New Discovery of Pattern of Purine Alkaloids in Wild Tea Trees*

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Abstract: The composition of purine alkaloids in kucha (*Camellia assamica* var. *kucha* Chang et Wang) were investigated from its one buds and two leaves including cultivated and wild samples in detail by qualitative TLC and quantitative HPLC, the results indicate that major purine alkaloids were 1, 3, 7, 9-tetramethyluric acid (theacrine) and caffeine. Theacrine in most of individuals inner variety was the major purine alkaloid and its content was from 1.3% to 3.4% dried weight. And theacrine in kucha was always companied with caffeine and theobromine. The concentration of theacrine was highest in apex buds, and then were rapidly decreased from young, mature to aged 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. In some individual trees, the theacrine content was changeable, sometime major purine was theacrine and otherwise was caffeine. Containing predominant theacrine in *kucha* is a new record not only in taxa of genus *Camellia* Section *Thea* (L.) Dyer but also is a new record in flowering plants.

Key Words: *Camellia assamica* var. *kucha*; 1, 3, 7, 9-tetramethyluric acid; theacrine
CLC number: Q946.88 **Document code**: A **Article ID**: 0529-6579 (2003) 01-0062-04

1 Introduction

Tea is a traditional beverage drunk for thousands years by Chinese. Drinking tea may excite nerve and digest helpfully because tea contains caffeine and other benefit compounds to human body. Two species, *Camellia sinensis* (L.) O. Kuntze and *C. assamica* (Mast.) Chang are used for tea manufacture. But in 1980s we found *C. ptilophylla* Chang, a species allied tea trees, contains theobromine about 4% dried weight and caffeineless. In taxonomy, *C. sinensis*, *C. assamica* and *C. ptilophylla* are belonged to genus *Camellia* Sect. *Thea* (L.) Dyer in which there are 32 species[1]. Predominant purine alkaloid is caffeine in tea and is theobromine in *C. ptilophylla*. In tea, about 0.05% theobromine and 0.000 2% theophylline dried weight are always existed accompanied with caffeine. In 1937, although Johnson firstly found natural 1, 3, 7, 9-tetramethyluric acid from abandon tea liquid after extracted caffeine, it is so lower quantity that never have been detected in routine determination of tea[2]. In 1999, Ye Chuang-xing (叶创兴) et al reported higher content of 1,3,7,9-tetramethyluric acid in *C. assamica* var. *kucha* Chang et Wang which was still lower quantity than caffeine in it[3].

In this present study, purine alkaloids in young shoot (one bud and two leaves) based on individual trees were determined. Theacrine was found as predominant purine alkaloid and caffeine was secondary purine alkaloid in kucha.

2 Experimental results and discussion

By qualitative and quantitative analysis based on extensive investigation of *C. assamica* var. *kucha*, individual trees were elected for determination of purine alkaloids in detail. The samples were from the cultivated and wild trees of *C. assamica* var. *kucha*, one of the trees was especially elected to detect its purine alkaloids in successive three year and successive growing season in one year. How we show that 1,3,7,9-tetramethyluric acid possibly become predominant purine alkaloid up to 3.4% dried weight in *C. assamica* var. *kucha*, caffeine and theobromine are its allied purine alkaloids.

Samples of *C. assamica* var. *kucha* were obtained according to one bud and two leave below bud from newly emeraged shoots; after fresh samples were steamed for 2.5 min kept them apart water and then put them into oven under 80 ℃ to dry. Ground samples were accurately weight and purine alkaloids were exacted with boiling water, lead acetate was added into the filtrate to remove tea polyphynol substances. Standards of purine alkaloids were caffeine, theophylline (SIGMA, USA), theobromine (Shanghai Biochemical Factory, China) and 1,3,7,9-methyluric acid (in our laboratory separated and purified from *C. assamica* var. *kucha*). Aliquots were analyzed

* 收稿日期: 2002－10－16
基金项目: 国家自然科学基金资助项目 (39570081)
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qualitatively by TLC 200 mm × 200 mm sheets of using aluminum and fluorescent silica gel (Fluka Chemic AG, Germany), the solvent system separated was consisted of ethyl acetate/methanol/chloroform (v (ethyl acetate) : v (methanol) : v (chloroform) = 5 : 0.15 : 5) which were compared with standards and were monitored using a Shimadzu UV-Vis monitor, type SPD-10A. The TCL result of one tree of *kucha* was showed as Fig. 1.

