Review

Determination of benzanthrone in environmental samples

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Abstract

This paper summarizes advances in the determination of benzanthrone and other polycyclic aromatic ketones in environmental samples in the last 20 years. Data on toxicity and mutagenicity are also reviewed. Methods of determination include liquid chromatography, high-performance liquid chromatography, gas chromatography and gas chromatography–mass spectrometry.

Contents

1. Introduction

7*H*-Benz[*de*]anthracen-7-one (benzanthrone) (Fig. 1) is a precursor of dyestuffs in industry [1] and its toxic effects have been recognized for a long time [2]. Its determination in airborne particulate matter became feasible only recently after the development of efficient chromatographic methods of analysis.

Fluoranthene (Fig. 1), a major component of polycyclic aromatic hydrocarbons (PAHs) emitted from combustion engines, can be obtained from benzanthrone by elimination of CO, since the elimination of CO is a well known reaction in the pyrolysis of some ketones [3]. Hence, it is
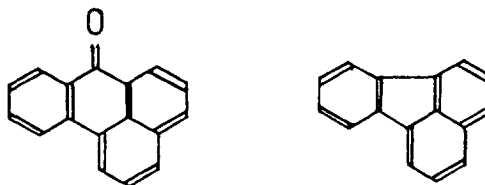

Fig. 1. Structural formulae of (left) benzanthrone (m.p. 170°C) and (right) fluoranthene (m.p. 110°C).

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not surprising that benzanthrone and other poly-
cyclic aromatic ketones always accompany PAHs
in particulate emissions of combustion sources.

Sublimation of polycyclic aromatic compounds
at 300°C reveals that both PAHs and polycyclic
aromatic ketones are not decomposed, whereas
diketones are less stable [4]. These data prove
considerable stability of polycyclic aromatic
ketones at increased temperature.

Benzanthrone is almost completely adsorbed
on particulate matter in the atmosphere. Only
3% of total benzanthrone was found in the gas
phase when high-volume sampling was done in
Portland, OR, USA, in 1984 [5]. In the same
sampling experiment, 28% of total chrysene and
94% of total fluoranthene were found in the gas
phase. These data indicate a lower volatility of
benzanthrone compared with these PAHs.

It is well known that the concentration of
benzo[a]pyrene shows a large short-term vari-
ation in the atmosphere. The average concen-
tration of benzo[a]pyrene in Essen (Germany)
during 50 weeks varied between 6 and 40 ng/m^3,
whereas short-term concentrations ranged from
0.2 to 299 ng/m^3 [6]. A similar concentration
range of three orders of magnitude can be
expected for benzanthrone, although it decays
slightly faster than benzo[a]pyrene in the atmos-
phere. It is normally lost during clean-up of
samples for PAHs [6].

Benzanthrone, the industrial precursor of
dyestuffs, which is also among the major poly-
cyclic aromatic ketones found in emissions from
all combustion sources, is the subject of this
review. Owing to the close structural relationship
between benzanthrone and other polycyclic aro-
matic ketones and PAHs, the purpose of this
review is to provide a literature survey on the
determination, properties and environmental oc-
currence of benzanthrone and its relationship to
other polycyclic aromatic compounds during
analytical procedures.

2. Liquid chromatography

Liquid chromatography routinely is used for
sample clean-up prior to the determination of

benzanthrone. Although compound separation
can also be done by HPLC, supplemental meth-
ods such as GC, GC–MS or LC–MS are applied
more frequently owing to the large number of
different PAHs other than benzanthrone in en-
vironmental samples.

The isolation of PAHs is effected by a pre-
column clean-up on silica columns, followed by
chromatography on Sephadex LH-20 with 2-pro-
panol as the eluent [6]. This clean-up separates
benzanthrone from PAHs owing to its higher
polarity, and it is then discarded with contami-
nants of similar polarity and thus escapes detec-
tion. Multi-step clean-up procedures have also
been developed (7), and benzanthrone will be
found in a column eluate not containing PAHs.

