THEACRINE (1,3,7,9-TETRAMETHYLURIC ACID) SYNTHESIS IN LEAVES OF A CHINESE TEA, KUCHA (*CAMELLIA ASSAMIC* VAR. *KUCHA*)

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Summary:

Theacrine (1,3,7,9-tetramethyluric acid) and caffeine were the major purine alkaloids in leaves of an unusual Chinese tea known as kucha (*Camellia assamica* var. kucha). Endogenous levels of theacrine and caffeine in expanding buds and young leaves in most of individuals inner variety were from 1.3% to 3.4% and 0.6 to 2.7% of dry weight, respectively, but the concentrations were lower in the mature leaves. The theacrine content were changed together with seasons but its characterization of theacrine as major purine alkaloid were not changed in identified individual trees with major theacrine. Our research indicated that young leaves radioactivity of [8-^{14}C]adenosine and [methyl-^{14}C]SAM was incorporated into caffeine and theacrine. The conversion of [8-^{14}C]caffeine to theacrine was also shown in kucha leaves. These results suggest that in kucha leaves theacrine is synthesized from caffeine by a three-step pathway with 1,3,7-methyluric acid as an intermediate. This is the first demonstration on a new theacrine biosynthetic pathway from adenine nucleotides.

Key words: Kucha; Chinese tea; Biosynthesis; Theacrine; Caffeine

Introduction:

As a part of research to discover new Chinese tea resources for beverage and medicinal uses, we found some tea plants grown in China accumulated large quantities of different purine alkaloids. Kucha (*Camellia assamica* var. *kucha*) is an unusual tea because as well as caffeine its leaves contain sizable amounts of theacrine (1,3,7,9-tetramethyluric acid). Since theacrine was discovered as a minor component of *C. sinensis* leaves by Johnson (1937), it has been detected along with related methyluric acids, such as liberine and methylliberine in several species of coffee including *Coffea liberica*, *Cof. Dewevrei* and *Cof. Abeokuta*.

In the present study, the levels of purine alkaloids in kucha leaves at different developmental stages were determined.

Materials and methods

Kucha (*Camellia assamica*, var. *kucha* Chang et Wang) were obtained from the experimental farm of the Zhongshan University, Guangzhou, China. The developmental stages of the leaves were categorized as (i) expanding buds, (ii) young leaves and (iii) mature leaves.

Theacrine levels and biosynthesis determined as shown previously (Ashihara, et al. 1996, Koshiishi et al. 2001).

Results and discussion

Common name	Expanding bud	(%)	Young	(%)	Mature	(%)
Theobromine	1.45 ± 0.1	(21)	0.04 ± 0.01	(1.0)	0.15 ± 0.01	(6.0)
Caffeine	2.7 ± 0.15	(39)	1.25 ± 0.01	(40)	0.78 ± 0.16	(34)
Theophylline	nd	(-)	nd	(-)	nd	(-)
Theacrine	2.8 ± 0.35	(40)	1.87 ± 0.01	(59)	1.38 ± 0.01	(60)
Liberine	nd	(-)	nd	(-)	nd	(-)
Methylliberine	nd	(-)	nd	(-)	nd	(-)
Weight	7.32 ± 0.01		46 ± 0.1		284 ± 5.2	

In kucha, theacrine was a major purine alkaloid and its content was from 1.3% to 3.4% dry weight in young leaves companied with caffeine (0.6%-2.7%), but the concentrations were lower in the mature leaves (Table above). However, methylliberine and libertine were not detected in any of the kucha leaf samples that were analysed in the present study.

Radioactivity from *S*-adenosyl-L-[methyl-^{14}C]methionine was incorporated into theacrine as well as theobromine and caffeine by leaf disks of kucha indicating that *S*-adenosyl-L-methionine acts as the methyl donor not only for caffeine biosynthesis but also for theacrine production.

When [8-^{14}C]adenosine, the most effective purine precursor for caffeine biosynthesis in tea (*Camellia sinensis*), was incubated with young kucha leaves for 24 h, up to 1% of total radioactivity was recovered in theacrine. However, pulse-chase experiments with [8-^{14}C]adenosine demonstrated much more extensive incorporation of label into caffeine than theacrine, possibly because of dilution of [^{14}C]caffeine produced by the large endogenous caffeine pool.

The pattern of metabolism of [8-^{14}C]adenosine was in keeping with the conversion of caffeine to theacrine. To further investigate this point, metabolism of [8-^{14}C]caffeine to theacrine and other potential methyluric acids was investigated in segments of kucha buds and leaves. During the course of a 4 h incubation 7.8% of the [8-^{14}C]caffeine taken up by kucha bud segments was converted to theacrine, with a lower incorporation in young leaves.

These results indicate that in kucha leaves theacrine is synthesized from caffeine in what is probably a three-step pathway with 1,3,7-methyluric acid acting an intermediate (Fig. 1)

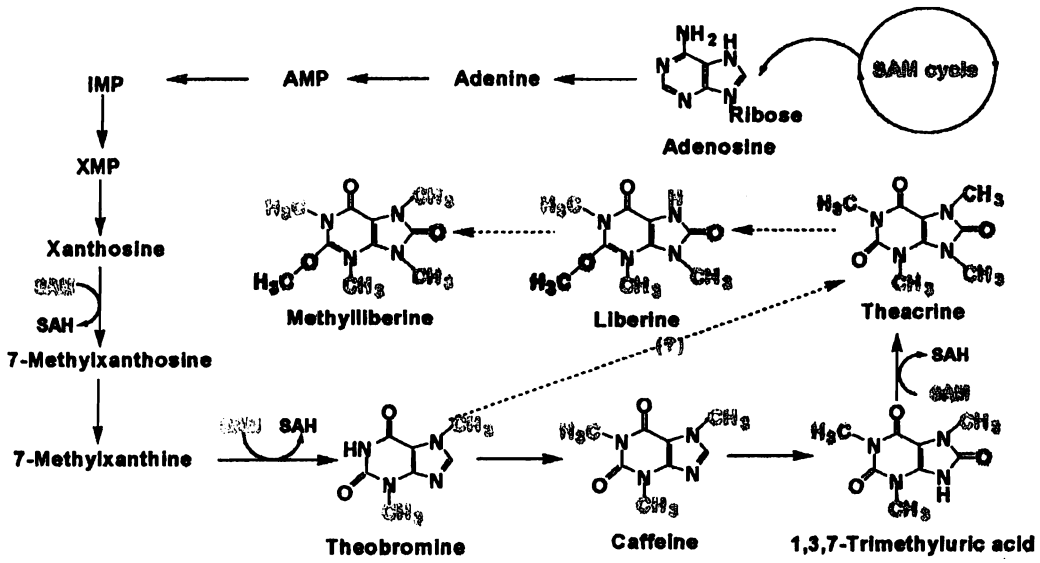

Fig. 1 The biosynthesis pathway of theacrine in kucha leaves

Traditionally, kucha leaves have been used as a cold cure, and there is also a belief that drinking kucha tea enhances longevity. The isolation of the kucha genes encoding enzymes for theacrine synthesis and their insertion into *C. sinensis*, would open up the possibility of producing genetically-modified tea that is rich in theacrine yet contains low levels of caffeine. Such a beverage would appeal to the general public who are consuming decaffeinated tea and coffee in increasing amounts.

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