Rapid Determination of Benzo[a]pyrene in Roasted Coffee and Coffee Brew by High-Performance Liquid Chromatography with Fluorescence Detection

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A rapid and reliable analytical method is presented for the determination of trace amounts of benzo[a]pyrene in roasted coffee, coffee brew, and spent grounds. Roasted coffee and spent grounds were extracted with acetone, followed by saponification and cyclohexane extraction. Coffee brew was extracted three times with cyclohexane, and the combined extracts were purified by chromatography on a silica gel column. The extracts were analyzed by HPLC with a 5-μm Vydac reversed-phase 201 TPB 5 column and fluorescence detection under isocratic conditions. The benzo[a]pyrene levels in 55 roasted coffee samples, commercially available in The Netherlands, ranged from not detectable (<0.1 μg/kg) to 0.5 μg/kg. Coffee brews were prepared by two different methods from an over-roasted coffee sample with an elevated benzo[a]pyrene level of 2 μg/kg. These brews yielded benzo[a]pyrene contents of approximately 1 ng/L, indicating benzo[a]pyrene extraction yields of about 1% for both coffee preparation methods.

Polycyclic aromatic hydrocarbons (PAH) are fused-ring compounds formed in both natural and manmade processes and are found widely distributed throughout the human environment.

The established carcinogenic activity of many PAH (Zedeck, 1980) and their extensive environmental occurrence have led to a widespread interest in the development of analytical methods for isolating and measuring various PAH in different matrices, such as air, water, soil, and foods.

The analytical methodology and the reported findings of PAH in foods, beverages, and related products have been reviewed by Howard and Fazio (1980).

The presence of PAH in foods originates mainly from endogenous formation by plants or other organisms, environmental pollution, food packaging, and food processing (Lo and Sandi, 1978). Lickint (1938) first indicated the possible formation of carcinogenic hydrocarbons during the roasting of coffee beans. Later, various studies were conducted on the PAH content of roasted coffee (Kuratsune and Hueper, 1960; Fritz, 1968; Bories and Gasc, 1980; Guyot et al., 1982), coffee soots (Kuratsune and Hueper, 1958), coffee oil (Strobel, 1973), and coffee substituents (Fritz, 1968). Levels of benzo[a]pyrene in normal roasted coffee ranging from 0.1 to 0.8 μg/kg have been obtained by various investigators using several analytical methods (Fritz, 1968; Lintas et al., 1979; Bories and Gasc, 1980; Guyot et al., 1982).

Although most investigators have reported rather low benzo[a]pyrene levels for normal roasted coffee, more than once there have been rumors about the occurrence of benzo[a]pyrene in roasted coffee in France and in The Netherlands. These rumors appear to have been based on erroneous results obtained by the application of inefficient and laborious sample preparation procedures and analytical methods that are susceptible to interferences.

Only a few authors have reported the determination of PAH in coffee brew (Kuratsune and Hueper, 1960; Fritz, 1969; Lintas et al., 1979; Guyot et al., 1982). In these studies most attention has been focused on the determination of benzo[a]pyrene, which is widely considered to be a general indicator of the presence of PAH in a sample. Levels of benzo[a]pyrene ranging from 0.006 to 0.3 μg/kg were found in coffee brews, indicating benzo[a]pyrene extraction yields varying from about 25 to 80%.

The apparent susceptibility to interferences, which manifests itself in false-positive results and interlaboratory variation, has demonstrated the need for a standardized, selective, and sensitive determination of trace amounts of benzo[a]pyrene in coffee samples.

In this paper a rapid method is described for the high-performance liquid chromatographic (HPLC) determination of benzo[a]pyrene in roasted coffee and coffee brew. The method consists of a simple but effective sample preparation procedure, in which the use of solvents and glassware is limited to minimize contamination problems. Determination of benzo[a]pyrene was done by means of a selective and sensitive HPLC procedure, using fluorescence detection.

EXPERIMENTAL SECTION

Reagents. All solvents were distilled in glass or were of HPLC grade. All other reagents were of analytical reagent quality. The silica gel used (Silica Woelm, 63-200 mesh, aktiv) was obtained from Woelm Pharma GmbH & Co. (D-3440 Eschwege, Germany). The silica gel was deactivated with 15% water before using, as described by Grimmer and Böhnke (1975).

