Chemosystematic Study of Diterpenoids in Green Coffee Beans

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SUMMARY

The diterpenic fraction of Variedad Colombia and nine other related genotypes of green coffee beans were studied by gas chromatography mass spectrometry. Eleven diterpenic compounds were identified and quantified. Cafestol , kahweol, 16-*O*-methylcafestol, 15,16-dehydrocafestol, 13,16-dehydrocafestol, 15,16-dehydrokahweol, 13,16-dehydrokahweol, 16-*O*-methylkahweol and the new diterpene 11,12-dehydrokahweol. The 11,12-dehydrokahweol was found in typical variety but not in Caturra variety, allowing to differentiate these two varieties of C. *arabica.* The 16-*O*-methylcafestol and 16-*O*-methylkahweol were found only in the genotypes of *C. canephora* allowing to discriminate these coffees of the rest of materials.

INTRODUCTION

The most studies of diterpenes in coffee have been related to thepresence of cafestol, kahweol and/or 16-*O*-methylcafestol. The cafestol was found in both *C. arabica* and *C. canephora* species (Nackunstz and Maier, 1987). The kahweol was detected in *C. arabica* at concentrations 100 times higher than in *C. canephora* (Lercker, 1995), while the 16-*O*-methylcafestol has been found more concentrated in *canephora* coffees than in *arabica* coffees (Speer and Montag, 1989), these data have been used as quality control for making possible to identify mixtures of these coffees (Speer et al., 1991). Diterpen glycosides also has been reported in *Coffea* but in low concentrations and its presence in some cases has been associated with the origin (Ducroix et al., 1975; Poisson, 1977; Prewo et al., 1990).

Following with the study of composition from Variedad Colombia and other related genotypes of coffee, this paper describes the isolation and analysis of diterpenes composition, in order to establish the principal differences among these coffee genotypes that allow evaluation of hybrid material. Special attention was given to the new diterpenoids tentatively identified by gas chromatography-mass spectrometry (GC-MS).

MATERIALS AND METHODS

Material. The study was made with green coffee beans of ten genotypes: Variedad Colombia (advanced generation of Caturra x Híbrido de Timor crossing), cultivar resistant to rust (*Hemileia vastatrix*), the first generation of the crossing of Caturra x Híbrido de Timor (F1), two varieties of *C. arabica*, Caturra and Típica; three introductions of Híbrido de Timor (1343, 832 and 2252) and three introductions of *C. canephora* (BP4, BP46 and Centro 1). These materials were planted in Cenicafé germplasm collection in Chinchiná, Caldas, Colombia. The fruits were processed under identical conditions and the seeds were sun dried and grounded in presence of liquid nitrogen.

*Diterpenic fraction extraction.*The diterpenic fractions of each genotype were extracted from 2 g of sample as describes Speer (Speer and Lebensm, 1989), but using terbutyl methylether as solvent and five hours soxhlet extraction. The eluate was concentrated under nitrogen current for subsequent with GC analysis.

Identification. The diterpenic fractions were analyzed with a Hewlett Packard 6890 GC-MS equipped with a HP-5MS column (30 m x 0.250 mm i.d., 0.25 µm film thickness). The temperature program was 150°C for 2 min followed by arise to 250ºC at a rate of 20ºC-min^{-1}. After 30 min the temperature was raised to 280ºC at a rate of 1ºC-min^{-1} and held for 3 min. Helium was used as carrier gas, split ratio 20:1; saver flow 15.0 $mL.min^{-1}$. The injector temperature was 280ºC and 1µL of each sample was injected. Mass spectra were taken at 70 eV. The relative retention times, were calculated with relation to the cafestol and the retention indexes were estimated according to the Kovats method, based on *n*-hydrocarbons. The most of diterpenic compounds were identified by comparison with mass spectra of standard compounds and/or mass spectra previously reported (Pettitt, 1987; Speer et al., 1989; Tewis et al., 1993; de Roos et al., 1997). Other compounds were tentatively identified by comparison of mass spectra with those of standard or structurally similar compounds.

The quantification of each compound was performed using the external standard method, integrating the area of each of the chromatographic peaks and relating them to cafestol acetate (1 $mg.mL^{-1}$).

