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FAILURE TO DETECT GLUCOSINOLATES IN COCOA

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Abstract—Dehulled cocoa beans and hulls of cocoa beans, *Theobroma cacao* L., have been examined for a supposed content of glucosinolates. Different methods of glucosinolate analysis have been used for investigations of the anion-containing fractions but no glucosinolates (detection limit 1 nmol/g) could be detected. The significance of the results obtained is briefly discussed.

INTRODUCTION

Glucosinolates co-occur with myrosinases (thioglucoside glucohydrolase, EC 3.2.3.1) in all of the plants of the order Capparales hitherto investigated [1–4]. Glucosinolate degradation, catalysed by myrosinase, yields characteristic products [3, 4] which are the basis of many analytical methods [3–6]. There have been occasional reports of the occurrence of glucosinolates and their degradation products in species which are systematically remote from the Capparales. In view of the chemotaxonomic significance of such occurrences it has been argued that unambiguous evidence for the presence of glucosinolates is necessary to support such claims [6, 7]. This is particularly true in cases where hydrolysis products alone have been identified and where these have been found only in trace amounts.

Volatile components of cocoa (*Theobroma cacao* L.) have been identified in a comprehensive study [8]. Some of these are often considered as characteristic degradation products of glucosinolates [9]. Based on these results the presence of glucosinolates in cocoa has been claimed [9], but there are no reports of other members of the Sterculiaceae containing such compounds. Recently sophisticated chromatographic and physiochemical methods have been developed for the analysis of intact glucosinolates (see Experimental) and these, together with methods for the analysis of myrosinase and the products of glucosinolate hydrolysis, have been employed in a re-investigation of these claims.

RESULTS AND DISCUSSION

After dehulling, both hulled and dehulled beans were extracted with boiling methanol–water (7 : 3) to minimize any myrosinase-induced hydrolysis and analysed for intact glucosinolates by glucose release [10], HPLC [11, 12], GC [13], coupled GC-MS [14] and HPLC-MS, paper chromatography and high voltage electrophoresis [3, 7]. Reference glucosinolates [11, 15] were isolated and used to optimize and calibrate these techniques. Extracts of beans analysed by HVE at pH 1.9 contained anions which behaved similarly to glucosinolates (mobility, response to silver nitrate reagent) but these were shown not to be glucosinolates by PC, GC and HVE at pH 6.5. HPLC analysis of the fractions revealed several components but none possessed any of the ions characteristic of glucosinolates when examined by HPLC-MS and all were unaffected by treatment with myrosinase. The same was true when the extracts were treated with sulphatase [13] and analysed by GC and GC-MS [14]. None of the extracts examined possessed myrosinase activity [16] nor contained discernible glucosinolate hydrolysis products, notably benzyl isothiocyanate, nitrile or thiocyanate.

Quantitative assessment, based upon the results of HPLC and GC analysis and the behaviour of benzyl glucosinolate [14] revealed a detection limit for this component of *ca* 1 nmol/g. According to Gill *et al.* [9] the products of benzyl glucosinolate were found in the samples of cocoa examined at the level of approximately 10 nmol/g, very much lower than that usually found in glucosinolate-containing species [4].

A bulked sample (1 kg) of cocoa beans was ground and extracted according to the procedure of Gill *et al.* [9]. A second sample was extracted with aqueous methanol [17] and, after removal of the organic solvent, treated with myrosinase and ascorbic acid. After workup [9] neither of these samples showed evidence of any benzyl or *iso*-butyl glucosinolate products when assayed by GC-MS.

In conclusion, the presence of glucosinolates in cocoa samples has not been confirmed although a variety of sensitive and specific methods have been used. Unequivocal proof of the absence of glucosinolates in plant material is difficult to obtain; however, on the basis of the analysis conducted in this study the levels of glucosinolates present must be much less than 1 nmol/g. It has been suggested that the presence of trace amounts of benzyl glucosinolate noted by others [9] may have resulted from contamination with papaya (*Carica papaya*) seeds, but no evidence has been found to support this. In conclusion, it is considered that claims for the occurrence of glucosinolates in the Sterculiaceae, and in particular cocoa, must be regarded as questionable.

EXPERIMENTAL

Plant material. Cocoa beans, free from debris, were obtained from commercial plantations in Malaysia, Ivory Coast, Brazil and the Dominican Republic and were provided by international confectionary companies.

Methods of analysis. These have been published previously and comprised glucose release [10], GC [13] and HPLC [11, 12] techniques, both alone and in combination with MS, PC and HVE [3, 7]. Combined GC-MS was used to monitor for individual products of glucosinolate hydrolysis [9] and myrosinase was determined by the coupled enzyme procedure [16].

Isolation procedure. Beans (100 g) were dehulled manually and the hulls, dehulled beans ground and stored at $-40°$ until required for analysis. To avoid myrosinase-catalysed glucosinolate breakdown, homogenization and extraction was carried out in boiling MeOH–H_2O (7:3) as described previously [17].

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