Biosynthesis and Biodegradation of Caffeine, Theobromine, and Theophylline in *Coffea arabica* L. Fruits

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The biosynthesis and biodegradation of the purine alkaloids in developing *Coffea arabica* fruits were studied with [2-^{14}C]caffeine, [8-^{14}C]theophylline, [8-^{14}C]adenine, [8-^{14}C]guanine, [2-^{14}C]xanthine, or L-[*methyl*-^{14}C]methionine dissolved in water. Both immature and mature coffee fruits degraded [2-^{14}C]caffeine to theobromine, theophylline, N^3-methylxanthine, N^7-methylxanthine, allantoin, allantoic acid, and urea. Immature fruit metabolized [8-^{14}C]theophylline and [2-^{14}C]xanthine more rapidly than the mature fruit. [8-^{14}C]Adenine was more effective than [8-^{14}C]guanine or L-[methyl-^{14}C]methionine as a precursor for the biosynthesis of N^7-methylxanthine, theobromine, and caffeine. These results indicate that (a) biosynthesis of caffeine occurs mainly during the green stage of fruit development through methylation of N^7-methylxanthine and theobromine and (b) biodegradation occurs through theophylline, which accumulates after the seed is full size and proceeds to ripen.

Caffeine (1,3,7-trimethylxanthine) is a major alkaloid of coffee, tea, cola, and guarana (Weevers, 1930), and other methylxanthines, e.g., theobromine (3,7-dimethylxanthine) and theophylline (1,3-dimethylxanthine), are found as minor components in these plants (Maravalhas, 1966; Franzke et al., 1968; Tabak et al., 1969). In vivo and in vitro studies with tea and coffee plants have shown that theobromine is synthesized from N^7-methylxanthine and transformed to caffeine (Suzuki and Takahashi, 1975, 1976a,b; Ogutuga and Northcote, 1970a,b; Looser et al., 1974; Roberts and Waller, 1979; Waller et al., 1981). In contrast, theophylline can be synthesized from N^1-methylxanthine in vitro (Suzuki and Takahashi, 1975; Roberts and Waller, 1979; Waller et al., 1981), but there is no definitive evidence for the in vivo process. Theophylline proved much less efficient (4%) as a precursor of caffeine than theobromine (100%) in a substrate specificity study (Roberts and Waller, 1979; Waller et al., 1981). N^3- and/or N^7-methylxanthine, allantoin, allantoic acid, urea, and CO_2 were found by Kalberer (1964, 1965) as biodegradation products of caffeine when radioactive (*N*-methyl-labeled as well as ring-labeled) caffeine was administered to old leaves of coffee plants. His report did not describe the dimethylxanthines but suggested that these are formed from caffeine by demethylation rather than from methylxanthines by methylation. Other papers also reported caffeine metabolism in tea and coffee plants (Spedding and Wilson, 1964; Baumann and Wanner, 1972), but they did not characterize the degradation products. Suzuki and Waller (1984) reported that biodegradation of caffeine occurred in mature, ripened, coffee fruits through theophylline and theobromine as the first biodegradation products. This paper describes experiments showing that biodegradation and biosynthesis reactions occur in the immature fruit more rapidly than in the mature fruit.

EXPERIMENTAL SECTION

Materials. Materials were obtained from the following sources: [2-^{14}C]xanthine (48 mCi/mmol), [8-^{14}C]adenine (50 mCi/mmol), and [8-^{14}C]guanine (53.4 mCi/mmol) from Le Commissariat à l'Énergie Atomique, Paris, France; L-[*methyl*-^{14}C]methionine (0.5 mCi/mmol) from Amersham; [8-^{14}C]theophylline (41.5 mCi/mmol) from New England Nuclear; paraxanthine, 3-methylxanthine, xanthine, allantoin, allantoic acid, uric acid, and Ehrlich reagent from Sigma Chemical Co.; 1-methylxanthine and 7-methylxanthine from Pfaltz and Bauer; urea from Fisher Scientific; solvents from Burdick and Jackson Co.; caffeine, theobromine, and theophylline from Dr. Bender—Dr. Hobein, AG, of Switzerland.

The [2-^{14}C]caffeine was synthesized from [2-^{14}C]xanthine (48 mCi/mmol) according to the methylation procedure of Heftmann (1971) and purified by repeated paper chromatography and thin-layer chromatography (TLC) in solvents described below to eliminate possible contamination by other radioactive methylxanthines. The sensitivity of the TLC method is 0.1–0.5 μg of alkaloid. Furthermore, the mass spectrum, compared with that of a standard, showed no other methylxanthines present. The caffeine was 99.9% radiochemically pure by TLC and the specific activity was 3.8 mCi/mmol (cold caffeine was added).

