Journal of Chromatography, 643 (1993) 1–10
Elsevier Science Publishers B.V., Amsterdam

CHROMSYMP. 2723

Review

Chromatographic techniques used to determine benz[*c*]acridines in environmental samples

Noboru Motohashi*

Meiji College of Pharmacy, 1-22-1, Yato-cho, Tanashi-shi, Tokyo 188 (Japan)

Kunihiro Kamata

Metropolitan Research Laboratory of Public Health, 24-1, Hyakunin-cho 3-chome, Shinjuku-ku, Tokyo 169 (Japan)

Roger Meyer

Allergan Herbert Laboratories, 2525 Dupont Drive, Irvine, CA 92715 (USA)

ABSTRACT

Benz[*c*]acridine and many of its related compounds have been shown to exhibit carcinogenic activity. Unfortunately, these compounds are continually being found in many natural and environmental samples in widely divergent geographical locations. A review of chromatographic methods for mainly benz[*c*]acridine and its analogues is presented.

* Corresponding author.

0021-9673/93/$24.00

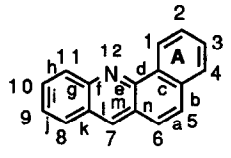
1. INTRODUCTION

Investigations into the composition of urban air are a recent phenomenon. It was not until the early 1950s that the 3,4-benzopyrene content of the urban atmosphere was reported [1,2]. Later, benzacridines of azaarenes were found in airborne particulates (Fig.1). Because of a lack of standard benzacridines with various attached alkyl groups, research in the biochemical and pollution fields lagged behind. This is important because the presence of a methyl group in a benzacridine molecule can cause a drastic change in its carcinogenic activity. For example, benz[*c*]acridine (**1**) and benz[*a*]acridine (**2**) are inactive as carcinogens on mouse skin, whereas their 7-methyl derivatives demonstrate very strong activity. Many of the other methyl derivatives also show carcinogenic activity [3,4]. In addition to skin adsorption, inhalation and ingestion may serve as routes of entry of these compounds. Consequently, the metabolism of these benz[*c*]acridines may lead to active carcinogens. Mutagenicity studies on the metabolites of these compounds, in bacteria and mammalian cells, indicate that the position of the nitrogen heteroatom can markedly affect the mutagenic activity [5]. Among dibenzacridines, dibenz[*a,j*]acridine (**4**) and dibenz[*a,h*]acridine (**5**) have been shown to be weak carcinogens [6,7], and dibenz[*c,h*]acridine (**6**) has also been shown to be carcinogenic [6].

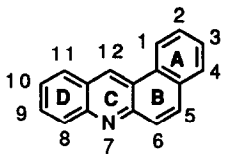
Many methods for the separation of benz[*c*]acridines have been investigated. For example, thin-layer chromatography (TLC) on silica gel 60 GF_{254} with benzene was used for the separation of a methyl-substituted benz[*c*]acridine mixture [8]. Reversed-phase high-performance TLC (RP-HPTLC) was also used for the same purpose [9]. Both gas–liquid chromatography (GLC) and high-performance liquid chromatography (HPLC) were applied for the separation of methyl-substituted benz[*c*]acridines [10]. Benz[*c*]acridines were separated by cation-exchange HPLC (CE-HPLC) with an ion-exchange column (Partisil 10 SCX) [11].

This paper reviews chromatographic methods for mainly benz[*c*]acridine (**1**) and analogues of these potentially carcinogenic and mutagenic benzacridines and dibenzacridines. Methods for the syntheses as standard benzacridines are not discussed here [12].

1. Benzacridines

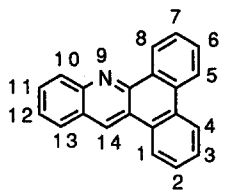


benz[*c*]acridine (**1**)
(1,2-benzacridine)

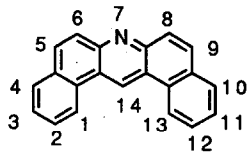


benz[*a*]acridine (**2**)
(3,4-benzacridine)

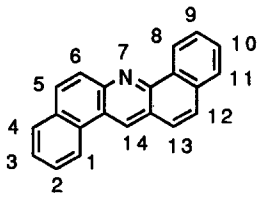
2. Dibenzacridines



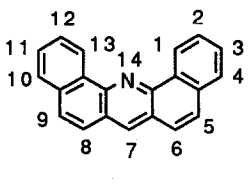
dibenz[*a,c*]acridine (**3**)
(1,2,3,4-dibenzacridine;
phenophenanthracridine)



dibenz[*a,j*]acridine (**4**)
(1,2,7,8-dibenzacridine;
3,4,6,7-dinaphthacridine;
β-naphthacridine)



dibenz[*a,h*]acridine (**5**)
(1,2,5,6-dibenzacridine;
α-N-β-dinaphth-
acridine)



dibenz[*c,h*]acridine (**6**)
(3,4,5,6-dibenzacridine;
α-naphthacridine;
α-N-a-dinaphthacridine)

Fig. 1. Structures of benzacridines and dibenzacridines.

