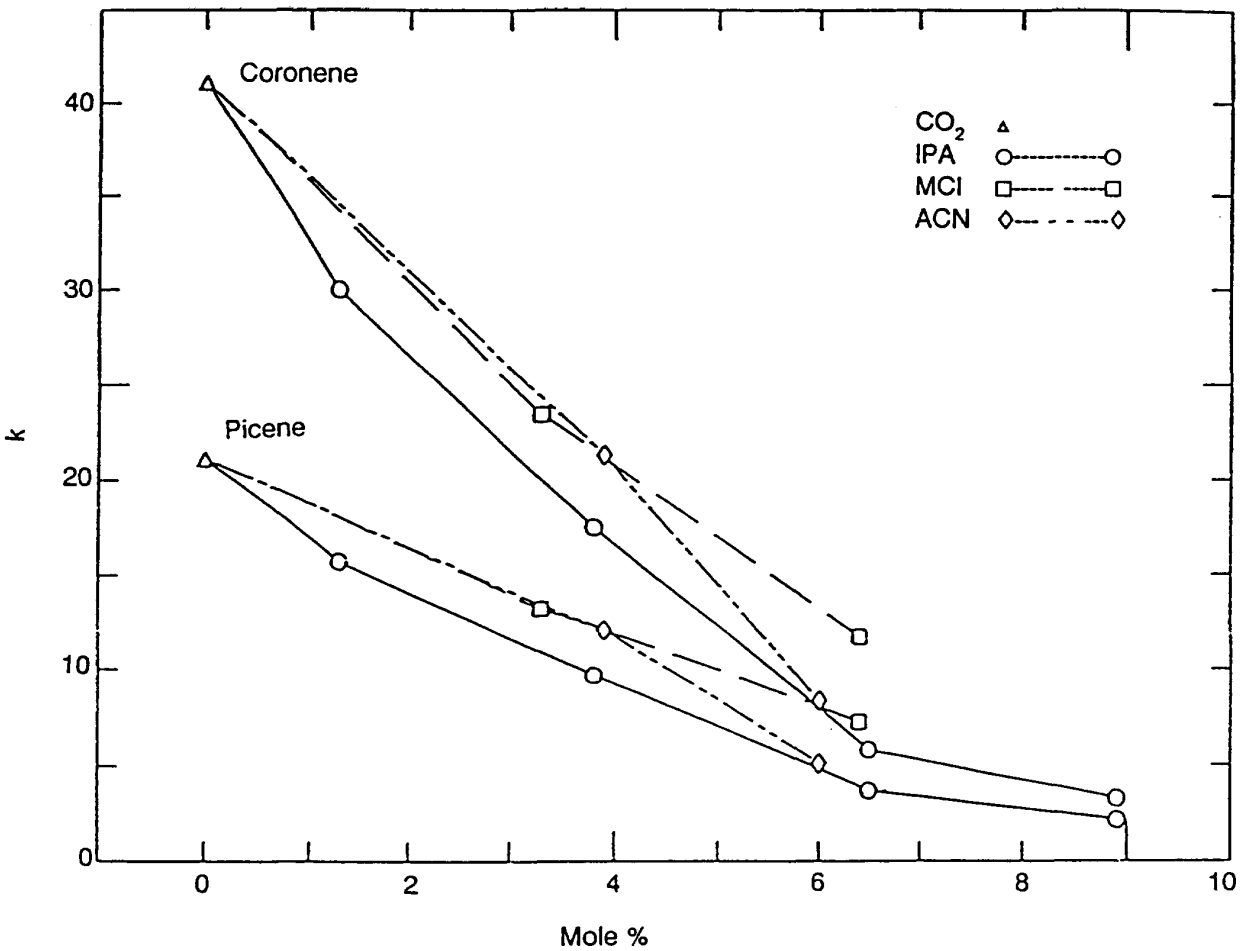

Figure 9. Retention of picene and coronene as a function of modifier mole fraction.

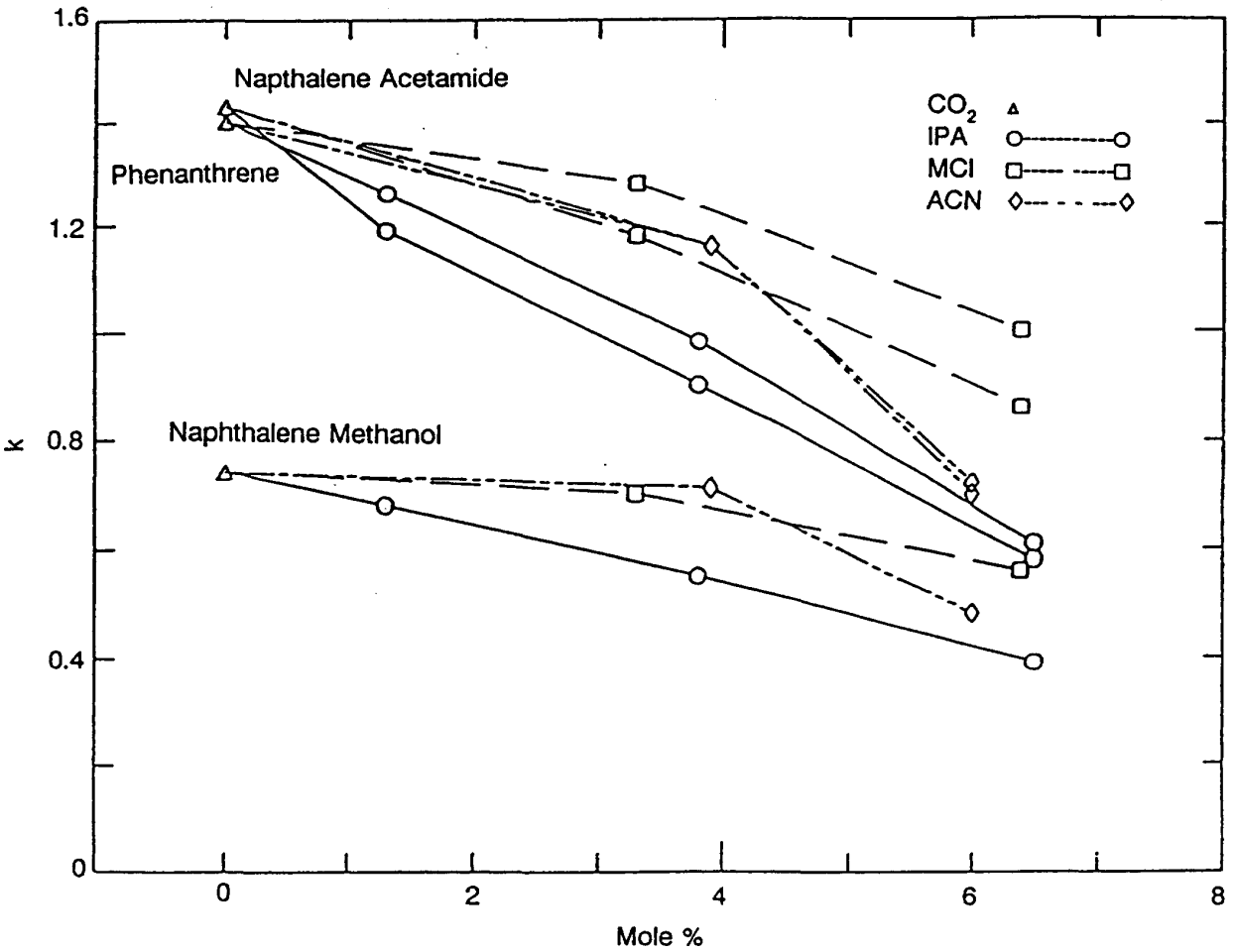

Figure 10. Retention of phenanthrene, naphthalene acetamide, and naphthalene methanol as a function of modifier mole fraction.

