CHROM. 6039

Determination of benzo[*a*]pyrene in bitumen and plants

Petroleum fractions, such as fuels and bitumen, are applied on soils for agricultural purposes. Fuels are used as herbicides[1] and as agglomerating agents in fertilizers, and bitumen is employed in an emulsion as a soil conditioner[2]. As the application of these mixtures always involves some risk of pollution, we decided to examine bitumen and plants grown on soils treated with petroleum fractions for polycyclic aromatic hydrocarbons. In this paper, the determination of benzo[*a*]pyrene (BaP) is described.

Polycyclic aromatic hydrocarbons (PAHs) have been determined in a wide range of materials. The isolation of PAHs as a group is usually performed by extraction with a solvent, such as benzene, cyclohexane or chlorinated hydrocarbons. In a recent review, the advantages and disadvantages of solvents have been considered[3]. In the same review, the separation of PAHs by different methods is compared: twenty-three methods involving liquid–liquid chromatography, fourteen paper chromatography, seventeen thin-layer chromatography (TLC) and nine gas chromatography (GC) are mentioned. TLC seems to be the most suitable method, although GC is often applicable.

Specific methods for the determination of BaP in bitumen, pitch and tar have been described, based mainly on liquid–liquid or liquid–solid chromatography[4–7]. These and other column chromatographic methods on magnesium oxide–Celite[8] and Sephadex[9] usually gave inadequate separation, for UV and fluorescences pectra comparable with those of pure BaP to be obtained. Complete separation was felt to be necessary to allow correct identification of components and for fluorimetry to be suitable for use in the determination. Correct identification is important as the carcinogenic properties of the various PAHs differ[10]. Fluorimetry affords the highest sensitivity, but is useless for mixtures, for which erratic results are produced[11a,11b,12].

The separation of PAHs from bitumen is hampered by the presence of a large excess of comparably high-boiling products. Therefore, GC was not suitable. We further tried TLC on silica gel[13], alumina[14], cellulose[15] and acetylcellulose[15] and combinations of pairs of them. None of these trials allowed sufficient purification to be achieved. SAWICKI's method, consisting of three consecutive runs on alumina, cellulose and acetylcellulose[11a,11b], was the best but was still inadequate.

Optimum results were obtained by preliminary distillation of bitumen to remove the higher molecular weight components[4] and analysis of the distillation fractions by three consecutive TLC runs on silica gel, cellulose (reversed)[16] and acetylcellulose. Combination of this procedure with other adsorbents, such as Sephadex and alumina, did not enhance the separation markedly. The band corresponding to BaP was removed immediately from the plate, extracted and spotted on to the next plate. Precautions were taken to avoid decomposition of the bands and standards by light. The fluorescence spectrum of the product isolated from bitumen in this way was essentially identical with the spectrum of pure BaP (Figs. 1–3). This procedure therefore permits the quantitative determination of BaP by fluorimetry. It was used also for the analysis of commercial BaP and plants suspected of eventual pollution by PAHs. These examples show that a less rigorous method of separation could easily lead to faulty conclusions.

