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Studies on the Indoles in the Common Reed

II. Changes of Indole Compounds During the Growth of Sprouts

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갈대의 Indole 化合物 硏究

II. 갈대幼芽의 生長過程中의 Indole 化合物의 變化

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SUMMARY

Methanolic extract of the common reed sprouts (*Phragmites communis* Trin.), unincubated or incubated at 27°C in the dark, was subjected to thin layer chromatography for the indole compounds.

Serotonin, tryptophan and tryptamine were the major indoles in the unincubated sprouts. At early stage of incubation of sprouts, however, serotonin and tryptamine decreased while other indoleamines increased. Tryptophan remained constant throughout the incubation periods. From the observation in the present study, it was speculated that serotonin be synthesized from tryptophan via tryptamine and in turn metabolized to bufotenine.

INTRODUCTION

Indoleamines are often psychotomimetic substances in animals and indole acetic acid as well as its metabolic precursors are frequently known as plant growth regulators. It has been generally considered that the formers are metabolic products of animals while the latters are those of plants. Indoleamines also have been found in plants recently, but their metabolism and physiological roles in plant are still left to be elucidated.

We have been working on the indoleamines in plants for some time and suggested that indoleamines might be closely related to the physiology of hydrophytes[1]. We also observed among hydrophytes that the common reed contained exceptionally high concentration of van Urk positive compounds, some of which were identified as described in the previous paper[2]. The present work has been aimed at understanding of a metabolic pathway of indoleamines observed in the time course study of indole compounds in the common reed.

MATERIALS AND METHODS

1. Plant Materials.

During the period of November, 1974 - April, 1975, rhizomes of common reed (*Phragmites communis* Trin.) growing wild on the shore of Seo Ho Lake, Su-won Si, Gyung Gi Do, were collected, sealed in black polyethylene film bags and transported to the laboratory. They were washed with tap water and again with distilled water. The internodes were cut off and each node was incubated in the sterile moist sand at 27°C in the dark. Distilled water was supplied ones a day. The grown sprouts were collected from the incubator at the end of each incubation period (Table 1).

2. Extraction of Indole compounds.

A slightly modified mothod based on the reports by Gmelin et al.[3] and Schneider et al.[4] was employed for the extraction of indole compounds. Twenty-five shoots were marcerated in a mortar with methanol (0.5ml per gram of the fresh material). The extracts were filtered through 1 G 4 glass filter. The methanolic extract was adjusted to 80% (percent methanol, v/v) by addition of distilled water. The extracts were then heated for 10 minutes at 70°C on a water bath, cooled and kept overnight in a refrigerator maintained at −16°C.[5] The precipitates formed were separated by centrifugation at 1000G for 15min. and discarded.

The supernatant was evaporated to dryness under a reduced pressure at 40°C and methanol was added for redissolving the indole compounds. An aliquot of the methanol extracts was subjected to thin layer chromatography.

Table 1. Incubation of tissues and extraction of indole compounds for thin layer chromatography.

Incubation periods (hrs)	Fresh weight* (g)	Methanol for extraction (ml)	Methanol for redissolving (ml)
0	0.56	2.8	0.22
	0.34	1.7	0.14
16	0.78	3.9	0.31
	0.85	4.25	0.34
38	1.11	5.55	0.44
	1.47	7.35	0.59
62	2.18	10.9	0.88
	2.86	14.3	1.14
86	4.38	21.9	1.76
	5.46	27.3	2.19
110	6.18	30.9	2.46
	7.12	35.6	2.85

*25 shoots

3. Thin Layer Chromatography and Quantification of Indoles during the Sprouting.

A solvent system, composed of iso-propanol: methylacetate: water (35:45:20), which was found satisfactory for effecting the separation of several indole compounds from the common reed tissue, was adopted in the present work. One tenth ml of the methanol extracts from each incubation were spotted on a single plate and developed to the length of 10 cm. Visualization was made by the van-Urk reagent and also with ultraviolet light. Ultraviolet light source used was UVSL-25, Ultraviolet products Inc.

