left to stand overnight. After treatment in conventional way, the reaction product was recrystallized from MeOH to yellow needles, m.p. 221~223.5°. IR λ_{max}^{Nujol} μ : 2.86(OH), 5.99(non-conj. CO). *Anal.* Calcd. for $C_{17}H_{14}O_5$: C, 68.45; H, 4.73; $(OCH_3)_2$, 20.81. Found : C, 68.10; H, 4.97; OCH_3, 21.29.

Aloe-emodin Trimethyl Ether—A mixture of 130 mg. of aloe-emodin, 7.5 cc. of *N* NaOH solution, and 1 cc. of Me_2SO_4 was stirred at room temperature for 1.5 hr. One-third portion each of a solution of 1.5 cc. of Me_2SO_4 in 7.5 cc. of *N* NaOH was added three times to the reaction mixture in an interval of 2 hr., during which stirring was continued. After being left overnight, the mixture was again stirred with another 0.5 cc. of Me_2SO_4 and 5 cc. of 2*N* NaOH solution for 8 hr. and finally mixed with 0.5 cc. of Me_2SO_4 and 3 cc. of 2*N* NaOH solution, and left to stand overnight. Treatment of the reaction mixture in a usual way and recrystallization of the product from MeOH afforded orange needles, m.p. 153~156°. IR λ_{max}^{Nujol} μ : 5.98(non-conj. CO). *Anal.* Calcd. for $C_{18}H_{16}O_5$: C, 69.22; H, 5.16; OCH_3, 29.77. Found : C, 69.39; H, 5.29; OCH_3, 29.86.

Aloe-emodin Triacetate—Acetylation of 70 mg. of aloe-emodin was performed with 2.75 cc. each of Ac_2O and pyridine in a conventional manner and after recrystallization from Me_2CO-MeOH mixture, 50 mg. of the triacetate was obtained as pale yellow needles, m.p. 174~177°. IR λ_{max}^{Nujol} μ : 5.64, 5.75, 5.93, 5.97(acetate and CO). *Anal.* Calcd. for $C_{21}H_{16}O_5$: C, 63.63; H, 4.07. Found : C. 63.91; H, 4.27.

Rhein Diacetate from Aloe-emodin Triacetate—To a solution of 553 mg. of aloe-emodin triacetate in 15 cc. each of AcOH and Ac_2O, a solution of 550 mg. of CrO_3 in 15 cc. of AcOH and 1 cc. of H_2O was added dropwise at 55° in the course of 30 min. The mixture was stirred at 65~70° for 3 hr. and poured into lukewarm water. The precipitate was collected and recrystallized from dioxane to 320 mg. of yellow granules, m.p. 247~247.5°(decomp.). IR λ_{max}^{Nujol} μ : 5.65(acetate), 5.88(COOH), 5.93 (CO). *Anal.* Calcd. for $C_{19}H_{12}O_8$: C, 61.96; H, 3.29. Found : C, 62.17; H, 3.49. The product was confirmed to be identical with authentic rhein diacetate, m.p. 250°(decomp.), by mixed m.p. determination and infrared spectrum.

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Summary

An unexpected finding is reported that aloe-emodin is soluble in aqueons sodium carbonate solution and is methylated with diazomethane. From these results, identity of rhabarberone and isoemodin with aloe-emodin is suggested.

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Takao Inoue and Yaeko Kawamura : Studies on Biogenesis of Tea Components. II.[1] Formation of Caffeine in Excised Tea Shoots.

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Biosynthesis of plant alkaloids has been studied by numerous workers. Dawson[2] early demonstrated that nicotine formed in the roots of Nicotiana but not in the leaves, while similar results were obtained with Datura alkaloid. Imaseki,[3] however, recently confirmed that Datura alkaloids are partly biosynthesized in the leaves in addition to the roots, using a tracer technique.

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1) Part I : Yakugaku Zasshi, **80**, 548 (1960).
2) R. F. Dawson : Am. J. Bot., **31**, 351 (1944); S. M. Peacock, Jr., D. B. Leyerle, R. F. Dawson : *Ibid.*, **31**, 463 (1944).
3) I. Imaseki : This Bulletin, **5**, 447 (1957).

In a previous work,[1] intact tea plants were fed with a nutrient solution containing $(^{15}NH_4)_2SO_4$, so as to examine incorporation of ^{15}N into caffeine and protein in young and mature leaves, stems, and roots. In the present work, excised shoots of tea tree were cultivated in $(^{15}NH_4)_2SO_4$ solution to investigats whether caffeine synthesis takes place in the leaves and ^{15}N content of isolated caffeine was determined after growing for 1 week.

Experimental

Material and Cultivation—*Thea sinensis* L. grown in Tea Experimental Station, Saitama Prefecture, was used as a plant material. It is desirable that cultivation is placed between mid April and mid May, when tea plants grow young leaves containing the largest amount of caffeine and also when the caffeine content reaches the maximum in mature leaves.

