Identification of Salsolinol as a Phenolic Component in Powdered Cocoa and Cocoa-Based Products

An important alkaloid in cocoa and cocoa-based products has been identified as 1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (salsolinol) using chromatographic techniques described in a previous communication (Riggin, R. M., McCarthy, M. J., Kissinger, P. T., *J. Agric. Food Chem.* **24**, 189 (1976)). Quantitative experiments using liquid chromatography with electrochemical detection indicate that salsolinol is present in powdered cocoa at the level of 40 ± 4 μg/g.

In a recent communication to this journal (Riggin et al., 1976) we described the identification of 1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (salsolinol) as a major metabolite of 3-hydroxytyramine (dopamine) in the ripening banana. This accidental discovery resulted from a concern for the influence of dietary factors on the urinary excretion of catecholamines in man. Recent work in the same area (Riggin and Kissinger, 1976) has led us to the equally fortuitous discovery that individuals consuming chocolate products excrete elevated amounts of salsolinol. Following up on this observation we have now established that this alkaloid is an important phenolic constituent in cocoa and cocoa-based products.

EXPERIMENTAL SECTION

Powdered confectioners cocoa (Hershey Foods Corporation) was purchased locally. Samples (100 mg) were prepared for analysis by shaking for 5 min with 5 ml of 0.1 M HCl. Two grams of ammonium sulfate was added and the suspension was centrifuged at 3500 rpm for 5 min. A standard addition of 20 μg of salsolinol was made to some samples prior to the shaking step. The supernatants were extracted with ethyl acetate and then hexane (5 ml each). The organic layers were discarded and the aqueous layer was adjusted to pH 8.5 and shaken with 100 mg of acid-washed aluminum oxide to adsorb catechols. The catechol fraction was desorbed into 1 M acetic acid (500 μl) as previously described for urinary catecholamines (Kissinger et al., 1975).

The isolated acetic acid extract was analyzed in the same manner as for a similar extract obtained from the banana (Riggin et al., 1976). The qualitative results (HPLC, TLC, GLC) were as described in the earlier report and strongly support our contention that salsolinol is a major catechol alkaloid in cocoa. As before, quantitative work was carried out using liquid chromatography with electrochemical detection (LCEC). Salsolinol was the predominant peak in these chromatograms and there was no detectable amount of its logical precursor dopamine. Results for powdered cocoa indicate a salsolinol concentration of 40 ± 4 μg/g in the single lot tested. In the LCEC experiments 20 μl of the extract was injected onto two different chromatographic systems (VYDAC SCX; pH 3.96 mobile phase: 0.01 M in acetate and 0.04 M in Na_2SO_4; and DuPont Zipax SCX; mobile phase: 0.01 M H_2SO_4 and 0.04 M Na_2SO_4). In both cases the unknown component was found to have the same retention time as authentic salsolinol.

Qualitative work supports the presence of salsolinol in many, if not all, chocolate products.

DISCUSSION

Salsolinol has apparently not been previously identified in cocoa. The mechanism of its formation is not known; however, there is as yet no evidence to suggest that the synthetic route follows the classical Pictet-Spengler reaction as in the banana (Riggin et al., 1976). Fundamental phytochemical studies are clearly needed. Perhaps such studies will be facilitated by the new approach to analysis of phenols recently developed in our laboratory (Felice et al., 1976). At this point it is unclear whether dietary salsolinol is of any pharmacological importance; however, it appears to be readily conjugated and cleared from the body (Riggin and Kissinger, 1976).

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Received for review February 9, 1976. Accepted April 8, 1976. Support for this research from the National Science Foundation, the National Institute of General Medical Sciences, and the Showalter Trust is gratefully acknowledged.