Liquid chromatography on Sephadex LH-20
with 2-propanol as eluent will elute benzanth-
rone just before chrysene and benz[a]anth-
racene, and this method alone is therefore not
suitable for the separation of benzanthrone from
PAHs.

A separation of organic compound classes in
synthetic fuels has been performed. Stepwise
elution of compounds from neutral alumina with
hexane, benzene, chloroform–0.75% ethanol
and tetrahydrofuran THF–10% ethanol was per-
formed. Benzanthrone was found in the chloro-
form fraction. This fraction was subjected to
column chromatography on silica gel, and ben-
zanthrone and amino-PAHs were found in the
benzene eluate [8].

Polycyclic aromatic compounds were isolated
from fish tissue by adsorption chromatography
on alumina and gel chromatography on
biobeads, and the detection limit was below 0.2
μg/kg [9]. Clean-up on an alumina column
yielded well defined compound classes [10].
Compound class separation was also achieved by
normal-phase HPLC [11]. Oxygenated and nitro-
substituted PAHs were determined by HPLC
with electrochemical detection [12].

A clean-up based on XAD-2 was developed in
order to isolate polar and non-polar polycyclic
compounds [13,14]. This method is based on the
polycyclic aromatic structure of solutes rather
than polarity. It provides a column eluate con-
taining all non-polar and slightly polar polycyclic

aromatic compounds in one fraction. Thus, nitroarenes, heterocycles, PAHs and benzanthrone are isolated together, while most interfering compounds are absent. The mixture of polycyclic compounds obtained by this clean-up often contains too many polycyclic compounds, and a direct determination of benzanthrone without a second clean-up can only be done by high-resolution GC.

A combination of gel chromatography on Bio-Beads SX-12 and normal-phase LC was used for clean-up of aromatic ketones and related compounds followed by capillary GC [15].

An HPLC method for mixtures of up to 600 organic compounds, followed by GC–MS, has been published [16]. Narrow-bore columns of 1 mm I.D. were used in HPLC for the separation of compounds, and subsequent off-line MS was performed [17]. HPLC in conjunction with diode-array UV detection and MS was found suitable for the analysis of complex mixtures of polycyclic aromatic compounds [18].

On-line LC–GC has also been applied to polycyclic compounds in airborne particulate matter and diesel exhaust particulate extracts [19,20]. LC–GC is used directly with crude extracts without prior sample clean-up. Compounds present in the sample are split up between different gas chromatograms. This is not satisfactory, because it should be the aim of GC analysis to produce one gas chromatogram representing total amounts of compounds to be determined.

3. Gas chromatography and gas chromatography–mass spectrometry

Diesel particulate matter has been analysed. Polycyclic compounds were separated by normal-phase HPLC with gradient elution. PAHs and polar polycyclic compounds are found in different fractions, and the concentration of benzanthrone is about one quarter of that of fluoranthene. Compound identification was achieved by GC–MS using non-polar stationary phases. Mass spectra for polar aromatic compounds were given [21]. Benzanthrone was also identified in

treated wood after supercritical fluid extraction and GC–flame ionization detection [22]. Liquid crystalline stationary phases were applied to the determination of polycyclic materials in airborne particulate matter. Benzanthrone and other polycyclic compounds were separated from PAHs by HPLC on silica gel prior to GC [23]. Mass spectra of benzanthrone and related compounds have been reported and fragmentation pathways investigated [24,25]. MS fragmentation and GC retention of benzanthrone among 80 other compounds frequently encountered in environmental samples has been described [26].

Polycyclic aromatic ketones and PAHs have been isolated from urban airborne particulate matter and both compound classes separated by LC on silica gel. A second clean-up by column chromatography on Sephadex LH-20 with 2-propanol as eluent was applied in order to separate oxygenated polycyclic compounds from aliphatic polar compounds. The sample originated from urban airborne particulate matter collected in Duisburg (Germany). Benzanthrone was the most abundant polycyclic aromatic ketone, and its concentration ranged from 0.46 to 3.66 ng/m^3. Many other polycyclic compounds were identified in addition to benzanthrone by GC–MS [27]. A characteristic profile of benzanthrone and other polycyclic compounds in aircraft turbine particulate emissions has been obtained by a selective clean-up and GC [28].