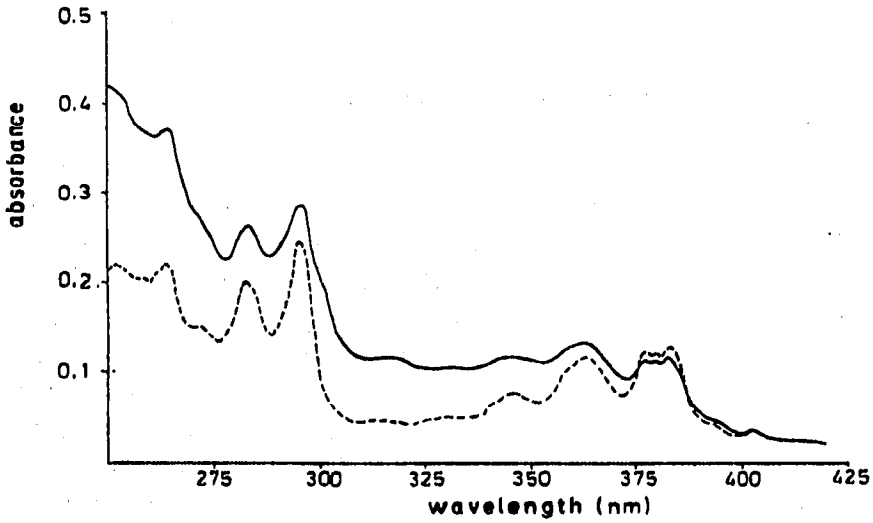
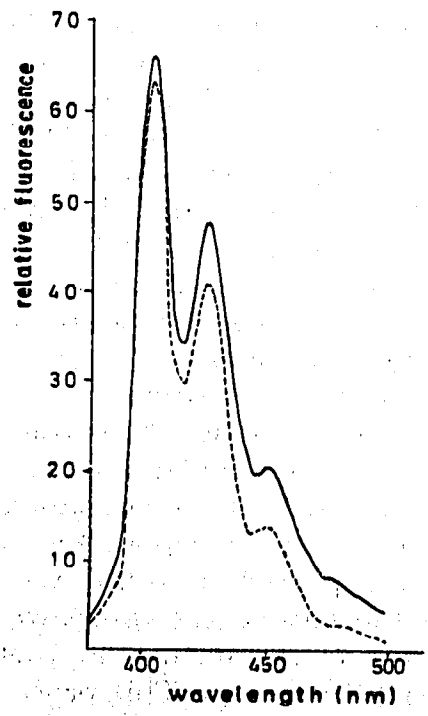


Fig. 1. UV spectrum of benzo[*a*]pyrene. ———, Isolated from bitumen; — — —, pure (1 p.p.m. in methanol).



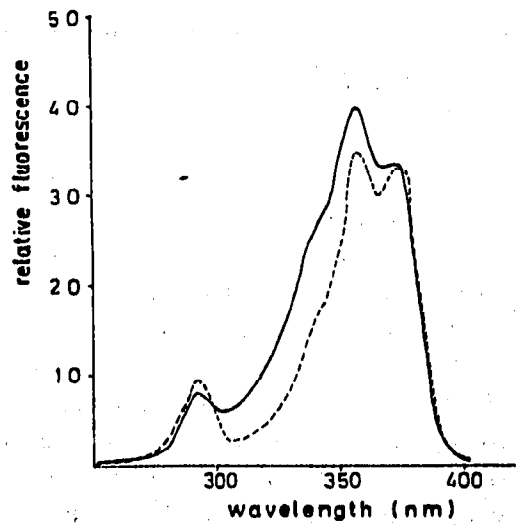


Fig. 2. Fluorescence spectrum of benzo[*a*]pyrene activation at 360 nm). ———, Isolated from bitumen; — — — pure (5 p.p.b. in methanol).

Fig. 3. Activation spectrum of benzo[*a*]pyrene (fluorescence at 420 nm). ———, Isolated from bitumen; — — —, pure (5 p.p.b. in methanol).

Experimental

UV spectra were recorded on a Beckman DB UV spectrometer. Fluorescence spectra were measured on a Farrand Optical fluorimeter (band width 5 nm, sensitivity 10 μA).

Bitumen. Bitumen (10 g) was fractionated by distillation at normal pressure, fractions being collected at 250–300°, 300–320°, 320–340° and 340–360°. A further fraction was distilled at 12 mm Hg, the total amount distilled being *ca.* 6 g.

Extraction of plants. Plants (2 kg) were chopped, air dried and extracted either in a Soxhlet apparatus or manually with pentane and hexane (total volume *ca.* 3 l). The extract after evaporation was ready for TLC analyses; further purification by the frequently-used dimethyl sulphoxide extraction or by column chromatography was not helpful. Extraction with dimethyl sulphoxide seems to be particularly suitable with fatty products (*e.g.*, foods)[17].

Thin-layer chromatography. TLC plates, 20 × 20 cm and 0.5 mm thick, were prepared in the usual way with Silica Gel G (Merck), cellulose MN 300 (Macherey, Nagel) and acetylcellulose MN 300 AC 30% (Macherey, Nagel), and air dried.

