Synthesis of potential theanine metabolites, related structures and their affinity for CB receptors

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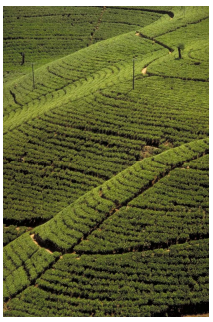
1. Introduction



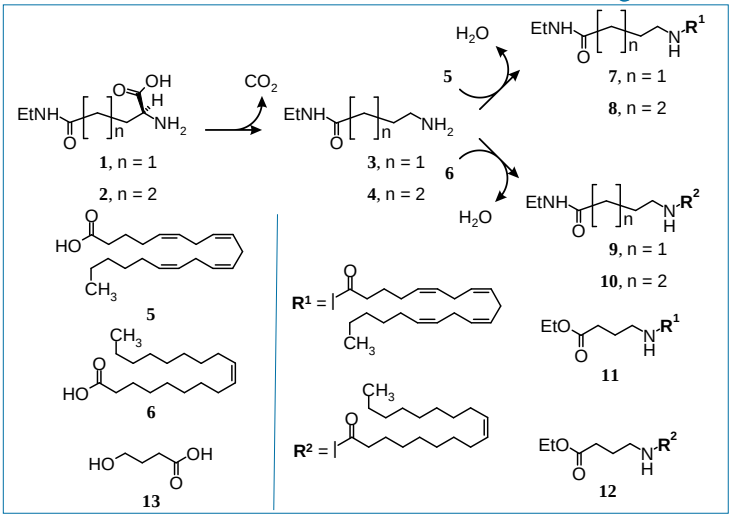
Tea plantation Sri Lanka [1]

Tea has many pharmacological effects which cannot be solely ascribed to its main alkaloid, caffeine. Our research focusses on the amino acid theanine (**1**) and its homologue 2-amino-5-ethylcarbamoyl-pentanoic acid (**2**). Both were found only in a fungus (*Xerocomus badius* Fr. Gilb.) [2] and in *Camellia* species. *C. sinensis* (L.) O. Kuntze contains up to 1.7 % of **1** in dried leaves [3,4]. We hypothesize that **1** and **2** are decarboxylated in vivo to GABA analogs, viz. 4-amino-N-ethyl-butyramide (**3**) and 5-aminopentanoic acid ethylamide (**4**) (see formula scheme in Box 2). Enzymatically catalyzed acylation of **3** and **4** by arachidonic or oleic acid (**5, 6**) would lead to the lipids **7**-**10**. For 4-arachidonyl- and 4-oleoylaminobutyric acid ethyl ester (**11**, **12**) we found binding to CB1 and CB2 receptors.

This mechanism of action could explain theanine effects like immunomodulation and influence on mood to be caused by modulation of the cannabinoid and GABA system [5,6].

The narcoleptic and anticataplectic agent Xyrem® contains γ–hydroxy butyric acid (**13**) [7]. **13** is also known as 'liquid ecstasy'. The arachidonyl and oleoyl amide of **13** is structurally related to compounds **11** and **12** and we suspect the acyl derivatives of **13** to interact with CB receptors, as well.

2. Possible metabolism of theanine and its homologues

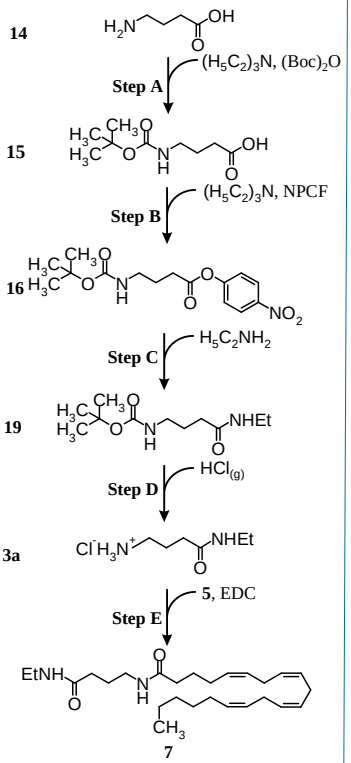


3. Synthesis of the potential metabolites

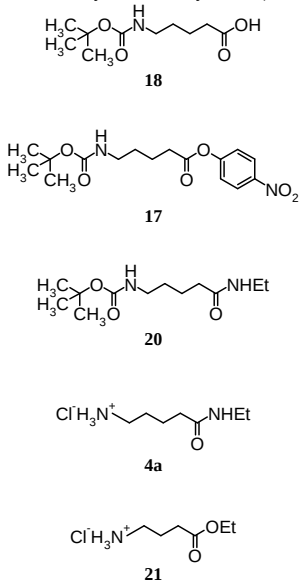
• **Step A** and **B:** 4-Aminobutyric acid (**14**) and di-*tert*-butyl dicarbonate (Boc_2O) reacted to give 4-*tert*-butoxyocarbonylamino-butyric acid (**15**) [8]. This was activated using 4-nitrophenyl-chloroformate (NPCF) to 4-*tert*-butoxyocarbonylamino-aminobutyryl-p-nitrophenyl ester (**16**) [8]. 5-Aminopentanoic acid gave 5-*tert*-butoxycarbonylaminopentanoic acid 4-nitrophenyl ester (**17**) via 5-*tert*-butoxycarbonyl-amino-pentanoic acid (**18**).