Plant Material. *Coffea arabica* L. trees were obtained as 6-week-old seedlings through the Plant Introduction Division of the U.S. Department of Agriculture (P.I. 394420) in 1976. They were grown at 23–32 °C in a greenhouse of Oklahoma State University, Stillwater, and have been producing coffee fruits since 1977.

Radioisotopic Feeding Experiments. Green (3–4 months old; 1.40–1.50 g fresh weight) or red (6–7 months old; 1.80–1.90 g fresh weight) fruits were excised, with small stocks, from the branches of coffee trees by cutting under distilled water. Each fruit was washed well with water and placed immediately in a vial of 0.1-mL capacity. Each coffee fruit was fed through the petiole with the appropriate radioactive compound (250–500 nCi in 50 μL of water except when otherwise stated) for 12 h, after which it was incubated at 23 °C in distilled water for various periods (four coffee berries were used in each incubation except as otherwise noted). After the incubation period, the samples (coffee fruit and the incubating water) were frozen at −20 °C and kept frozen until extraction.

Analytical Procedures. The analytical procedures used for the caffeine metabolites were those of Suzuki and Waller (1984).

RESULTS

[2-^{14}C]Caffeine Biodegradation in Fruits. Slow degradation of caffeine occurs in mature *C. arabica* fruits (Suzuki and Waller, 1981, 1984), but this is likely to occur in young fruits also. Table I shows the results of administering [2-^{14}C]caffeine (0.25 μCi in 50 μL of water) to both

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Table I. Biodegradation: Distribution of ^{14}C-Labeled Compounds after Incubation of Coffee Fruits with [2-^{14}C]Caffeine[a]

	nCi/fruit						
	radioactivity uptake	radioactivity recovered	distribution of radioactivity				
			Cf	Tb	Tp	MX	AA	urea
immature fruit	220	(A) 30	13	0.15	0.05	0.04	0.30	7.11
		(B) 130	102	0.02	0.01	0.03	0.73	0.68
mature fruit	240	(A) 110	104	0.56	0.53	0.45	1.06	0.94
		(B) 70	62	0.10	0.04	0.40	5.43	0.60

[a] Each fruit was placed in a 0.1-mL vial with its petiole immersed in [2-^{14}C]caffeine solution (250 nCi in 50 μL of water) for 12 h and then incubated in water for 80 h. A = aqueous incubating water; B = acid-soluble extracts; Cf = caffeine; Tb = theobromine; Tp = theophylline; MX = N^3-methylxanthine plus N^7-methylxanthine; AA = allantoin plus allantoic acid.

Table II. Biodegradation: Distribution of ^{14}C-Labeled Compounds after Incubation of Coffee Fruits with [8-^{14}C]Theophylline[a]

	nCi/fruit							
	radioactivity uptake	radioactivity recovered	distribution of radioactivity					
			Cf	Tp	3-MX	AA	urea	X
immature fruit	440	(A) 80	1.3	31	1.1	0.9	5.7	26
		(B) 80	0.3	11	1.7	42	12	nil
mature fruit	460	(A) 100	0.9	43	4.2	4.1	3.1	33
		(B) 170	0.3	31	2.7	120	7.5	nil

[a] Same as Table I but with [8-^{14}C]theophylline (500 nCi in 50 μL of water) instead of [2-^{14}C]caffeine. Absorption was for 12 h and then incubation for 36 h in water. 3-MX = N^3-methylxanthine; X = unknown compound.

immature and mature fruits. Some 14 and 46% of the absorbed isotope were found in the water when the immature and mature fruits, respectively, were incubated for 80 h. The recovery of isotope in the acid fractions of the immature and mature fruits was 59 and 29%, respectively. In both cases, most of the ^{14}C was found in the caffeine; however, it was located primarily in the acid-soluble fraction from the immature fruit and in the incubating water from the mature fruit. There was also a little radioactivity in theophylline, theobromine, 3-methylxanthine, 7-methylxanthine, allantoin, allantoic acid, and urea from the immature fruit (Table I), indicating that the immature fruit can degrade caffeine like the mature fruit.