Benzo[a]pyrene was obtained from the Community Bureau of Reference (BCR), Commission of the European Communities, Brussels, Belgium. Benzo[a]pyrene standard solutions of about 1 μg/mL and 10 ng/mL were prepared by diluting with methanol aliquots of a stock standard solution of benzo[a]pyrene (about 1 mg/mL) in methanol. The stock standard solution was stored in the dark at 4 °C and was stable for at least 1 month. *Caution*: Benzo[a]pyrene is a known carcinogen. All work using benzo[a]pyrene should be performed in a properly functioning fume hood, and gloves should be worn to minimize exposure.

Samples. Various brands of roasted coffee commercially available in The Netherlands were either obtained through coffee roasting companies or purchased from local food retail outlets. The commercial coffee samples were received in the original packaging and included brands of all major coffee roasting companies in The Netherlands.

One specially prepared over-roasted coffee sample was supplied by Douwe Egberts Research and Development (Utrecht, The Netherlands).

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Preparation of Coffee Brews. Brewed coffee was prepared by two different methods.

Method 1 (Filtration Method). The reservoir of an electric coffee maker (Philips HD 5147) was filled with 1 L of tap water at room temperature. Then 50 g of ground coffee was placed in the plastic filter holder lined with a filter paper (Melitta No. 2), and the coffee maker was switched on. The volume of the collected coffee brew was measured.

Method 2 (Traditional Method). Exactly 50 g of ground coffee was weighed into a 2-L conical flask. Exactly 1 L of boiling tap water was added to the sample. The flask was covered with a watch glass and heated in a water bath maintained at 90 °C for 15 min. Subsequently, the mixture was filtered by the plastic filter holder of the coffee maker (method 1) lined with a filter paper (Melitta No. 2). The volume of the collected coffee brew was measured.

Extraction Procedure for Roasted Coffee. A 15–20-g portion of ground coffee was extracted in a Soxhlet apparatus for 6 h with 200 mL of acetone, with a preextracted extraction thimble. The solvent was removed under reduced pressure, and the residue was saponified with 1.4 g of potassium hydroxide in 50 mL of methanol–water (9:1, v/v) under reflux. After completion of the saponification (30 min), 100 mL of cyclohexane was added slowly through the condenser. After about 5 min the mixture was cooled by adding 120 mL of cold tap water through the condenser. After separation of the layers (overnight), a 50-mL aliquot of the clear cyclohexane layer, which represents 50% of the original sample, was concentrated to near dryness on a rotary evaporator. The last traces of solvent were removed with a stream of nitrogen and gentle heating of the concentrate. The residue was dissolved in 2 mL of methanol.

Extraction Procedure for Coffee Brew. Coffee brew obtained by means of method 1 (filtration method) or method 2 (traditional method) was transferred into a 1-L brown glass bottle with screw cap and made to a final volume of 900 mL with tap water. The coffee brew was extracted with 100 mL of cyclohexane by rotation of the closed bottle on a roller mixer at 100 rpm for 4 h. The mixture was transferred to a 1.5-L separatory funnel. To break the emulsion, 30 mL of ethanol was added and the mixture was gently swirled. After separation of the layers, the lower aqueous phase was drawn off into the 1-L brown glass bottle. The extraction was repeated twice with a 100-mL and a 50-mL portion of cyclohexane, each time for 2 h. The cyclohexane extracts were filtered into a 300-mL round-bottomed flask through a 2-cm layer of anhydrous sodium sulfate that had been prewashed with diethyl ether and placed in a glass filter funnel (G4). The combined cyclohexane extracts were concentrated to approximately 2 mL under reduced pressure. The concentrate was subjected to purification by chromatography on a silica gel column as described by Grimmer and Böhnke (1975). The eluate was concentrated to near dryness on a rotary evaporator. The last traces of solvent were removed with a stream of nitrogen and gentle heating of the concentrate. The residue was dissolved in 1 mL of methanol.

Extraction Procedure for Spent Grounds. Spent grounds were dried in an oven at 105 °C to constant weight. The residue was further treated as described under Extraction Procedure for Roasted Coffee.