Bitumen fractions (0.5 g) in benzene were spotted as a line on ten silica gel plates, which were developed with a pentane–diethyl ether (95:5) mixture. Pure BaP was spotted as an internal standard and a relatively broad band corresponding to it was scraped off. In some instances it was necessary to repeat this separation once, when a heavy background was present after the first separation. Concentrated extracts of plants were spotted in the same way. The removed band was extracted with a dichloromethane–methanol (4:1) solution. The extract was concentrated to a small volume and methanol was removed by consecutively adding benzene and evaporating, three times, in order to obtain narrower spotting bands.

The concentrated extract was spotted as a line on the cellulose plate, which was developed by the method of WHITE AND HOWARD[16]. The plate was inverted and all of it, except for the spotted line, was immersed in a 20% solution of dimethylformamide in diethyl ether. After draining off the excess solution, the plate was developed in the usual manner with isooctane. Separation was improved by this technique. Narrow bands were obtained and the one corresponding to BaP was removed and extracted with hot methanol. The extract, concentrated as before, was spotted on the acetylcellulose plate and developed with ethanol–toluene–water (17:4:4).

All the solvents used were of pure reagent grade and were distilled before use. BaP from various commercial sources was purified by TLC in the same manner as the samples (see below).

Standards were kept in the dark and renewed frequently. Extracts were not evaporated completely. Elution was also performed in the dark and the bands were removed from the plate immediately in the UV box. Extractions were carried out quickly.

Results

Commercial benzo[a]pyrenes. None of three commercial benzo[*a*]pyrenes, called here A, B and C, gave only one band in the three separations mentioned. All of them gave one band on silica gel. A showed four minor impurity bands on cellulose and only one band on acetylcellulose. B gave one impurity band on cellulose and three

on acetylcellulose. C gave one band on cellulose but three strong bands on acetylcellulose. Even the fluorescence spectrum of C was quite different from that of pure BaP. Hence commercial BaP must be purified for use as a testing material.

Bitumen. Distillation fractions obtained at 250–300°, 300–320°, 320–340° and 340–360° and the 12 mm Hg fraction were analysed without any pre-treatment. Fractions up to 340° after separation on silica gel gave some bands on cellulose, but none of these bands corresponded to BaP. The 340–360° fraction gave a very weak BaP band on cellulose and acetylcellulose. The vacuum-distilled fraction produced a broad band on silica gel, which on cellulose gave at least six bands. Its BaP band on acetylcellulose was split into three new bands, one of them corresponding to BaP. The UV and fluorescence spectra of this band are identical to those of BaP (see figures).

The addition of 10 p.p.m. of BaP to a bitumen gave an increase of BaP content as determined of 5.1–5.5 p.p.m. Recovery is therefore about 50%, presumably owing to the fact that some BaP remains in the residue during the preliminary distillation. Bands on various plates depend largely on the origin and treatment of the bitumen. The BaP content corrected for the recovery factor varied between 3 and 5 p.p.m.

Plants. Grass, grown on soil that had been treated with fuel, was examined for BaP. The silica gel chromatogram under UV illumination contained a small blue band corresponding to BaP. After transfer to the cellulose plate, various bands were obtained, one of them corresponding to BaP. This band was transferred to an acetylcellulose plate and then gave three bands, none of which corresponded to BaP. This clearly shows that less extensive separation of PAHs can lead to erroneous results.

Beets, grown on a soil which had been conditioned with a bitumen emulsion, gave two main bands on silica gel. The band corresponding to BaP on cellulose was split into three medium bands, and its BaP band on acetylcellulose yielded two very weak bands, one of which corresponded to BaP. However, the blank showed an identical pattern, so traces of PAHs in this instance are presumably due to atmospheric pollution.

An analogous result was obtained with carrots. The cellulose plate contained five medium bands. As a consequence of the large extent of carotenoid extraction, separation was poor and cellulose chromatography was repeated on the BaP band. On acetylcellulose, a trace of BaP was present, as it was in the blank.

Conclusion

Successive TLC separations on silica gel, cellulose (reverse) and acetylcellulose allow complete separation of BaP from different mixtures, as plant extracts or bitumen. Examples showed that this extensive separation is necessary to avoid false positive results. It further allows determinations to be made by fluorimetry, which is about 200 times more sensitive than UV spectrometry.

Although this method gives a satisfactory determination of BaP, this is only part of the problem of carcinogenicity, which can depend on the presence of several polycyclic aromatic hydrocarbons[10]. More work must be carried out to establish reliable methods for other highly carcinogenic components.

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