

CAFFEINE FROM GREEN BEANS OF *MASCAROCOFFEA*

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Abstract—Unequivocal evidence is presented for the presence of 0.55–0.81% caffeine in the beans of population A213 of *Coffea kianjavatensis*, one taxon of the *Mascarocoffea*, which traditionally have been viewed as caffeine-free.

INTRODUCTION

Coffees belong to the family Rubiaceae, subfamily Cinchonoideae, tribe Coffeae. The Coffeae have recently been restricted to two genera, *Coffea* (with three subgenera) and *Psilanthus* (with two subgenera) [1]. Our previous chemotaxonomic studies [2, 3] on the seeds from 27 species of *Coffea* and 4 species of *Psilanthus* have tended to support Robbrecht and Puff's morphological classification [1], although it was recognised that seeds from a considerable number of other species had yet to be analysed. Among these omissions were the geographically restricted species from Madagascar and neighbouring islands which in 1947 Chevalier [4] designated as *Mascarocoffea*. Chevalier *et al.*, believed that the seeds of *Mascarocoffea* were caffeine-free, and essentially this belief has been substantiated, with traces of caffeine being found in only one of some 20 taxa examined more recently [5]. We now report data obtained independently by groups working in Guildford and Montpellier showing the presence of significant quantities of caffeine in the beans from one Madagascan *Mascarocoffea*.

RESULTS AND DISCUSSION

Bean samples were prepared by the Research Centre of Kianjavato, FO.FI.FA., Madagascar, and supplied after drying to ca 10% moisture content. The data reported below were obtained independently late in 1988, but it was not until mid-1990 that both groups realised that identical conclusions had been reached. Thereafter, it seemed sensible to prepare this joint publication.

Using different methods of extraction and HPLC both groups observed a 276 nm absorbing peak which could not be resolved from authentic caffeine added as a spike. Comparison with caffeine standards suggested a putative caffeine content in excess of 0.5% db. No record could be found of the seeds of *C. kianjavatensis* having been analysed for caffeine, a situation no doubt arising due to acceptance of Chevalier's assertion that the seeds of *Mascarocoffea* were caffeine-free. Accordingly, both groups sought confirmation by spectroscopic methods, and the material corresponding to the peak in question

was collected by preparative chromatography of the concentrated extract prepared either at reduced pressure (Guildford), or by partitioning into chloroform (Montpellier). The isolates so obtained were subjected to mass, UV, NMR and FTIR spectroscopy.

Coffea kianjavatensis, in common with many other coffees, has a significant content of caffeoylquinic acids (5.9% db), and dicaffeoylquinic acids (0.69% db), but only traces of feruloylquinic acids. Two other 276 nm absorbing components, previously referred to as components A and C [2], and which may be related to caffeine, are under investigation. In each case the data obtained for the isolate were indistinguishable from those obtained for authentic caffeine. The wavelengths of maximum absorption were 209 and 273 nm in EtOH (Guildford) and 273 nm in H₂O (Montpellier). The ¹H NMR spectrum in DMSO-*d*₆ showed four singlets at δ 8.02 (1H, H-8), 3.87 (3H, N-7 Me), 3.41 (3H, N-3 Me) and 3.22 (3H, N-1 Me). The IR spectrum (CCl₄ solution) showed two carbonyl peaks at 1711 and 1667 cm⁻¹. Mass spectroscopy gave a [M]⁺ at *m/z* 194.12 (100%) together with fragment ions at *m/z* 165 (5%), [M - NMe]⁺, *m/z* 137 (7.1%), [M - C₂H₃NO]⁺, *m/z* 109 (58%, C₅H₇N₃), *m/z* 82 (29%, C₄H₆N₂), *m/z* 67 (38%, C₄H₄N) and *m/z* 55 (49%). The mean caffeine content of the beans (four replicates) obtained in Guildford by HPLC was 0.53%; the corresponding mean value obtained in Montpellier was 0.81% by GC. These values are considerably greater than the 0.07% and 45 mg kg⁻¹ previously observed in *C. mauritiana* [5, 6] and similar to those for several east African species placed in *Coffea* subgenus *Coffea* [2].

Our observations indicate that Chevalier's description of *Mascarocoffea* as caffeine-free is no longer tenable if all Madagascan species are included. Therefore, there is a need for further investigations of these geographically isolated species to ascertain their correct position within the classification proposed by Robbrecht and Puff [1].

EXPERIMENTAL

Materials. Ripe fruits from *C. kianjavatensis* (A213) were collected from previously identified plants growing in Madagas-

car. The seeds were released by the wet process and dried in the shade to ca 10% moisture content.

Methods, Montpellier. Grinding and H₂O extraction of beans and GC analysis for caffeine were as described in ref. [7]. Prep and purification of a 70% MeOH extract was as described in ref. [8] and analytical reverse-phase HPLC was performed as described for cocoa phenols in ref. [9]. MS were obtained at 70 eV in EI mode; source temp. was 250° with desorption from 20° to 250°.

Methods, Guildford. Grinding and extraction of beans and analytical and prep. reverse-phase HPLC were as previously described [2, 10-12]. ¹H NMR spectra were obtained at 300 MHz. Samples were dissolved in DMSO-*d*₆ and examined at room temp. and peak positions recorded rel. to TMS as int. standard. UV spectra were recorded in EtOH at room temp. FTIR spectra were recorded in CCl₄.

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