HPLC Analysis. HPLC analyses were carried out with a Waters Associates Model 6000 A pump, coupled to a Perkin-Elmer Model 650-10 LC dual-beam monochromator fluorescence detector. The fluorescence detector was operated with an excitation wavelength of 381 nm (slit width 10 nm) and an emission wavelength of 407 nm (slit width 10 nm). Separations were achieved on a 5-μm Vydac reversed-phase 201 TPB 5 column (4.6-mm i.d. × 25-cm length) operated at ambient temperature. The mobile phase of methanol and water (90:10, v/v) was used at a flow rate of 2.0 mL/min and was continuously degassed with helium. Aliquots of 20 μL (extracts of roasted coffee or spent grounds) or 100 μL (extracts of coffee brew) were injected into the HPLC column utilizing a Waters Associates U6K variable-volume syringe loading injector. Chromatograms were displayed on a Kipp & Zonen Model BD-40 strip chart recorder using a chart speed of 0.5 cm/min. Quantitation was performed by comparing sample peak heights with those obtained for standard solutions.

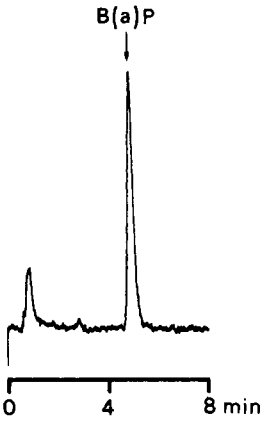

Figure 1. Chromatogram of benzo[*a*]pyrene standard. Amount injected: 160 pg of benzo[*a*]pyrene.

Recovery Experiments. The recovery of benzo[*a*]pyrene from roasted coffee was determined by applying with a microsyringe 20-μL aliquots of benzo[*a*]pyrene standard solutions (1.00 or 1.30 μg/mL), containing 20.0 or 26.0 ng of benzo[*a*]pyrene, to a 20-g portion of ground roasted coffee. To another 20-g portion nothing was added. The recovery of benzo[*a*]pyrene from coffee brew was determined by adding a 10-μL aliquot of a benzo[*a*]pyrene standard solution (1.32 μg/mL), which corresponds to 13.2 ng of benzo[*a*]pyrene, to 1 L of coffee brew prepared by a household coffee maker (coffee to water ratio 50 g/L). To another 1-L portion of coffee brew, prepared in the same way, nothing was added.

The samples were analyzed as described above. Percent recoveries were based on the difference between the total amount in the spiked vs. unspiked samples.

RESULTS AND DISCUSSION

Chromatography. Figure 1 shows a typical chromatogram of a benzo[*a*]pyrene standard solution. The detection limit, twice the base-line noise, was estimated to be 10 pg/injection, which corresponds to 0.1 μg of benzo[*a*]pyrene/kg for roasted coffee and spent grounds and 0.1 ng of benzo[*a*]pyrene/kg for coffee brew. In Figure 2 are shown a chromatogram obtained for a ground roasted coffee sample containing less than 0.1 μg of benzo[*a*]pyrene/kg and a chromatogram for the same coffee sample fortified with 0.5 μg of benzo[*a*]pyrene/kg of coffee.

Peak identification was established by comparing the retention times of peaks in the sample chromatograms with those in the standard chromatograms. Further confirmation of identity can be obtained by various methods that rely either on comparison of the chromatographic characteristics of the eluting components with benzo[*a*]pyrene reference material or on the characteristics of the detection system (Parris, 1984).

Table I. Recovery Study of Benzo[a]pyrene Added to Ground Roasted Coffee

amt in roasted coffee, μg/kg	amt added, μg/kg	amt found, μg/kg	recovery,[a] %
0.21	1.00	1.18	97
0.21	1.00	1.18	97
0.21	1.00	1.10	89
0.10	1.00	0.94	84
0.10	1.00	0.95	85
0.10	1.00	0.89	79
0.10	1.00	1.03	93
0.10	1.00	0.95	85
0.20	1.30	1.33	87
0.20	1.30	1.21	78
0.20	1.30	1.37	90

[a] Average 88 ± 6% standard deviation (n = 11).

Table II. Percent Recovery of Benzo[a]pyrene Added to Coffee Brew

amt in coffee brew, ng/L	amt added, ng/L	amt found, ng/L	recovery, %
0.3	13.2	15.1	112
0.3	13.2	15.3	114