2. URBAN AIR

2.1. Urban air particulates

Over the years, many researchers have presented methods for and results of air pollution studies. For example, benzacridines in air particulates from the effluents of air pollution sources were separated by column chromatography (CC) and TLC. By the use of the appropriate bands in their spectra, the benzacridines were determined by a baseline technique in combination with spectrofluorimetry of TLC fractions. Air pollution effluents of coal used for room heating showed that benz[*c*]acridine (**1**), benz[*a*]acridine (**2**), dibenz[*a,h*]acridine (**5**) and dibenz[*a,j*]acridine (**4**) were measured at concentrations of 15 and 18, 26 and 7.70, 17 and <0.12, and 2 and <0.015 mg per 1000 m^3 of gas, respectively [13]. Another study of an air pollution source of industrial effluents showed also benz[*c*]acridine (**1**), benz[*a*]acridine (**2**), dibenz[*a,h*]acridine (**5**) and dibenz[*a,j*]acridine (**4**). These compounds were determined at concentrations of 60, 18, 0.7 and 1.8 mg per 1000 m^3 of gas, respectively [13].

Another study, using a Technicon time-flow fraction collector, demonstrated the CC separation of organic airborne particulate fractions. For detection of benzacridines in a 1-year composite airborne particulate sample from downtown Nashville, TN, USA, TLC was used with dimethylformamide (DMF)–water (35:65) on cellulose after alumina CC fractions of the basic fraction were collected. The fluorescent spot of benzacridines was treated with trifluoroacetic acid fumes. By this method, benz[*c*]acridine (**1**), benz[*a*]acridine (**2**), dibenz[*a,h*]acridine (**5**) and dibenz[*a,j*]acridine (**4**) were identified. Additionally, some of the alkyl derivatives of benz[*c*]acridine (**1**) and dibenz[*a,j*]acridine (**4**) could be carcinogenic (Table 1) [14].

Azaarenes, including benzacridines, were determined by gas chromatography (GC) with a glass capillary column coated with SE-52. The instrument was equipped with dual-channel flame ionization and electron-capture detectors, with a dual electrometer connected to the dual channels. Samples of *ca.* 0.3–0.5 g of dust collected from 1000–2000 m^3 of air were extracted in a Soxhlet extractor for 12 h with 100 ml of cyclohexane. Benz[*c*]acridine (**1**), 8,10-dimethylbenz[*c*]acridine (**7**), 10-methylbenz[*c*]acridine (**8**), 10-methylbenz[*a*]acridine (**9**) and 1,10-dimethylbenz[*a*]acridine (**10**) in the extracts were detected by GC on SE-52 [15].

Benz[*c*]acridine (**1**), benz[*a*]acridine (**2**), dibenz[*a,h*]acridine (**5**), dibenz[*a,j*]acridine (**4**) and their alkylated counterparts were found as potentially carcinogenic benzacridines in six downtown urban atmospheres in the USA [16].

A simple TLC procedure was used to determine benz[*c*]acridine (**1**) in pollutants associated with airborne particulates. Benz[*c*]acridine (**1**) was separated from crude benzene-soluble compounds using pentane–diethyl ether (19:1) on TLC plates coated with equal volumes of aluminium oxide G and silica gel G. Benz[*c*]acridine (**1**) was measured spectrofluorimetrically ($\lambda_{em.}$ 290 nm, $\lambda_{em.}$ 470 nm) in trifluoroacetic acid. Crude benzene-soluble compounds of particulates collected from 51 American cities in January–June, 1966, were analysed and concentration

TABLE 1

BENZACRIDINES IN AN AVERAGE AMERICAN URBAN ATMOSPHERE

Compound	Benzene-soluble fraction (μg/g)	Airborne particulates (μg/g)	In 1000 m^3 of air (μg)
Benzacridines			
Benz[*c*]acridine (**1**)	50	4	0.6
Benz[*a*]acridine (**2**)	20	2	0.2
Dibenzacridines			
Dibenz[*a,h*]acridine (**5**)	7	0.6	0.08
Dibenz[*a,j*]acridine (**4**)	4	0.3	0.04

ranges of 0–1.5 μg per 1000 m^3 of air for benz[*c*]acridine (**1**) were determined [17].

Airborne particulate samples from a residential town were collected on Whatman GFA glass-fibre filters and extracted in a Soxhlet extractor with benzene. The benzene solution was separated into a basic fraction. For separation of the residue, GC was performed on a 5-m packed column containing 4% Dexsil 300 on Gas Chrom Q (100–120 mesh) support. From the relative retention times on the column and gas chromatography–mass spectrometry (GC–MS), azabenz[*a*]anthracenes such as benz[*c*]acridine (**1**) and benz[*a*]acridine (**2**), methylbenzacridines and dibenz[*a,h*]acridine (**5**) were detected down to a concentration of 4 ppm [18].