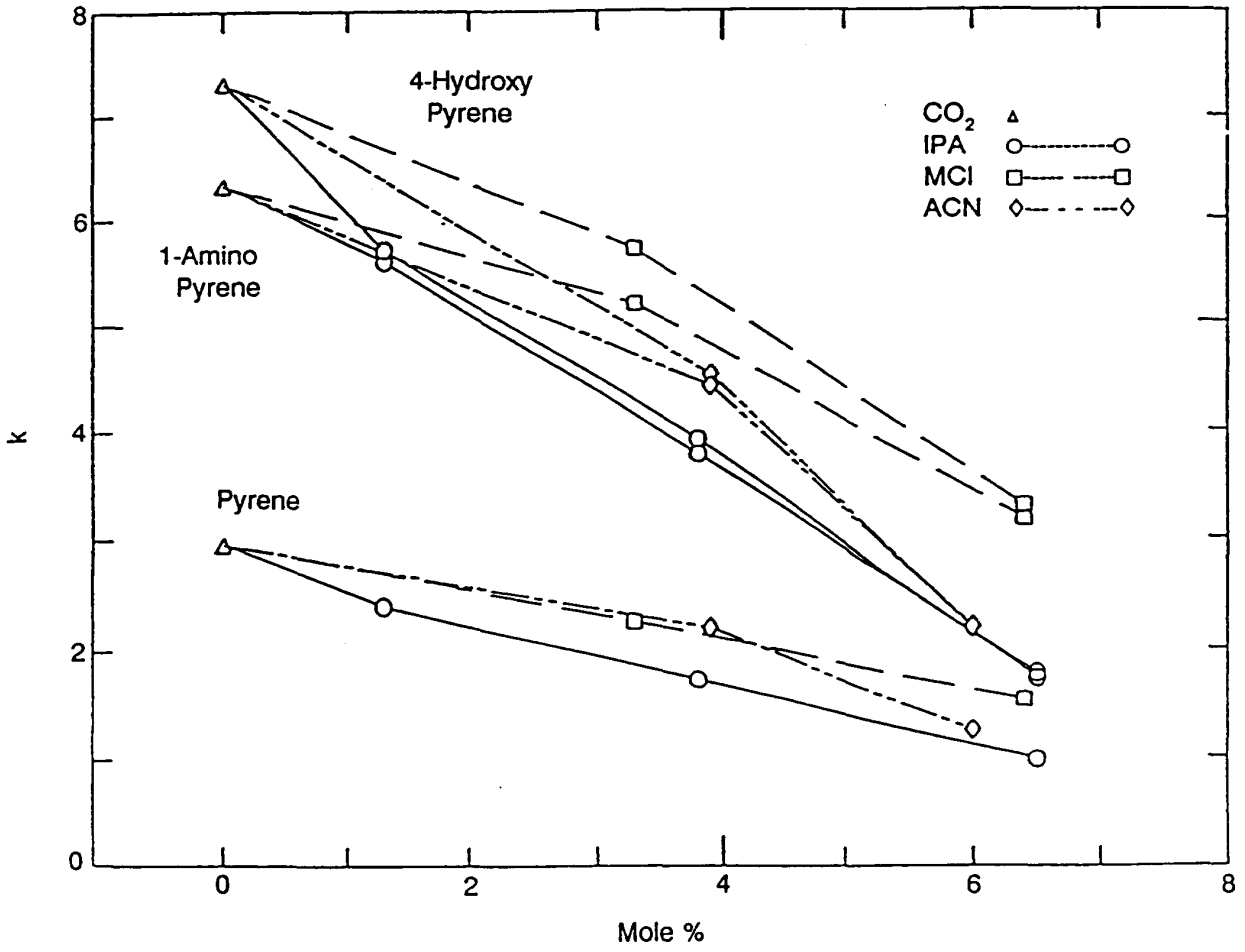

Figure 11. Retention of pyrene, 4-hydroxypyrene, and 1-aminopyrene as a function of modifier mole fraction.

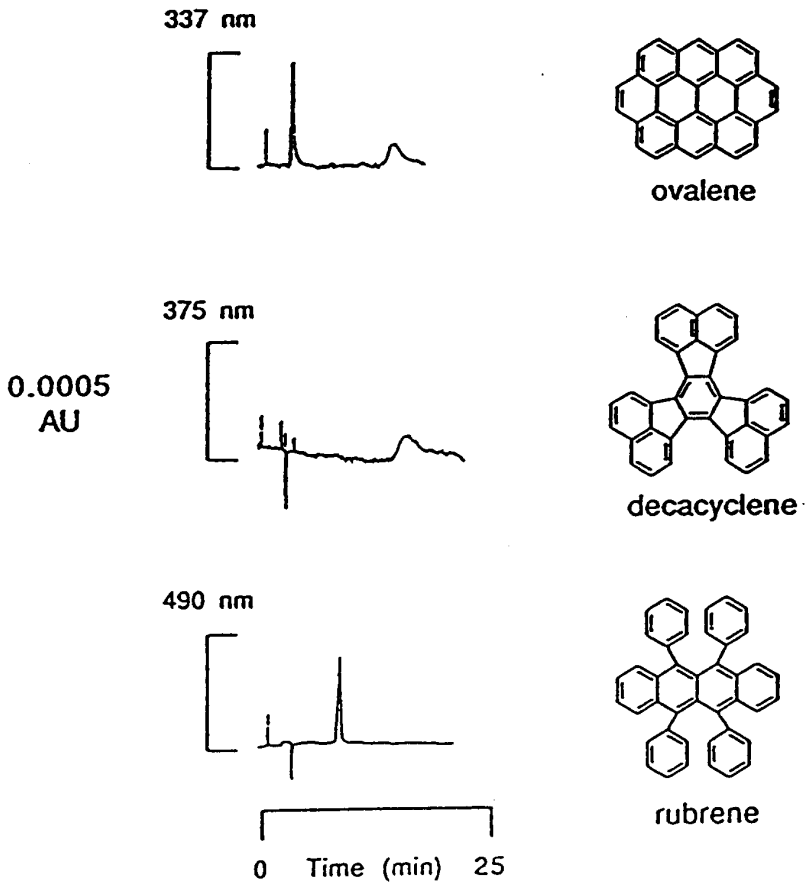

Figure 12. SFC chromatograms of large PAC with an 8.9 mole percent IPA/CO_2 mobile phase. Conditions: 205 atm, 120°C.

Table 2. Physical Properties of SFC Modifiers

	o-Dichlorobenzene	Dimethyl formamide	Dimethyl sulfoxide	Propylene carbonate	Pyridine	Toluene
Molecular Weight	147.0	73.10	78.13	102.09	79.10	92.14
B.P., °C	180.48	153.0	189.0	241.7	115.25	110.62
T_c, °C	424.15	374.0	459.0	504.8	346.85	318.65
P_c, atm	40.5	44.2	57.2	53.4	55.6	41.0
V_c, mL mole^{-1}	360	265	212	251	254	316
Acentric Factor	0.272	0.360	0.293	0.449	0.24	0.263
Dipole Moment, debye	2.27 @24°C	3.86 @25°C	3.9 @25°C	---	2.37 @25°C	0.31 @20°C
Solvent Group	7	3	3	6	3	7
Polarity Index	~ 2.7	6.4	7.2	6.1	5.3	2.4
Liquid Density, g mL^{-1} @ 20°C	1.3058	0.9487	1.0958 @25°C	1.189	0.9832	0.8669
Dielectric Constant	9.93 @25°C	36.71 @25°C	46.68 @20°C	69.0 @23°C	12.4 @20°C	2.38 25°C

measurements reduced this number to two, propylene carbonate and dimethyl formamide.

Chromatographic evaluations of propylene carbonate and dimethyl formamide (DMF) as CO_2 modifiers indicated that both of these modifier mixtures dramatically enhance the solubility of PAC in CO_2. Coronene and ovalene test solutes were separated at 150°C on a 100% methylpolysiloxane column using each of these modifier mixtures and by pressure-programming from 100 atm at 3 atm min^{-1}. Under these conditions, a 6.0 mole % propylene carbonate in CO_2 mixture eluted coronene at 111 atm and ovalene at 120 atm. With 9.0 mole % DMF in CO_2, coronene eluted at 117 atm and ovalene at 120 atm. However, the DMF mixture began to remove the protective polyimide coating of the fused silica column that was exposed to the mobile phase. No loss in chromatographic efficiency was observed with either modifier mixture.

To evaluate the selectivity of liquid crystalline stationary phases for large molecules, six high molecular weight (MW = 323) isomeric naphthylcarbazole ethanes, which cannot be eluted by GC, were separated on three different stationary phases in SFC. Figures 13 and 14 show chromatograms of these six isomers on *n*-octyl and polyethyl ether substituted polysiloxane stationary phases, respectively. Figures 15 and 16 illustrate the efficacy of the liquid crystalline stationary phase for the selective separation of high molecular weight heterocyclic linked compounds related to those possible in coal-derived heavy ends.