RESULTS AND DISCUSSION

A total of 11 compounds were identified and quantified in diterpenic fractions analyzed by GC and GC-MS. Table 1 shows these results together with relative retention times to cafestol and Kovats indices. These compounds presented the same basic structure but with differences among them in the position, number of double bonds and/or in the radical on C-16. Cafestol, 16-*O*-methycafestol and kahweol were identified in *C. canephora* and *C. arabica*. The isomers of the dehydrocafestol: 15,16-dehydrocafestol and 13,16-dehydrocafestol and the isomers of the dehydrokahweol: 15,16-dehydrokahweol and 13,16-dehydrokahweol, were tentatively identified in this study for first time in green coffee beans. These 4 compounds had been found in low amounts in roasted coffee and were reported as dehydration products of cafestol and kahweol during the roasting process (Tewis et al., 1993). The 16-*O*-methylkahweol was previously reported in green beans of *C. stenophylla* (de Roos et al., 1997) but it is the first report in *C canephora*. The three remaining compounds, 11,12-dehydrokahweol, 16-*O*-isobutylcafestol and 16-*O*-isobutylkahweol were found for the first time in this work, their spectra are shown in the Figure 1.

The main differences among genotypes were: the presence of kahweol, 13,16-dehydrokahweol and 15,16-dehydrokahweol in *C. arabica*, (Caturra and Tipica varieties), but not in *C. canephora* accessions; the presence of 16-*O*-methylcafestol and 16-*O*-methylkahweol in *C. canephora* but not in *C. arabica.* It was also found significant difference within the varieties of *C. arabica* studied. The main compound of Tipica variety, 11,12-dehydrokahweol was absent in Caturra, while kahweol, one of the highest peaks of Caturra variety, was detected in low concentration in Tipica. Both compounds would differentiate between the two varieties of *C. arabica*. These results correspond well with earlier studies on discrimination of these two varieties (Guerrero et al., 2001) and all this information would contribute in the selection to obtain hybrid materials from them. Additionally the presence of the 11,12-dehydrokahweol in the Variedad Colombia and F1,

could be attributed to the inheritance of the Híbrido de Timor, which might come from the natural crossing of *C. arabica* x *C. canephora* (Moreno, 1982).

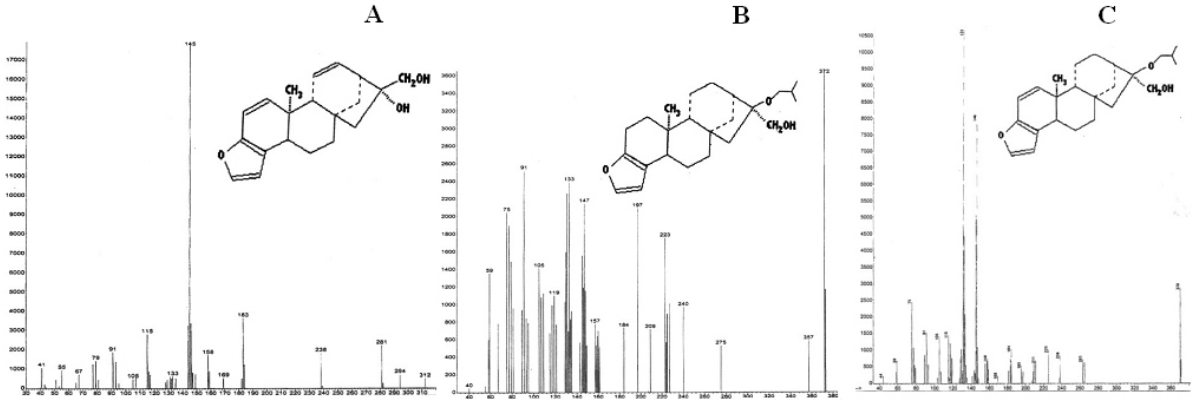

Figure 1. Mass spectrums of new diterpenes found in green coffee beans (A) 11,12-dehydrokahweol, (B) 16-*O*- isobutylcafestol and (C) 16-*O*-isobutylkahweol.

Table 1. Diterpenes identified by GC-MS in genotypes of green coffee beans comparing Retention Times Relatives to cafestol (tR') and Kovats indices (KI). Variety (V.), Hibrido de Timor (HT), Canephora (CAN), and First generation of Caturra x Híbrido de Timor (F1).