The 100 tea shoots, 30 cm. long, having young and mature leaves, were excised, 10 shoots were dried immediately as a control, and 90 shoots were cultivated in 1.18 g./L. of $(^{15}NH_4)_2SO_4$ solution (N : 250 p.p.m., 8.5 atom% ^{15}N excess) for 1 week from April 27 to May 3. At the end of cultivation, the material was removed from the solution and the leaves were gathered, dividing into three groups of (I) young leaves, (II) mature leaves grown in autumn of the previous year, and (III) older leaves. The yields of dried leaves were (I) 7.9 g., (II) 28.4 g., and (III) 34.2 g. The amount of the solution absorbed was about 1.35 L.

Procedure—(1) Isolation of caffeine : The ground leaves were heated with 20 volumes of water and 2 volumes of heavy MgO for 0.5 hr. and the leaves were extracted once more with 10 volumes of water. The filtrate was combined, concentrated *in vacuo*, and extracted with $CHCl_3$. Caffeine obtained by distillation of $CHCl_3$ solution was repeatedly recrystallized from water and used for ^{15}N determination.

(2) Determination of caffeine, total N, protein N, and ^{15}N concentration in each fraction was made as described in the previous paper.[1]

Results and Discussion

The results obtained by these experiments are shown in Tables I and II.

Table I. Nitrogen Content and ^{15}N Concentration of Non-alkaloidal Nitrogen (mg./10 shoots) $(^{15}NH_4)_2SO_4$·····8.5 atom% N excess

		Control		Feeding			Absorbed			Absorbing ratio[a] (%)	Ratio of total N absorbed per mg. (%)
		%	Ratio	%	mg.	Ratio	^{15}N% excess	mg.	Ratio		
(I) Young leaves	Total N	4.86	100	7.07	62.1	100	1.066	7.79	100	12.5	
	Protein N	3.25	67	3.89	34.1	55	0.916	4.03	52	11.9	86
	Nonprotein N	—	19	—	17.0	27	—	2.42	30	14.2	
(II) Mature leaves	Tatal N	3.76	100	3.34	105.3	100	0.168	2.08	100	1.97	
	Protein N	2.43	65	2.45	77.2	73	0.164	1.33	64	1.72	6
	Nonprotein N	—	31	—	18.2	17	—	0.68	33	3.74	
(III) Old leaves	Total N	2.94	100	3.27	124.3	100	0.211	3.08	100	2.48	
	Protein N	2.38	81	2.21	84.0	68	0.204	2.04	66	2.43	8
	Nonprotein N	—	17	—	33.9	27	—	0.97	31	2.86	

a) The ratio of N absorbed to the total amount of N in each fraction

The young leaves absorbed the largest amount of the culture solution because in the stage of growth and the content of total N increased markedly by $^{15}NH_4$-N feeding, while total N and protein N in mature and old leaves hardly changed. The large incorporation of ^{15}N in young leaves showed that 10~15% of protein, non-protein N, and caffeine are formed from nitrogen absorbed during the experimental period. In young leaves, protein N seemed rather to decrease in view of variation in ratio of protein N to total N at the start and the end of experiment, and this result suggested that protein has decomposed gradually to non-protein fraction together with biosynthesis from $^{15}NH_4$-N.

The incorporation of ^{15}N into caffeine was also found in mature and old leaves as well as young leaves and it was proved that caffeine can be biosynthesized in the leaves of tea plant.

Table II. Analysis of Caffeine
(mg./10 shoots) $(^{15}NH_4)_2SO_4$ ····· 8.5 atom% ^{15}N excess

	(I) Young leaves	(II) Mature leaves	(III) Old leaves
(%)[a]	4.34	1.09	0.58
(mg.)	38.1	34.3	22.5
Caffeine-N (mg.)	11.0	9.9	6.5
$\frac{\text{Caffeine-N}}{\text{Total N}} \times 100$	17.7	9.4	5.2
^{15}N% excess	1.123	0.063	0.112
Newly formed caffeine (mg.)	5.04	0.25	0.30
$\frac{\text{Caffeine newly formed}}{\text{Amount of caffeine}} \times 100$	13.2	0.73	1.33
$\frac{\text{Caffeine-N newly formed}}{\text{Total N absorbed}} \times 100$	18.6	3.4	2.9

a) Content of caffeine in the leaves at the start of the experiment : (I) 2.35%, (II) 0.56%, (III) 0.23%.

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Summary

The excised shoots of tea plants were cultivated for 1 week in $(^{15}NH_4)_2SO_4$ solution. ^{15}N was incorporated considerably more into protein and caffeine of young than into those of mature and old leaves. It was proved by these results that formation of caffeine can take place in the leaves of tea plant.

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Makoto Shirasaka and Masako Tsuruta : Microbiological Transformation of Steroid. VII.[1] Hydroxylation of Steroid by *Gibberella saubinetii*; 6β- and 15α-Hydroxylation.

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15α-Hydroxylation of steroids by microörganisms is a comparatively common reaction now and the substrate steroids used in such a case are usually progesterone and deoxycorticosterone. Only *Hormodendrum viride*[2] and *Helminthosporum sativum*[3] are known to effect 15α-hydroxylation of Reichstein's compound S. The microörganisms examined to date have only been tested with one kind of a substrate, except in the case of *Fusarium lini*[4] which was tested with four kinds of steroid, androstenedione, testosterone, progester-

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