Airborne particulates in Taiyuan area (province) of China were analysed for organic pollutants by flexible quartz capillary column GC. One component was found at a concentration of 1.5–20 μg/m^3. This compound, with a molecular mass of 229 and one nitrogen atom, showed carcinogenicity and was tentatively identified as benz[*c*]acridine (**1**) [19].

Azaarenes were extracted from airborne particulate samples by toluene with sonication. The basic azaarenes in the toluene phase were then extracted with 8.25 *M* phosphoric acid. After this step, the toluene phase still contained various arene molecules. The phosphoric acid phases were combined and adjusted to pH 14 with 11 *M* potassium hydroxide. The azaarenes were extracted from this alkaline phase with dichloromethane. By capillary column GC (utilizing an Ultra-1 fused-silica column with a nitrogen-sensitive detector), benz[*a*]acridine (**2**), dibenz[*a,h*]acridine (**5**) and dibenz[*a,j*]acridine (**4**) were detected in the air of a suburban residential area and in a busy street in Copenhagen, Denmark, in the Februarys of 1976–82 at concentrations of 0.09 and 0.17, 0.2 and 0.08 and , 0.2 and 0.07 ng/m^3, respectively. Remarkably, the concentrations of dibenz[*a,j*]acridine (**4**) and dibenz[*a,h*]acridine (**5**) were 2.7 times higher in the residential area than in the busy street. This suggests that perhaps the heating of homes is a major source of dibenz[*a,j*]acridine (**4**) and dibenz[*a,h*]acridine (**5**), and that the emission rate of these compounds is much higher in combustion gases from home furnaces and stoves than that in car exhausts [20].

The azaarene fraction of an urban atmospheric particulate matter extract collected in Tokyo has been measured by HPLC on Zorbax ODS using acetonitrile–water (7:3). Benz[*a*]acridine (**2**), dibenz[*a,c*]acridine (**3**), dibenz[*a,j*]acridine (**4**), and dibenz[*a,h*]acridine (**5**) were detected at average concentrations of 3.3, 0.43, 0.29, and 0.36 mg/g, respectively [21].

GC–MS was used for the detection of azaarenes in the air of Calcutta, India. Benz[*a*]acridine (**2**) (1.06–4.76 ng/m^3), dibenz[*a,j*]acridine (**4**) (7.27–13.16 ng/m^3) and dibenz[*a,h*]acridine (**5**) (7.27–13.16 ng/m^3) were detected at higher levels than those found in European or American cities [22].

2.2. Additional air pollution source effluents

Samples of urban airborne particulates or air pollution source effluents were extracted with benzene–diethylamine (4:1) in a Soxhlet extractor. The residue, dissolved in dichloromethane, was placed on an alumina TLC plate and developed with pentane–diethyl ether (19:1). Benz[*c*]acridine (**1**) was determined spectrofluorimetrically ($\lambda_{ex.}$ 288 nm, $\lambda_{em.}$ 472 nm). The detection limit of benz[*c*]acridine (**1**) was 40 ng/ml [23].

2.3. Automobile exhaust

A basic fraction was chromatographed using pentane containing increasing 8% multiples of diethyl ether, up to 64%, on an alumina TLC column. Carcinogenic benzacridines, such as dibenz[*a,h*]acridine (**5**), dibenz[*a,j*]acridine (**4**) and the alkyl benz[*c*]acridines were detected in automobile exhaust at concentrations lower than 0.3 μg per gram of the benzene-soluble fraction, indicating that the carcinogenic effect of automobile exhaust in terms of these compounds is negligible compared with some other sources of pollution [24].

In Japan, benzacridines were detected in both diesel and gasoline engine vehicle exhausts in air samples taken in a road tunnel. The base extracted tars were collected and fractionated with a Soxhlet extractor. The solution was chromatographed on a two-dimensional TLC plate coated with alumina G + Kieselghur G [2:1% (w/w) and 26% acetylated cellulose]. 7,9-Dimethylbenz[*c*]acridine (**11**), 7,10-dimethylbenz[*c*]acridine (**12**), and dibenz[*a,h*]acridine (**5**) were identified. The average emission rates (μg/h per vehicle) of the three benzacridines

TABLE 2

AVERAGE EMISSION RATES OF BENZACRIDINES FROM DIESEL AND GASOLINE ENGINE VEHICLES

Compound	Average emission rate (μg/h)	
	Diesel	Gasoline
Benzacridines		
7,9-Dimethylbenz[*c*]acridine (**11**)	2.1	0.18
7,10-Dimethylbenz[*c*]acridine (**12**)	6.0	1.2
Dibenzacridine		
Dibenz[*a*,*h*]acridine (**5**)	54	6.5

from heavy-duty diesel engine vehicles were detected in larger amounts than those from light-duty gasoline engine cars, especially for 7,9-dimethylbenz[*c*]acridine (**11**). (Table 2) [25].