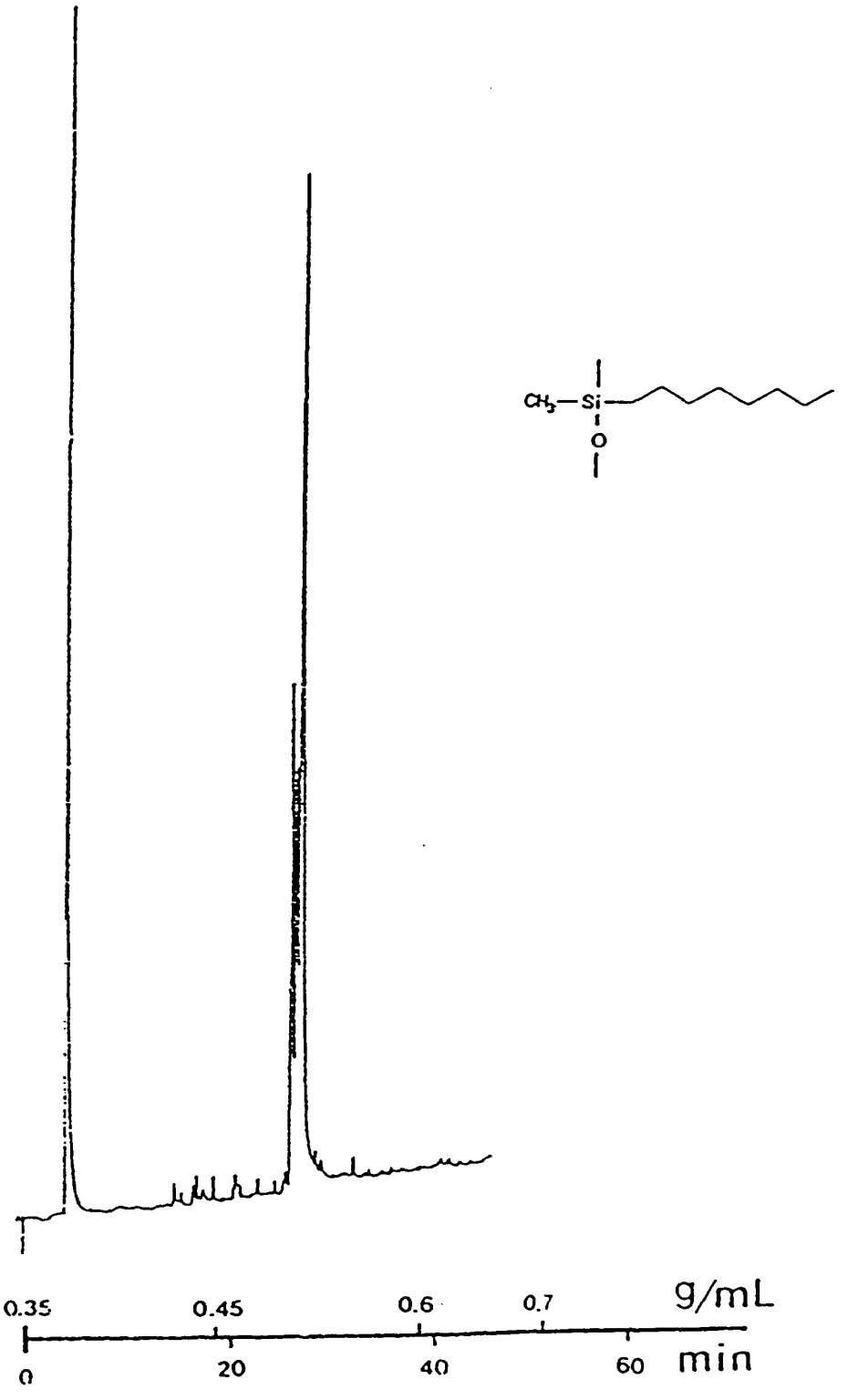

Figure 13. Supercritical fluid chromatogram of six naphthylcarbazole isomers on an *n*-octyl stationary phase and CO_2 mobile phase at 125°C. Density programmed from 0.35 g/mL to 0.7 g/mL at 0.0075 g/mL min^{-1} after a 5 min isoconfertic period.

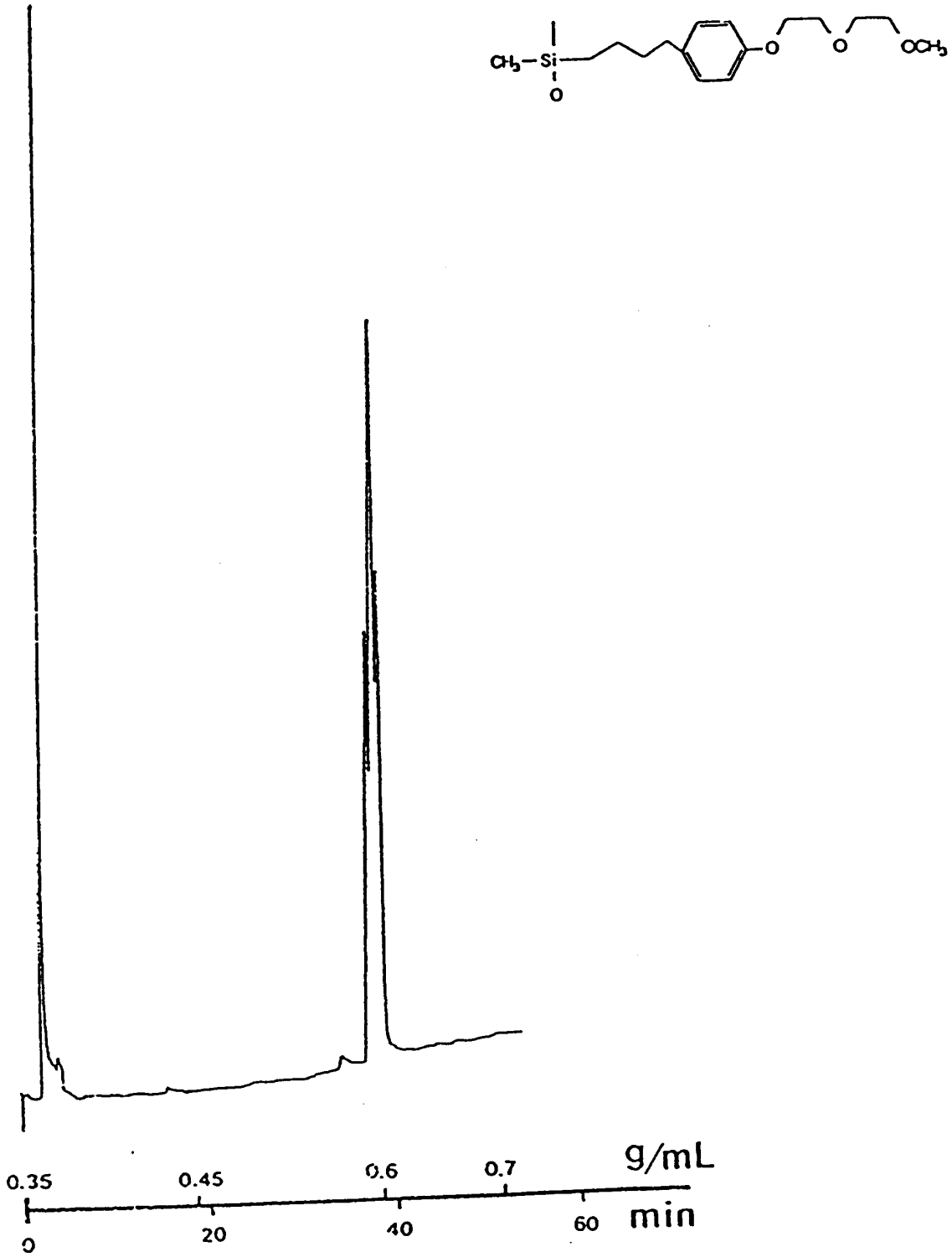

Figure 14. Supercritical fluid chromatogram of six naphthylcarbazole isomers on a polyethylether stationary phase. Conditions are given in Figure 13.

