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Determination of 7-methylbenz[*c*]acridines by capillary gas chromatography with electron-capture detection

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ABSTRACT

A sensitive gas chromatographic procedure for the determination of 7-methylbenz[*c*]acridines (7-methyl-BAcs) is presented. The 7-methyl-BAcs are easily oxidized to 7-formylbenz[*c*]acridines (7-formyl-BAcs) with periodic acid in dimethyl sulfoxide. The 7-formyl-BAcs are then reacted directly with *p*-fluoroaniline by way of Schiff base formation. The Schiff bases are analyzed by gas chromatography using a 25-m HP-5 column with electron-capture detection. The electron-capture response for the Schiff bases is very sensitive and amounts down to 20 pg are easily detected. Mass spectral data for the Schiff bases obtained under electron inpact conditions are also presented.

INTRODUCTION

The analytical study of 7-methylbenz[*c*]acridines (7-methyl-BAcs) has received considerable interest as a number of these compounds, such as 7-methylbenz[*c*]acridine and 7,10-dimethylbenz[*c*]acridine, are known carcinogens in some experimental animals and are suspected carcinogens or mutagens in other species [1–5]. 7-Methyl-BAcs have been identified in a wide variety of sources, such as tobacco smoke [6,7], automobile exhausts [8,9], coal liquefaction products [10,11], petroleum products [12,13], shale oil [14], creasote oil [15], coal tar [16,17] and lake [18] and marine sediments [19]. The determination of 7-methyl-BAcs in these samples has proved to be difficult because of their low concentrations and because the widely varying samples contain a vast number of potentially interfering compounds. Analytical techniques are required that are not only extremely sensitive but also highly selective.

The determination of 7-methyl-BAcs has been carried out by different chromatographic techniques, including high-performance liquid chromatography [6,20–25] and gas chromatography (GC) [6,9,10,13,16–21,26–28]. With the latter, the best separations of 7-methyl-BAcs are achieved with capillary column GC. Generally, successful GC

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methods have used flame ionization (FID) or nitrogen-specific detection (N-FID). Although these methods are routinely used, they lack the sensitivity required for many environmental samples.

GC with electron-capture detection (ECD) has been demonstrated to be a very sensitive tool for the determination of low concentrations of a variety of compounds. Compounds with low or no electron-capture response can usually be made electron-capture sensitive by means of derivatization. However, in order to detect 7-methyl-BAcs by ECD, it is necessary to employ a derivatizing agent with the desired detectable functionality.

The purpose of this paper is to demonstrate the selectivity and highly sensitive detection of 7-methyl-BAcs by GC–ECD. It was found that the method presented here is over 100 times more sensitive than the N-FID method for the determination of 7-methyl-BAcs. Resolution differences of the Schiff bases in capillary columns with three different stationary phases are discussed. The mass spectral data for the 7-methylbenz[*c*]acridine oxide (formyl type) and Schiff base forms obtained with electron impact (EI) ionization are also presented.

EXPERIMENTAL

Materials

The following 7-methyl-BAcs were synthesized according to the literature and purified as described [20]: 7-methylbenz[*c*]acridine, 5,7-dimethylbenz[*c*]-acridine, 7,9-dimethylbenz[*c*]acridine, 7,10-dimethylbenz[*c*]acridine, 7,11-dimethylbenz[*c*]acridine, 7,9,10-trimethylbenz[*c*]acridine and 7,9,11-trimethylbenz[*c*]acridine. Stock standard solutions were prepared by dissolving each 7-methyl-BAc in dimethyl sulfoxide (DMSO) to give a concentration of 100 μg/ml. Working standard solutions were prepared by diluting the stock standard solutions with DMSO.

Periodic acid, DMSO, and *p*-fluoroaniline were purchased from Nacalai Tesque (Kyoto, Japan) and 1-chloro-9,10-diphenylanthracene (used as an internal standard) from Aldrich (Milwaukee, WI, USA). The internal standard solution for GC–ECD was prepared by dissolving 1.0 μg of 1-chloro-9,10-diphenylanthracene in 1 ml of methanol. Solvents used were of analytical-reagent grade.