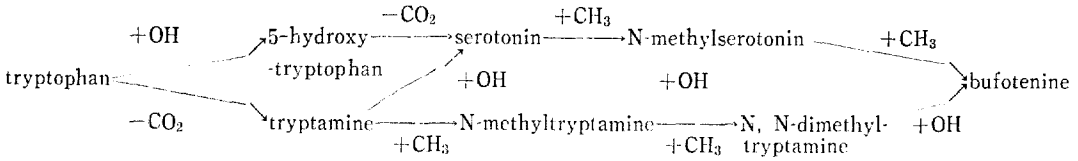
Quantitative comparison was performed with densitometry on a thin layer platevisualized by van-Urk reagent. The densitometer used was Densitorol DMU-2 (Toyo Inc., Filter; 625nm, Sensitivity; 1.0, Chart ratio; 1 : 1.0).

RESULTS AND DISCUSSION

In the previous paper, it was observed that tryptophan, serotonin, tryptamine, N-methylserotonin, bufotenine, and N,N-dimethyltryptamine appeared in the sprout of the common reed. Thin layer chromatograms representing the changes of these compounds in the coures of incubation of the sprout were shown in Figure 1. Densitometric expression of the chromatograms is shown in Figure 2. The resolution of each chromatogram and densitogram was not so sentive as to lead to quantitative and decisive results. We made, however, some numeric comparison according to the relative area of each densitogram to find a clue to the possible metabolic pathway of indole amines (Table 2)

Considering the chemical relationship between

the indoleamines, we may postulate the following pathways.



In the common reed sprout, the major indoles were serotonin, tryptophan and tryptamine in the unincubated sprout. The relative change in amount of tryptophan was not so marked during incubation. Serotonin and tryptamine decreased in amount with the slight increase of other indoleamines during the period

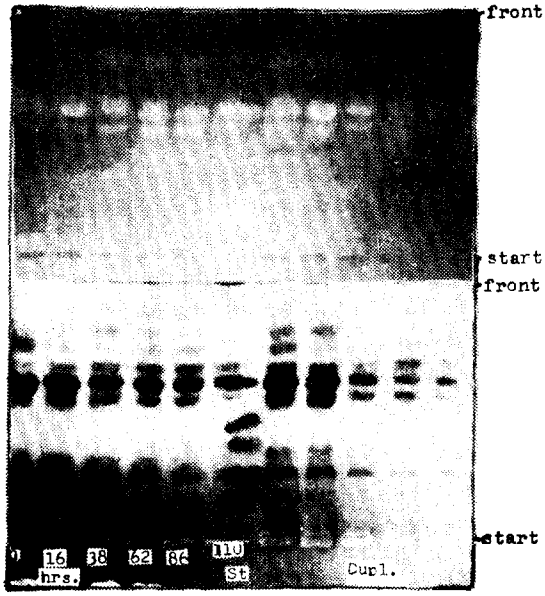


Figure. 1. Thin layer chromatograms representing the changes of indole compounds in the course of incubation of common reed sprouts. Visualization was made under UV_{250} light (upper) and with van-Urk reagent (lower). The figures indicate the incubation periods and standards are serotonin, indole butyric acid, indole acetic acid, tryptophan, and 5-hydroxytryptophan with decreasing Rf values. Solvent system was isopropanol: methylacetate: 7N-NH_4OH(35: 45 : 20).

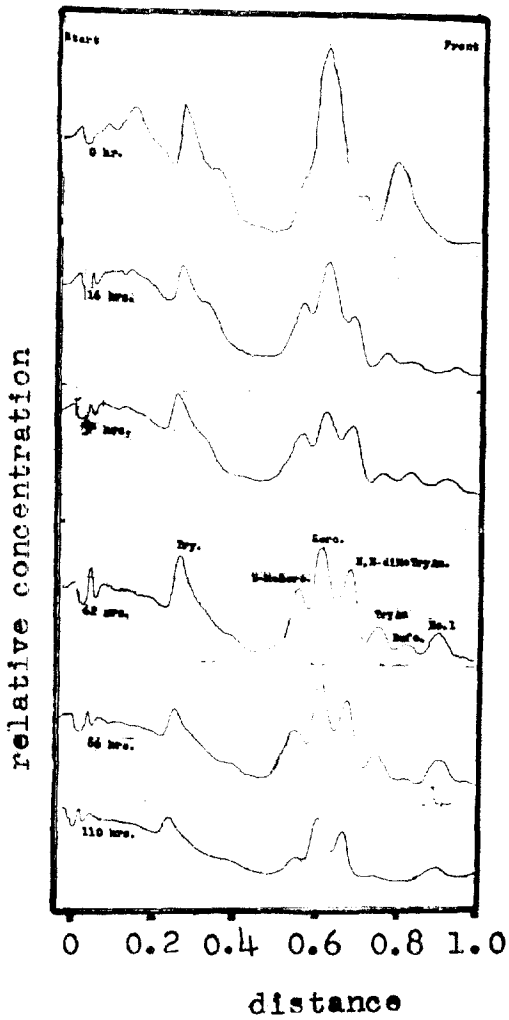


Figure 2. Densitometric interpretation of the thin layer chromatograms. The scanning was carried out after 24 hours following van-Urk reagent treatment.