• **Step C:** Aminolysis of **16** and **17** by ethylamine (2 M in THF) in dichloromethane lead to (3-ethylcarbamoylpropyl)carbamic acid *tert*-butyl ester (**19**) and (4-ethylcarbamoylbutyl)carbamic acid *tert*-butyl ester (**20**). They were purified by MPLC on silica gel, eluting with 3% triethylamine in ethyl acetate, and recrystallization from hexane and ethyl acetate to yield colourless needles.

• **Step D:** The *tert*-butoxycarbonyl group was removed in chloroform by gaseous hydrogen chloride. After evaporation of the solvent, 4-amino-N-ethylbutyramide HCl (**3a**) and 5-amino pentanoic acid ethylamide HCl (**4a**) were obtained.



• **Step E**: Using the coupling reagent N-(3-dimethylaminopropyl) - N' - ethyl - carbodi-imide (EDC), **3a**, **4a** and 4-amino-butyric acid ethyl ester HCl (**21**) were condensed in pyridine with arachidonic acid and oleic acid to give **7**, **8**, **11**, **9**, **10** and **12**. The solvents were evaporated and the residues suspended in 0.1 M HCl. After extraction with chloroform, the compounds were purified by MPLC (silica gel, eluent 3 % triethylamine in ethyl acetate).



4. Pharmacology of theanine

Pharmacokinetics

• It was shown in animals that theanine was absorbed from the small intestine by Na^+ coupled active transporters.

• Theanine crossed the blood-brain barrier via the leucine-preferring transport system.

• The metabolic fate of theanine is not fully known and difficult to investigate.

Pharmacological activities

• Theanine has a relaxing effect on mood and could thus antagonize caffeine stimulation.

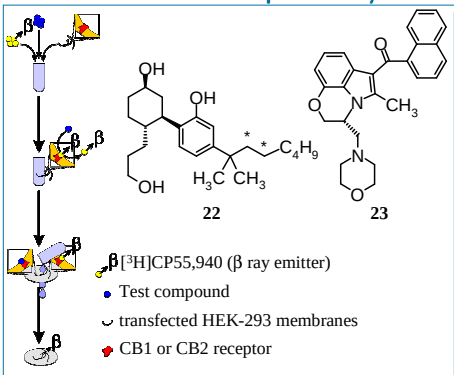
• Tea has a promotional effect on the generation of T-cells.

• Theanine acts positively as a modulator in cytostatic therapies[9].

• The pharmacologic effects have not been fully elucidated.

Dried tea leaves [1]

5. Cannabinoid receptor assay



• The incubation was started by addition of a suspension of transfected HEK-293 membrane cells (CB1 or CB2) to a mixture of [^{3}H]CP55,940 (**22**) and the test compound and was carried out for 30 minutes.

• The reaction was terminated by rapid vacuum filtration. The filters were washed. Radioactivity bound to the membranes on the filters was determined by scintillation couting.

• Non-specific binding was defined and determined as the amount of **22** bound in the presence of WIN55,212-2 (**23**) [10,11].

Results are presented in the table below.

Compound	CB1, extent of displacement		CB2, extent of displacement	
	1 µM	10 µM	1 µM	10 µM
7	**44 %**	**100 %**	**59 %**	**94 %**
8	48 %	100 %	48 %	91 %
9	30 %	100 %	0 %	48 %
10	34 %	62 %	5 %	26 %
11	**57 %**	**100 %**	**36 %**	**85 %**
12	27 %	66 %	0 %	17 %

6. Conclusion and outlook

• In the literature, both absorption of theanin and penetration of the blood-brain barrier was demonstrated in animal models. The metabolism of theanine is largely unknown [9].

• **We synthesized a potential theanine metabolite and related compounds (3 and 4). They are analogs of GABA. Fatty acid derivatives of 3 and 4 had affinity to CB receptors. The theanine derivative 7 and the related GABA ester 11 were most potent.**

• **According to this mechanism, pharmacological effects of theanine on mood and T-cell generation may be due to interaction with the CB system in the brain and immune system.**

• Now the acyl derivatives need to be looked for in vivo after oral application of theanine.

7. References

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