Extraction and Cleanup Method. The efficiency of the extraction and cleanup method was tested by recovery studies. Samples of roasted coffee and coffee brew were spiked with known amounts of benzo[a]pyrene, and the recovery of benzo[a]pyrene from these samples was determined. The results of the recovery experiments are presented in Tables I and II. In view of the low benzo[a]pyrene levels in the investigated samples, the recovery values are quite satisfactory. As higher aromatic PAH are ubiquitous, samples that are analyzed for minute amounts of benzo[a]pyrene can easily be contaminated during the analysis. Therefore, in the investigated sample preparation procedure, the use of solvents and glassware was limited as much as possible. For instance, in the extraction procedure for ground roasted coffee samples, the same flask was used for the Soxhlet extraction, the evaporation of the solvent, and the saponification procedure. In the extraction procedure for roasted coffee and spent grounds, a saponification step is performed, which is followed by a cyclohexane extraction of the aqueous mixture obtained. This extraction is carried out by the addition of cyclohexane through the condenser while the alkaline solution is still boiling and heating of the resulting mixture for about 5 min. This simple extraction procedure appeared to be very effective compared with a repeated cold extraction of the mixture. Not only is this method less time consuming but less solvent and glassware are required.

The sensitivity and the selectivity of flame ionization detectors generally used for the GC analysis of benzo[a]pyrene are considerably inferior to that of fluorescence detectors applied in the HPLC determination. Consequently, the GC sample preparation procedure is more laborious, involves more cleanup steps and more glassware, and requires a larger sample amount and further concentration of the final sample extract. The amount of solvent required in the HPLC sample preparation procedure to obtain an extract that is suitable for chromatographic analysis is about 2% of that employed in the GC sample workup. Therefore, less contamination of the sample will occur if the HPLC sample preparation procedure is used.

It appeared that for the determination of benzo[a]pyrene in both ground roasted coffee and spent grounds the column cleanup step as described by Grimmer and Böhnke (1975) can be omitted. In the extracts obtained for these sample types, no impurities that interfere with the benzo[a]pyrene determination were found to be present.

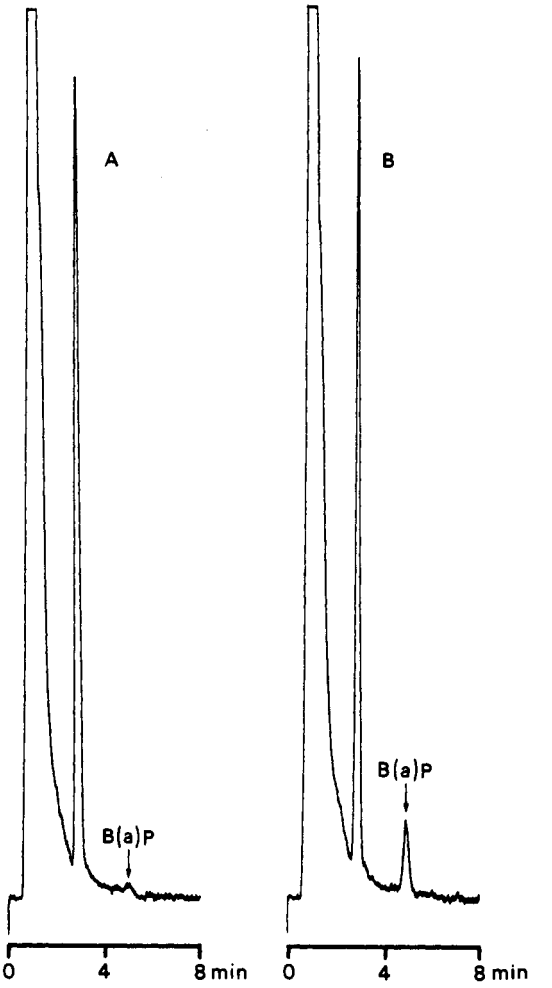

Figure 2. Chromatograms: (A) extract from roasted coffee sample containing <0.1 μg of benzo[a]pyrene/kg; (B) extract from the same roasted coffee sample fortified with 0.5 μg of benzo[a]pyrene/kg. Benzo[a]pyrene peaks represent injected amounts of <10 pg of benzo[a]pyrene (A) and 50 pg of benzo[a]pyrene (B).

Omitting this cleanup step improves the speed of the analysis. In addition, the possible introduction of interferences during silica gel column cleanup and a loss in benzo[a]pyrene that can occur during the column cleanup by irreversible adsorption or photoreaction are prevented if this step is omitted.

As benzo[a]pyrene is photosensitive, it is important to protect the sample from light during the sample preparation procedure, especially during the silica gel column cleanup. Therefore, in this investigation the sample preparation procedure was carried out under shaded lighting and the silica gel column was wrapped with an aluminum foil.

