(+)-*Thalifarazine* (**2**) This compound was obtained as yellowish white amorphous powders 8 mg, $[\alpha]_D^{24}$ +48° (MeOH, *c* 0 1) This alkaloid was characterized by spectral (UV, IR, ^{1}H NMR and MS) analyses and comparison with literature data [10]

Biological evaluation The cytotoxicity assay was carried out according to a procedure described in literature [12]

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PLANT GROWTH INHIBITING PROPERTIES OF PHALAENOPSINE T FROM *PHARAENOPSIS* SPP

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Key Word Index—*Phalaenopsis* spp, Orchidaceae, pyrrolizidine alkaloid, phalaenopsine T, growth inhibitory activity

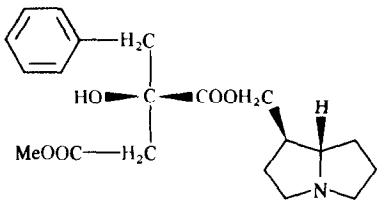
Abstract—A pyrrolizidine alkaloid, phalaenopsine T was identified as a growth inhibitory compound contained in *Phalaenopsis* spp using the lettuce seedling and *Calanthe* seedling tests

INTRODUCTION

In continuing studies on growth inhibitors of plants, we have already reported the isolation of eucomic acid and hydroxyeucomic acid from *Cattleya trianaei* [1, 2] and *p*-coumaroyl ester of 2,3-dihydroxy-1,2-propanedicarboxylic acid and diferuloylsucrose from *Lilium longiflorum* [3. 4] In the case of *Phalaenopsis* spp, the growth of the shoot tip is occasionally inhibited during the shoot tip culture The growth inhibitor was detected by the lettuce seedling and the *Calanthe biscolor* (Orchidaceae) seedling test and localized in the alkaline fraction The following report deals with the isolation of a constituent with significant plant growth inhibiting activity which was shown to be identical with the pyrrolizidine alkaloid, phalaenopsine T[5]

RESULTS AND DISCUSSION

A methanol extract (Fr 1) of sliced fresh leaves of *Phalaenopsis* spp was extracted with ether (Fr. 2) and ethylacetate (Fr. 3) successively under acidic condition and then chloroform (Fr 4) and *n*-butanol (Fr 5) successively under alkaline conditon The inhibiting assay for individual fraction was carried out using the lettuce



seedling test [6] The result clearly suggests that the inhibitory effect was in fraction 4 as shown in Table 1. The growth inhibitory fraction (Fr. 4) was repeatedly purified by column chromatography on Sephadex LH-20, silica gel, alumina and finally recrystallization by monitoring for growth inhibitory activity by the lettuce seedling growth test to give colourless crystalline, compound **1**, mp 99–104° The FDMS showed a molecular ion at 361 (M^+). The ^{13}C NMR spectrum of **1** resembled that of eucomic acid dimethyl ester [1] indicating phenyl carbons at 127.2, 128 2, 130.2 and 134.8 ppm, carbomethoxyl carbon at 51.8 ppm, benzyl methylene carbon at 42.9 ppm and aliphatic tertiary carbon at 75 8 ppm. In addition, two methylene carbons neighbouring nitrogen atom at 54 4 and 54 8 ppm and three other methylene carbons at 26 0, 30 6 and 31.8 ppm were observed ^1H NMR spectrum of **1** indicated phenyl protons as a singlet at 7.27 ppm. From these results **1** might be phalaenopsine T or phalaenopsine La which are diastereoisomers. Finally **1** was identified as phalaenopsine T comparing the $[\alpha]_D$ with that of literature [5].

The growth inhibitory effect of **1** was investigated in the lettuce and *Calanthe biscolor* seedling tests (Table 2). In the lettuce seedling test **1** inhibited does dependently the growth of both hypocotyl and root. The *Calanthe* seedling test also showed the dose dependent effect on shoot, root length and total weight. Significant inhibition was observed at 1000 ppm with all seedlings dying during the 52-day-culture. In the medium containing 500 ppm and 100 ppm of **1**, 2/5 and 1/7 seedlings die, respectively.

