

Levels of Cafestol, Kahweol, and Related Diterpenoids in Wild Species of the Coffee Plant *Coffea*

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Coffee beans of commercially important *Coffea* species contain the diterpene cafestol, which raises serum cholesterol in humans. Coffee diterpenes also have shown anticarcinogenic properties in experimental animals. We determined levels of cafestol and other diterpenes in nine wild African *Coffea* species, in a search for a species without the cholesterol-raising cafestol. In addition, data on diterpene profiles might assist in the taxonomic grouping of *Coffea* species. Each *Coffea* species we investigated contained cafestol. Kahweol concentrations may be related to the geographical distribution of the *Coffea* species. 16-*O*-Methylcafestol was only present in *Coffea canephora*, *Coffea stenophylla*, and *Coffea liberica* var. *dewevrei*. A new diterpene was found in *C. stenophylla* and tentatively identified as 16-*O*-methylkahweol. Another four new diterpenes, which seem to contain an additional double bond compared to kahweol, were found in *Coffea salvatrix* and *Coffea pseudozanguebariae*, suggesting that these two species are closely related. In conclusion, cafestol is universally present in *Coffea* species. Amounts of kahweol, 16-*O*-methylcafestol, 16-*O*-methylkahweol, and the four newly found diterpenes present in various coffee beans may prove useful in the taxonomic classification of *Coffea* species.

Keywords: *Coffea* diterpenes; African coffee beans; cholesterol; taxonomy

INTRODUCTION

All coffee plants belong to the tribe Coffea of the Rubiaceae subfamily Cinchonoideae. *Coffea* is one of the two genera of the Coffea tribe, and it has three recognized subgenera. *Coffea* subgenus *Coffea* contains about 85 species, including all the commercially useful species. The beans of these commercially important species have been analyzed extensively. The unsaponifiable lipid fraction of these beans is rich in two diterpenes specific for coffee: cafestol and kahweol (Figure 1) (Viani, 1988; Urgert et al., 1995). The total diterpene content ranges from 1.3% to 1.9% (w/w) in green beans of *Coffea arabica* (commonly known as Arabica) and from 0.2% to 1.5% in beans of *Coffea canephora* (commonly known as Robusta) (Viani, 1988; Ratnayake et al., 1993).

Cafestol potentially raises serum cholesterol in humans (Weusten-van der Wouw et al., 1994; Urgert et al., 1997). We estimated in a meta-analysis of 11 experiments with cafestol-rich preparations that each 10 mg of cafestol consumed per day (equivalent to 2–3 cups of coffee brewed without the use of a paper filter) raises serum cholesterol by 0.15 mmol/L (Urgert and Katan, 1997). As *C. arabica* and *C. canephora* represent 99% of the world coffee market (Debry, 1994), coffee beans of commercial blends will inevitably contain the cholesterol-raising compound cafestol. Filtered coffee does not contain cafestol or kahweol, as the diterpenes are retained by a paper filter (Van Dusseldorp et al., 1991). Diterpene levels in instant coffee are also negligible (Urgert et al., 1995). However, levels of cafestol and

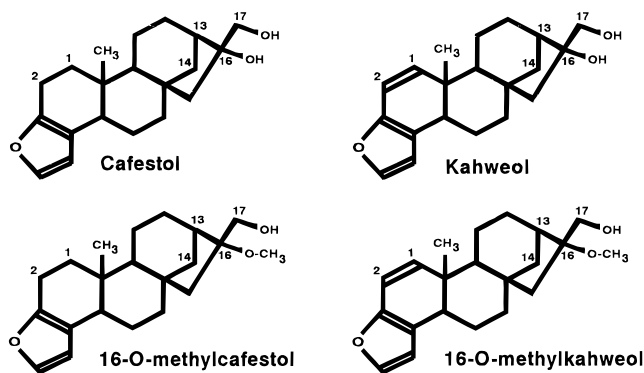


Figure 1. Structures of the diterpene alcohols cafestol, kahweol, 16-*O*-methylcafestol, and 16-*O*-methylkahweol. Diterpenes occur in coffee beans mainly esterified to fatty acids at the C16 or C17 position.

kahweol are high in Turkish/Greek coffee—brewed by boiling very finely ground coffee in water—and in cafetière coffee (also known as ‘French press’ or ‘plunger’ coffee)—brewed by pouring hot water onto coffee grounds in a glass jug after which a metal screen strainer is pushed down to separate the grounds from the brew (Urgert et al., 1995, 1996).