Derivatization procedure

Periodic acid (30 mg) was added to a 2.0-ml aliquot of the DMSO solution (0.02–10 μg of each 7-methyl-BAc) in a 10-ml reaction vial. The reaction was allowed to proceed for 50 min at 100°C and, after cooling, the solution was transferred into a 200-ml separatoring funnel with 50 ml of water. To the separating funnel 5 ml of 0.05 *M* sodium thiosulfate was added and then the contents were back-extracted twice with 50-ml portions of diethyl ether with vigorous shaking for 5 min. The combined diethyl ether portion was washed with 25 ml of water, transferred into a round-bottomed flask and evaporated to dryness at 40°C under reduced pressure in a rotary evaporator. After addition of 2 ml of a methanolic solution of *p*-fluoroanaline (1 mg/ml), the solvent mixture was evaporated to dryness at 40°C. The flask was then heated for 20 min at 60°C. After cooling, 1 ml of the internal standard solution was added to the reaction mixture. The mixture was then analyzed by GC–ECD under the described conditions.

Gas chromatography (GC)

GC was carried out on a Varian Model 3600 gas chromatograph equipped with a ^{63}Ni electron-capture detector and a thermionic specific detector. The column was a 25 m × 0.2 mm I.D. fused-silica capillary coated with HP-5 (Hewlett-Packard) at a film thickness of 0.33 μm. The injector temperature was 300°C. A 1-μl volume of the reaction mixture was injected (splitless) at a detector temperature of 350°C and an oven temperature of 280°C. Helium as carrier gas at a flow-rate of 2.0 ml/min and nitrogen as make up gas at a flow-rate of 20 ml/min were used. The GC–thermionic specific detection (TSD) conditions were the same as the GC–ECD conditions except that the make-up gas was helium.

Gas chromatography–mass spectrometry (GC–MS)

EI ionization mass spectra were obtained on an Automass 50 mass spectrometer (JEOL) interfaced with a Hewlett-Packard Model 5890 gas chromatograph. The operating conditions for all GC–EI-MS experiments were as follows: electron energy, 70 eV; filament emission current, 0.3 nA; source temperature, 140°C; injection port temperature, 280°C; column, HP-1 (25 m × 0.2 mm I.D.), 0.33-μm film thickness; initial column temperature, 230°C for 2

min, increased at 10°C/min to a final temperature of 300°C, held for 20 min; carrier gas, helium at a flow-rate of *ca.* 1 cm^3/min.

RESULTS AND DISCUSSION

Buu-Hoi *et al.* [29] found that selenium dioxide oxidation was specific for the conversion of a methyl group at the *meso*-position into a formyl group with excellent yields. For instance, 7-methyl-, 7,9-dimethyl- and 7,9,10-trimethylbenz[*c*]acridine readily formed 7-formylbenz[*c*]acridine (64% yield), 7-formyl-9-methylbenz[*c*]acridine (50% yield) and 7-formyl-9,10-dimethylbenz[*c*]acridine (45% yield), respectively. However, these reactions require a higher yield for analytical techniques. Seven oxidation reagents (selenium dioxide, periodic acid, potassium dichromate, vanadium pentoxide, potassium permanganate, copper oxide and hydrogen peroxide) were tested for the conversion of 7-methyl-BAcs into 7-formyl-BAcs. Each yield of 7-formyl-BAc using these oxidation reagents was determined by integrating the GC–TSD area counts. It was found that the best yield of 7-formyl-BAcs was obtained by oxidation with periodic acid, and this reagent was able to attack only the methyl group on position 7. The benz[*c*]acridines without a 7-methyl group, such as benz[*c*]acridine, 8-methylbenz[*c*]-acridine, 9-methylbenz[*c*]acridine, 10-methylbenz[*c*]acridine and 11-methylbenz[*c*]acridine, are not oxidized by periodic acid.

Further, eleven organic solvents (DMSO, methanol, chloroform, hexane, tetrahydrofuran, acetonitrile, acetone, N,N-dimethylformamide, carbon tetrachloride, carobn disulfide and 1,4-dioxane) were tried to determine the differences of the reaction system in each solvent. The result was that DMSO proved to be the best solvent in periodic acid oxidation. The combination of periodic acid and DMSO to convert 7-methyl-BAcs to 7-formyl-BAcs gave essentially 100% yields.

