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.

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Received for review March 17, 1986. Accepted November 24, 1986.

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

U.S. Customs Service, Technical Services Division, Washington, D.C. 20229.

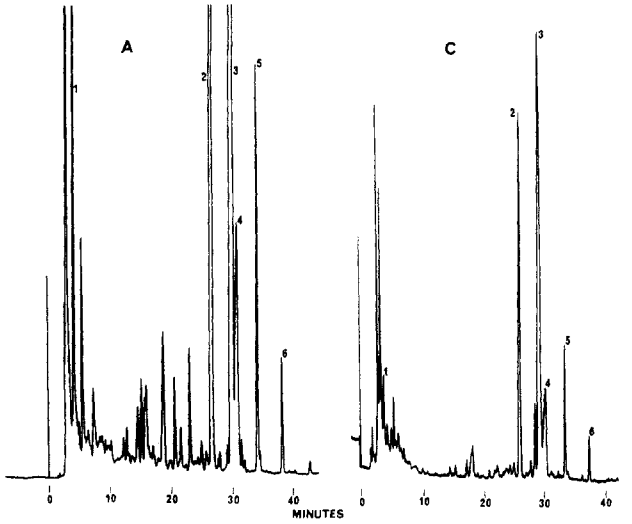

Figure 1. HPLC of diterpene ester fractions: A = Arabica; C = Canephora. Key: 1, cafestol + kahweol; 2, kahweol linoleate; 3, kahweol palmitate; 4, cafestol palmitate + kahweol oleiate; 5, kahweol stearate; 6, kahweol eicosanoate.

with 15 mL of acetonitrile overnight on a wrist-type shaker. A portion of this extract was filtered through a 0.45-μm filter, and 10 μL of the filtrate was injected. For isolation of the individual diterpene compounds, a 30-g sample was extracted with petroleum ether in a Soxhlet apparatus for 3 h. After evaporation of the petroleum ether, under nitrogen, the residue was taken up in a suitable volume of acetonitrile, filtered through a 0.45-μm filter, and made ready for injection. After collection of the individual peaks, a small amount was taken from each for analysis by solid-probe mass spectrometry. The remaining portions were saponified with 10% methanolic KOH at 60 °C for 2 h. The resulting free diterpenes were silylated with SYLON BTZ (Supelco, Inc.), and the fatty acids were methylated with BF_3 in methanol.

RESULTS AND DISCUSSION

In Figure 1 are typical HPLC chromatograms of Arabica (A) and Canephora (C) samples. As can be seen, both coffees have the same set of diterpene esters in very similar ratios. The total amount of esters is, however, very much less in Canephoras (approximately 50-fold difference). It is of interest that, of the six diterpene esters identified, only one is a cafestol derivative. Cafestol predominates in the unesterified portion of both coffee types, at a level approximately 10 to 1 over kahweol.

Kahweol is considered to be present in very small amounts in Canephoras, and at least one worker has stated that it is absent (Wurziger et al., 1979). In the Canephoras utilized in this study, kahweol as a percentage of cafestol ranged from 1.0% (Philippines) to 7.0% (Uganda). In no case was it absent.

The new diterpene appeared only in the Canephoras and the Excelsa coffees. Expressed as a percentage of cafestol, its concentration ranged from 18% (Angola) to 65% (Philippines). In the one Excelsa sample its relative concentration was 12%. Under the HPLC conditions employed, only one ester derivative could be identified, that of the palmitate. By GC, the relative retention time of its silyl derivative, compared to the disilyl derivative of cafestol, was 1.34. The tentative identification of this compound is based on comparisons of its ^1H NMR and mass spectra to the corresponding spectra for cafestol.

Figure 2 contains the proposed structure, 16-methoxycafestol (A), along with the structures of cafestol (B) and kahweol (C). Overall the ^1H NMR spectra of cafestol and

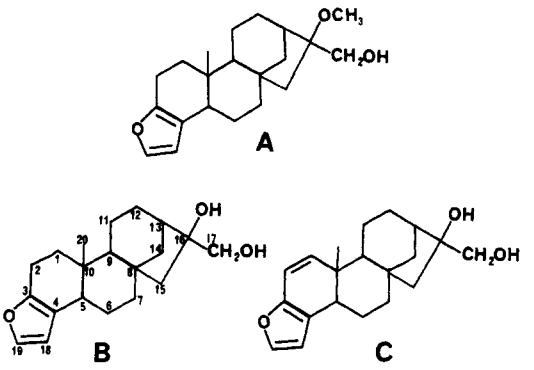

Figure 2. Diterpenes: A = 16-methoxycafestol; B = cafestol; C = kahweol.

