7-Methylxanthine Is Not Involved in Caffeine Catabolism in *Coffea dewevrei*

Paulo Mazzafera

Departamento Fisiologia Vegetal, IB, Universidade de Campinas, CP 6109, 13081-970 Campinas, SP, Brazil

To investigate the role of 7-methylxanthine as an intermediary compound in the degradation of theobromine to xanthine in coffee, leaves and immature fruits of *Coffea dewevrei* were incubated with [8-^{3}H]caffeine plus allopurinol. *In vivo* inhibition of xanthine degradation occurred in fruits and leaves fed with allopurinol since marked incorporation of radioactivity in xanthine and 3-methylxanthine was observed. Nevertheless, HPLC analysis did not reveal any radioactivity in 7-methylxanthine. The results suggest that in coffee theobromine is degraded to xanthine via 3-methylxanthine and 7-methylxanthine is exclusively involved in the caffeine biosynthesis pathway.

INTRODUCTION

Theobromine (3,7-dimethylxanthine) is located at an interesting position in the caffeine (1,3,7-trimethylxanthine) metabolic pathway in *Coffea arabica*, being the immediate precursor and one of the first products of caffeine catabolism. Nevertheless, caffeine is mainly degraded via theophylline (1,3-dimethylxanthine), 3-methylxanthine, xanthine, and uric acid, and this last compound undergoes degradation via a urea pathway [Suzuki and Waller, 1984a; for a review, see Suzuki et al. (1992)].

Theoretically, the theobromine coming from caffeine breakdown could be reused for the synthesis of caffeine or it could be demethylated to 3-methylxanthine or 7-methylxanthine. Previous investigation has shown that 7-methylxanthine is the precursor of theobromine in coffee fruits (Roberts and Waller, 1979; Suzuki and Waller, 1984a), but there is a lack of evidence proving that this monomethylxanthine is a product of theobromine degradation.

Although low levels of radioactivity were detected, Suzuki and Waller (1984a,b) obtained consistent data showing that theobromine and 3-methylxanthine were products of caffeine degradation in experiments in which fruits of *C. arabia* were fed with ^{14}C-labeled caffeine. Thus, they could not conclude if 7-methylxanthine was a product of the theobromine breakdown.

Unlike *C. arabica*, fruits and leaves of *Coffea dewevrei* are very efficient for [8-^{3}H]caffeine degradation, accumulating substantial amount of theobromine (Mazzafera et al., 1991). However, even when fruits and leaves were fed with [8-^{3}H]caffeine, of a higher specific activity than ^{14}C-labeled caffeine, it was not possible to detect radioactivity in 7-methylxanthine in the HPLC analysis. On the other hand, marked amounts of radioactivity were incorporated in theobromine, 3-methylxanthine, and xanthine.

With the purpose of studying the specific metabolic step theobromine → 7-methylxanthine → xanthine in coffee, *C. dewevrei* was used as a model, feeding fruits and leaves with [8-^{3}H]caffeine only or with [8-^{3}H]caffeine plus allopurinol. Allopurinol, an analogue of purine, is a strong inhibitor of xanthine oxidase (Schubert and Boland, 1990) and has been used previously in investigations of ureide metabolism in legumes growing symbiotically with N_2-fixing bacteria (Fujihara and Yamaguchi, 1978).

MATERIALS AND METHODS

Immature fruits were used because they are metabolically more active than fruits in advanced stages of maturation (Suzuki and Waller, 1984a; Mazzafera et al., 1991). Leaves of the second pair of leaves and 35-week-old fruits were collected from a *C. dewevrei* tree ca. 50 years old at the Experimental Station of Instituto Agronômico, Campinas, Brazil. After washing in running tap water, the leaves were quickly dried with absorbent tissue and the petioles immersed in vials with 1 mL of 0.1 M sodium phosphate buffer, pH 6.5, containing 0.5% ascorbic acid and 2.2×10^6 dpm of [8-^{3}H]caffeine (22.2 Ci/mmol; Amersham). When present, allopurinol was added to the concentration of 1 mg/mL. During the incubation period the absorbed solution was being replaced by buffer containing ascorbic acid. At the end of the experiment, radioactivity was counted in the remaining solution in the vials. Eighty-four percent of the [8-^{3}H]caffeine was absorbed by the leaves for the allopurinol treatment, and 89% was absorbed by the control leaves.