The relationship between the amount of periodic acid in DMSO and yield of 7-formyl-BAcs was examined by changing the concentration from 0.01 to 100 mg per 10 μg of 7,9,11-trimethylbenz[*c*]acridine. Of all compounds tested, 7,9,11-trimethylbenz[*c*]acridine proved to be the most difficult to oxidize in this manner. The yield of 7-formyl-9,11-dimethylbenz[*c*]acridine increased with increasing strength of the periodic acid (between 0.01 and 20 mg) but the increased values became constant in the range 20–100 mg. From these results, the actual amount of periodic acid was chosen to be 30 mg. Fig. 1 shows the effects of the reaction time and reaction temperature on the yield of 7-formyl-9,11-dimethylbenz[*c*]acridine (the reaction time was shortened with increase in reaction temperature). Therefore, the reaction temperature and time adopted were 100°C and 50 min, respectively.

The reaction of aldehydes to form 2,4-dinitrophenylhydrazones is widely used in carbonyl group analysis, which can be followed by GC–FID [30] and GC–ECD [31,32]. The reaction between 2,4-dinitrophenylhydrazine and carbonyl compounds is extremely specific. Schiff base (2,4-dinitrophenylhydrazone) formation by aldehydes is very easy but the resulting solubility of the base is low in many solvents. In spite of the poor solubility, Schiff bases give two peaks in GC. The coupling reaction of aniline with an aldehyde forms a Schiff base in high yield. Because GC–ECD is much more sensitive to

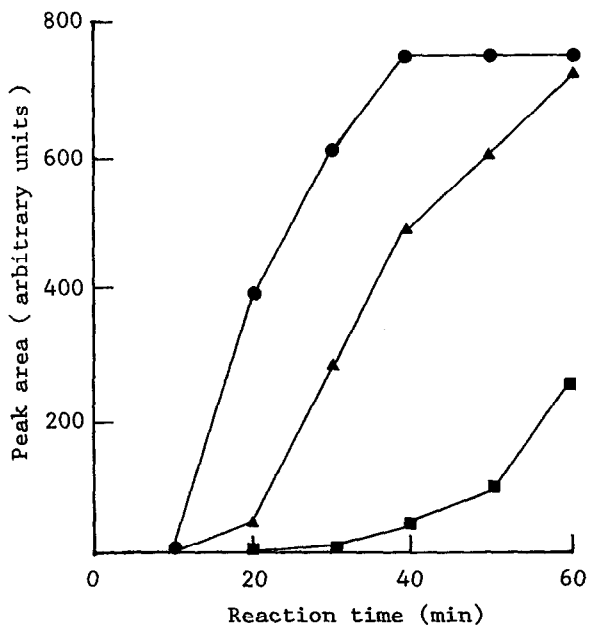

Fig. 1. Effect of reaction time and reaction temperature on the oxidation product after addition of periodic acid to 7,9,11-trimethylbenz[*c*]acridine in DMSO. To 10 μg of 7,9,11-trimethylbenz[*c*]acridine were added 30 mg of periodic acid in 2 ml of DMSO and the product was analyzed by GC–TSD, (●) at 100°C, (▲) at 80°C and (■) at 60°C.

the fluoro compounds than non-fluoro compounds, the use of fluorinated Schiff bases makes it possible to determine these compounds with much lower detection limits. In this work, *p*-fluoroaniline was chosen as a derivatizing agent to form Schiff bases with 7-formyl-BAcs. The reaction of 7-formyl-BAcs with *p*-fluoroanaline easily gives a Schiff base.

To improve the reaction efficiency of Schiff base formation with 7-formyl-BAcs and *p*-fluoroanaline, reaction times and temperatures were investigated. The reaction efficiency could be improved by increasing both the reaction time and the reaction temperature. However, the presence of solvent during the reaction resulted in a poor yield. Therefore, the solvent was first evaporated to dryness at 40°C and then formation of the Schiff base was carried out at 60°C for 20 min. The relationship between *p*-fluoroaniline concentration and yield of Schiff base with 7-formyl-BAcs was examined by varying the amount of *p*-fluoroaniline between 0.1 and 5.0 mg for amounts of 7-formyl-BAcs corresponding to 10 μg of 7-methyl-BAcs. The yield of Schiff base increased with increasing amount of *p*-fluoroaniline (0.1–1.0 mg), but remained constant in the range 1.0–5.0 mg. Consequently, the amount of *p*-fluoroaniline chosen was 2.0 mg.