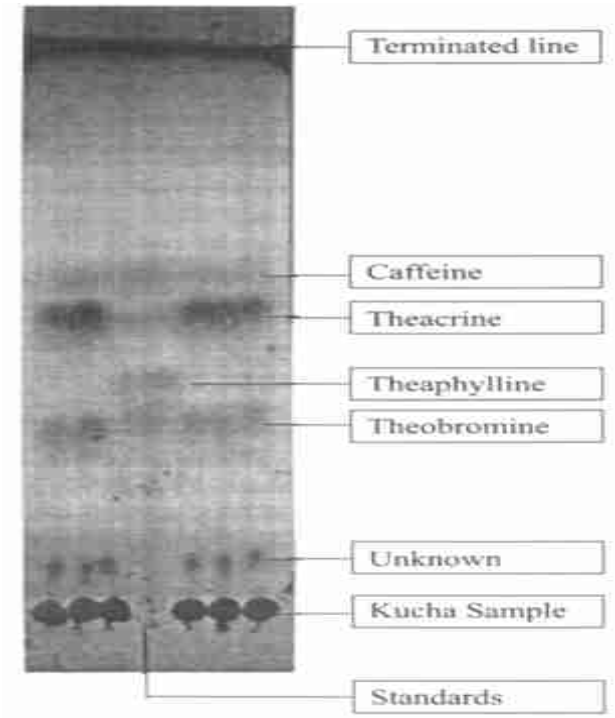

Fig. 1　TCL chromatography

Aliquots were analyzed quantitatively by HPLC. Analysis was carried out with Shimadzu HPLC system, Type LC 10A on a ODS-Hypersil column (250 mm × 4.6 mm i.d.) packed with a 5 μm ODS Hypersil support (Shandon, Runcorn, Cheshire, UK), elute a flow rate of 1 mL/min with a 25 min, 0 ~ 40% gradient of methanol in 50 mmol/L sodium acetate[4,5], pH 5.0. The absorbance at 270 nm and 290 nm were monitored using a Shimadzu UV-Vis monitor, type SPD-10A. Content of each purine alkaloid separated was calculated following the formula:

$$M = \frac{FA'}{m} \times 250$$

$$F = \frac{c}{a}$$

M is content of purine alkaloid, mg/g; A' is peak area; m is sample dried weight; F is rectify factor; c is concentration of standard; a is peak area of standard.

Tab. 1 indicates clearly that the major purine alkaloids accumulated in 9 trees detected in leaves of *C. assamica* var. *kucha* were 1,3,7,9-tetramethyluric acid, caffeine and theobromine.

Tab. 1　Analysis of silica gel thin layer chromatography[1)]

No	Theacrine	Caffeine	Theobromine	Theophylline
K002	d	d	d	nd
K003	d	d	d	nd
K005	d	d	d	nd
K008	d	d	d	nd
K010	d	d	d	nd
K011	d	d	d	nd
K013	d	d	d	nd
K017	d	d	d	nd
K018	d	d	d	nd

1) d, detected; nd, no detected.

Tab. 2 show that the major purine alkaloid was theacrine in six of nine trees, sometime was theacrine,