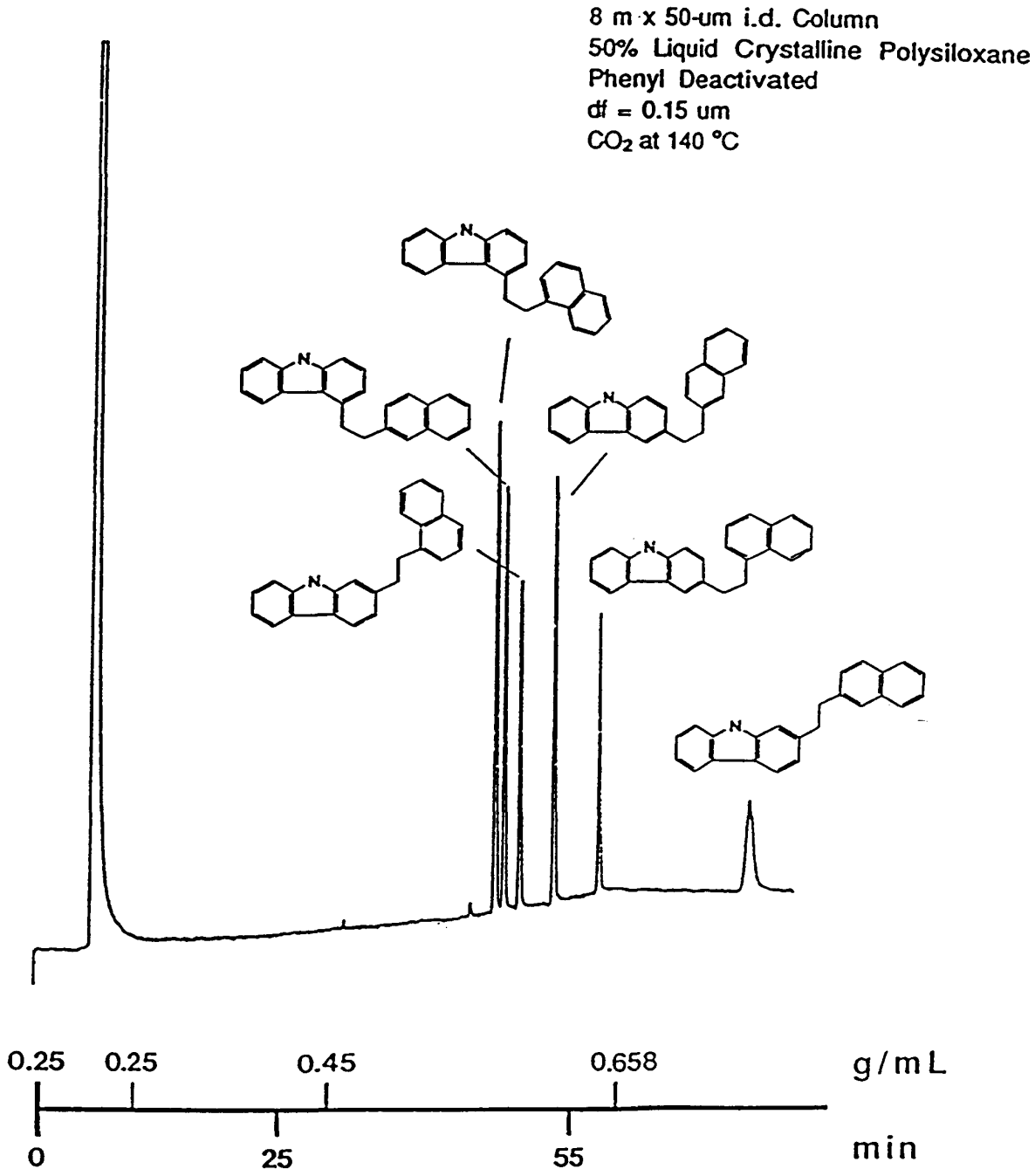

Figure 15. Supercritical fluid chromatogram of six naphthylcarbazole isomers on a liquid crystalline stationary phase and CO_2 mobile phase at 140°C. Density programmed from 0.25 g/mL to 0.658 g/mL at 0.008 g/mL min^{-1} after a 10-min isoconfertic period.

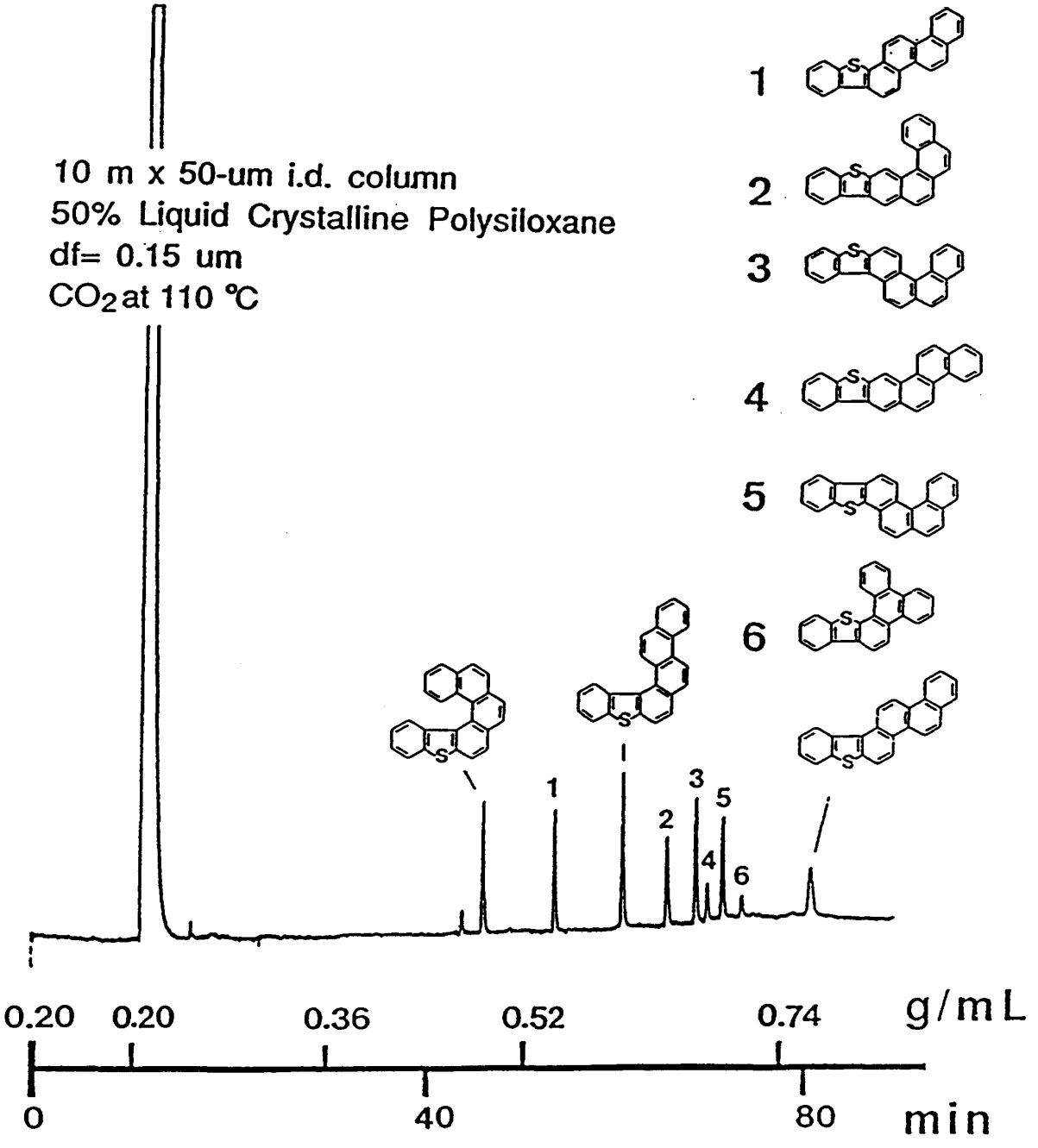

Figure 16. Supercritical fluid chromatogram of six-ring S-PAC isomers on a liquid crystalline stationary phase and CO_2 mobile phase at 110°C. Density programmed from 0.20 g/mL to 0.74 g/mL at 0.008 g/mL min^{-1} after a 10-min isoconfertic period.

F. Development of an Interface Between a Double Focussing Mass Spectrometer and a Supercritical Fluid Chromatograph

The majority of studies to date on capillary SFC/MS have involved quadrupole systems, mainly because of simplicity, low cost, and capacity for rapid scanning. However, a sector MS can provide high resolving power and expanded mass range, which are areas in which quadrupole systems are more limited. We recently reported the direct coupling of capillary SFC to a double focussing mass spectrometry (HRMS) (see Appendix 8), in which the direct insertion probe, originally designed for the direct analysis of solid samples, was used to assist vaporization of the SFC effluent from the frit restrictor without modification of the MS source chamber or the pumping system. This section describes the construction and operation of a direct heated probe for use with a frit restrictor for sample transfer and depressurization. Both electron impact (EI) and chemical ionization (CI) results are reported.