HPLC methods were developed for detecting benzacridines in atmospheric aerosols and particulates emitted by diesel and gasoline engines with a view to speed and high sensitivity (pg) by using fluorimetric detection ($\lambda_{ex.}$ 313 nm, $\lambda_{em.}$ 375 nm and $\lambda_{ex.}$ 366 nm, $\lambda_{em.}$ 425 nm) (Table 3) [26].

By GC with a 25-m cross-linked SE-54 fused-silica capillary column, benz[*c*]acridine (**1**) and benz[*a*]acridine (**2**) in diesel fuel were detected at concentrations of < 1 ppm [27].

TABLE 3

CONCENTRATIONS OF BENZACRIDINES AND DIBENZACRIDINES IN ATMOSPHERIC AEROSOLS FROM DIESEL AND GASOLINE ENGINES

Compound	Concentration (ng/m^3)	
	Diesel	Gasoline
Benzacridines		
Benz[*c*]acridine (**1**)	5.80	0.07
Benz[*a*]acridine (**2**	6.10	0.19
2-Methylbenz[*a*]acridine (**13**)	1.12	0.01
7-Methylbenz[*a*]acridine (**14**)	0.49	0.01
Dibenzacridines		
Dibenz[*a*,*h*]acridine (**5**)	0.82	
Dibenz[*a*,*j*]acridine (**4**)	0.84	
Dibenz[*c*,*h*]acridine (**6**)	0.72	

3. PETROLEUM DISTILLATES

3.1. Coal-tar pitch

Alumina CC was used for the separation of airborne particulate samples polluted by coal-tar pitch. The collected coal-tar polluted atmosphere was Soxhlet extracted with benzene. The basic fraction was separated and then chromatographed on an alumina column with pentane–diethyl ether, pentane–acetone, diethyl ether and then methanol. The fractions were separated further using DMF–water (35:65) on TLC plates coated with MN-300 G cellulose powder. Benz[*c*]acridine (**1**), alkylated benz[*c*]acridines, dibenz[*a*,*h*]acridine (**5**), benz[*a*]acridine (**2**), alkylated benz[*a*]acridines and dibenz[*a*,*j*]acridine (**4**) were all detected in coal-tar pitch in air pollution source effluents [28].

Dibenz[*a*,*h*]acridine (**5**) was detected spectrofluorimetrically ($\lambda_{ex.}$ 308 nm, $\lambda_{em.}$ 450 nm) from a CC fraction obtained from air polluted with coal-tar pitch. Prior to the scan, the sample was separated by cellulose TLC with DMF–water (35:65). The spots were treated with trifluoroacetic acid fumes before scanning [29].

A basic fraction of coal-tar pitch was analysed by two-dimensional TLC [solvent 1 = cyclohexane–ethyl acetate (19:1); solvent 2 = DMF–water (35:65)] on alumina–cellulose (2:1). Benz[*c*]acridine (**1**), benz[*a*]acridine (**2**) and dibenz[*a*,*h*]acridine (**5**) were detected in the coal-tar pitch [30].

A method for the rapid determination of benz[*c*]acridine (**1**) in urban airborne particulates and air pollution source effluents involves preliminary separation by one-dimensional TLC using pentane–diethyl ether (19:1) on alumina or two-dimensional TLC using pentane–diethyl ether (19:1) followed by DMF–water (35:65) on alumina–cellulose (2:1). Either preliminary separation step was followed by direct spectrofluorimetric detection, elution and spectrofluorimetric detection, or elution and filter fluorimetric detection [31].

After pretreatment by alkali extraction, cation-exchange chromatography on Amberlyst 15, LC on a polar bonded-phase silica OPN–Poral C and co-oxidation chromatography on $FeCl_3$–Chromosorb W for each azaarene, benz[*c*]acridine (**1**) was determined by GC on a 40-m glass support-coated open-tubular (SCOT) capillary column coated with

SP-2250 (50% methyl-, 50% phenylsilicone) stationary phase and GC–MS [32–34].

Exhaustive Soxhlet extractions of pitch performed near the boiling points of the solvents *n*-hexane and subsequently benzene yielded the following extracts: *n*-hexane solubles (HS), benzene solubles (BS) and *n*-hexane insolubles (HI). The concentrates were separated by gel permeation chromatography on a glass column filled with Sephadex LH-20 with tetrahydrofuran as eluent. The eluates were analysed using a UV detector at 254 nm. The basic concentrates were characterized by mass spectrometry using direct introduction of a sample into the ion source. From analysis of the HS-extract, benzacridine of average molecular mass 238 was detected at a concentration of 8.5% (v/v), and from analysis of BS and HI extracts, benzacridine of average molecular mass 239 was detected at a concentration of 7.1% (v/v) [35].