[8-^{14}C]Theophylline Metabolism in Fruits. Results obtained from feeding coffee fruits with labeled theophylline are given in Table II. Uptake of the radioisotope was 88 and 92% efficient with the immature and mature fruits, respectively. Some 18 and 22% of the isotope absorbed by the immature and mature fruits were respectively found in the water after the incubation period of 36 h. The recovery of isotope in the acid-soluble fraction of the immature fruit samples was 18%. This value is lower than for the mature fruit (37%), indicating that the immature fruit can metabolize theophylline more rapidly than can the mature fruit. In contrast with the results of experiments with [2-^{14}C]caffeine (Table I), the radioactivity of theophylline in the fruit decreased significantly. Allantoin (plus allantoic acid) and an unidentified compound or unidentified compounds were the major products of theophylline metabolism in immature fruit, and 3-methylxanthine, urea, and caffeine were also identified as products.

Metabolism of [2-^{14}C]Xanthine in Fruits. In the experiments described above, we could not isolate xanthine and uric acid, but this may have been due to the rapid turnover of the compounds in coffee fruits. To examine this hypothesis, experiments were conducted in coffee fruits by feeding [2-^{14}C]xanthine. Rapid biodegradation of xanthine to urea via uric acid, allantoin, and allantoic acid in tea leaves has been reported (Suzuki and Takahashi, 1975). In these experiments, three fruits were used in each administration (12 h) and incubation (24 h; Table III). Uptake of the radioactivity was about 97% efficient. The recovery of radioactivity in the acid-soluble fraction

Table III. Biodegradation: Distribution of ^{14}C-Labeled Compounds after Incubation of Coffee Fruits with [2-^{14}C]Xanthine[a]

	nCi/fruit					
	radioactivity uptake	radioactivity recovered	distribution of radioactivity			
			Xan	All	Ala	urea
immature fruit	490	80	nil	40	14	9
mature fruit	480	180	nil	143	13	3

[a] Same as Table I but [2-^{14}C]xanthine (500 nCi in 50 μL of water) substituted for [2-^{14}C]caffeine. Incubation was for 24 h in water. Xan = xanthine; All = allantoin; Ala = allantoic acid.

and incubating water was 16% for the immature fruit samples and 38% (mature fruits), which indicates that metabolism of xanthine proceeds faster in the green fruit. The [2-^{14}C]xanthine supplied was completely metabolized during the first 12-h absorption period. Allantoin, allantoic acid, and urea were all identified as products of xanthine metabolism in coffee fruits.

Biosynthesis of Caffeine by Fruits. To clarify further the role of dimethylxanthines in caffeine metabolism, experiments were conducted in the biosynthesis of caffeine in coffee fruits by feeding L-[*methyl*-^{14}C]methionine, [8-^{14}C]adenine, and [8-^{14}C]guanine separately. Among these precursors, adenine has proven the best precursor for the biosynthesis of caffeine in tea plants in a pulse–chase labeling study (Suzuki and Takahashi, 1976b, 1977).