Details of the capillary SFC and HRMS equipment used in this study are described in detail in Appendices 8-10. A Finnigan-MAT Model 8430 double focusing mass spectrometer (San Jose, CA) in which a reverse Nier-Johnson geometry design is used, was coupled to a Lee Scientific Model 501 supercritical fluid chromatograph (Salt Lake City, UT). SFC grade CO_2 (Scott Specialty Gases, Plumsteadville, PA) was delivered by the SFC pump. A schematic diagram of the new direct heated probe developed in this study is shown in Figure 17.

The stainless-steel probe body was designed and built in-house. The probe body consists of an access assembly welded to a 9" long stainless steel tube. The access assembly was fabricated from 2 3/4" o.d. round stainless steel bar stock. This was hollowed out and machined to provide a knife-edge seal with a 2 3/4" o.d. high vacuum, high voltage feed-through (Insulator Seal Inc., Hayward, CA).

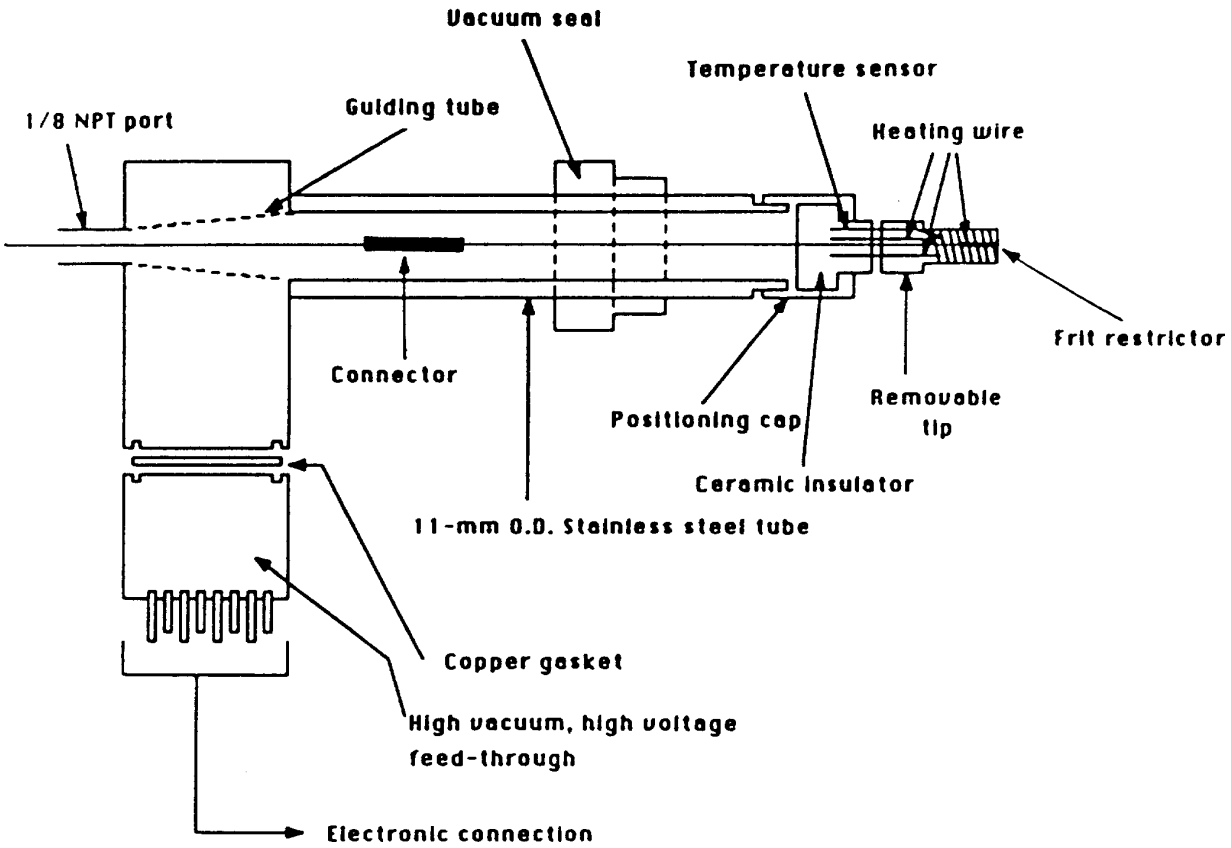

Figure 17. Schematic diagram (not to scale) of the direct heated probe SFC/HRMS interface.

Opposite from the feed-through, the access assembly was machined to accept a 7/16" o.d. stainless steel tube and a 1/8" NPT fitting. At the 1/8" NPT fitting port, a Swagelock fitting was attached to provide Swagelock connection to the Finnigan-MAT flexible transfer line. A stock 7/16" o.d. x 9" long stainless steel tube was machined to an outer diameter of 11 mm, and its surface was polished to provide a vacuum seal using a stock Finnigan-MAT gasket assembly. This tubing was then welded to the access assembly at the 7/16" port. The electrical wires for supplying current for heating at the tip were placed directly inside the stainless steel body and run through the high vacuum, high voltage feed-through. These wires were connected to the existing direct insertion probe interface heater outlet for accurate temperature control. The tip was made from brass and was wrapped with heating wire. A 2-mm i.d. hole was drilled through the center of the tip body in order to feed the frit restrictor.

A well-deactivated 6 m x 50 μm i.d. fused silica capillary column (Polymicro Technologies, Phoenix, AZ) coated with a crosslinked 0.25-μm film of 25% biphenyl polysiloxane stationary phase was used throughout this study. Standard compounds were obtained from various sources: solanesol was obtained from R J Reynolds Tobacco Co. (Winston-Salem, NC), valinomycin was purchased from Sigma Chemical Co. (St. Louis, MO), and benzylpenicillin-1'-ethoxycarbonyloxy-ethyl ester and raclopride were obtained from Astra Lakemedel AB (Södertälje, Sweden). These samples were dissolved in methylene chloride, ethanol, or benzene and injected into the SFC using a split ratio of 20:1. Experimental conditions, both for the SFC and the HRMS, differed according to the requirements of the different samples. These are cited where appropriate in the text.

It is well known that the cooling effect which results from the expansion of the supercritical fluid from the outlet of the SFC into the MS ion source

chamber, is one of the most important factors affecting SFC/MS performance. Heat is required at the restrictor to prevent precipitation and to assist in the vaporization of the solute. Moderate heating of the fluid just prior to expansion and heating of the restrictor itself has been shown to be important in improving the detection limits for nonvolatile compounds. The direct heated probe interface developed in this study produced a heated region of 1 cm in length at the probe tip, which is about the same length as the frit restrictor which is positioned in the probe tip.

The preservation of the environment in which the ionization of gas phase molecules takes place is the major requirement for good MS performance. The interface designed for coupling the chromatograph to the MS should not only maintain the integrity of the chromatographic separation, but should also meet the unique requirements of the MS ion source, and the proper transfer of the solute from the chromatograph to the MS. The influence of temperature in the fluid expansion area on ion source pressure has been discussed previously. Using the direct heated probe interface with a tip temperature of 350°C, a vacuum of 10^{-6}-10^{-5} torr was achieved during a typical density programmed SFC run with a 50 μm i.d. capillary column and a mobile phase linear velocity of approximately 1 cm/sec. No additional ion source pumping was necessary.

In this new interface design, heat is also applied to the column just prior to the restrictor. This has also assisted in the detection of solutes when a flame ionization detector was used. Since changes in fluid density result in changes in fluid solvating power, and fluid density is mainly controlled by pressure and temperature, the heat applied to the frit restrictor should not exceed the necessary temperature required for compensating for the cooling

effect. In this study, a tip temperature of 300°C to 350°C was found to be optimum.

The performance of the SFC/MS interface was evaluated using several compounds that possessed one or more of the properties of low volatility, moderate to high polarity, and thermal lability. None of the compounds were derivatized before analysis.

Solanesol (see Figure 18 for the structure) is a polyisoprenoid alcohol. Recently, solanesol has been suggested as a stable tracer in environmental tobacco smoke. Analytical methods that have been developed for the determination of solanesol include analysis by GC of its trimethylsilyl derivative, reversed phase LC, and magic angle spinning NMR with the nuclear overhauser enhancement (NOE) technique. The mass spectrum of 10 ng of underivatized standard solanesol obtained using SFC/HRMS is shown in Figure 19. The molecular ion was detected (m/z=631) as well as the typical fragment of $[M-18]^+$ (m/z=613), which is normally seen in mass spectra of alcohols. The fragment ions at m/z=543, 475, 407, 339, 271, 203, and 135, clearly indicate the stepwise loss of a repeating unit of 68 amu in the solanesol structure.