COMPOUND	t_R	KI	CATURRA	TIPICA	V. COLOMBIA	F1	HT1343	HT2252	HT832	CANCEN1	CANBP4	CANBP46
13,16 - dehydrokahweol	0.75	1573.89	+	++	++	+++	++	++	+	—	—	—
15,16 - dehydrokahweol	0.77	1591.45	+	++	++	+++	+	+	+	—	—	—
13,16 - dehydrocafestol *	0.78	1600.82	++	++	+	++	+	+++	++	++	++	+
15,16 - dehydrocafestol	0.80	1620.73	+	+	++	++	—	—	+	+	++	+
11,12 - dehydrokahweol	0.81	1626.58	—	+++	++	++	—	—	++	—	—	—
16-O-isobutilkahweol	0.88	1658.13	+	++	+	+	—	—	—	+	—	—
16-O-isobutilcafestol	0.93	1726.11	—	+	—	—	—	—	—	++	+	+
16-O-methylkahweol	0.95	1743.68	—	—	—	—	—	—	—	+	+	+
Kahweol	0.96	1765.23	+++	+	—	+	+++	+	+++	—	—	—
Cafestol	1.00	1798.01	++++	+++	—	—	++	++++	—			
16-O-methylcafestol **	1.00	1798.01	—	—	—	—	—	—	—	++++	+++	++++

*< 10 mg / g of green coffee oil (+); 10-30 mg / g (++) ; 30-70 mg / g (+++) ; 70-110 mg / g (++++); --- = Undetected; * Overlapped with ptalate in HT 2252 coffee; ** 16-O-methylcafestol is overlapped with cafestol in C. canephora coffees.*

The composition of Variedad Colombia and the F1 were similar. In both, the most important diterpenes were the dehydrokahweol and dehydrocafestol, which might indicate that the selection made by agronomic characters, did not affect the composition of this fraction, confirming the results previosly reported (Guerrero et al., 2001). Furthermore, differences were found among Híbrido de Timor accessions, the introduction 2252 exhibited high concentration of cafestol and low concentration of kahweol while in 1343 and 832 introductions the cafestol was not presented or it was found in low concentrations and the kahweol was the major compound and there were not found important differences between *C. canephora* accessions, the three presented the same compounds with the 16-*O*-methylcafestol overlapped with the cafestol.

On the other hand the 16-*O*-isobutylcafestol was detected only in low concentrations in Típica variety and *C. canephora* accessions while the 16-*O*-isobutylkahweol was not found in Híbrido de Timor accessions and in *C. canephora* introductions BP4 and BP46 but it was detected in the other genotypes.

Mass spectral analysis. The 11,12-dehydrokahweol was the main compound of Típica variety, its spectra (Figure 1), showed $[M]^{+\bullet}$ 312, $[M\text{-}H_2O]^{+\bullet}$, $[M\text{-}CH_2OH]^{+}$, common fragmentation of this kind compounds and one important fragment (*m/z* 238), resulted from the loss of 74 amu, which allows to establish the presence of a double bound in 11-12 position. This was confirmed by spectral comparison with a compound of similar structure and double bound in the same position (Herz, 1982). The 16-*O*-methylkawheol presented in its spectra $[M]^{+\bullet}$ 328, $[M\text{-}CH_2OH]^{+}$, $[M\text{-}CH_3OH]^{+\bullet}$, $[M\text{-}CH_2OH\text{-}\ CH_3OH]^{+\bullet}$ and as main fragments *m/z* 131, *m/z* 145 and *m/z* 146 which were contained in kawheol spectra indicating C1-C2 unsatured. The fragment *m/z* 59 confirms *O*-sustitution in C16 as in 16-*O*-methylcafestol spectra.

The compounds 16-*O*-isobutylkahweol ($M^{+\bullet}$ 370) and the 16-*O*-isobutylcafestol ($M^{+\bullet}$ 372) had mass spectra a little different to other diterpenes. These compounds did not show neither of two fragments $[M\text{-}H_2O]^{+\bullet}$ nor $[M\text{-}CH_2OH]^{+}$ which may indicate *O*-sustitution in C-16. Additionally the fragment *m/z* 59 confirms this substitution. In the 16-*O*-isobutylkahweol spectrum was found main ionic fragments presented in the kahweol spectrum and the 16-*O*-isobutylcafestol presented *m/z* 133 and *m/z* 147 fragments as in the cafestol spectrum.

In conclusion, the results of this study allowed to differentiate between *C. arabica* Caturra and Típica varieties, and also among all Hibrido de Timor accessions and differentiate *C. canephora* accesssions from the other genotypes. There were not found important differences in the composition between First generation of the crossing of Caturra x Híbrido de Timor and Variedad Colombia. Furthermore it was possible to identify tentatively three new furanekaurenic diterpenes, which are also discriminating compounds. This information would be used for evaluation of hybrid material, very important in plant breeding programs.

ACKNOWLEDGEMENTS

The authors thank COLCIENCIAS for its financial support.

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