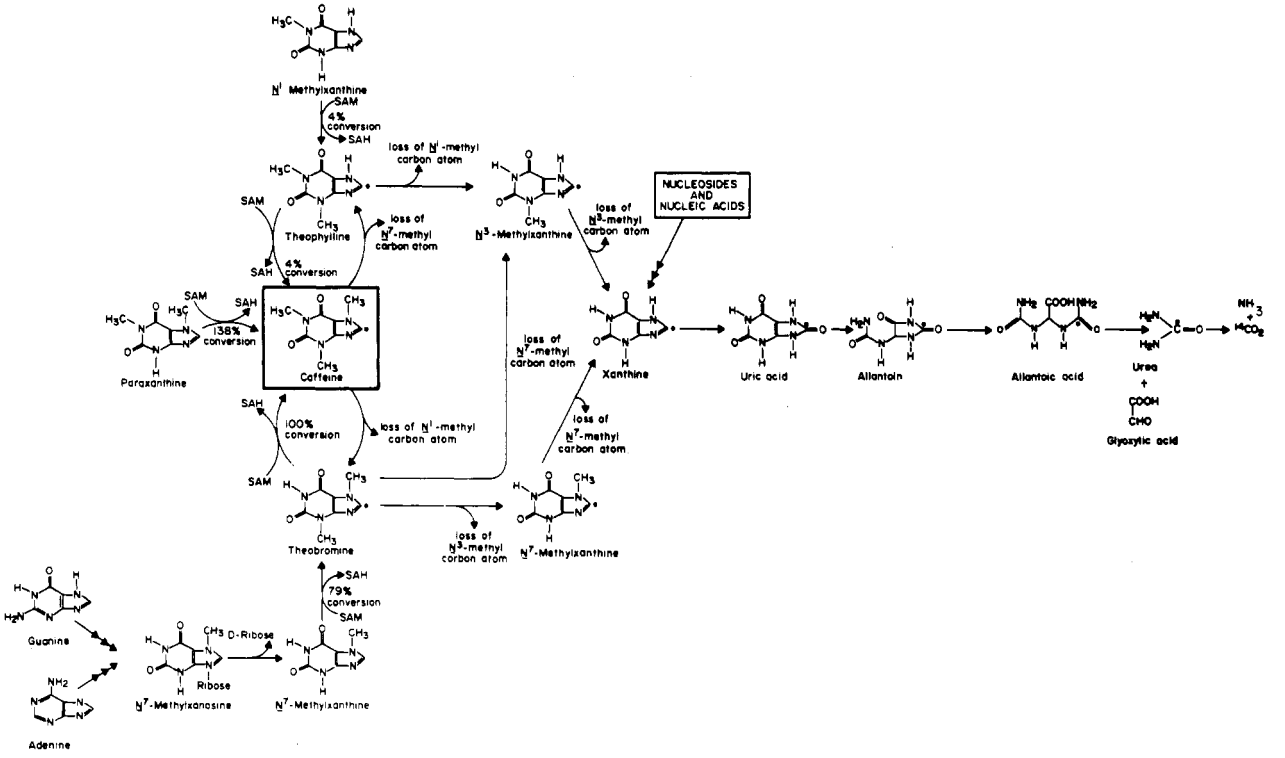
The results of these experiments are summarized in Table IV. Uptake of [8-^{14}C]adenine, [8-^{14}C]guanine, and L-[*methyl*-^{14}C]methionine was 91, 97, and 92% efficient, respectively. Some 73, 33, and 12% of [8-^{14}C]adenine, [8-^{14}C]guanine, and L-[*methyl*-^{14}C]methionine absorbed were incorporated into caffeine in immature fruits, respectively. In all cases, among the methylxanthines the radioactivity was incorporated only into 7-methylxanthine, theobromine, and caffeine. Other methylxanthines, i.e., 3-methylxanthine, 1-methylxanthine, theophylline, and paraxanthine, were not detected as metabolites of [8-^{14}C]adenine, [8-^{14}C]guanine, and L-[*methyl*-^{14}C]methionine in coffee fruits. Incorporation of radioactivity from L-[*methyl*-^{14}C]methionine into caffeine and theobromine was higher in the green fruit than in the red fruit. This might

Table IV. Biosynthesis: Incorporation of ^{14}C-Labeled Precursors into Methylated Xanthine in Coffee Fruits[a]

precursor	nCi/fruit radioactivity uptake	nCi/fruit radioactivity incorporated into 7-MX	Tb	Cf
immature fruit				
[8-^{14}C]adenine	440	4.5 (1.0)[b]	6.9 (1.6)	147.2 (33.4)
[8-^{14}C]guanine	480	2.8 (0.6)	3.8 (0.8)	48.0 (10.0)
L-[*methyl*-^{14}C]methionine	450	trace (-)	0.7 (0.2)	17.1 (3.8)
mature fruit				
L-[*methyl*-^{14}C]methionine	460	trace (-)	0.6 (0.1)	4.3 (0.9)

[a] Same as Table I but [8-^{14}C]adenine, [8-^{14}C]guanine, or L-[*methyl*-^{14}C]methionine (500 nCi in 50 μL of water) substituted for [2-^{14}C]-caffeine. Administration was for 12 h and then incubation for 36 h in distilled water. 7-MX = N^7-methylxanthine. [b] Numbers in parentheses represent percent of the radioactivity incorporated.

Scheme I. Metabolism of Caffeine by *C. arabica* L. Fruit[a]



[a] (•) represents ^{14}C label from [8-^{14}C]caffeine and [8-^{14}C]theophylline in the biodegradation part. The rates of conversion are taken from Roberts and Waller (1979). Note: the left half of the diagram relates to the biosynthesis and the right half represents the biodegradation of caffeine.

be expected since the green fruit is actively biosynthesizing the caffeine.

Plant Residue. The plant residue left from extraction was assayed for its radioactivity and contained only 0.1–2.0% of the total activity for the experiments conducted above.