Figure 20 shows the EI spectrum (10 ng) of raclopride (see Figure 18 for structure) obtained from a density programmed SFC/HRMS run. The molecular weight (m/z = 346) as well as rich structural information was obtained from this analysis. The most intense peak is at m/z=98; and with the appearance of the m/z=248 peak, it is suggested that cleavage of the C-C bond, which generates the ethylpyrrolidinyl ion, is dominant. The fragile fragment (m/z=248) is further broken down to form a more stable ion of m/z=219.

The total ion chromatogram and EI spectrum of benzylpenicillin-1'-ethoxycarbonyloxyethyl ester (10 ng) from a typical SFC/HRMS run are given in

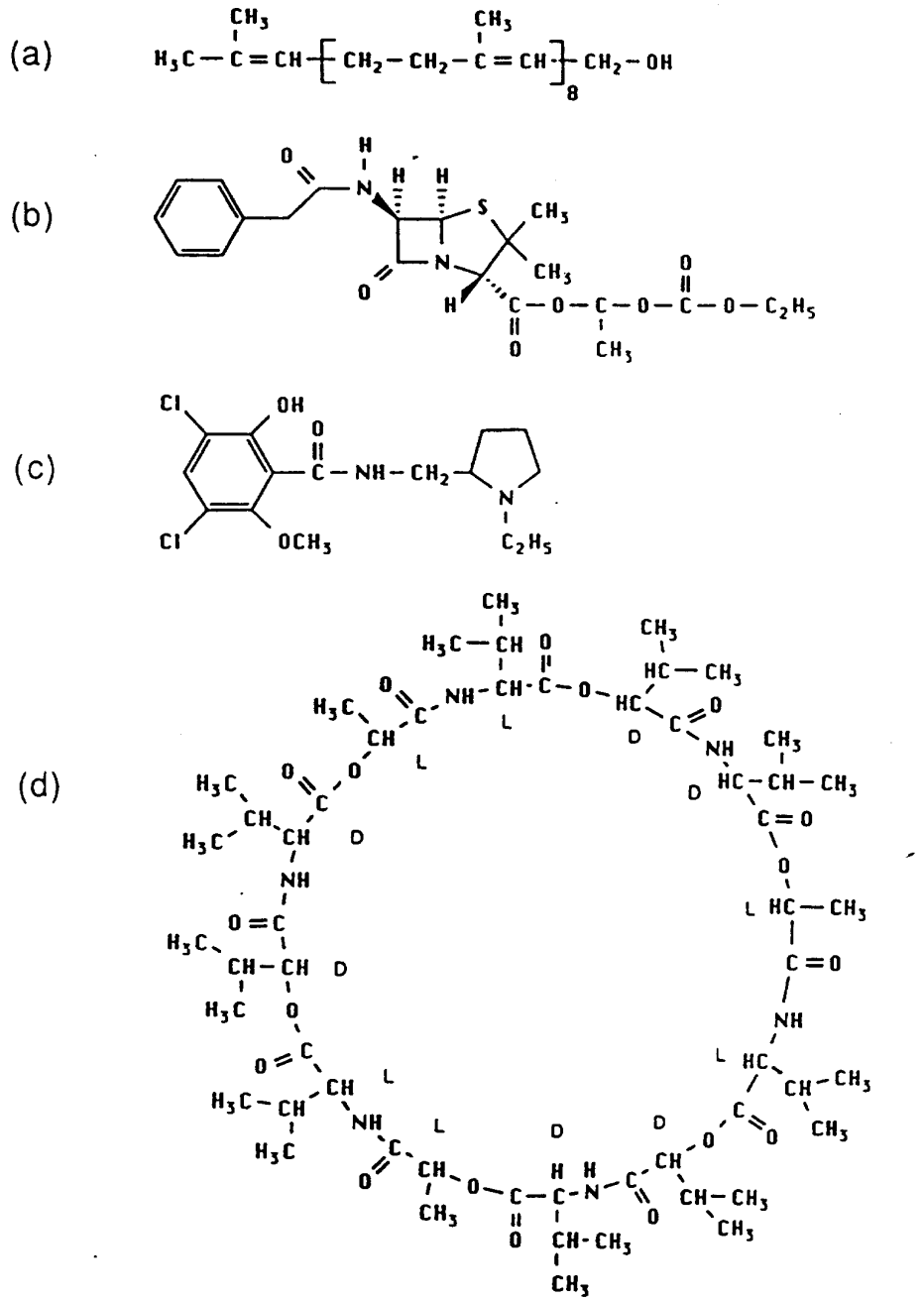

Figure 18. Chemical structures of (a) solanesol, (b) raclopride, (c) benzylpenicillin-1'-ethoxycarbonyloxyethyl ester, and (d) valinomycin.

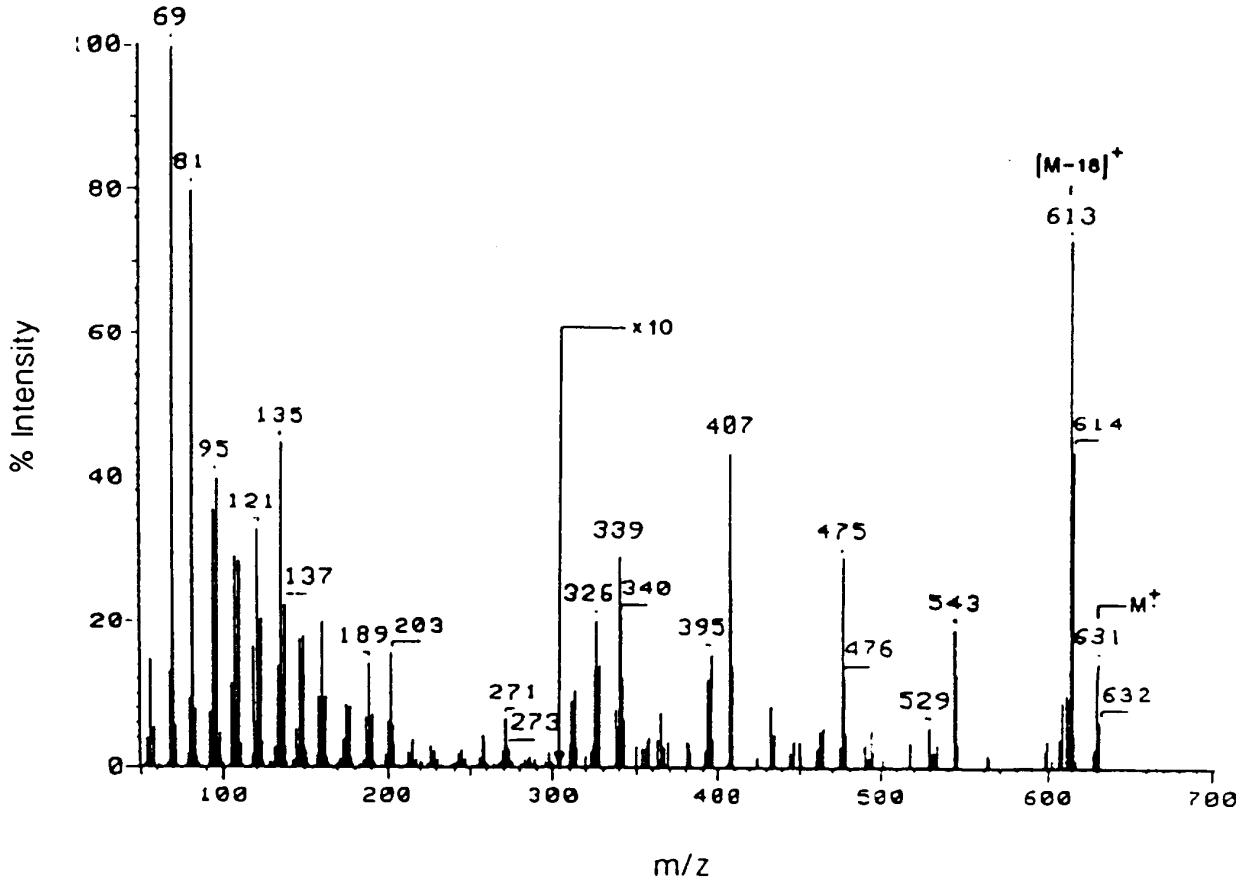

Figure 19. EI spectrum of solanesol (10 ng) obtained from SFC/HRMS run. SFC conditions: 100°C, linear density program from 0.25 g/mL to 0.7 g/mL at 0.009 g/mL/min after an initial 5-min isoconfertic period. MS conditions: 320°C interface probe tip temperature, 250°C source temperature, scan from 50 to 700 amu at 0.5 sec/decade.