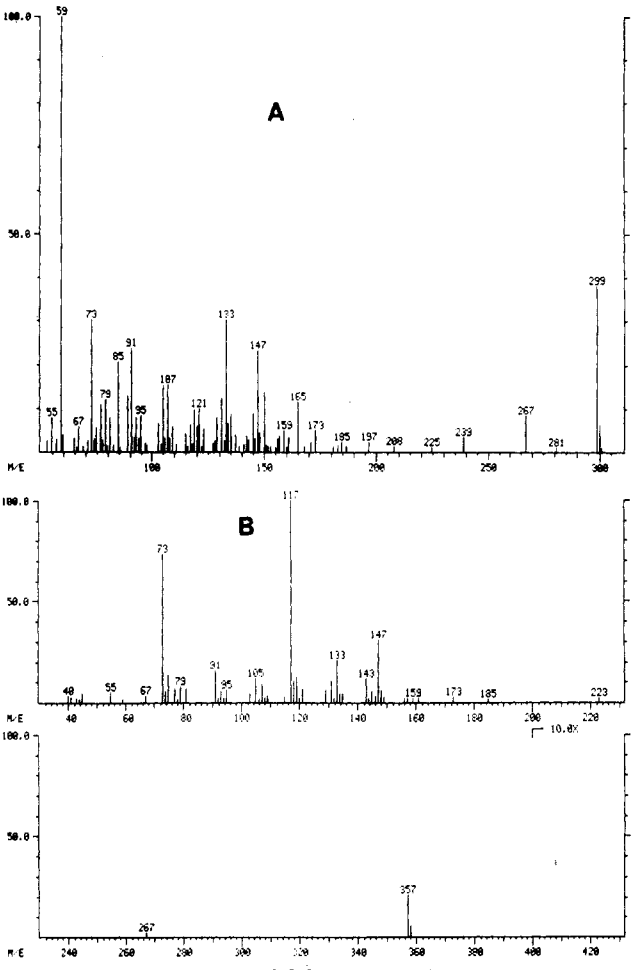

Figure 3. Mass spectra of silyl derivatives: A = methoxycafestol; B = cafestol.

the new diterpene are similar, with two exceptions. In the cafestol spectrum, an AB quartet occurs at 3.75 ppm. This has been ascribed (Lam et al., 1982) to intramolecular hydrogen bonding involving the hydroxy groups at C16 and C17, preventing free rotation. In the corresponding spectrum of the new diterpene this quartet is now a singlet (3.76 ppm). The spectrum of the new diterpene has a signal at 3.17 ppm, which does not appear in the cafestol spectrum. Both observations are consistent with the addition of a methoxy group to cafestol at either the C16 or C17 position.

In Figure 3, the mass spectra of the silyl derivatives of cafestol and the new diterpene are shown. The peaks at m/z 133 and 147 in both spectra probably arise from ring scission across bonds C9-C10 and C6-C7 to yield furan-containing fragments. Kahweol, with a double bond at

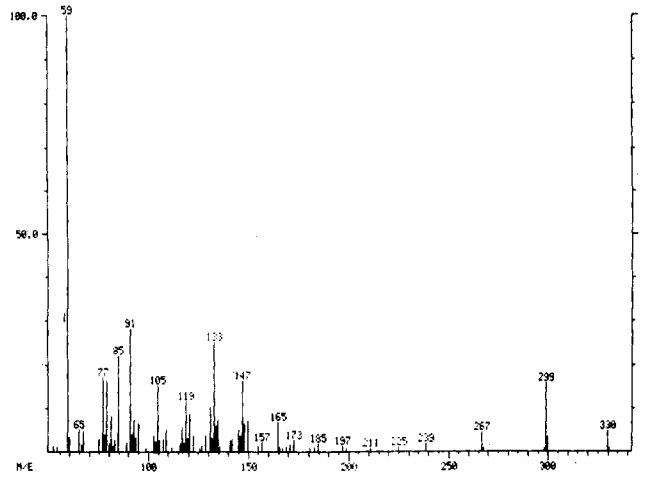

Figure 4. Solid-probe mass spectrum of 16-methoxycafestol.