Tab. 2　Analysis of *C. assamica* var. *kucha* samples

Time	No.	$\frac{m}{\text{mg}}$	w/%		
			Theacrine	Caffeine	Theobromine
A	K002	120	1.991±0.111	0.839±0.052	0.298±0.102
	K005	496	2.214±0.013	1.069±0.214	1.582±0.043
	K008	509	2.060±0.001	1.340±0.036	0.752±0.021
	K011	486	1.923±0.004	0.952±0.042	1.111±0.004
	K017	500	2.020±0.101	1.490±0.118	0.647±0.014
	K018	440	1.529±0.008	1.528±0.129	0.448±0.068
B	K002	125	1.991±0.112	0.256±0.023	0.281±0.009
	K005	496	2.200±0.021	1.201±0.001	1.432±0.013
	K008	204	1.981±0.016	1.912±0.048	0.768±0.015
	K011	180	3.084±0.066	0.351±0.038	0.409±0.008
	K013	167	1.866±0.007	1.253±0.020	0.044±0.005
	K017	132	2.626±0.004	1.846±0.048	0.420±0.034
	K018	218	2.606±0.227	0.475±0.054	0.111±0.006
C	K017	175	1.866±0.011	0.898±0.017	0.146±0.005
	K018	215	1.553±0.004	0.922±0.046	0.170±0.101
D	K002	204	1.501±0.010	0.461±0.026	0.538±0.041
	K005	200	2.128±0.017	0.379±0.026	1.222±0.031
	K008	166	2.217±0.010	1.596±0.130	1.371±0.023
	K010	152	2.191±0.039	2.136±0.024	0.953±0.042
	K011	170	2.284±0.024	0.618±0.030	1.710±0.005
	K017	170	2.092±0.004	1.674±0.040	0.685±0.003
	K018	275	1.751±0.017	2.076±0.081	0.682±0.046
E	K002	184	1.832±0.022	0.987±0.028	1.649±0.132
	K003	200	2.965±0.007	0.781±0.011	0.860±0.012
	K010	243	1.736±0.002	1.363±0.060	0.791±0.014
	K011	500	1.802±0.012	0.696±0.027	0.643±0.045
	K013	500	1.305±0.001	2.256±0.001	0.554±0.002
	K017	266	1.721±0.004	1.379±0.071	0.548±0.045
	K018	289	1.195±0.003	2.076±0.016	0.251±0.021
F	K002	144	1.890±0.027	1.360±0.050	1.289±0.054
	K003	114	1.622±0.005	1.606±0.039	1.099±0.070
	K005	144	2.108±0.005	1.406±0.102	2.080±0.037
	K008	116	2.207±0.201	2.057±0.009	1.545±0.012
	K010	111	2.032±0.029	1.593±0.148	1.495±0.151
	K011	140	1.883±0.030	1.541±0.017	1.790±0.004
	K013	500	1.321±0.001	2.267±0.001	0.445±0.103
	K017	180	1.612±0.003	2.277±0.007	0.697±0.003
	K018	137	1.552±0.001	2.773±0.004	0.548±0.002

1) time, A: 19980927; B: 19981013; C: 19981228; D: 19990415; E: 20000715; F: 20000817

sometime was caffeine in two of other three trees. Major purine alkaloid was theacrine in 32 of 38 samples, second purine alkaloid was caffeine in six samples of three trees. The experiment result indicates that major purine alkaloid is theacrine in most of individual trees of *C. assamica* var. *kucha*.

Tab. 3 indicates that the character contained major purine alkaloid was definite from No. K011, one tree of *C. assamica* var. *kucha* in Sun Yat-sen University Gardens.

Tab. 3 Analysis of *kucha* samples from No. K011 during growing seasons

Time	$\frac{m}{mg}$	w/%		
		Theacrine	Caffeine	Theobromine
20000415	500	3.444±0.001	0.460±0.003	0.795±0.001
20000515	500	1.821±0.003	0.501±0.003	0.763±0.001
20000617	500	1.934±0.000	0.562±0.003	0.795±0.001
20000715	246	1.802±0.012	0.696±0.027	0.643±0.045
20000817	140	1.883±0.030	1.541±0.017	1.790±0.004
20000914	505	2.190±0.001	0.721±0.000	.130±0.008
20001011	234	1.591±0.001	1.220±0.003	1.924±0.008

Tab. 4 shows the changes of theacrine and caffeine inner *C. assamica* var. *kucha* in which major purine alkaloid was theacrine, and indicates the character of major theacrine would be not changed under various seasons. In spring, content of theacrine was highest, in autumn it was lowest.