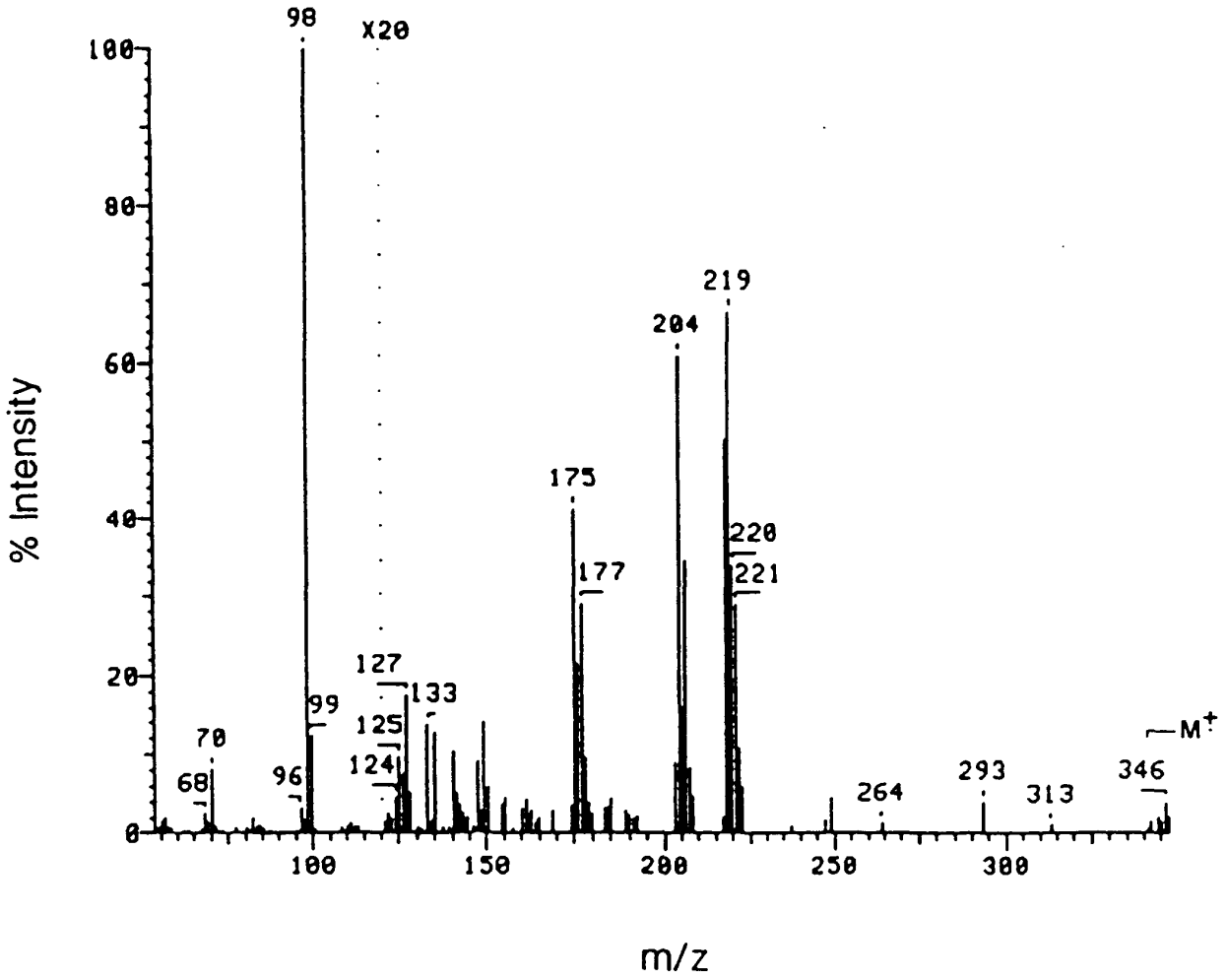

Figure 20. EI spectrum of raclopride (10 ng) obtained from SFC/HRMS. SFC conditions: 80°C, linear density program from 0.2 g/mL to 0.7 g/mL at 0.015 g/mL/min after an initial 10-min isoconfertic period. MS conditions: same as described in Figure 19, except scan from 40 to 350 amu.

Figure 21 and 22, respectively. The total-ion-chromatogram indicates the excellent sensitivity (signal-to-noise) obtained in the scanning mode for only 10 ng injected. In the mass spectrum, structural information as well as the molecular weight (m/z=450) are obtained. Previously, LC/MS using thermospray or direct liquid introduction was the only technique for definitive analysis of such compounds. However, it is difficult to obtain EI-type spectra from LC/MS, regardless of the type of interface used. This limits the structural information available in the spectra. In contrast, EI spectra at low nanogram levels using SFC/HRMS is easily obtainable.

In many cases, higher sensitivity or more simplified spectra can be obtained by carefully selecting a reagent gas for chemical ionization or by using more advanced MS techniques, such as tandem MS. Figure 23 shows the spectrum resulting from negative ion chemical ionization (NICI) SFC/HRMS of valinomycin (10 ng) with methane as a reagent gas. The molecular ion (m/z=1112) is the most intense ion in the spectrum. Typical methane CI behavior is also observed by the presence of ions at m/z=1127 and 1097, which represent $[M+15]^-$ and $[M-15]^-$ respectively.

The direct heated probe SFC/HRMS interface adequately handles the typical flow rates generated from capillary SFC systems, and in most cases, the chromatographic integrity is preserved. However, in the analysis of valinomycin, a relatively low ionization efficiency and poor total ion current peak shape were observed. It is believed that this resulted from insufficient heat transfer to the restrictor, which resulted in poor vaporization of this large molecule. By reducing the dimensions of the tip i.d. to provide more intimate contact could improve heat transfer and eliminate this problem. This and other alternatives are presently under investigation.

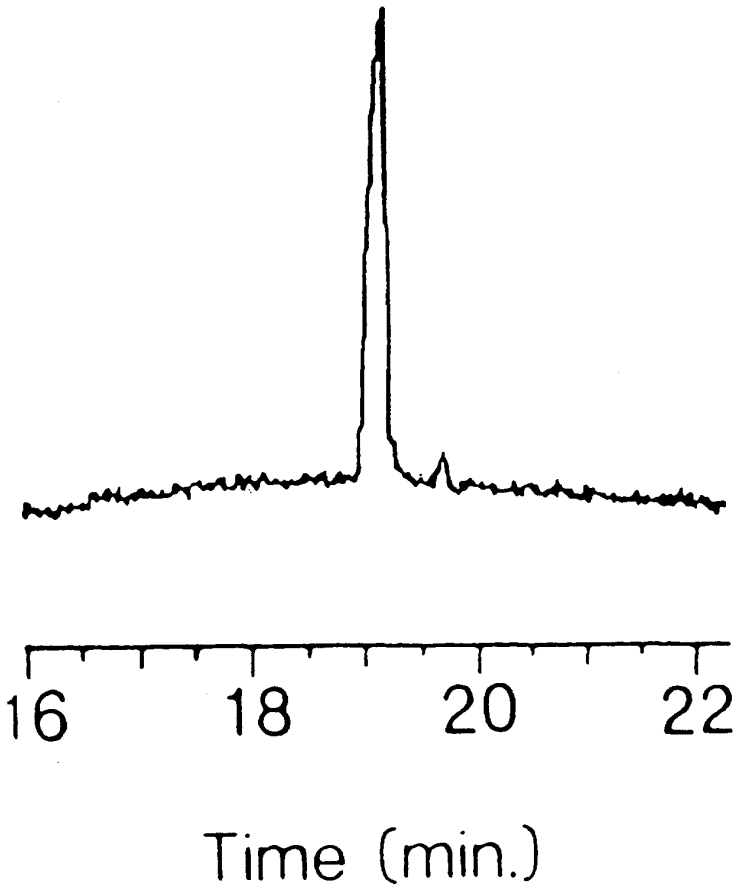

Figure 21. Total ion chromatogram (EI) of benzylpenicillin-1'-ethoxycarbonyloxyethyl ester (10 ng) obtained from SFC/HRMS. SFC conditions: 80°C, linear density program from 0.15 g/mL to 0.7 g/mL at 0.015 g/mL/min after an initial 5-min isoconfertic period. MS conditions: same as described in Figure 19, except scan from 30 to 500 amu.