Intake of diterpene-rich coffee is associated with a higher risk of heart disease (Tverdal et al., 1990), but there are also tentative indications for a protective effect against cancer. Some epidemiological studies revealed a lower incidence of colon cancer with increasing coffee intake (as reviewed by the IARC Working Group, 1991). Moreover, intake of coffee diterpenes reduced the frequency of adenocarcinoma of the colon induced by 1,2-dimethylhydrazine in rats (Gershbein, 1994). A biological mechanism has not been established (Rosenberg, 1990), but the effect might be mediated by glutathione *S*-transferase, as both cafestol and kahweol raised the

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Table 1. Levels of Diterpenes in Green Beans of Various Wild *Coffea* Species^a

species	sample ^b	origin	bean length × width (mm)	amount (mg/100 g of bean mass)				
				cafestol	kahweol	16- <i>O</i> -methylcafestol	16- <i>O</i> -methylkahweol	unknown a, b, c, and d
<i>C. canephora</i> (Robusta)	IFA25	Ivory Coast	8 × 6	239 ± 3	5 ± 0	154 ± 3		
<i>C. canephora</i> (Robusta)	IF461	Ivory Coast	8 × 5	250 ± 1	8 ± 0	102 ± 0		
<i>C. stenophylla</i>		Ivory Coast	8 × 5	292 ± 6	700 ± 8	54 ± 1	77 ± 2	
<i>C. stenophylla</i>	L04L10A18	Ivory Coast	7 × 5	341 ± 11	783 ± 10	65 ± 1	95 ± 2	
<i>C. liberica</i> var. <i>liberica</i>		Ivory Coast	9 × 6	283 ± 11	154 ± 5			
<i>C. liberica</i> var. <i>liberica</i>	bulk	Ivory Coast	11 × 8	273 ± 7	152 ± 3			
<i>C. liberica</i> var. <i>dewevrei</i>		Central African Republic	8 × 6	334 ± 10	54 ± 2	99 ± 5		
<i>C. liberica</i> var. <i>dewevrei</i>	005402	Central African Republic	7 × 5	616 ± 4	95 ± 2	19 ± 0		
<i>C. congensis</i>	031627	Central African Republic	8 × 6	344 ± 11	1065 ± 24			
<i>C. racemosa</i>		Mozambique	5 × 4	436 ± 10	505 ± 10			
<i>C. racemosa</i>	bulk	Mozambique	5 × 4	459 ± 9	580 ± 11			
<i>C. salvatrix</i>	bulk	Mozambique	5 × 4	488 ± 15	875 ± 22			478 ± 5
<i>C. pseudozanguebariae</i>	D15L39A39	Kenya	4 × 3	394 ± 2	824 ± 5			320 ± 1
<i>C. sessiliflora</i>	L05L17A2	Kenya	5 × 4	542 ± 3	702 ± 4			

^a Mean levels ± SD. For each species sample, duplicate analyses were performed. ^b Bulk: beans obtained from a number of coffee plants of the same species. Beans from the other samples were obtained from a single plant.

activity of this detoxifying enzyme in the liver and mucosa of the small bowel in mice and rats (Lam et al., 1987).

Determination of diterpene contents in commercial coffee species has proven useful in the grouping of *Coffea* strains. A diterpene typically present in Robusta but not in Arabica is 16-*O*-methylcafestol (Figure 1). Kahweol occurs in Arabica but barely in Robusta. 16-*O*-Methylcafestol and kahweol are therefore used as indicators of the proportion of Robustas in commercial blends (Speer and Montag, 1989).