DISCUSSION

Of considerable interest is how the catabolism and accumulation of purine alkaloids, including the methylxanthines, in the mature and immature coffee fruits occur (Waller and Suzuki, 1982). The radioactivity absorbed by the fruits is largely recovered as caffeine in the fruits and in the incubating water rather than as biodegradation products of caffeine (Table I). It is possible that the degradation of caffeine to dimethylxanthine (theophylline and theobromine) is slow even in the mature fruit and that it is a rate-limiting step. The degradation of theophylline and xanthine occurs more rapidly in the immature fruit than in the mature fruit (Tables II and III). Thus, the increase of theophylline (Suzuki and Waller, 1984) is probably due to a slowdown of theophylline catabolism at the red-brown-black fruit stage. In contrast, theobromine production is associated mainly with caffeine biosynthesis (Table IV), although it is also involved in caffeine biodegradation; Roberts and Waller (1979) have shown that the green fruit had about 150 times as much biosynthetic activity as measured by *N*-methyltransferase activity as did the yellow-red partially ripe fruit. Theophylline may be degraded via N^3-methylxanthine to xanthine, but it is not known whether theobromine is degraded via N^3-methylxanthine and N^7-methylxanthine to xanthine. We could not isolate xanthine in the caffeine and theophylline breakdown experiments nor uric acid at all (Tables I and II), primarily because so little was present in the tissues. Each has been shown to occur in coffee plants (Kalberer, 1964, 1965; Waller et al., 1981, 1983). In contrast with our observation on caffeine degradation in *C. arabica* fruits is the conclusion on caffeine degradation in suspension cultures of *C. arabica* made by Frischknecht and Baumann (1980) that caffeine can be considered as a metabolic end product in suspension cultures of *C. arabica*. We speculate that the degradation of caffeine to dimethylxanthine(s) is slow but steady while further degradation proceeds somewhat faster, finally to produce CO_2. Frischknecht and Baumann (1980) and Baumann and Frischknecht (1982)

detected theobromine as a minor product in the culture system they used, and the end product, CO_2 from total breakdown of the caffeine molecule, has been reported in *C. arabica* leaves (Kalberer, 1964, 1965).

Table IV shows clearly that adenine is the most effective biosynthetic precursor of caffeine in coffee fruits as in tea leaves (Suzuki and Takahashi, 1976b, 1977). Adenine and guanine may be converted into purine nucleotides via the pathway of purine salvage, followed by methylation of purine nucleotides (and/or nucleosides). The pathway of purine salvage in higher plants has been described (Suzuki and Takahashi, 1976b; Ross, 1981), and the precursor of 7-methylxanthine biosynthesis has been reported to be 7-methylxanthosine (Baumann et al., 1978; Roberts and Waller, 1979; Waller et al., 1981). However, at present, it cannot be determined decisively which purine nucleotide has the most important role in caffeine biosynthesis.

A summary of the pathway of caffeine metabolism in caffeine-producing plants, particularly in *C. arabica*, which is in agreement with the above observations, is shown in Scheme I. Although it appears to be less complicated than the multiple pathways of caffeine catabolism in animals and in microorganisms (Burg, 1975; Kihlman, 1977; Arnaud and Welsch, 1981), it is still too early to rely on such a general statement. The control mechanisms for caffeine in caffeine-producing plants is not known. Some *Coffea* species (*Coffea liberica*, etc.) may have different pathways of caffeine metabolism; for instance, methylated uric acids are considered to be the first products of caffeine metabolism by Wanner et al. (1975) and Citroreksoko et al. (1978). Methylation–demethylation reactions of caffeine and/or dimethylxanthines are probably the simplest and most investigated processes; a few alkaloids are known for which the biodegradation route is the reverse of the biosynthetic pathway (Waller and Nowacki, 1978; Waller and Dermer, 1981; Poulton, 1981). In any case it is to be emphasized that the old belief that alkaloids, including caffeine, are inert end products of plant metabolism is erroneous; they undergo a variety of degradations, some at appreciable rates.

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Registry No. Caffeine, 58-08-2; theophylline, 58-55-9; adenine, 73-24-5; guanine, 73-40-5; xanthine, 69-89-6; theobromine, 83-67-0; N^3-methylxanthine, 1076-22-8; N^7-methylxanthine, 552-62-5; allantoin, 97-59-6; allantoic acid, 99-16-1; methionine, 63-68-3; urea, 57-13-6.

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