A comprehensive presentation of polycyclic aromatic ketones in environmental samples has been given, and methods for the characterization of polycyclic aromatic ketones have been reviewed [29,30]. Benzanthrone and other oxygenated polycyclic aromatic compounds were sampled from Diesel exhaust, separated from PAHs by clean-up on silica gel and identified by GC–MS [31]. Condensate from brown coal combustion flue gas in residential heating also contained benzanthrone, among other oxygen-containing polycyclic compounds [32]. The seasonal variation of organic aerosols in California has been investigated by GC–MS [33]. Extracts from gasoline exhaust particulates were separated into five fractions according to polarity, and individual compounds were identified by GC–MS

[34]. During the determination of benzanthrone and other polycyclic aromatic ketones by capillary GC, random overlap with PAHs may occur, and stationary phases of different polarities should be employed in compound identification by GC [35].

Polar polycyclic aromatic compounds have been characterized by GC–MS with negative-ion chemical ionization [36]. The sample used was NBS standard reference material SRM 1650, which originated from Diesel exhaust particulate matter and was fractionated by normal-phase HPLC prior to GC–MS.

4. Mutagenicity

Numerous investigations into the mutagenicity of benzanthrone have been performed in the past 15 years. Benzanthrone was found to be mutagenic in a test with *Salmonella typhimurium* strains after activation, and it is suspected that its oxidation in air may also lead to mutagenic products [37]. The Ames test for mutagenicity was performed with 30 polycyclic compounds from urban air particulate matter [38]. Soot and 70 associated polycyclic compounds were tested for mutagenicity [39]. The mutagenicity of indoor air pollutants was measured [40,41]. Weak mutagenicity was found for keto derivatives of PAHs [42]. Dilute wood smoke was reacted with sub-ppm levels of O_3 and NO_2 in a Teflon chamber. Aromatic ketones contributed 4% of total mutagenicity before reaction and 16–30% after reaction [43]. Residential wood combustion contributed to Contra Costa County community airborne mutagens in a winter inversion [44]. The polar neutral fraction of urban airborne particulate matter was found to be more mutagenic in the Ames assay than aliphatic or aromatic hydrocarbons [45].

Polar neutral compounds isolated from airborne particulate matter originating from residential heating and exhaust gases were more mutagenic than PAHs [46]. A mutagenicity-directed approach was undertaken to the determination of genotoxic components in coastal sediments. Fractionation was carried out by normal-

and reversed-phase LC and gel permeation chromatography (GPC), and the Ames test for mutagenicity was performed [47]. A bioassay-directed fractionation and interseasonal study was also undertaken [48].

5. Toxicity

Since benzanthrone is also an industrial chemical, data on its toxicity have been reported. More than six hepatic microsomal metabolites were isolated [49]. Algal cancer was observed in the marine alga *Porphyra tenera* in the presence of benzanthrone in Fukuoka, Japan [50]. The effect of benzanthrone on the bladder of guinea pigs has been investigated, and localized damage was observed at a dose of 25 mg/kg [51]. The long-term effect of benzanthrone in rats at a biweekly administration rate of 25 μg/kg has been described [52]. Benzanthrone was administered to mice, rats and rabbits, and a low acute and a strong subchronic toxicity to liver and blood were observed [53]. The health status of workers in the production of benzanthrone was surveyed, and disorders of the liver were attributed to combined effects of several chemicals [54]. The interaction of benzanthrone with serum proteins was found to be strongest at pH 7–8 [55]. The toxicity of PAHs and related compounds to *Daphnia magna* was investigated, and PAHs were classified as toxic, moderately toxic and non-toxic [56]. Photoinduced toxicity to larvae was reported [57]. Bioelimination and organ retention of benzanthrone in scorbutic and non-scorbutic guinea pigs was investigated, and ascorbic acid facilitated the bioelimination of benzanthrone [58,59]. Ascorbic acid also reversed the effects of benzanthrone in mice [60]. Bioelimination of benzanthrone was slower in guinea pigs than in rats [61].