Fruits were fed with [8-^{3}H]caffeine as described before (Mazzafera et al., 1991). Each fruit received 2.2×10^6 or 4.4×10^6 dpm of [8-^{3}H]caffeine and during the incubation 0.1 M sodium phosphate buffer, pH 6.5, was periodically dropped on the small cut made in the peduncle. For the treatment of fruits with allopurinol, the compound was dissolved in buffer (25 mg/mL) and continuously dropped on the small cut. Each fruit received approximately 50–60 μg of allopurinol.

After 8 h of incubation, leaves and fruits were frozen in liquid nitrogen, freeze-dried, and extracted according to the method of Petermann and Baumann (1983). Endogenous compounds and labeled metabolites were analyzed as described previously (Mazzafera et al., 1991), except that a Waters HPLC system was used and the HPLC gradient was performed in 25 min in 45% of methanol.

Twenty fruits were used in the tracer experiments, and two replicates were made for the extractions of [8-^{3}H]caffeine metabolites. Samples from two replicates were analyzed in the experiments with leaves, and four leaves composed each replicate. All incubations were carried out at room temperature (25 °C) and under constant light (300 microeinstein m^{-2} s^{-1}).

RESULTS AND DISCUSSION

The demethylation of caffeine to theophylline appears to be the limiting step for caffeine degradation in fruits of *C. arabica* (Suzuki and Waller, 1984a,b). After 90 h of incubation, Suzuki and Waller (1984a) observed that more than 70% of the [8-^{14}C]caffeine absorbed by the fruits was recovered as caffeine. Only 5% of [8-^{14}C]caffeine was metabolized in fruits incubated for 24 h (Suzuki and Waller, 1984b). However, when fruits were incubated with

0021-8561/93/1441-1541$04.00/0

Table I. Distribution of Radioactivity of [8-^{3}H]Caffeine Metabolites in Leaves and Fruits of *C. dewevrei* after 8 h of Incubation

	distribution of radioactivity,[a] %							
	Xan	3mx	7mx	Thb	Thp	Caf	recovered, %	applied[b]
fruits								
[8-^{3}H]caf + Allo	6.9	5.0	ND[c]	31.9	2.9	53.2	21.0	18600
[8-^{3}H]caf	2.4	4.0	ND	25.9	2.6	64.8	24.2	18800
leaves								
[8-^{3}H]caf + Allo	12.9	8.5	ND	16.5	13.2	48.4	7.3	3248
[8-^{3}H]caf	1.0	4.0	ND	23.0	10.2	54.8	4.7	3955

[a] [8-^{3}H]caf, [8-^{3}H]caffeine; Caf, caffeine; Thp, theophylline; Thb theobromine; 3mx, 3-methylxanthine; 7mx, 7-methylxanthine; Xan, xanthine; Allo, allopurinol. [b] Applied radioactivity is expressed as 10^6 dpm/mg of dry weight, and recovered radioactivity is expressed as percent of applied radioactivity recovered after 8 h of incubation. [c] ND, not detected.

Table II. Distribution of Radioactivity of [8-^{3}H]Caffeine Metabolites in Fruits of *C. dewevrei* after 24 h of Incubation

	distribution of radioactivity,[a] %							
	Xan	3mx	7mx	Thb	Thp	Caf	recovered, %	applied[b]
[8-^{3}H]caf	ND[c]	10.0	ND	48.9	ND	ND	3.6	26500
[8-^{3}H]caf + Allo	3.2	15.0	ND	72.6	2.0	6.4	5.2	26800

[a] [8-^{3}H]caf, [8-^{3}H]caffeine; Caf = caffeine; Thp, theophylline; Thb, theobromine; 3mx, 3-methylxanthine; 7mx, 7-methylxanthine; Xan, xanthine; Allo, allopurinol. [b] Applied radioactivity is expressed as 10^6 dpm/mg of dry weight, and recovered radioactivity is expressed as percent of applied radioactivity recovered after 24 h of incubation. [c] ND, not detected.