In order to minimize contamination during the sample preparation procedure, furthermore, it is necessary to rinse all glassware thoroughly with diethyl ether before use.

For the extraction of benzo[a]pyrene from heterogeneous materials, such as ground roasted coffee and spent grounds, two procedures can be used (Jacob and Grimmer, 1979). For this sample type an extraction with acetone is generally effective. In case an incomplete extraction is suspected, an alkaline digestion of the matrix followed by extraction of the resulting homogeneous aqueous mixture with cyclohexane is recommended. The suitability of both extraction procedures was briefly investigated in a comparative study. Four ground coffee samples were extracted by means of the two procedures. The extracts obtained were analyzed by means of HPLC. As demonstrated in Table III, with this sample type the values obtained by the

Table III. Comparison of Extraction Procedures for the Determination of Benzo[a]pyrene in Roasted Coffee

roasted coffee sample	benzo[a]pyrene content, μg/kg: acetone extrctn	benzo[a]pyrene content, μg/kg: saponifícn–extractn[a]
A	0.46	0.40
B	0.47	0.24
C	0.52	0.38
D	0.18	0.22

[a] Alkaline saponification followed by extraction with cyclohexane.

Table IV. Investigation of the Completeness of the Cyclohexane Extraction of Benzo[a]pyrene from Coffee Brew[a]

extractn no.	benzo[a]pyrene content,[b] ng/L	extractn no.	benzo[a]pyrene content,[b] ng/L
1	0.40	3	0.06
2	0.06	4	<0.06

[a] Extractions were performed according to the procedure described in the Experimental Section, under Extraction Procedure for Coffee Brew. [b] Benzo[a]pyrene content calculated on the basis of the coffee brew extracted.

Table V. Benzo[a]pyrene Levels in Ground Roasted Coffee Commercially Available in The Netherlands

benzo[a]pyrene range, μg/kg	no. of samples
≤0.10	7
0.11–0.20	28
0.21–0.30	11
0.31–0.40	7
0.41–0.50	2
mean 0.20	total 55
median 0.17	

two extraction methods were generally similar. As the saponification procedure is more laborious and time consuming, acetone extraction was chosen for the roasted coffee and spent grounds analyses reported in this investigation.

The completeness of the cyclohexane extraction of benzo[a]pyrene from coffee brew was verified. A coffee brew sample was repeatedly extracted with cyclohexane. The results of these extraction experiments are listed in Table IV. From these data it is apparent that three cyclohexane extraction steps are required to ensure a complete extraction of benzo[a]pyrene from coffee brew.

To verify the purity of the reagents, blank tests were carried out in parallel with the sample workup, using exactly the same procedure as is used during the determination on a sample. If necessary (coffee brew samples) the benzo[a]pyrene levels found were corrected for the results of the blank determination.

Roasted Coffee. A total of 55 ground roasted coffee samples, commercially available in The Netherlands, were purchased and analyzed between 1980 and 1984. These products included brands of all major coffee roasting companies in The Netherlands. All samples were analyzed in duplicate, and the results are presented in Table V. The benzo[a]pyrene levels in the roasted coffee samples analyzed range from approximately 0.1 to 0.5 μg/kg. The results of this study are in good agreement with previously published data for normal roasted coffee that vary from 0.1 to 0.8 μg/kg (Fritz, 1968; Lintas et al., 1979; Bories and Gasc, 1980; Guyot et al., 1982).

Coffee Brew. For all roasted coffee samples investigated in this study, benzo[a]pyrene levels of less than 0.5 μg/kg were obtained. In view of these very low benzo[a]pyrene levels, these samples were considered not very suitable for an investigation of the benzo[a]pyrene content of coffee brew. Guyot et al. (1982), however, have reported higher values (up to 3.2 μg/kg) for heavily roasted coffee. Also for a partially burnt coffee sample, investigated in a previously reported study on coffee roasting and benzo[a]pyrene (van der Stegen and van Overbruggen, 1982), a benzo[a]pyrene content of 22.7 μg/kg was found. The samples investigated in the framework of this study on coffee roasting and benzo[a]pyrene were analyzed in the laboratory of TNO-CIVO by means of an HPLC method that is similar to the method applied in the present investigation.