The use of *p*-fluoroaniline with the Schiff base of 7-formyl-BAcs led to orders of magnitude greater sensitivity and selectivity when coupled with ECD. Whereas nanogram amounts can be determined using GC–TSD, amounts about 100 times lower can only be determined using GC–ECD.

The Schiff bases were very stable under normal laboratory conditions and no thermal decomposition products were observed in the GC analysis.

The structure of each Schiff base was confirmed by GC–MS. The mass spectra of 7-methylbenz[*c*]acridine, 7-formylbenz[*c*]acridine and the Schiff base are shown in Fig. 2. The mass spectrum corresponding to the peak obtained by oxidation of 7-methylbenz[*c*]acridine is shown in Fig. 2B with ion peaks of *m/z* 257 (M^+) and 229 $[M-28]^+$. The parent peak is at *m/z* 243 for 7-methylbenz[*c*]acridine (see Fig. 2A) and at *m/z* 257 for 7-formylbenz[*c*]acridine. All 7-formyl-BAcs generally produced the same prominent fragmentation pattern and each mass spectrum was dominated by a single unique $[M-28]^+$ fragment ion in the high-molecular-weight region.

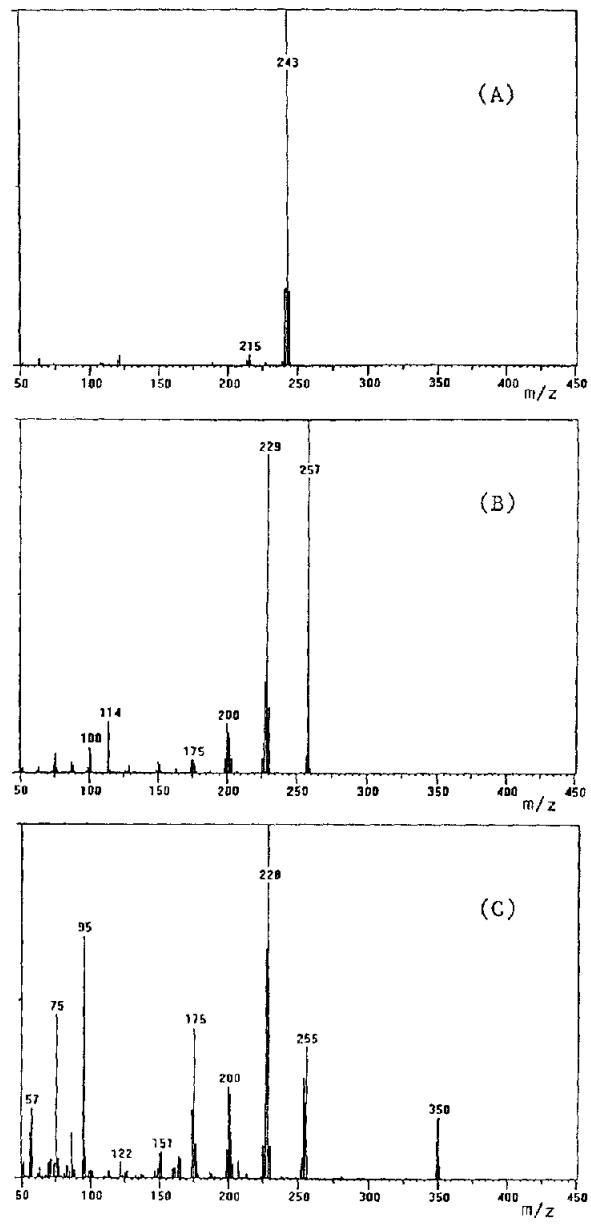

Fig. 2. EI mass spectra of (A) 7-methylbenz[*c*]acridine, (B) its oxidation product (7-formylbenz[*c*]acridine) and (C) its Schiff base.