[2-^{14}C]xanthine for only 24 h, all of the recovered radioactivity was found in degradation products of xanthine.

The percentages of radioactivity of Tables I and II are related to compounds involved in the caffeine catabolism that could receive the ^{3}H from the degradation of [8-^{3}H]-caffeine. The polar compounds detected previously by Mazzafera et al. (1991) were also detected in some extracts, but these data are not shown.

The results in Table I confirm previous investigations and show that fruits and leaves of *C. dewevrei* are very efficient for caffeine degradation when compared with data obtained for *C. arabica* (Mazzafera et al., 1991). As observed before (Mazzafera et al., 1991), fruits of *C. dewevrei* accumulated more radioactivity in theobromine than theophylline, but a similar disproportionate ratio was not observed in leaves.

Less radioactivity was recovered in leaf extracts (4.7%) than in fruits (24.2%), indicating higher efficiency for caffeine degradation (Table I). However, when leaves were fed with [8-^{3}H]caffeine plus allopurinol, more radioactivity was recovered. A 10-fold increase of radioactivity in xanthine and a 2-fold increase in 3-methylxanthine were also observed in leaves treated with allopurinol, suggesting that the allopurinol had an inhibitory effect in xanthine oxidation and in the previous metabolic step, the conversion of 3-methylxanthine to xanthine. Although fruits treated with allopurinol have a higher percentage of labeled xanthine than the control, this was not as pronounced in leaves. Radioactivity remaining as caffeine and recovered as theobromine and theophylline was not significantly affected by allopurinol. It is interesting to note that caffeine represents a higher percentage of radioactivity in the controls despite a lower recovery of radioactivity.

HPLC chromatograms of standards (Figure 1A) and extracts from leaves of *C. dewevrei* incubated either with [8-^{3}H]caffeine (Figure 1B) or with [8-^{3}H]caffeine plus allopurinol (Figure 1C) are shown in Figure 1. An example of the sensitivity of the method used for the analysis of the ^{3}H-labeled metabolites is given in Figure 1B, where the detected radioactivity for the peak corresponding to 3-methylxanthine was ca. 1700 dpm. Thus, considering that considerable amounts of [8-^{3}H]caffeine were supplied to leaves (2×10^6 dpm/leaf) and fruits ($2–4 \times 10^6$ dpm/fruit) and the sensitivity of the method used for the analysis

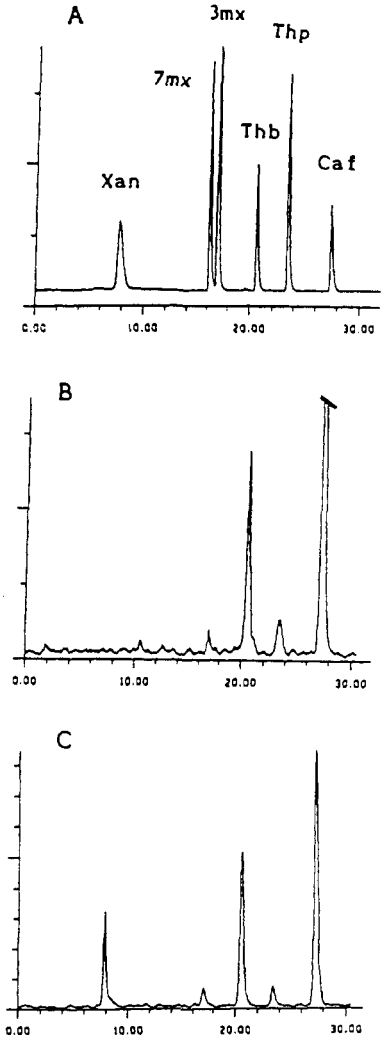

Figure 1. HPLC chromatograms of standards (A) and extracts from leaves of *C. dewevrei* treated with [8-^{3}H]caffeine (B) or [8-^{3}H]caffeine plus allopurinol (C). The standard peaks were detected in an UV monitor and the labeled metabolites in a radioactivity monitor. Caf, caffeine; Thp, theophylline; Thb, theobromine; 3mx, 3-methylxanthine; 7mx, 7-methylxanthine; Xan, xanthine.