3.2. Creosote

Creosote oil was chromatographed using methanol–dichloromethane (1:4) on a silica gel–alumina column to obtain fractions enriched in benzacridines. The benzacridines were characterized by GC with a thermionic (nitrogen–phosphorus-specific) detector and a GC–MS system. Two benzacridines (isomers with a molecular mass of 229 but with different retention indices of 392.0 and 397.9) were detected at concentrations of 4900 and 600 μg/g, respectively. Three methylbenzacridines (isomers with a molecular mass of 243 but with different retention indices of 408.0, 409.9 and 416.6) were detected at concentrations of 71, 110 and 98 μg/g, respectively. The reference compound, dibenz[*a,j*]-acridine (**4**) (molecular mass 279; calculated retention index 489.6), showed a concentration of 0.62 μg/g with a measured retention index of 488.9 [36].

After selective enrichment including liquid–liquid acid (base) partioning, CC on Sephadex LH 20 and ion-exchange chromatography, basic fractions of a creosote oil were analysed by HPLC with fluorescence detection and subsequent capillary column GC with a thermionic detector. Additionally, a basic fraction was separated by HPLC and RP-HPTLC. The subfractions were followed by GC–MS and spectrofluorimetry. The concentration of benz[*c*]acridine (**1**), 9-methylbenz[*c*]acridine (**15**) and 10-methylbenz[*c*]acridine (**8**) found in creosote oil were 7.7, 18.4 and 192.7 μg/g, respectively [37].

3.3. High-boiling petroleum distillates

A base fraction was separated through a column containing Cellex-P cation-exchange cellulose. The non-reactive bases of the eluate from the Cellex-P column were passed through an acidic alumina column. The weakly held bases of each fraction were then placed on a basic alumina column. Subfractions showed partial fluorescence excitation and fluorescence emission spectra of benz[*c*]acridine (**1**) and benz[*a*]acridine (**2**) together with the corresponding spectra of a typical Wilmington petroleum sample. Comparison of the petroleum sample spectra with the spectra of benz[*c*]acridine (**1**) and benz[*a*]acridine (**2**) suggested that the petroleum sample contains a mixture of the two benzacridines [38].

3.4. Coal liquefaction products

Basic fractions in coal liquefaction products were analysed by GC with flame ionization detection (FID). The GC conditions consisted of a 40-m SGE glass SCOT capillary column coated with SP-2250 (50% methyl-, 50% phenylsilicone) stationary phase using hydrogen as the carrier gas for FID. Benz[*c*]acridine (**1**) was subsequently identified by GC–MS [39].

The azaarene fraction from a neutral alumina adsorption step was separated on a silicic acid adsorbent using benzene and diethyl ether eluents to provide an enriched azaarene fraction. A Hewlett-Packard (HP) Model 5880 gas chromatograph with a DB-5 fused-silica capillary column was used, with FID and ^{63}Ni electron-capture detection (ECD). Qualitative analysis of each fraction was also performed on an HP 5982A capillary column GC–MS system operated in the electron impact mode. Benz[*c*]acridine (**1**) was measured at 578, 1237 and 2357 ppm on 371–399°C, 399–427°C and 427–454°C fraction cuts from a solvent refined coal liquefaction process, respectively (SRC II) [40].

The basic fraction from asphaltene of a flash pyrolysis tar of Millmerran coal in Australia was fractionated by adsorption chromatography on silica gel. The column was eluted successively with light

petroleum (b.p. 40-60°C), light petroleum (b.p. 40-60°C)–toluene, toluene, chloroform and methanol. Benz[*c*]acridine (**1**) and dibenz[*a,h*]acridine (**5**) were identified in the basic fraction from the asphaltene on the basis of the similarity of their fluorescence emission and excitation spectra with those of known compounds. In contrast, the basic fraction from New Waterfield hydrogenation oil in South Africa was separated using Amberlyst 15 cation-exchange resin and fractionated by chromatography on acidic and then basic alumina. Benz[*a*]-acridine (**2**) was identified by comparison of the fluorescence excitation spectra in both neutral and acidic media with the reported UV spectra [41].

4. TOBACCO (SMOKE)

Hueper *et al.* [42] presented evidence that cigarette smoking is only one of many factors (including air pollution) that play a significant role in causing lung cancer. Wynder and Hoffman [43] cited evidence that cigarette smoke plays the major role in the etiology of lung cancer.

For analysis of tobacco pyrolysed at 850°C for azaarenes of the atmosphere, a scheme was designed to fractionate the tobacco smoke condensate. First, for bases, GC was applied using a stainless-steel column containing 15% Carbowax 20M on 60–80-mesh Chromosorb W. Second, TLC of azaarenes was carried out on glass plates coated with silica gel G using ethyl acetate–methanol–formic acid (80:10:10) and benzene–methanol (95:5) as eluents. Benzacridines were detected in tobacco and nicotine pyrolysates and tobacco smoke condensates [44].