In relation to the reaction mechanism of benzaldehyde, MacCollum and Meyerson [33] studied its synthesis with labelled-deuterium and Natalis and Franklin [34] measured the energetic values. They both concluded that the direct loss of the aldehyde

substituent with retention of charge on the fragment with oxygen occurs only to a small extent, just like the removal of carbon monoxide (CO) from the molecular ion to give $[C_6H_6]^+$ (*m/z* 78). From their conclusions, for instance, 7-formylbenz[*c*]acridine similarly showed that the $[M-28]^+$ fragment ion was formed by removal of one carbon monoxide function from 7-formylbenz[*c*]acridine. From the peak at *m/z* 229, $[M-CO]^+$, it was concluded that the product, 7-formylbenz[*c*]acridine, has a formyl substituent.

The mass spectrum corresponding to the peaks of the Schiff base is shown Fig. 2C. The molecular ion peak (M^+) with postulated *m/z* 350 and the prominent fragment ion peaks, *m/z* 255, $[M-95]^+$, and 228, $[M-122]^+$, were observed and were useful for structure assignments. The *m/z* 255 fragment ion was assigned as loss of the fluorobenzene radical, $C_6H_4F\cdot$, from the molecular ion. The base peak at *m/z* 228, $[C_{17}H_{10}N]^+$, came from the molecular ion by dissociation of $CHN-C_6H_4F$.

The proposed reaction mechanism for 7-methylbenz[*c*]acridine is shown in Fig. 3.

The chromatographic resolution of the Schiff bases was attempted on capillary columns with three different stationary phases (DB-17, HP-5 and HP-1). The retention times for seven Schiff bases on the three columns are given in Table I. All the columns were found to be suitable for the analysis of Schiff bases, but the less polar HP-1 and HP-5 columns were better than the DB-17 column. The elution order of Schiff bases was the same on all three columns. The HP-1 and HP-5 columns have similar

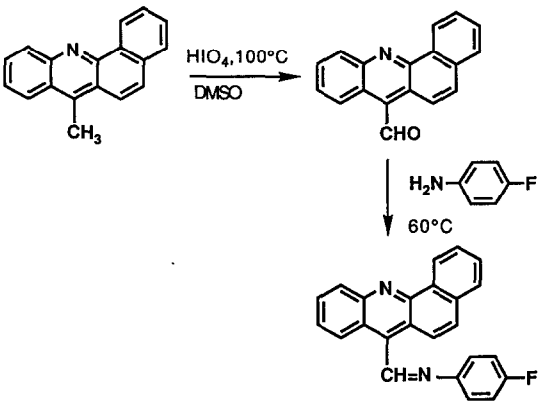

Fig. 3. Oxidation of 7-methylbenz[*c*]acridine by periodic acid and its Schiff base.

TABLE I

RETENTION TIMES OF SCHIFF BASES OF 7-METHYLBENZ[*c*]ACRIDINES ON THREE CAPILLARY COLUMNS

7-Methylbenz[*c*]acridine	Retention time (min)		
	HP-1[a]	HP-5[b]	DB-17[c]
7-Methylbenz[*c*]acridine	24.71	23.66	35.23
5,7-Dimethylbenz[*c*]acridine	31.27	28.79	42.80
7,9-Dimethylbenz[*c*]acridine	29.07	27.60	39.58
7,10-Dimethylbenz[*c*]acridine	31.90	30.22	42.86
7,11-Dimethylbenz[*c*]acridine	28.52	26.91	37.77
7,9,10-Trimethylbenz[*c*]acridine	41.64	39.22	57.25
7,9,11-Trimethylbenz[*c*]acridine	33.08	30.85	41.49

[a] 25 m × 0.20 mm I.D.; column temperature 270°C.
[b] 25 m × 0.20 mm I.D.; column temperature 280°C.
[c] 30 m × 0.25 mm I.D.; column temperature 280°C.