of the extracts, we believe that if 7-methylxanthine were a precursor of xanthine, as known for 3-methylxanthine, some radioactivity could be found in this compound, at least in samples from the treatments with allopurinol.

In another attempt to check if 7-methylxanthine could be a product of theobromine breakdown, we extended to 24 h the incubation period for fruits (Table II). When the [8-^{3}H]caffeine metabolism was allowed to proceed for 24 h, all of the metabolic steps between caffeine and xanthine were affected by allopurinol. Caffeine was extensively degraded in fruits of both the control and allopurinol treatments. Theobromine accumulated the highest level of radioactivity, but as before, no radioactivity was detected in 7-methylxanthine, even in fruits incubated with allopurinol.

The low degradation rate of caffeine in fruits of *C. arabica* is a limiting factor for the detection of 7-methylxanthine as a product of theobromine demethylation. However, since the compounds detected from caffeine degradation in fruits of *C. arabica* were found in leaves and fruits of *C. dewevrei* (Table I; Suzuki and Waller, 1984a,b; Mazzafera et al., 1991), indicating a strong metabolic similarity, it is proposed that 7-methylxanthine is not a catabolite of caffeine in *arabica* fruits.

ACKNOWLEDGMENT

I thank Dr. Göran Sandberg for the laboratory facilities and CNPq—Brazil for granting a fellowship during a leave of absence at The Swedish University of Agricultural Science, Umeå.

LITERATURE CITED

Fujihara, S.; Yamaguchi, M. Effects of allopurinol [4-hydroxypyrazolo(3,4-d)pyrimidine] on the metabolism of allantoin in soybean plants. *Plant Physiol.* **1978**, *62*, 134–138.

Mazzafera, P.; Crozier, A.; Magalhães, A. C. Caffeine metabolism in *Coffea arabica* and other species of coffee. *Phytochemistry* **1991**, *3*, 3913–3916.

Petermann, J. B.; Baumann, T. W. Metabolic relations between methylxanthine and methyluric acids in *Coffea* L. *Plant Physiol.* **1983**, *73*, 961–964.

Roberts, M. F.; Waller, G. R. N-methyltransferases and 7-methyl-N^9-nucleoside hydrolase activity in *Coffea arabica* and the biosynthesis of caffeine. *Phytochemistry* **1979**, *18*, 451–455.

Schubert, K. R.; Boland, M. J. The ureides. In *Intermediary Nitrogen Metabolism*; Miflin, B. J., Lea, P. J., Eds.; Academic Press: New York, 1990; pp 197–282.

Suzuki, T.; Waller, G. R. Biosynthesis and biodegradation of caffeine, theobromine, and theophylline in *Coffea arabica* L. fruits. *J. Agric. Food Chem.* **1984a**, *32*, 845–848.

Suzuki, T.; Waller, G. R. Biodegradation of caffeine: Formation of theopohylline and theobromine from caffeine in mature *Coffea arabica* fruits. *J. Sci. Food Agric.* **1984b**, *35*, 66–70.

Suzuki, T.; Ashihara, H.; Waller, G. R. Purine and purine alkaloid metabolism in *Camellia* and *Coffea* plants. *Phytochemistry* **1992**, *31*, 2575–2584.

Received for review March 8, 1993. Revised manuscript received May 28, 1993. Accepted July 12, 1993.*

* Abstract published in *Advance ACS Abstracts*, September 1, 1993.