6. Environmental occurrence

As benzanthrone is not only an industrial chemical but also accompanies PAHs in combustion related particulate emissions, it is found
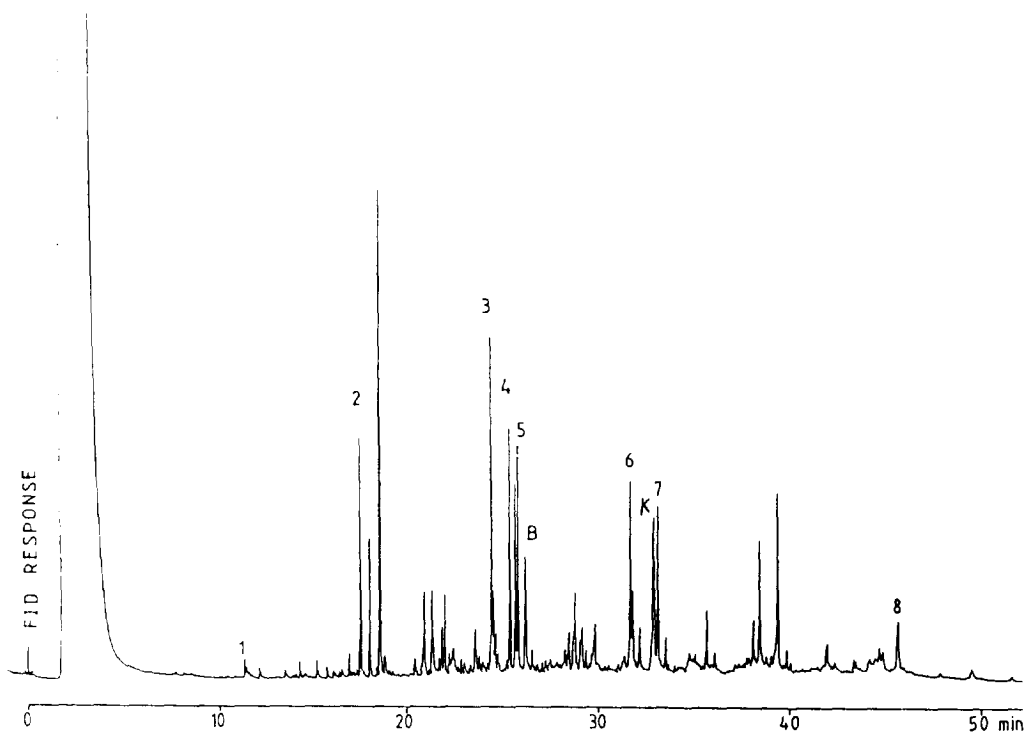
Fig. 2. Gas chromatogram of polycyclic aromatic compounds isolated from aircraft turbine particulate emissions (Pratt and Whitney JT3D3, gas turbine). Stationary phase, OV-1; column, 60 m × 0.03 mm I.D., temperature, programmed from 110 to 280°C at 4°C/min. Y Ordinate scale: peak 2 (fluoranthene) represents 4.5 ng. Peaks: 1 = phenanthrene; 2 = fluoranthene; 3 = benzo[ghi]fluoranthene; 4 = cyclopenta[cd]pyrene; 5 = chrysene; 6 = benzo[b]fluoranthene; 7 = benzo[a]pyrene; 8 = coronene; B = benzanthrone; K = 6-H-benzo[cd]pyrene-6-one.

virtually everywhere in the environment. Most interesting is the fact that it was also found in a meteorite [62]. A characteristic fingerprint of benzanthrone among other polycyclic aromatic emissions is displayed in Fig. 2. This sample was obtained from gas turbine particulate emissions and also reveals the quantitative ratio of benzanthrone to PAHs. The concentrations of benzanthrone in air particulate matter and surface soil are listed in Table 1.