C1-C2, shows analogous peaks at m/z 131 and 146. At the high end of the spectrum, cafestol, which forms a disilyl derivative (MW 460), has a peak at m/z 357 corresponding to a loss of a 103 radical [$-CH_2O - Si(CH_3)_3$]* arising from the silyl ether on the primary alcohol at C17. A corresponding M – 103 peak at m/z 299 occurs in the spectrum of the new diterpene. This is evidence for the fact that the new diterpene can only form a monosilyl derivative (MW 402) and that silylation occurs at C17. The m/z 59 ion, which is the base peak in the spectrum of the new diterpene's silyl derivative, is also the base peak in the spectra of both its free alcohol (Figure 4) and its esterified derivative (not shown). The peak at m/z 299 is also prominent in all three spectra. These observations can be explained by assuming that in the diterpenes the CH_2OH group at C16 is readily lost whether or not derivatized. With the proposed structure for the new diterpene (MW 330), a loss of M – 31 would result in an ion at m/z 299. In cafestol this loss results in a peak at m/z 285. Once this occurs in the new diterpene, further fragmentation could give rise to the m/z 59 ion $[C_3H_7O]$* containing carbons C15, C16, and the methoxy group.

The addition of 16-methoxycafestol would bring to four the number of diterpenes identified in coffee. A third diterpene, cafestol-2-one, was found by Richter and Spiteller (1979) and isolated as the 11-*O*-β-D-glucopyranoside. It is interesting to speculate that the 16-methoxy derivative of kahweol might also exist in Canephoras. Although given the small amount of kahweol, it would undoubtedly be present at very low levels.

Registry No. Kahweol linoleate, 108214-29-5; kahweol palmitate, 81760-45-4; cafestol palmitate, 81760-46-5; kahweol oleate, 108214-30-8; kahweol stearate, 108214-31-9; kahweol eicosanoate, 108214-32-0; 16-methoxycafestol, 108214-28-4.

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Received for review May 21, 1986. Revised manuscript received December 10, 1986. Accepted March 20, 1987.

Mineral Metabolism and Bone Strength of Rats Fed Coffee and Decaffeinated Coffee

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Two studies were conducted to determine effects of ingestion of nutritionally complete diets with 6.6% coffee or 6.6% decaffeinated coffee on growth, mineral metabolism, hematological status, and bone strength of weanling rats (study 1) and anemic young (50-day-old) rats (study 2). Rats fed coffee had elevated concentrations of iron in their livers, kidneys, and tibias (study 1) and absorbed iron more efficiently (study 2) than control rats. In both studies, rats fed coffee had elevated liver copper levels and elevated tibia concentrations of zinc, calcium, magnesium, and phosphorus. Rats fed decaffeinated coffee also had elevated zinc tibia levels. These differences in bone mineral levels were not associated with differences in bone strength or elasticity.

In 1982 the average American coffee drinker consumed approximately 3.4 cups of coffee/day (Diamond, 1983). Recently, consumption figures have dropped slightly, but coffee continues to be a popular beverage (International Coffee Organization, 1985).

Morck et al. (1983) found that human subjects incorporated less ^{59}Fe into their red blood cells when coffee was added to a standardized, radiolabeled meal. They believed this effect represented a change in iron absorption. However, coffee and one of its components, caffeine, have been found to influence a number of physiological functions (i.e., thermogenesis, gastrointestinal motility, GI secretions, kidney function) that could ultimately affect utilization of dietary iron for heme synthesis (Von Borstel, 1983; Acheson et al., 1980; Feldman et al., 1981; Cohen and Booth 1975; Sunano and Miyazaki, 1973; Wald et al., 1976; Palm et al., 1984; Yeh et al., 1986; Massey and Berg, 1985). The practical importance of several of these factors can

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0021-8561/87/1435-0551$01.50/0