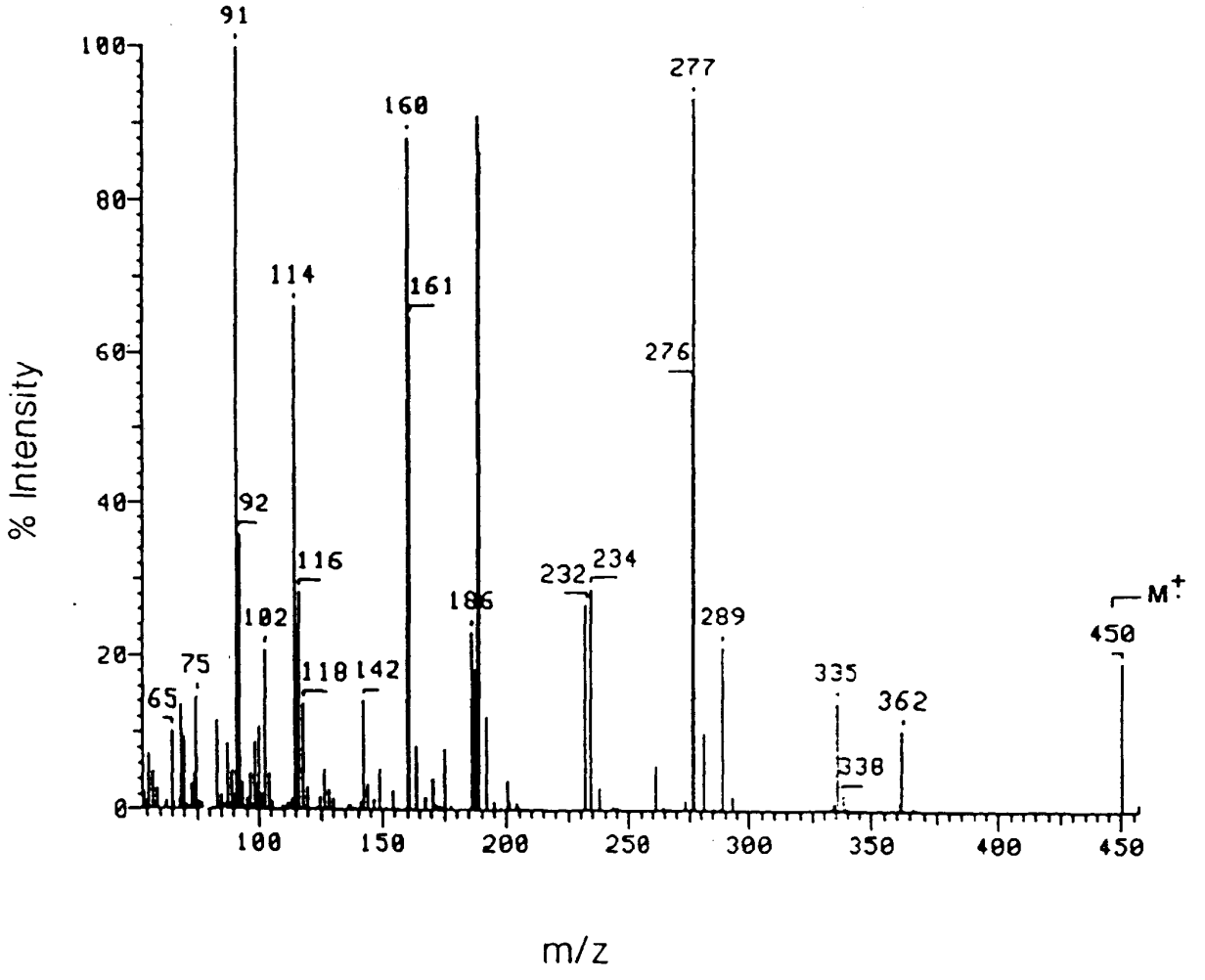

Figure 22. EI spectrum of benzylpenicillin-1'-ethoxycarbonyloxyethyl ester. Conditions: Same as described in Figure 21.

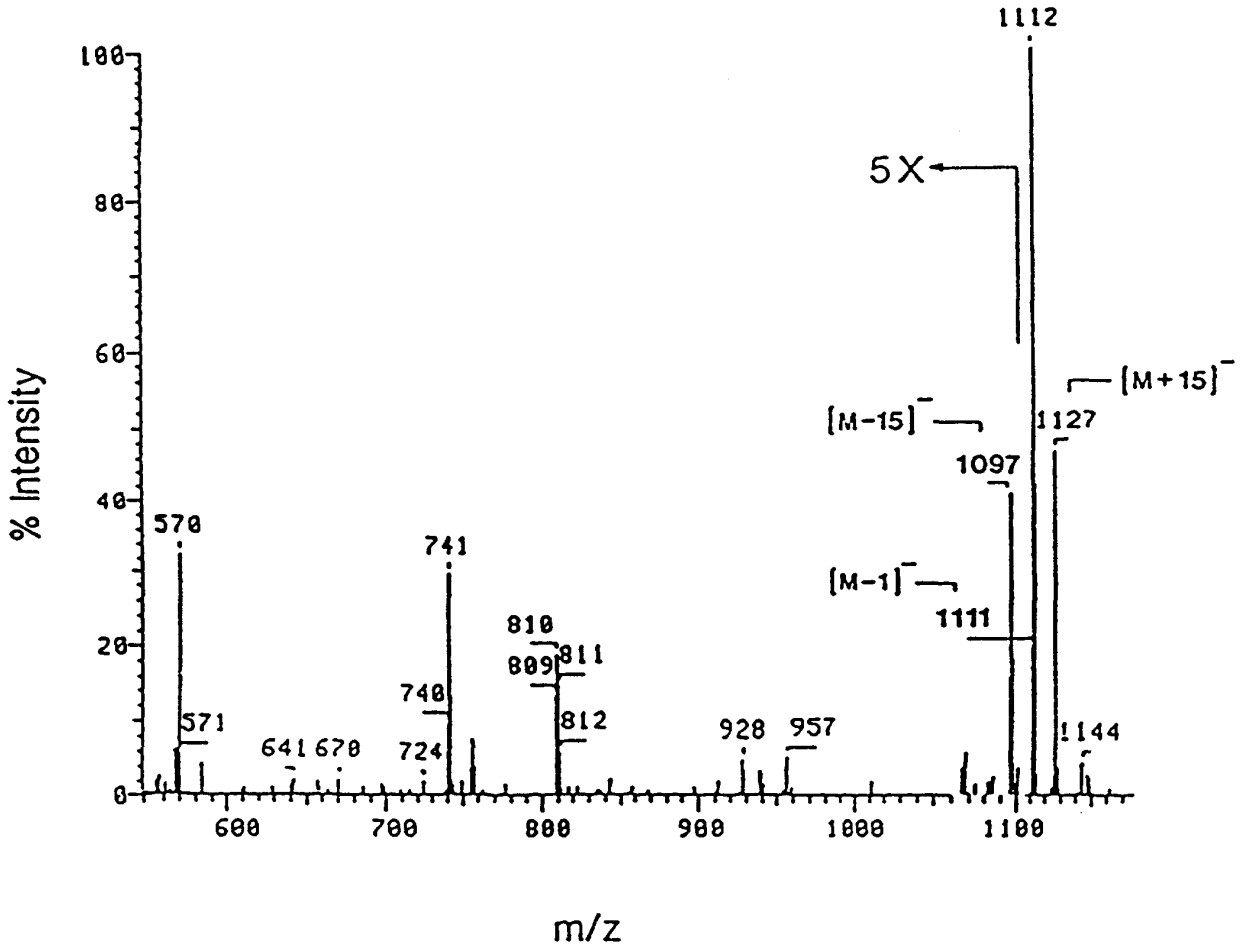

Figure 23. Negative ion chemical ionization (NICI) mass spectrum of valinomycin (10 ng) obtained by SFC/HRMS. SFC conditions: 120°C, linear density program from 0.45 g/mL to 0.6 g/mL at 0.005 g/mL/min with an initial 5-min isoconfertic period. MS conditions: NICI mode with methane as the reagent gas, 350°C interface probe tip temperature, 280°C source temperature, and scan from 500 to 1200 amu.

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