Snook *et al.* [45] investigated benzacridine concentration in the basic fraction of cigarette smoke condensate. After gel chromatography on Bio-Beads S-X12 of cigarette smoke condensate in benzene, each individual gel fraction was submitted to GC on a column packed with 6% OV-17 on 100–120-mesh Chromosorb G/HP. The separated components of GC cuts were determined by GC–MS. No benzacridines were detected under these chromatographic conditions.

For extraction, basic compounds were collected by S-Sepharose ion-exchange chromatography, Sephadex LH-20 and again with S-Sepharose. GC with a fused-silica column of SE-54 with N-FID was used for the detection of particulate-bound azaarene extracts from mainstream smoke and sidestream smoke. Benz[*c*]acridine (**1**), benz[*a*]acridine (**2**) and 7-methylbenz[*c*]acridine (**16**) were detected in mainstream and sidestream smoke when compared with standard benz[*c*]acridines [46].

HPLC with a reversed-phase ODS column was used for azaarene extract separation. The best isocratic elution was with acetonitrile–water (75:25). Benz[*c*]acridine (**1**) and 9-methylbenz[*c*]acridine (**15**) were detected in cigarette smoke condensates [47].

5. SEDIMENTS

5.1. Lake sediment

Major peaks with estimated concentrations of identified benzacridines in two surface sediments and street dust were compared using GC and GC–MS. It was found that the levels of benz[*c*]acridine (**1**) and benz[*a*]acridine (**2**) were almost the same in Lake Zurich samples, whereas benz[*a*]acridine (**2**) was much more abundant than benz[*c*]acridine (**1**) in Lake Lucerne and street dust samples. A similar but more dramatic variation was observed for dibenz[*a,c*]acridine (**3**; 1,2,3,4-dibenzacridine) and dibenz[*a,j*]acridine (**4**, 1,2,7,8-dibenzacridine) (Table 4) [48].

5.2. River sediment

Samples were subjected to Soxhlet extraction with dichloromethane. The extract was preseparated on a Sephadex LH-20 column eluted with 2-propanol. The fraction containing benzacridines was analysed by fused-silica capillary GC and GC–MS. Benz[*c*]acridine (**1**; M_r 229), benz[*a*]acridine (**2**; M_r 229), dibenz[*a,c*]acridine (**3**; M_r 279) and dibenz-[*a,h*]acridine (**5**; M_r 279) were all identified from GC retention times and by molecular mass methods by comparison with standard samples [49].

5.3. Marine sediment

Organic extracts of marine sediments from Eagle Harbor, Puget Sound, WA, USA, were eluted using methanol–dichloromethane (1:4) on a silica gel–alumina column to obtain fractions enriched in benz-

TABLE 4

BENZACRIDINES AND DIBENZACRIDINES IDENTIFIED AND DETERMINED IN TWO SURFACE SEDIMENTS AND STREET DUST

Compound	Estimated concentration (ng/g)		
	Lake surface sediment		Street dust
	Zurich	Lucerne	
Benzacridines			
Benz[*c*]acridine (**1**)	45	3.9	525
Benz[*a*]acridine (**2**)	50	0.8	220
Dibenzacridines			
Dibenz[*a,c*]acridine (**3**) or dibenz[*a,h*]acridine (**5**)	35	5.0	260
Dibenz[*a,j*]acridine (**4**)	37	0.7	56

acridines. The benzacridines were characterized by GC with a thermionic (nitrogen–phosphorus-specific) detector and GC–MS. Two benzacridines (isomers of molecular mass 229 with retention indices of 392.0 and 398.3) were detected at concentrations of 7.7 and 8.5 μg/g, respectively. Three methylbenzacridines (isomers of molecular mass 243 with retention indices of 408.0, 410.0 and 416.6, respectively) were detected at concentrations of 1.0, 1.2 and 1.4 μg/g, respectively. The reference compound, dibenz[*a,j*]acridine (**4**; M_r 279; calculated retention index 489.6), showed a concentration of 0.62 μg/g with a measured retention index of 488.9 [36].

5.4. Groundwater

Groundwater samples contaminated by coal-tar wastes was analysed for azaarenes. GC with a wall-coated open-tubular, fused-silica capillary column coated with SE-54 and MS were utilized for the separation and identification of the basic extract after isolation by partitioning. Each isomer of benzacridines and dibenzacridines was identified in the oily-tar phase of groundwater [50].