Table VI. Determination of Benzo[a]pyrene in Ground Over-Roasted Coffee, Coffee Brew, and Spent Grounds

sample type	preparation (method)	benzo[a]pyrene content,[a] μg/kg
ground over-roasted coffee		2.00 ± 0.15 (5)
coffee brew	filtration (1)	0.0008 ± 0.0004 (2)
spent grounds[b]	filtration (1)	2.56 ± 0.40 (4)
coffee brew	traditional (2)	0.0010 ± 0.0004 (2)
spent grounds	traditional (2)	2.40 ± 0.39 (4)

[a] Mean and standard deviation. The number in parentheses is the number of analyses. [b] Spent grounds were dried prior to analysis.

The benzo[a]pyrene content of an over-roasted coffee sample (supplied by Douwe Egberts Research and Development, Utrecht, The Netherlands) was determined, as well as brewed coffee prepared from that sample by two different methods, using a coffee to water ratio of 50 g/L. [This ratio will produce rather strong coffee brew by Dutch standards; in The Netherlands more common practice is the ratio of 40 g/L.] Both the coffee brews and the spent grounds were analyzed for benzo[a]pyrene. As demonstrated in Table VI, very low benzo[a]pyrene levels are found in brews prepared by two different methods from an over-roasted coffee sample containing 2.0 μg of benzo[a]pyrene/kg. Also from the results obtained for the determination of benzo[a]pyrene in spent grounds (Table VI), it is apparent that nearly all the benzo[a]pyrene present in the roasted coffee is retained in the spent grounds. The elevated benzo[a]pyrene content of the spent grounds compared to that of the roasted coffee is due principally to the removal of soluble solids, volatile aroma components, and water during the brewing process and the drying of the spent grounds. The benzo[a]pyrene levels in coffee brew found in the present work indicate benzo[a]pyrene extraction yields of about 1% for both preparation methods. This value is significantly lower than previously published benzo[a]pyrene extraction yields that vary from about 25 to 80%. These high benzo[a]pyrene extraction yields were obtained, however, by rather insensitive ultraviolet and/or fluorescence techniques (Fritz, 1969; Grimmer and Hildebrandt, 1966) or HPLC with UV detection (Guyot et al., 1982). This latter HPLC detection system is less sensitive and specific compared with the fluorescence detector used in this study and therefore more susceptible to interferences.

CONCLUSION

A relatively rapid and reliable method has been developed and applied to the determination of trace levels of benzo[a]pyrene in roasted coffee, coffee brew, and spent grounds. The results of this study indicate that benzo[a]pyrene levels in roasted coffee commercially available in The Netherlands are generally below 0.5 μg/kg. For coffee brews prepared from an over-roasted coffee sample with an elevated benzo[a]pyrene level of 2 μg/kg, benzo[a]pyrene contents of approximately 1 ng/L were found. It is apparent from these results that the benzo[a]pyrene content of coffee brew, prepared from normal roasted

coffee with a benzo[a]pyrene level ≤0.5 μg/kg, will be less than 1 ng/L. The daily total food and beverage intake of benzo[a]pyrene by humans is estimated to range from 0.25 to 2.5 μg (Dennis et al., 1983; Fritz, 1983). These figures indicate that coffee contributes very insignificant quantities to the daily human intake of benzo[a]pyrene.

In this study only the occurrence of benzo[a]pyrene in roasted coffee and coffee brew has been investigated. Future studies are planned in which the determination of various PAH in both coffee and tea samples will be investigated.

Registry No. Benzo[a]pyrene, 50-32-8.