We wished to establish the level of cafestol and other diterpenes in samples of wild *Coffea* species because there might exist a *Coffea* species which does not contain the cholesterol-elevating diterpene cafestol. Availability of such a species might help to breed new commercial species free from cafestol. We also speculated that other diterpenes, which might have anticarcinogenic properties, could be present in such species. Finally, data on diterpene profiles might assist in the taxonomic grouping of *Coffea* species. The morphological criteria traditionally used by taxonomists to distinguish the species in *Coffea* are subject to much variation, and additional information on biochemical and molecular diversity of species within subgenus *Coffea* is needed (Clifford et al., 1989; Anthony et al., 1993; Lashermes et al., 1997). We therefore determined the identity and levels of various diterpenes in coffee beans of nine wild *Coffea* species from Africa.

METHODS

Origin of the Samples. All samples were provided by the Orstom Station de Génétique du Caféier, Man, Ivory Coast, where the plants were cultivated. Beans of *Coffea liberica* var. *liberica*, *Coffea racemosa*, and *Coffea salvatrix* were collected from a number of plants, whereas beans of the other species were collected from a single plant. About 100 beans were collected per sample. They were sent to the Wageningen laboratory in a sealed plastic bag and stored at room temperature until analysis. *C. canephora* samples represent the *Coffea* species which is used for Robusta coffee production.

Chemical Analyses. Analysis of diterpenes was performed as described by Urgert et al. (1995). Briefly, grounded green beans combined with the internal standard 5 α -cholestane were dissolved in ethanolic potassium hydroxide and saponified at 80 °C for 60 min. After cooling, lipids were extracted by diisopropyl ether and washed by demineralized water. The ether phase was dried, and the residue was dissolved in dried pyridine. We added a mixture of hexamethyldisilazane and

trichloromethylsilane to silylate hydroxyl groups. After incubation at room temperature, excess pyridine was removed. The sample was diluted in hexane. We injected 1 μ L of this sample splitless into a gas chromatograph equipped with a 25 m × 0.22 mm fused silica and CP Sil 5 column. The initial oven temperature was 70 °C for 2.5 min followed by a rise to 200 °C at a rate of 40 °C/min. After 10 min the temperature was raised to 235 °C at a rate of 6 °C/min and then to 285 °C at a rate of 30 °C/min, at which it was held for 6.75 min. Other conditions: carrier gas, hydrogen; pressure, 100 kPa; makeup gas, nitrogen; splitless injection, after 2.5 min with an injector purge flow of 100 mL/min at 300 °C; flame ionization detector temperature, 305 °C. Authenticity and purity of peaks were verified on a combined gas chromatography mass spectrometer. The coefficients of variation for a control pool of boiled coffee were 2.5% within and 9.5% between runs over a 6-month period for cafestol, and 2.3% and 7.2% for kahweol, respectively.

We compared gas chromatography traces and mass spectra of unknown compounds before and after hydrogenation in order to investigate the presence of double bonds. For this purpose, we dissolved a fat fraction containing about 100 mg of diterpenes and 50 mg of Pd–Pb–CaCO₃ catalyst (5%:5%:90%, w:w; Merck, Germany, no. 40687248) in 2.5 mL of ethanol. Hydrogen was supplied under vigorous stirring at a flow of 10 mL/min for 15–30 min. Samples were then centrifuged for 10 min. The ethanol phase was collected, evaporated, and silylated as described by Urgert et al. (1995).

RESULTS AND DISCUSSION

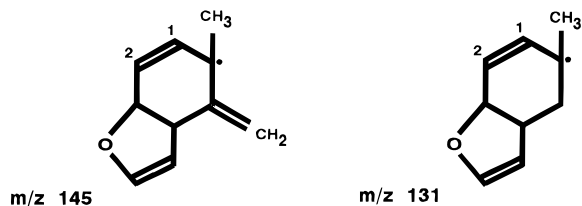
Levels of Cafestol, Kahweol, and 16-*O*-Methylcafestol. Cafestol was present in all species (Table 1). Its amount ranged from 239 mg/100 g of bean mass in *C. canephora* to 616 mg/100 g of bean mass in *C. liberica* var. *dewevrei*. This is the same range as found in commercial *Coffea* species (Viani, 1988; Urgert et al., 1995). As all species exhibited a high cafestol content with a large range of variation, this component is not discriminant for taxonomy.