Biosynthetic aspects of serotonin in plants was already discussed in the previous paper.[8] Serotonin may be formed through two main routes from tryptophan: one is via 5-hydroxytryptophan, and the other via tryptamine. The former was found in animal tissues[9] and several plant tissues,[8,10] and the latter only in plants such as *Piptadenia pregrina*.[6] We may assume that serotonin is

Table. 2. Relative area of the densitogram of indoles in the sprout of the common reed during incubation.*

Incubation (hours) / Indoles	0	16	38	62	86	110
tryptophan	24.1	27.9	29.3	21.3	24.8	37.5
serotonin	48.8	34.7	23.8	29.3	25.7	27.0
N-methylserotonin	4.4	18.2	19.0	16.1	17.7	10.2
bufotenine	—	3.1	5.3	2.9	1.7	—
tryptamine	16.4	5.8	4.8	7.7	8.3	2.5
N, N-dimethyl-tryptamine	6.2	10.4	17.9	22.6	21.8	22.9

* The figures represent the percentage in which the area of each indole compound was divided by the total area of indole compounds.

derived from tryptophan via tryptamine which was detected in a large amount in unincubated sprouts. But it is not clear whether or not serotonin is formed via 5-hydroxytryptophan, considering that hydroxylation of tryptophan is the limiting step in the synthesis of serotonin.

Two routes are possible in bufotenine biosynthesis. Fellow et al.[6] suggested that bufotenine was derived from serotonin through N-methylserotonin but Fish et al.[7] reported that synthesis of bufotenine from N, N-dimethyltryptamine was also present in a plant tissue. The amount of serotonin was large while that of N-methylserotonin and bufotenine was negligible in the unincubated sprout. After the 16 hour incubation N-methylserotonin, a methylated derivative of serotonin, was increased in amount with the decrease of serotonin and after 38 hours bufotenine was slightly increased. Thereafter the quantity of N-methylserotonin as well as that of bufotenine was decreased gradually. Consequently it implies that serotonin may be derived from serotonin by way of N-methylserotonin in the common reed. It may be suggested that bufotenine is possibly biosynthesized from serotonin that was derived from tryptophan via tryptamine. Bufotenine was also slightly increased with the increase of N, N-dimethyltryptamine was increased gradually until 110 hours. Therefore derivation of bufotenine from N,N-dimethyltryptamine may be unlikely in the common reed.

As for N,N-dimethyltryptamine, the amount was small and tryptamine was present relatively in large amount in the unincubated sprouts, After 16 hours the amount of N,N-dimethyltryptamine was greatly increased while that of tryptamine was rapidly decreased. Thereafter the quantity of both compounds was maintained relatively constant. It may be assumed that tryptamine is metabolized to N,N-dimethyltryptamine.

요 약

갈대(*Phragmites communis* Trin)의 幼芽를 27°C의 暗所에서 110시간 동안 砂耕 培養하였다. 幼芽가 자라는 동안 一定한 시간마다 試料를 채취하여 methanol로 處理하여 그 抽出物에 대하여 thin layer chromatography를 實施하였다. 그 결과 培養하지 않은 幼芽에서는 indole化合物 중에서 serotonin, tryptophan, tryptamine이 多量으로 나타났으나, 培養 過程 중에 이들 化合物의 減少와 더불어 다른 여러가지 indoleamine類의 生合成이 顯著하였다. 즉 培養 過程 중에 tryptophan의 量에는 큰 變化가 없으나, serotonin 및 tryptamine이 감소하면서, 이들의 methyl化 유도체들이 增加하였다. 그 增減의 相互關係를 관찰하여 본 결과, bufotenine은 tryptophan에서 tryptamine을 거쳐 serotonin, N-methylserotonin의 經路로 合成될 것임을 시사하였다.

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