Benz[*c*]acridine (**1**) and benz[*a*]acridine (**2**) in groundwater from Beijing, China, were separated by TLC with a mobile phase of pentane–diethyl ether (19:1) on silica gel G and identified by TLC

TABLE 5

BENZ[*c*]ACRIDINES AND BENZ[*a*]ACRIDINES IDENTIFIED IN GROUNDWATER IN BEIJING

Sampling point	Sampling point depth (m)	Benz[*c*]acridine (**1**, μg/l)[a]			Benz[*a*]acridine (**2**, μg/l)[b]		
		1	2	3	1	2	3
S1	46 (control)	0.059	0.023	0.083	D[d]	D	D
S2	70	0.360	0.049	0.090	D	D	D
S3	60–70	0.130	0.045	N.D.[c]	D	D	D
S4	12	0.039	0.010	N.D.	D	D	D
S5	60–70	Trace	0.015	0.102	Trace	D	D
S6	River water	0.069	0.056	0.193	D	D	D

[a] Recovery 65 ± 3%.
[b] Qualitative determination.
[c] N.D. = not detected.
[d] D = detected.

and MS. Concentrations of 0.01–0.40 μg/l of benz[*c*]acridine (**1**) were determined by spectrofluorimetry at 384 nm with recoveries of 65 ± 3% (Table 5) [51].

6. FOOD (HAM)

A method for the determination of benzacridines in meat was investigated by liquid–liquid partitioning (dimethylformamide–water–cyclohexane). Extraction of benzacridines was accomplished with sulphuric acid, re-extraction after neutralization by cyclohexane or, alternatively, by non-adsorbing ion-exchange chromatography. Further purification was performed by CC on Sephadex LH-20. Benzacridines were separated by capillary column GC and measured by comparing the corresponding peak areas with those of an internal standard such as 10-azabenzo[*a*]pyrene. The detection limit of this method ranges from 0.1 to 0.4 ng for benzacridines. The relative standard deviations (R.S.D.s) for reference benz[*c*]acridine (**1**), 8,10-dimethylbenz[*c*]-acridine (**7**), dibenz[*c,h*]acridine (**6**), dibenz[*a,h*]-acridine (**5**), and dibenz[*a,j*]acridine (**4**) in comparison with 10-azabenzo[*a*]pyrene as internal standard were 13.6, 4.0, 5.5, 5.2 and 5.8% for the analysis, respectively. The R.S.D.s for benz[*c*]acridine (**1**), dibenz[*c,h*]acridine (**6**), dibenz[*a,h*]acridine (**5**) and dibenz[*a,j*]acridine (**4**) in spiked meat samples were 10.4, 22.3, 10.7 and 25.4%, respectively [52,53].