Tab. 4 The variation of theacrine content in different seasons

Time	w(Theacrine)/%	w(Caffeine)/%
April	2.09	1.17
May	1.91	1.16
June	1.93	1.35
July	2.01	1.04
August	1.96	1.59
September	1.62	0.99
October	1.77	1.30

Tab. 5 shows the contents of terminate bud and the leaves below bud from upper to lower in *C. assamica* var. *kucha*. In generally speaking, theacrine was highest, and its concentration decreases rapidly from terminate buds to aged leaves. The development stages of the leaves were categorized as young, mature and aged. Young leaves were the most recently emerged; mature leaves comprised the fully expanded second leaf below the apex; aged leaves were from one year old shoots near the base of the branches.

Tab. 5 Comparison of contents of main purine alkaloids in buds various leaves[1)]

Location	w(Theacrine)/%	w(Caffeine)/%
Buds	2.80	2.70
Young leaves	2.75	0.55
Mature leaves	1.25	0.70
Aged leaves	0.09	nd[1)]

1) nd, not detected

Tab. 6 shows assayed results in samples picked in random from 10 trees in wild forestry, major purine alkaloids was theacrine in eight trees, in one of them, the contents were almost equal between theacrine and caffeine.

Tab. 6 Composition of purine alkaloids inner *C. assamica* var. *kucha* leaves from primitive location[1)]

No.	$\frac{m}{mg}$	w/%		
		Theacrine	Caffeine	Theobromine
1	450	2.173±0.011	1.086±0.005	0.399±0.018
2	490	2.470±0.027	1.027±0.006	0.747±0.015
3	252	3.659±0.019	0.963±0.016	0.944±0.061
4	338	3.451±0.025	0.570±0.035	0.402±0.035
5	296	0.275±0.004	0.268±0.005	0.056±0.005
6	366	3.252±0.030	0.592±0.006	0.539±0.017
7	268	1.968±0.002	0.540±0.034	0.256±0.018
8	398	0.937±0.051	1.397±0.205	0.202±0.028
9	323	1.682±0.573	1.324±0.169	0.406±0.180
10	423	2.372±0.633	0.706±0.147	0.491±0.008

1) Time is 20000426.

3 Summary

Data obtained from qualitative and quantitative analysis in *C. assamica* var. *kucha* indicates that major purine alkaloid is 1,3,7,9-tetramethyluric acid in most of individualities inner the variety. 1,3,7,9-tetramethyluric acid has been artificial synthesis in 1890s, in 1937, natural 1,3,7,9-tetramethyluric acid was found by Johnson T B firstly but it is never detectable major purine alkaloid in routine analysis of tea. Higher content of 1,3,7,9-tetramethyluric acid possibly means that *Camellia assamica* var. *kucha* will be used for helpful beverage and needed progressively to research. After 1970s, Baumann et al[6—9] continuously reported theacrine were found in leaves from species of genus coffea but it was lower content and it was catabolized as the intermediated metabolite. Recently, Zheng et al[10] reported theacrine was biosynthesized from caffeine in *Camellia assamica* var. *kucha* Chang et Wang.

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一种野生茶树的新嘌呤碱模式

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摘　要: 通过单株定性和定量的分析, 发现中国特有的变种苦茶 *Camellia assamica* var. *kucha* Chang et Wang 其芽叶嘌呤碱依含量高低分别由 1,3,7,9-四甲基尿酸、咖啡碱、可可碱组成, 其中 1,3,7,9-四甲基尿酸的含量占芽叶干质量的 1.3%～3.4%, 其次是咖啡碱, 其质量分数为 0.26%～2.8%, 再其次是可可碱, 质量分数为 0.1%～2.1%。苦茶嘌呤碱各组成成份在植株间存在差别, 但多数植株 1,3,7,9-四甲基尿酸始终表现为优势嘌呤碱; 少数植株咖啡碱表现为优势嘌呤碱, 而 1,3,7,9-四甲基尿酸是第二位的嘌呤碱。在调查植株间嘌呤碱时还发现有的植株时而表现为 1,3,7,9-四甲基尿酸是优势嘌呤碱, 时而表现为咖啡碱是优势嘌呤碱。这些现象产生的真正原因仍然有待于研究, 但苦茶嘌呤碱的组成模式是有花植物中尚未发现的。

关键词: 苦茶 *Camellia assamica* var. *kucha*; 1,3,7,9-四甲基尿酸; 嘌呤碱的新分布模式
中图分类号: Q946.88　　文献标识码: A