Environmental Division

Separation of Mutagenic Components in Synthetic Crudes

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Mutagenic, basic constituents of a synthetic coal oil and a shale oil were isolated from the crude mixtures. In arriving at an efficient isolation procedure, several liquid chromatographic packing-eluent combinations were tried and the fractions bioassayed to determine the distributions of the mutagenic components. The most effective separation was achieved using a sequential elution scheme with first an alumina-benzene combination followed by a Sephadex LH-20 gel-isopropanol-acetone system. About 75-80% (wt) of an ether soluble base is eluted with benzene through alumina (activity I). Analysis of this fraction has revealed a wide range of alkyl substituted quinolines and pyridines. Material remaining on the alumina column was eluted with ethanol, dried and placed on the Sephadex column. Isopropanol (~250 ml) and acetone (~600 ml) were used in that order to elute the material quantitatively. About 12% (wt) of the ether soluble base is eluted with the isopropanol while the rest (~10%) is eluted with the acetone. Additional alkyl pyridine compounds are eluted with isopropanol while the acetone fractions are predominantly mutli-ring nitrogen heterocyclic compounds, according to mass spectral analyses.

Bioassay data show excellent isolation of the mutagenic activities into the acetone fractions. Negligible activity is found in the sum of the other (90% wt) fractions.
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The development and utilization of bioassays to pinpoint dangerous chemicals, especially carcinogens, is an important component of the ORNL Synthetic Fuels Life Sciences Program. Recent studies (1,2) have demonstrated that microbial mutagenicity assays have especially useful features, e.g., they are inexpensive, fast, reasonably reproducible and yield results which correlate well with the carcinogenic potentials of pure compounds. Less certainty exists concerning the ability of such tests to reliably identify hazardous components of complex mixtures.

During the past two years we have worked with colleagues in the Biology Division to design and carry out experiments to establish and validate bioassays for incorporation into a general toxicity screening program at ORNL. A data base of biological activities determined using the microbial mutagenesis assay has been generated for a wide variety of fossil-derived materials. Our experimental role in these mutually designed studies has been to fractionate crude materials into distinguishable chemical classes and identify constituents of biologically active fractions. Ether-soluble base (ESB) fractions of several crudes and aqueous wastes which were fractionated by an extractive separation procedure (3) exhibit high biological activities as measured by the Ames microbial mutagenesis test. For this reason, the ESB was chosen for subfractionation to isolate and identify the biologically active components.

A subfractionation procedure was developed to isolate the mutagenic components of the ESBs of a shale oil and a coal-derived crude oil. No particular guidance was available in the literature. Biologically active compounds might fall into several chemical classes and a successful subfractionation scheme had to be worked out in parallel with the bioassays of subfractions to determine if active components are being separated.

Biologically active subfractions were sought using liquid chromatography with several packing-solvent combinations. Bioassays indicated the extent of separation of the active components and, in effect, served as the chromatographic detector. We found that the best isolation was achieved by a two-step process: (a) an alumina (activity I) column eluted with benzene, followed by (b) a Sephadex LH-20 column eluted first with isopropanol and then acetone. Columns were constructed by modifying standard burets and were eluted by gravity flow.

The effective isolation of mutagenic components in the acetone fraction is apparent from the data in Table 1. Since the acetone subfraction contains a relative

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weight percent of about 10% of the ESB, and the ESB fraction represents only approximately 5 weight percent of the starting material, this represents an overall isolation of the principal mutagenic components into about one-half percent of the weight of the crude oil. The benzene and isopropanol eluates contain negligible biological activities but contain 90% of the material. The non-active components are separated extremely well from the active components by this elution sequence.

Mass balance calculations indicate quantitative recoveries of the ESB components. Biological activities are recovered nearly quantitatively as well. If the weight percentages of each of the three fractions are multiplied by their specific activities and these are summed, the additive activities of the subfractions yield predicted specific activities of 27,000 rev/mg for the synthoil ESB and 2,300 rev/mg for the shale oil ESB. These compare with 30,000 and 2,500 for the synthoil and shale oil ESBs, respectively, before subfractionation. Calculated recoveries are 90% and 93% of the original activities, respectively. The loss of ~10% of the biological activity in these cases might have some significance, i.e. absence of synergistic activity following fractionation might account for the difference. However, the reproducibility of bioassays also falls in this range. A general point worthy of note is that the mutagenic activity of the synthoil-derived ESB is comparable to that of pure benzo(a)pyrene while that from the shale oil is an order of magnitude smaller.

Gas chromatography and combined gas chromatography-mass spectrometry (GC/MS) has been applied to each of the subfractions. Compounds ranging from low molecular weight diazenes and pyridines in the benzene fractions to heterocyclic azacoronenes in the acetone fraction have been observed. Ninety percent of the peaks in the gas chromatographic (GC) profiles of all fractions have been tentatively identified by GC/MS. The highly mutagenic acetone subfraction contains principally aza-arenes such as azabenzoperylenes, azaindopyrenes, and azacoronenes. These compounds may be the components producing the high biological activity. Mutagenesis testing of pure compounds representative of high molecular weight aza-arenes is required to confirm this suspicion.

Detailed characterization of the acetone subfraction requires analysis on an analytical scale liquid chromatographic column and capillary column GC. This is being pursued, but positive identifications of components will continue to be limited because of the small amounts that can be isolated and the lack of commercially available compounds for reference purposes.

References

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Table 1. Distribution of Mutagenic Activity Among Subfractions of Synfuel Ether Soluble Base Fractions[a]

Subfraction	Synthoil Base Fraction			Shale Oil Base Fraction		
	Relative Weight (%)	Specific[b] Activity (rev/mg)	Relative[c] Activity (%)	Relative Weight (%)	Specific[b] Activity (rev/mg)	Relative[c] Activity (%)
Benzene	76	850	2	78	0	0
Isopropanol	12	0	0	12.5	226	1
Acetone	12	220,000	88	9.2	25,000	92
TOTAL	100		90	99.7		93
Ether Soluble Base Fraction		30,000			2,500	

[a]Data from J. L. Epler, et al., Biology Division.

[b]rev/mg = revertants/mg, the number of histidine revertants from Salmonella strain TA98 by use of the plate assay with 2 x 10^8 bacteria per plate. Values are derived from the slope of the induction curve extrapolated to a milligram value.

[c]percent of relative activity of each subfraction referred to mutagenic activity of the original base fraction, calculated by: specific activity of each subfraction (rev/mg) x % of relative weight of each subfraction/specific activity of original base fraction (rev/mg).