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CAFFEINE AND THEOBROMINE IN GREEN BEANS FROM *MASCAROCOFFEA*

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Key Word Index—*Coffea*; *Mascarocoffea*; Rubiaceae; Cinchonoideae; coffee beans; alkaloids; caffeine; theobromine; chemotaxonomy.

Abstract—The presence of theobromine (up to 0.14%) and of caffeine (up to 0.76%) has been established for the first time in beans from two populations of *Coffea lancifolia* (A320 and A405) and in one population of *C. kianjavatensis* (A213). This indicates that the *Mascarocoffea*, once thought to be purine-free, includes some members storing methyl xanthines in seeds, but the virtual absence of caffeine from another *C. kianjavatensis* (A602), and of both caffeine and theobromine from three populations of *C. homollei* (A574, A743 and SZ108) indicates the biochemical diversity of these taxa.

INTRODUCTION

Mascarocoffea, the wild coffees of Madagascar and neighbouring islands, have been divided morphologically into seven botanical 'Séries' [1, 2], but only *ca* 20 taxa therein have been studied from a chemical point of view. Chevalier [1] believed the *Mascarocoffea* were caffeine-free, and until recently this belief had been substantiated by many investigations [3–8] which found not more than 0.07% caffeine, and that only in a single taxon, *C. mauritiana* [3, 4]. Recently, however, it has been established that the beans of at least one population (A213) of *C. kianjavatensis* contain caffeine at up to 0.81% db [9]. This observation indicated the need for our previous chemotaxonomic studies [10, 11] of *Coffea* spp. and *Psilanthus* spp. to be extended to the seven 'Séries' of the *Mascarocoffea*, and a comprehensive study of over 100 samples has commenced.

RESULTS AND DISCUSSION

Aqueous extracts prepared from ground green beans were analysed for their caffeine contents by GC. Observation of a second purine-like peak during confirmatory HPLC led to the isolation of the chloroform-soluble components by preparative HPLC and their characterization by physical methods.

The retention time, UV and EI mass spectra (70 eV) of the two components were indistinguishable from authentic caffeine and theobromine. Caffeine *m/z* 194 (100); 109 (61); 55 (52); 67 (32); 82 (28); 137 (8). Theobromine *m/z* 180 (100); 55 (58); 67 (51); 109 (39); 82 (38); 137 (8). The quantitative GC and HPLC data for caffeine and the HPLC data for theobromine are presented in Table 1. The values for caffeine obtained by HPLC are slightly, but not significantly, smaller than those obtained by GC, but both sets are consistent with the data (not shown)

Table 1. Purine content (% db) of green beans from *Mascarocoffea*

	Taxa and number of entry						
	C. kianjavatensis		*C. lancifolia*		*C. homollei*		
	A213	A602	A320	A405	A574	A743	SZ108
Caffeine GC	0.81	*	0.69	0.59	nd	nd	nd
Caffeine HPLC	0.75	≈0.05†	0.63	0.55	nd	nd	nd
Theobromine HPLC	0.07	0.14	0.06	0.06	nd	nd	nd

*Insufficient sample for GC analysis.
†Lack of sample limits precision of this result.
nd: Not detectable, i.e. less than 0.01%.

obtained by the analysis of phenolic extracts prepared according to Fleuriet and Macheix [12].

This short communication constitutes the first report of theobromine, in at least some populations from two taxa of the *Mascarocoffea*. We further demonstrate that members of the 'Série Verae' of *Mascarocoffea* are biochemically diverse as there are significant quantities of: (i) caffeine in the green beans of *C. lancifolia* (A320, A405) and *C. kianjavatensis* (A213), but it is lacking from *C. kianjavatensis* (A602) and *C. homollei* (A574, A743 and SZ108); (ii) theobromine in beans from *C. lancifolia* (A320, A405), *C. kianjavatensis* (A213, A602), but not in beans of the three populations of *C. homollei* investigated.

EXPERIMENTAL

Materials. All bean samples were supplied by the Research Centre of Kianjavato, FO. FI. FA., Madagascar. *Coffea kianjavatensis, C. lancifolia* and *C. homollei* belong to the 'Série verae' of the *Mascarocoffea* [1, 13, 14]. All reagents were normal commercial items of good quality.

Methods. Ground green coffee (1 g) was refluxed in boiling water containing MgO for 30 min [15]. After filtration and the addition of int. standard (5-aminoquinoline), caffeine was determined on a Hewlett Packard 5710 A gas chromatograph equipped with a NPFID detector. A 1 m glass column packed with Chromosorb AWHMDS and coated with 3% carbowax 20 M, operating at 215° with a 40 ml min^{-1} N_2 flow rate was used for separation.

For theobromine and caffeine analysis by HPLC the aqueous phase as obtained above was extracted ×4 (v/v) with $CHCl_3$. The pooled extracts were dried over Na_2SO_4 and evapd to dryness, dissolved in 10 ml MeOH and analysed by HPLC using external standards for quantification. Analytical HPLC: separation was achieved on a Lichrospher 5 RP18 column (4.6 × 250 mm) using a 1 ml min^{-1} flow rate and a gradient from 5% MeOH in 2 mM H_3PO_4 to 100% MeOH in 48 min. Prep. HPLC was similarly performed, but using a Lichrosorb 10 RP18 (10 × 250 mm) column and a 2 ml min^{-1} flow rate. Mass spectra: EI mode at 70 eV. The source temperature was 250° with desorption over the range 20° to 250°.

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