Levels of kahweol were lowest in *C. canephora*. One sample of this *Coffea* species contained 5 mg and another one 8 mg of this diterpene per 100 g of bean mass. A similarly low concentration of kahweol in *C. canephora* was reported by Viani (1988). Levels of kahweol were also low in two other species: about 75 mg/100 g in *C. liberica* var. *dewevrei* and about 150 mg/100 g in *C. liberica* var. *liberica*. Kahweol levels in the remaining species ranged from 505 to 1065 mg/100 g (Table 1). The kahweol contents of *Coffea* species may be related to their geographical distribution: some

Table 2. Atomic Masses of Molecular Ions and Diterpene Specific Ionic Fragments as Trichloromethylsilane Derivatives of Various Coffee Diterpenes

diterpene	molecular mass (amu)	ionic fragment masses (amu)			
cafestol	460				
kahweol	458	145 ^a	131 ^a		
16- <i>O</i> -methylcafestol	402	299 ^b			
16- <i>O</i> -methylkahweol	400	297 ^b	145 ^a	131 ^a	
diterpenes a , b , and c	456	366 ^c	353 ^b	276 ^c	263 ^c
diterpene d	458				
diterpene e	458	368 ^c	355 ^b	278 ^c	265 ^c

^a See Figure 2. ^b Ionic fragment mass, probably produced by the loss of a trichloromethylsilane-O-CH₂ fragment. ^c Ionic fragment mass, probably produced by the loss of a trichloromethylsilane-OH fragment.

**Figure 2.** Proposed structures of two fragments found in the mass spectra of both kahweol and the new diterpene 16-*O*-methylkahweol.

species widely distributed in West and Central African forests, such as *C. canephora* and *C. liberica*, had a low kahweol content. Other species endemic in the same Guineo-Congolese area, namely, *C. stenophylla* from the Ivory Coast and *C. congensis* from the Central African Republic, contained 5–200 times more kahweol (Table 1). Ecological and genetic isolation of these species may explain a drift in biochemical components. *C. racemosa*, *C. salvatrix*, *C. sessiliflora*, and *C. pseudozanguebariae*, originating from East Africa, all showed high levels of kahweol.

16-*O*-Methylcafestol was present in *C. canephora* (Robusta) species, as reported previously (Speer and Montag, 1989), but was also found in *C. stenophylla* and *C. liberica* var. *dewevrei*. Amounts ranged from 19 mg/100 g in one sample of *C. liberica* var. *dewevrei* to 154 mg/100 g in one sample of *C. canephora* (Table 1).

Identification and Levels of 16-*O*-Methylkahweol. We found a new diterpene in *C. stenophylla*, with a silylated molecular weight of 400 atomic mass units (amu) (Table 2). A fragment with a mass of 297 amu probably resulted from the loss of a trichloromethylsilane-O-CH₂ group of 103 amu. Main fragments of the mass spectrum of this new component were 2 amu lower than the corresponding fragments of 16-*O*-methylcafestol, which showed ionic fragments of *m/z* 402 and 299. Moreover, the new component contained ionic fragments of *m/z* 131 and 145, which were also present in the spectrum of kahweol (Table 2). These masses have been described earlier (Lercker et al., 1995) and probably represent the fragments shown in Figure 2.

The unknown diterpene could be converted by catalytic hydrogenation into 16-*O*-methylcafestol. Because of the 2 amu difference in molecular weight with 16-*O*-methylcafestol, and the ionic fragments corresponding with kahweol, we tentatively identified this diterpene as 16-*O*-methylkahweol (Figure 1). The relative positions of 16-*O*-methylcafestol and 16-*O*-methylkahweol are indicated in Figure 3 on gas chromatograms of *C. salvatrix* and *C. pseudozanguebariae*.