Table 1
Contents of benzanthrone in airborne particulate matter and surface soil

Sample	Location	Concentration	
		ng/m^3 air	μg/kg
Urban air particulate matter [5]	Oregon, USA	1.7	
Urban air particulate matter [27]	Duisburg, Germany	0.46–3.66	
Surface soil [77]	Nagoya, Japan		10
Exhaust particulate matter [21]	In-use VW Diesel engines, USA		243–1281

Owing to its widespread distribution in the environment, there is a comprehensive literature available on the environmental occurrence of benzanthrone. Oxygenated arenes were identified in urban air in Toronto, Canada [63]. Diesel exhaust contains polycyclic aromatic ketones in the most mutagenic fractions separated from diesel soot extracts, and benzanthrone was identified among other oxygen-containing derivatives of PAHs such as anthracenedione, 4H-cyclopenta[def]phenanthren-4-one and fluorenones [64]. Polar subfractions of Diesel exhaust account for 65% of direct-acting mutagenicity in the Ames assay [65]. Polycyclic ketones were also identified in urban airborne particulate matter from St. Louis, MO, USA [66]. Emission rates of benzanthrone were found to be twice as high from gasoline engines than Diesel engines in Japan [67]. Emissions of polycyclic compounds from rubber combustion were characterized [68]. Ambient airborne particulate matter was investigated in the Philadelphia industrial area, and mutagenicity testing was done [69]. Polycyclic aromatic ketones were also present in the pot room of an aluminium production plant [70]. Higher mutagenic activity is found for PAHs in Diesel exhaust after oxidation [71]. An interesting area is the gas/particle distribution of atmospheric organic compounds including polycyclic compounds [5,72]. The analysis of organic matter in coke oven emissions also revealed polycyclic compounds [73]. Sources of fine organic aerosol were systematically investigated [74–76]. Benzanthrone was also determined in urban surface soil from Japan, and it was found that it had originated from air particulate matter precipitated from the atmosphere [77].

7. Conclusion

The determination of benzanthrone is mostly done by multi-stage clean-up procedures and characterization by instrumental methods involving MS in addition to HPLC or GC. Owing to the complex analytical procedure, fewer data have been published on the environmental oc-

currence of benzanthrone and other related polycyclic aromatic ketones than on parent PAHs. It is recommended that any determination of polycyclic compounds should include polycyclic aromatic ketones of molecular masses 230 and 254, since they are always abundant in emissions from combustion sources, and obviously contribute to the total mutagenicity after being derivatized by secondary reactions.

Minor components among polynuclear aromatic compounds and the atmospheric reaction products of primary emissions including benzanthrone form an exceedingly large group of slightly or strongly polar polynuclear aromatic compounds. Multi-step clean-up procedures must be applied, and determination of selected compounds poses a challenge to current efficient analytical methods. An increase in mutagenic activity after atmospheric reactions has been reported [71]. The ratio of benzanthrone and benzo[a]pyrene to benzo[e]pyrene decreases during atmospheric degradation, and this ratio thus gives an important indication whether such atmospheric reactions have occurred or not.

Profile analysis of polycyclic aromatic ketones must be performed if the ratio of benzanthrone to minor polycyclic aromatic compounds is to be determined. A multi-step clean-up procedure is performed, and PAHs are absent from the profile of polycyclic aromatic ketones obtained by capillary GC [27].

The ratio of benzanthrone to benzo[e]pyrene and other PAHs is most rapidly revealed by clean-up on XAD-2 and profile analysis by capillary GC. Quantitative ratios of benzanthrone to all other major polycyclic compounds, including PAHs, are directly available from the chromatogram, whereas some overlap may occur among minor components [13].

HPLC is an efficient method for sample clean-up but cannot provide comprehensive information on related compounds that occur together with benzanthrone at similar concentration. Combined techniques such as GC–MS, LC–GC or LC–MS were all applied successfully and allow direct compound identification without sample clean-up.

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