REFERENCES

1 R. E. Waller, *Br. J. Cancer*, 6 (1952) 8.
2 P. Kotin., H. L. Falk and P. Mader, *Arch. Ind. Hyg. Occup. Med.*, 9 (1954) 153.
3 G. M. Badger, *Br. J. Cancer*, 2 (1948) 309.
4 A. Lacassagne, N. P. Buu-Hoi, R. Daudel and F. Zajdela, *Adv. Cancer Res.*, 4 (1956) 315.
5 A. W. Wood, R. L. Chang, W. Levin, D. E. Ryan, P. E. Thomas, R. E. Lehr, S. Kumar, M. Schaefer-Ridder, U. Engelhardt, H. Yagi, D. M. Jerina and A. H. Conney, *Cancer Res.*, 43 (1983) 1656.
6 A. Dipple, in C. E. Searle (Editor), *Chemical Carcinogens (ACS Monograph*, No. 173), American Chemical Society, Washington, DC, 1976, pp. 174–179.
7 M. L. Lee, M. V. Novotny and K. D. Bartle, *Analytical Chemistry of Polycyclic Aromatic Compounds*, Academic Press, New York, 1981.
8 N. Motohashi and K. Kamata, *Yakugaku Zasshi*, 103 (1983) 795.
9 K. Kamata and N. Motohashi, *J. Chromatogr.*, 396 (1987) 437.
10 K. Kamata and N. Motohashi, *J. Chromatogr.*, 319 (1985) 331.
11 K. Kamata and N. Motohashi, *J. Chromatogr.*, 498 (1990) 129.
12 R. M. Acheson, *Acridines (The Chemistry of Heterocyclic Compounds, a Series of Monographs*, Vol. 9), Interscience, New York, 2nd ed., 1973.
13 E. Sawicki, J. E. Meerker and M. J. Morgan, *Int. J . Air Water Pollut.*, 9 (1965) 291.
14 E. Sawicki, S. P. McPherson, T. W. Stanley, J. Meeker and W. C. Elbert, *Int. J. Air Wat. Pollut.*, 9 (1965) 515.
15 G. Alberini, V. Cantuti and G. P. Cartoni, *Gas Chromatogr., 1966 Int. Symp. Anal. Instrum. Div. Instrum. Soc. Am.*, 6 (1967) 258.
16 E. Sawicki, *Arch. Environ. Health.*, 14 (1967) 46.
17 T. W. Stanley, M. J. Morgan and E. M. Grisby, *Environ. Sci. Technol.*, 2 (1968) 699.
18 W. Cautreels and K. V. Cauwenberghe, *Atmos. Environ.*, 10 (1976) 447.
19 A. Zhang and Y. Liu, *Huanjing Huaxue*, 4, No. 5 (1985) 21.
20 T. Nielsen and P. Clausen, *Anal. Chim. Acta*, 187 (1986) 223.
21 T. Yamauchi and T. Handa, *Environ. Sci. Technol.*, 21 (1987) 1177.
22 D. Chakraborti, L. V. Vaeck and P. V. Espen, *Int. J. Environ. Anal. Chem.*, 32 (1988) 109.
23 E. Sawicki, T. W. Stanley and W. C. Elbert, *J. Chromatogr.*, 26 (1967) 72.
24 E. Sawicki, J. E. Meerker and M. Morgan, *Arch. Environ. Health*, 11 (1965) 773.
25 T. Handa, T. Yamauchi, K. Sawai, T. Yamamura, Y. Koseki and T. Ishii, *Environ. Sci. Technol.*, 18 (1984) 895.
26 P. Masclet, M. A. Bresson, S. Beyne and G. Mouvier, *Analusis*, 13 (1985) 401.
27 P. T. Williams, K. D. Bartle and G. E. Andrews, *Fuel*, 65 (1986) 1150.
28 E. Sawicki, T. W. Stanley and W. C. Elbert, *J. Chromatogr.*, 18 (1965) 512.
29 E. Sawicki, T. W. Stanley and W. C. Elbert, *J. Chromatogr.*, 20 (1965) 348.
30 E. Sawicki, T. W. Stanley, S. P. Mcpherson and M. Morgan, *Talanta*, 13 (1966) 619.
31 E. Sawicki, T. W. Stanley and W. C. Elbert, *Prepr. Pap. Natl. Meet. Div. Water Air Waste Chem. Am. Chem. Soc.*, 6, No. 2 (1966), 111.
32 P. Burchill, A. A. Herod, J. P. Mahon and E. Pritchard, *J. Chromatogr.*, 265 (1983) 223.
33 P. Burchill, A. A. Herod, J. P. Mahon and E. Pritchard, *J. Chromatogr.*, 281 (1983) 109.
34 P. Burchill, A. A. Herod and E. Pritchard, *Fuel*, 62 (1983) 11.
35 J. Cerny, J. Mitera and P. Vavrecka, *Fuel*, 68 (1989) 596.
36 C. A. Krone, D. G. Burrows, D. W. Brown, P. A. Robisch, A. J. Friedman and D. C. Malins, *Environ. Sci. Technol.*, 20 (1986) 1144.
37 N. Motohashi, K. Kamata and R. Meyer, *Environ. Sci. Technol.*, 25 (1991) 342.
38 J. F. Mckay, J. H. Weber and D. R. Latham, *Anal. Chem.*, 48 (1976) 891.
39 P. Burchill, A. A. Herod and E. Pritchard, *J. Chromatogr.*, 246 (1982) 271.

40 D. W. Later, R. A. Pelroy, D. D. Mahlum, C. W. Wright, M. L. Lee, W. C. Weimer and B. W. Wilson, in M. Cook and A. J. Dennis (Editors), *Polynuclear Aromatic Hydrocarbons, 1982 International Symposium, 7th*, Battelle Press, Columbus, OH, 1983, 771.
41 J. R. Kershaw, *Fuel*, 62 (1983) 1430.
42 W. C. Hueper, P. Kotin, E. C. Tabor, W. W. Payne, H. Falk and E. Sawicki, *Arch. Pathol. Lab. Med.*, 74 (1962) 89.
43 E. L. Wynder and D. Hoffman, *J. Air Pollut. Control Assoc.*, 15 (1965) 155.
44 I. Schmeltz, W. S. Schlotzhauer and E. B. Higman, *Beitr. Tabakforsch. Int.*, 6 (1972) 134.
45 M. E. Snook, P. J. Fortson and O. T. Chortyk, *Beitr. Tabakforsch. Int.*, 11 (1981) 67.
46 G. Grimmer, K.-W. Naujack and G. Dettbarn, *Toxicol. Lett.*, 35 (1987) 117.
47 K. Kamata, N. Motohashi, R. Meyer and Y. Yamamoto, *J. Liq. Chromatogr.*, 15 (1992) 1907.
48 S. G. Wakeham, *Environ. Sci. Technol.*, 13 (1979) 1118.
49 S. Sun, F. He and and X. Wang, *Huanjing Kexue Xuebao*, 5, No. 2 (1985) 219.
50 W. E. Pereira, C. E. Rostad, J. R. Garbarino and M. F. Hult, *Environ. Toxicol. Chem.*, 2 (1983) 283.
51 S. Lei, Y. Bian, S. Liu and X. Zhao, *Huanjing Kexue Xuebae*, 5, No. 1 (1984) 33.
52 G. Grimmer, *Pure Appl Chem.*, 55 (1983) 2067.
53 G. Grimmer and K.-W. Naujack, *J. Assoc. Off. Anal. Chem.*, 69 (1986) 537.