Identification and Levels of Four Other Unknown Diterpenes. Gas chromatograms of diterpenes

from beans of *C. salvatrix* and *C. pseudozanguebariae* were practically the same. Both had four unknown peaks which were unique for these two *Coffea* species (Figure 3). Mass spectra of three of these unknown diterpenes, **a**, **b**, and **c**, strongly resembled each other; each had a mass of 456 amu. As this is 2 amu less than that of kahweol, the unknown components may contain one more double bond than kahweol. The position of this additional double bond is probably different for each of the three diterpenes **a**, **b**, and **c**, as the retention times of these diterpenes differed. Gas chromatography traces also suggested a fourth unknown peak **d**, which was overlapped by the kahweol peak. This peak had a mass of 458 amu, the same as kahweol. We therefore determined kahweol and **d** by comparing levels of cafestol and **e** before and after hydrogenation, because during hydrogenation kahweol is converted into cafestol and **d** is converted into **e**.

In the mass spectra of the diterpenes **a**, **b** and **c**, ionic fragments were present at *m/z* 366 and 353 (Table 2), which were probably produced by the loss of a trichloromethylsilane-OH fragment of 90 amu and a trichloromethylsilane-O-CH₂ fragment of 103 amu. These two fragments were also found in the spectrum of kahweol. Unlike kahweol, the mass spectra of the diterpenes **a**, **b**, and **c** also showed ionic fragments at *m/z* 276 and 263, which were probably derived from the loss of two additional trichloromethylsilane-OH fragments. This suggests that both in the newly found diterpenes **a**, **b**, and **c** and in kahweol the trichloromethylsilane-O-CH₂ fragment at the C17 position is easily cleaved off, while in **a**, **b**, and **c** a second trichloromethylsilane-O-CH₂ fragment is also easily separated. Therefore, it seems likely that in the newly found diterpenes the second hydroxyl group is not situated at C16, as it is in kahweol.

The four peaks **a**, **b**, **c**, and **d** disappeared after catalytic hydrogenation of the silylated samples, yielding a single new peak **e** with a retention time slightly shorter than that of kahweol (Figure 3). This hydrogenated diterpene **e** had the same mass as kahweol, i.e., 458 amu, and it showed ionic fragments of *m/z* 368, 355, 278, and 265, which are all 2 amu heavier than the fragments in the spectra of the original diterpenes **a**, **b**, and **c**. It thus appears that one double bond of the unknown components can easily be hydrogenated, while the other double bond is inaccessible to H₂, perhaps because of steric hindrance. We suggest that the latter double bond is situated in between C13 and C14, as all other possibilities yield double bonds that are easily accessible to H₂ or that are structurally impossible. As only one peak **e** arose after hydrogenation, the position of the second hydroxyl group is probably the same for diterpenes **a**, **b**, and **c**. Diterpene **d** probably contains one double bond which can easily be hydrogenated and is not situated in between C1 and C2.

We could not identify the origin of the new peaks eluting between 24.44 and 26.38 min, which appeared after hydrogenation of silylated samples of *C. salvatrix* and *C. pseudozanguebariae* (Figure 3) and also of silylated samples of *C. stenophylla*.

The presence of all four diterpenes in both *C. salvatrix* and *C. pseudozanguebariae* is in agreement with their ecological and botanical affinities (Hamon et al., 1984). These closely related species from Mozambique and Kenya are differentiated by their caffeine contents; *C. pseudozanguebariae* is the first caffeine-free species discovered in Africa (Hamon, 1984).