LITERATURE CITED

Bories, G.; Gasc, N. *Ann. Falsif. Expert. Chim. Toxicol.* **1980**, *No. 789*, 487.
Dennis, M. J.; Massey, R. C.; McWeeny, D. J.; Knowles, M. E. *Food Chem. Toxicol.* **1983**, *21*, 569.
Fritz, W. *Nahrung* **1968**, *12*, 799.
Fritz, W. *Dtsch. Lebensm.-Rundsch.* **1969**, *65*, 83.
Fritz, W. *Nahrung* **1983**, *10*, 965.
Grimmer, G.; Hildebrandt, A. *Dtsch. Lebensm.-Rundsch.* **1966**, *62*, 19.
Grimmer, G.; Böhnke, M. *J. Assoc. Off. Anal. Chem.* **1975**, *58*, 725.
Guyot, B.; Lahmy, S.; Vincent, J.-C. *Cafe, Cacao, The* **1982**, *XXVI*, 199.
Howard, J. W.; Fazio, T. *J. Assoc. Off. Anal. Chem.* **1980**, *63*, 1077.
Jacob, J.; Grimmer, G. In *Environmental Carcinogens. Selected Methods of Analysis. Vol. 3. Analysis of Polycyclic Aromatic Hydrocarbons in Environmental Samples*; Egan, H., Castagenaro, M., Bogovski, P., Kunte, H., Walker, E. A., Davis, W., Eds.; IARC Publications No. 29; International Agency for Research on Cancer: Lyon, 1979; Chapter 8.
Kuratsune, M.; Hueper, W. C. *JNCI J. Natl. Cancer Inst.* **1958**, *20*, 37.
Kuratsune, M.; Hueper, W. C. *JNCI J. Natl. Cancer Inst.* **1960**, *24*, 463.
Lickint, F. *Med. Klin.* **1938**, *45*, 1499.
Lintas, C.; DeMatthaeis, M. C.; Merli, F. *Food Cosmet. Toxicol.* **1979**, *17*, 325.
Lo, M.-T.; Sandi, E. In *Residue Reviews*; Gunther, F. A., Ed.; Springer-Verlag: New York, 1978; Vol. 69, p 35.
Parris, N. A. *Instrumental Liquid Chromatography*, 2nd ed.; Elsevier: Amsterdam, 1984; Chapter 13.
Strobel, R. G. K. In *ASIC, 7th International Scientific Colloqium on Coffee*; Association Scientific Internationale du Café (ASIC): Paris, 1973; p 128.
van der Stegen, G. H. D.; van Overbruggen, G. J. J. In *ASIC, 10th International Scientific Colloqium on Coffee*; Association Scientific Internationale du Café (ASIC): Paris, 1982; p 347.
Zedeck, M. S. *J. Environ. Pathol. Toxicol.* **1980**, *3*, 537.

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Identification of the Diterpene Esters in Arabica and Canephora Coffees

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The distribution of the fatty acid esters of cafestol and kahweol was determined in a series of Arabica and Canephora coffee beans. It was found that the distribution is similar in both coffee types. The majority of the esters are kahweol derivatives, cafestol represented only as the palmitate. In addition, evidence is presented for the existence of another diterpene in Canephoras, tentatively identified as 16-methoxycafestol.

This paper was part of a project on determination of the geographical origin of coffee beans. That the composition of certain chemical classes in coffee oil might vary with geography was suggested by Tiscornia et al. (1979). They found that African and American Arabicas could be distinguished by differing percentages of certain sterols.

The principal diterpenes of coffee, cafestol and kahweol, have been known since the 1930s (Bengis and Anderson, 1932). They are present both in the free form and with fatty acids esterified. While the fatty acids associated with the diterpenes have been determined (Folstar et al., 1975), the actual esters have not been well characterized. Lam et al. (1982), working with an Arabica coffee, found six distinct compounds but only identified the palmitate esters of cafestol and kahweol. This paper presents the identities of six diterpene esters and describes the evidence for the occurrence of an additional diterpene, tentatively identified as 16-methoxycafestol.

EXPERIMENTAL SECTION

HPLC. A Waters Associates gradient liquid chromatograph was used. Both the analytical column (25 cm × 4.6 mm) and the semipreparative column (25 cm × 10 mm) were 5-μm, C-8 bonded-phase types from Supelco Inc. A linear gradient, of 35-min duration, going from 80% acetonitrile/H_2O to 100% acetonitrile was employed. Flow rates were 1.0 mL/min for the analytical column and 3.5 mL/min for the semipreparative column. Detection was by UV at 280 nm.

GC–MS. Mass spectra were taken on a Finnigan Model 3300 gas chromatograph–mass spectrometer equipped with an INCOS data system. All separations were performed on a DB-17 bonded phase, fused silica capillary, 15 m, from J&W Scientific. For the analysis of the silyl derivatives of the diterpenes, the column temperature was 250 °C, while for the methyl esters of the fatty acids it was programmed from 140 to 250 °C at 4 °C/min. Spectra of the isolated diterpene esters were obtained with use of Finnigan's ballistically heated solid probe attachment.

NMR. Spectra were run on a Varian FT80A NMR spectrometer, with Me_4Si as the reference standard.

Sample Preparation. Samples of green coffee beans were obtained from the International Coffee Orginization. After obvious defects (black beans, broken beans, etc.) were removed, the beans were ground, in 10-g portions, in a Tekmar mill for 1 min. For comparison among samples of different origin by analytical HPLC, 10 g was extracted

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