Fate of the Radioactive Carbon of Theanine Labeled with Ethylamine-1-¹⁴C in Tea Seedlings

Sir:

Recently, we succeeded in the isolation of theanine (γ -glutamylethylamide) synthesizing enzyme from tea seedlings^{1,2)} and elucidated the mechanism of theanine synthesis. However, the physiological significance of theanine remained to be explained.

The authors wish to report the metabolism of theanine in tea seedlings. Theanine is rapidly formed in large quantities during germination of tea seeds. When ethylamine-1-¹⁴C was fed to these seedlings (eleven-day old), only ethylamine part of theanine was labeled as described previously,³⁾ and all the radioactivity incorporated existed in theanine.

The seedlings with labeled theanine in roots and cotyledons of this stage was called 0-day and then further grown on moisten cotton at 25°C under fluorescent light. Seventy-per cent alcohol extract of various parts of seedlings was passed through an Amberlite IR-120 (H⁺) column (1×17cm) to separate amino acids and the other compounds. The seedlings were used for an analysis.

For first seven days, the seedlings containing labeled theanine were grown under 3000 lux and then divided into two groups with different illuminations (150 and 4000 lux). Radioactivity transported from roots and cotyledons

TABLE I. EFFECTS OF ILLUMINATION ON THE METABOLISM OF THEANINE

days	lux	distribution of radioactivity (%) ^{*1}		distribution of radioactivity in shoots (%) ^{*2}	
		cotyledons and roots ^{*3}	shoots	IR-120	
				adsorbed	not adsorbed ^{*4}
7	3000	99	1	71	29
	150	96	4	78	22
14	4000	95	5	47	53
	150	93	7	76	24
21	4000	77	23	33	67
	150	83	17	83	17
28	4000	48	52	10	90

^{*1} All the radioactivity in a whole seedling (7×10^5 CPM in average) was regarded as 100%. Radioactivity was measured by a gas flow counter or a liquid scintillation counter.

^{*2} All the radioactivity in shoots was regarded as 100%.

^{*3} Almost all the radioactivity existed in theanine.

^{*4} Nearly all the radioactive compounds passed through an Amberlite IR-120 (H⁺) had the properties that they were adsorbed on an Amberlite IR-45 (OH⁻), an Amberlite IRA-400 (carbonate form) and a Dowex-1×8 (acetate form), but not eluted with 2N ammonium carbonate.

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FIG. 1. Compounds Adsorbed on an Amberlite IR-120 Column with 28-day Seedlings.

Compounds adsorbed on an Amberlite IR-120 column eluted with 1N ammonia and the eluate was concentrated to dryness in a rotary evaporator at 40°C. The dried residue was dissolved in a definite amount of water.

(a) Autoradiogram (exposure time, one month).

(b) Chromatogram treated with ninhydrin after the preparation of the autoradiogram.

Solvents used: I, phenol-water (4:1, NH₃); II, *n*-butanol-acetic acid-water (4:1:1).

Filter paper: Toyo No. 51 A, 36×36 cm.

1) Theanine

to young shoots increased with days (Table I). For twenty-eight days, almost all the radioactivity remained in a whole seedling. All the radioactive compounds adsorbed on an Amberlite IR-120 column corresponded to

FIG. 2. Compounds Passed through an Amberlite IR-120 Column with the Shoots of 28-day Seedlings.

The fraction passed through an Amberlite IR-120 column was concentrated to dryness in a rotary evaporator at 40°C. The dried residue was dissolved in a definite amount of 75% alcohol. By this procedure no loss of the radioactivity was found.

(a) Autoradiogram (exposure time, three weeks).

(b) Chromatogram treated with cold ammoniacal silver nitrate solution after the preparation of the autoradiogram. Solvents used: I, 2% acetic acid; II, *n*-butanol acetic acid-water (4:1:1).

Filer paper: Toyo No. 51 A, 36×36 cm.

1) epigallocatechin, 2) gallo catechin, 3) epicatechin, 4) catechin, 5) epigallocatechin gallate, 6) epicatechin gallate. Colours developed by the spray reagents of alcoholic AlCl_3 , vanillin-*p*-toluenesulfonic acid, *p*-diazobenzenesulfonic acid, Mohr's salt and $\text{FeCl}_3 \cdot \text{K}_3\text{-Fe}(\text{CN})_6$ were in complete agreement with authentic catechins.

only theanine even after twenty-eight days (Fig. 1). The greater part (approximately 90%) of the radioactivity passed through this column was found in catechins as shown in Fig. 2. By the anaerobic alkaline degradation of these radioactive catechins according to Zaprometov's method,⁴⁾ phloroglucinol, protocatechuic and gallic acids were obtained. However, the radioactivity was found only in phloroglucinol. Therefore, N-ethyl carbon of theanine seemed to be metabolized finally to ring A of catechins in tea leaves. In this case turnover rate of theanine to catechins in 4000 lux-seedlings was stimulated rather than in 150 lux-seedlings (Table I).

These results are in agreement with the fact that the catechins content is higher in unshaded tea leaves than in shaded leaves⁵⁾ in contrast to the case of theanine,⁶⁾ and catechins were synthesized only in young tea leaves.⁴⁾

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