

CHROM. 13,187

Note

Inadvertent formation of polynuclear aromatic hydrocarbons by the flame sealing of glass ampules

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(Received July 28th, 1980)

In the course of general chemical analysis of organic materials or as a method of storage or shipment of high purity reagents it is common practice to seal liquid samples in glass ampules. Borosilicate glass ampules are sealed with a small flame applied to the external surface of the glass neck. During the course of mass spectroscopic investigations of certain hexane extracts of fish oil which had been sealed in ampules for transport, a number of polynuclear aromatic hydrocarbons (PAHs) were found in these extracts. Further investigations showed that the flame sealing of the glass ampules led to PAH formation. We wish to alert other investigators to this problem and describe how the formation of PAHs during sealing can be avoided.

EXPERIMENTAL

Apparatus

A Waters Scientific high-performance liquid chromatograph consisting of two M-6000 A pumps, a No. 660 solvent programmer and a U6-K injector, was used throughout. Schoeffel SF 770 fluorescence and FS 970 UV detectors were used coupled to a Spectra-Physics System I computing integrator. UV and fluorescence chromatograms were recorded on Omniscribe and Linear recorders respectively at 10 mV full scale. The analytical column used was a VYDAC 201 TP reversed phase (25 cm × 3.2 mm I.D.; 10 μm).

Chromatographic analysis

Linear gradient elution from 70–100% (v/v) acetonitrile (Caledon Labs., Georgetown, Canada, HPLC grade) in glass-distilled water was used throughout. Inlet pressure was 1200 p.s.i.g.; flow-rate used was 1 ml/min. Wavelengths used were: UV, 265 nm; fluorescence, excitation at 280 nm, emission at >389 nm. Injection volumes varied from 1–10 μl. Individual PAHs were identified and quantitated by external and internal standardization as well as by the method of standard additions

when necessary. Peak areas and retention times were obtained from the System I computing integrator.

Sample preparation

Pyrex glass ampules, 10 cm \times 18 mm O.D., were washed with glass-distilled acetone, then hexane (Caledon, distilled-in-glass) and dried in a stream of dry nitrogen (Canadian Liquid Air, Halifax, Canada). A 2-ml volume of the test solvent was carefully placed in the bottom of the ampule, avoiding any wetting of the neck of the ampule. The ampule was then sealed using a propane/oxygen blowtorch (Fisher Scientific, Pittsburgh, PA, U.S.A.; No. 2-716), care being taken to avoid flaming the open end. After sealing, the ampules were allowed to cool standing with the sealed end uppermost (*ca.* 15 min). They were then shaken to rinse the walls with the enclosed solvent and, after opening, 100 μ l of dimethyl sulfoxide (DMSO) (purified) were added to the ampule and the test solvent was evaporated under a stream of dry nitrogen into the 100 μ l DMSO prior to HPLC analysis. Appropriate blanks were run throughout.

RESULTS AND DISCUSSION

The extremely heterogeneous mixture of PAHs formed during the sealing of hexane in a borosilicate ampule at room temperature is shown in Fig. 1A. It has been shown that combustion of organic materials results in the formation of very complicated mixtures of alkylated and non-alkylated PAHs, the relative amount of non-

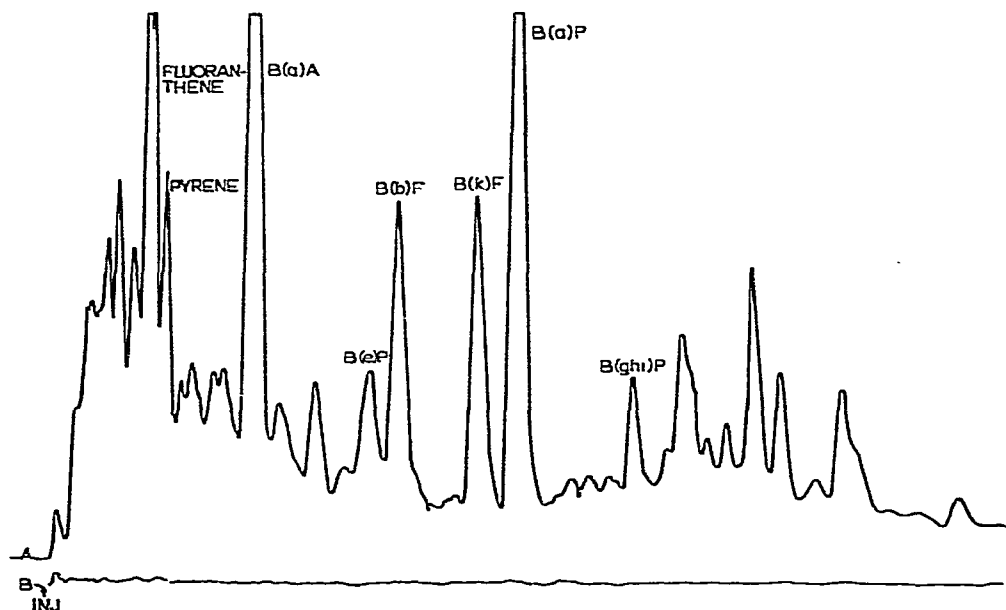


Fig. 1. A, chromatogram of a heterogeneous mixture of PAHs formed during the sealing of hexane in a borosilicate ampule at room temperature. B, chromatogram when ampule and contents are chilled before flame sealing.