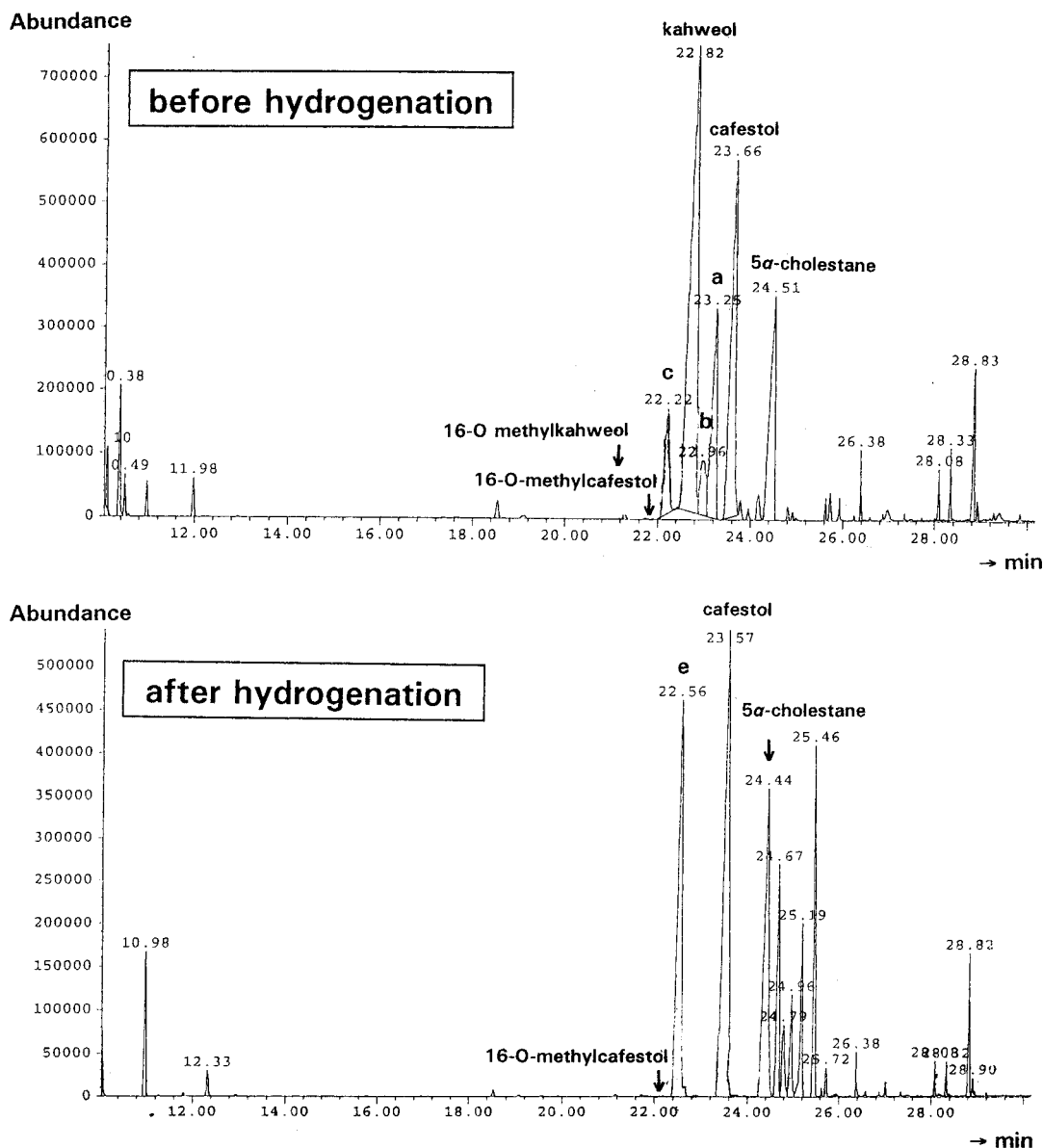


Figure 3. Gas chromatograms of diterpenes isolated from *C. salvatrix* and *C. pseudozanguebariae* before and after catalytic hydrogenation. The relative positions of 16-*O*-methylcafestol and 16-*O*-methylkahweol are also indicated, although these diterpenes are not present in *C. salvatrix* and *C. pseudozanguebariae*.

Diterpenes present in coffee beans might possibly have anticarcinogenic properties, but this benefit will be overshadowed by the cholesterol-raising effect of cafestol, which seems universally present in coffee beans of *Coffea* species. As previously reported for kahweol and 16-*O*-methylcafestol, 16-*O*-methylkahweol and the other newly found diterpenes present in the coffee beans may also prove useful in the taxonomic classification of *Coffea* species.

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