TABLE II

LINEAR REGRESSION DATA FOR SCHIFF BASES OF 7-METHYLBENZ[*c*]ACRIDINES

7-Methylbenz[*c*]acridine	Linear range of calibration graph (μg)	Linear equation of regression line[a]	Linearity (*r*)
7-Methylbenz[*c*]acridine	0.05–0.50	$y = 7.498x - 0.041$	0.9970
5,7-Dimethylbenz[*c*]acridine	0.05–0.50	$y = 8.580x + 0.001$	0.9992
7,9-Dimethylbenz[*c*]acridine	0.05–0.50	$y = 9.063x + 0.006$	0.9997
7,10-Dimethylbenz[*c*]acridine	0.05–0.50	$y = 7.533x + 0.014$	0.9998
7,11-Dimethylbenz[*c*]acridine	0.05–0.50	$y = 5.297x + 0.012$	0.9997
7,9,10-Trimethylbenz[*c*]acridine	0.05–0.50	$y = 8.643x + 0.181$	0.9958
7,9,11-Trimethylbenz[*c*]acridine	0.05–0.50	$y = 6.593x - 0.056$	0.9973

[a] y = Peak-area ratio; x = amount of 7-methylbenz[*c*]acridine.

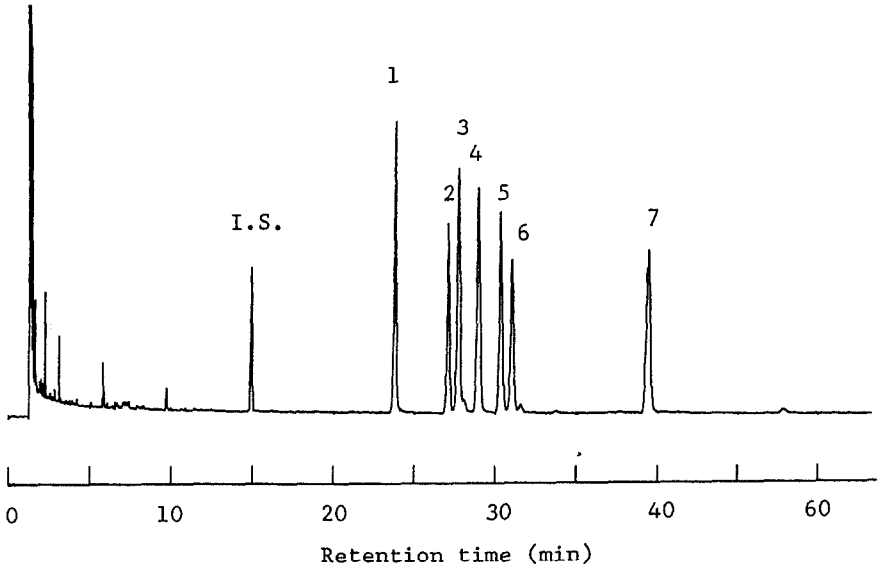

Fig. 4. GC–ECD of the Schiff bases of 7-methylbenz[*c*]acridine on the HP-5 column. For GC conditions, see Experimental. Peaks: 1 = 7-methylbenz[*c*]acridine; 2 = 7,11-dimethylbenz[*c*]acridine; 3 = 7,9-dimethylbenz[*c*]acridine; 4 = 5,7-dimethylbenz[*c*]acridine; 5 = 7,10-dimethylbenz[*c*]acridine; 6 = 7,9,11-trimethylbenz[*c*]acridine; 7 = 7,9,10-trimethylbenz[*c*]acridine; I.S. = 1-chloro-9,10-diphenylanthracene (internal standard).

resolving powers, the Schiff bases of 7-formyl-9-methylbenz[*c*]acridine and 7-formyl-11-methylbenz[*c*]acridine showed better resolution on the HP-5 than on the HP-1 column.

A typical chromatogram of seven Schiff bases is shown in Fig. 4. Each Schiff base displayed a single, symmetrical peak.

In order to test the linearity of the calibration graph, different amounts of 7-methyl-BAcs were prepared as previously described. A linear relationship was confirmed and the reproducibility was found to be satisfactory (Table II). The detection limit of 7-methyl-BAcs in this procedure was 20 pg (signal-to-noise ratio = 2).

CONCLUSION

An improved method for the determination of 7-methyl-BAcs by GC–ECD based on Schiff bases formed by the reaction of 7-formyl-BAcs with *p*-fluoroaniline was established. This method is satisfactory for the sensitive and selective determination of 7-methyl-BAcs.

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