TABLE I

POLYNUCLEAR AROMATIC HYDROCARBON CONCENTRATION, g/ml TEST SOLVENT

Abbreviations used: fluor = fluoranthene; chry = chrysene; B(a)A = benz[*a*]anthracene; B(e)P = benzo[*e*]pyrene; B(b)F = benzo[*b*]fluoranthene; B(k)F = benzo[*k*]fluoranthene; B(a)P = benzo[*a*]pyrene; B(ghi)P = benzo[*g,h,i*]perylene; ND = none detected.

PAH	Pentane	Hexane	Heptane	Nonane	Benzene	Acetone	Dichloro- methane	Ethanol	Methanol	<i>n</i> -Propanol	<i>n</i> -Butanol	Aceto- nitrile
Fluor	3994	2368	202	ND	606	22	13	ND	ND	ND	ND	ND
Pyrene	11,358	10,332	ND	59	ND	161	ND	ND	ND	ND	ND	ND
Chry	4867	3900	247	21	1125	55	43	ND	ND	ND	ND	ND
B(a)A	16,422	12,082	64	ND	272	27	25	ND	ND	ND	ND	ND
B(e)P	6641	6166	417	19	1115	98	ND	ND	ND	ND	ND	ND
B(b)F	723	690	65	2	126	13	ND	ND	ND	ND	ND	ND
B(k)F	164	119	12	0.3	12	2	ND	ND	ND	ND	ND	ND
B(a)P	662	630	53	0.4	38	15	3	ND	ND	ND	ND	ND
B(ghi)P	3879	1523	193	1.5	120	65	ND	ND	ND	ND	ND	ND

alkylated PAHs increasing with increasing combustion temperature¹. We have quantitatively determined nine common PAHs which are formed in the flame sealing process. We do not mean to imply that only non-alkylated PAHs are formed. In fact, due to the relatively low temperature of the melting glass, a relatively large percentage of alkylated PAHs should be formed. It is likely that oxygenated PAHs as well as a variety of other organic compounds are present in these ampules. (Fig. 18 illustrates also that chilling of the ampule and contents before flame sealing prevents PAH formation during sealing.)

Table I shows the concentrations in $\mu\text{g}/\text{ml}$ of test solvent of nine individual non-alkylated PAH compounds formed when sealing various solvents in ampules at room temperature. It is evident that the concentration, *i.e.* formation, of PAHs decreases as solvents vary from pentane (low boiling) to nonane (high boiling). This decrease in PAH formation is probably due to the decreased volatility of the solvent as the chain length of the alkane increases. The situation is obviously more complicated than this since each alkane solvent tested formed a characteristic PAH distribution. Benzene, a volatile aromatic solvent also forms PAHs when flame sealed in ampules at room temperature. The amount formed fell between hexane and heptane, as did the boiling point, suggesting that aromaticity of the solvent did not play an important role in PAH formation. Acetone and dichloromethane formed lesser amounts of PAHs presumably due to the presence of oxygen or chlorine in the starting solvent. The decomposition of the test solvents was also obvious from the discoloration that occurred. The nature of these decomposition products, particularly those from dichloromethane, is being further investigated. A number of solvents (acetonitrile, *n*-butanol, *n*-propanol, ethanol and methanol) did not form PAHs when sealed at room temperature. This is somewhat surprising since the boiling points of these solvents are in the range of those of the solvents forming PAHs.

We have been unable to find any report documenting formation of PAHs from organic solvents under the conditions described here. The amount of PAHs formed (up to 50 μg PAH/ml pentane) is surprising, especially when contrasted with the ease at which their formation can be prevented, by simply prechilling the ampule and contents in wet ice prior to sealing. This discovery has importance in at least three areas of chemistry:

(1) Trace residue analysis of foods, etc., at mg/kg or $\mu\text{g}/\text{kg}$ levels, since it is common to store cleaned up extracts in such ampules. These extracts are often in hexane, pentane or benzene, all solvents which formed PAHs in our study. Re-analysis of these stored extracts would show higher PAH levels than the original analysis and could lead to confusion if tolerance levels are approached. For example, the allowable level of benzo[*a*]pyrene in meat and cheese in the Federal Republic of Germany is 1 $\mu\text{g}/\text{kg}$ (ref. 2). Analysis of sealed extracts for certain organochlorine compounds such as polychlorinated biphenyls could be in error since many PAHs are electron-capture sensitive and depending on chromatographic conditions, could co-elute with the organochlorine compounds.

(2) Environmental baseline and monitoring levels. Contaminants in environmental samples such as seawater are often present at below ppb (10^9) levels. Formation of PAHs at the levels reported here can exceed environmental levels and erroneous reporting of PAHs could occur.

(3) Pure chemical and intercalibration studies. Since it is common to ship

high-purity chemicals, check sample standards and samples, sealed in glass ampules, the formation of PAHs could result in contamination of high-purity compounds and apparent changes in levels of organics in check sample standards and samples, especially those concerned with determination of trace amounts of PAHs (e.g. petroleum hydrocarbon intercalibration programs).

REFERENCES

- 1 *Particulate Polycyclic Organic Matter*, National Academy of Sciences, Washington, DC, 1972.
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