In our previous investigations we isolated the inhibitory compound found in *Cattleya trianaei* and suggested that the inhibition might have occurred due to the disorder of a related metabolic pathway resulting from of eucomic acid[1] Since phalaenopsine T has eucomic acid residue in a molecule, the previous hypothesis may be acceptable. Moreover, in the bioassay **1** may produce dehydroxyeucomic acid and trachelanthamidine by saponification and those compounds induce growth inhibition.

EXPERIMENTAL

Mp: uncorr. ^1H NMR spectra were measured at 100 MHz with TMS as int. standard. ^{13}C NMR spectra were taken on ppm scale with TMS as int standard. Dragendorff reagent, 10% H_2SO_4 and UV were used for detection CC was carried out with Sephadex LH-20, silica gel 60 and alumina.

Bioassay procedure The lettuce seedling growth test was done as previously described[6] with modification. The *Calanthe* seedling test was carried out on the agar medium containing 2 g of Hyponex (The Hyponex Company, Inc), 2 g of peptone and 25 g of sucrose/1 under 16 hr light cycle for 52-day-culture.

Table 1 Effect of individual fraction* on the growth of lettuce seedling

Growth rate (%)	Control	Fr 1	Fr 2	Fr 3	Fr. 4	Fr 5	Fr 6
Hypocotyl length	100	161 5	103 6	150.3	87 6	89 9	133.1
Root length	100	102.7	114 4	79 5	58.5	25 1	61 2

*1000 ppm of sample was assayed

Table 2. Effect of phalaenopsine T on the growth of lettuce seedlings (A) and *Calanthe biscolor* seedlings (B)

	Growth rate (%)	Phalaenopsine T (ppm) 1000	500	100	10	0
A	Hypocotyl length	43 6	69.1	81 8	98 2	100
	Root length	30 6	79.3	80.8	87.3	100
B	Hypocotyl length	0*	48 8**	78 8***	75 1	100
	Root length	0	11 3	54 9	71 8	100
	Total weight	0	34.8	55 1	52 9	100

*All seedlings died
**2/5 seedlings died.
***1/7 seedling died

Isolation of **1**. Fresh leaves and stem (550 g) of *Phalaenopsis* cv musashino (cryde × maliburiver) were homogenized with MeOH and stored at room temp overnight The MeOH extract (FR 1; 12 g) was suspended with 0 1 M HCl (100 ml, pH 2–3) and extracted with Et_2O(Fr 2, 1 23 g) and EtOAc(Fr 3, 0 46 g), successively. The aq layer was adjusted to pH 9 with NH_3 and extracted with $CHCl_3$ (Fr. 4, 0 24 g) and *n*-BuOH (Fr 5, 1 02 g), successively. The Fr 4 was repeatedly subjected to column chromatography to give a pale yellow powder (140 mg) which gave colourless crystalline **1** after recrystallization from EtOH Mp 99–104° (lit. [5], mp 104 5–105°), $[\alpha]_D^{22} - 24\ 5$ ($CHCl_3$, *c* 0 57), (lit [5], $[\alpha]_D^{20} - 15°$), FDMS *m/z* 361 (M^+), ^{1}H NMR ($CDCl_3$) 2 76, 3 04 (2H, *dd*, $J = 16$ Hz), 2 98 (2H, *d*, $J = 1\ 2$ Hz), 3.65 (3H, *s*), 3 99, 4 20 (2H, *ddd*, $J = 6$, 6, 11 Hz), 7.25 (5H, *s*), ^{13}C NMR ($CDCl_3$). 26 0, 30 6, 31 8, 42 9, 44 8, 45 5, 51 8, 54 4, 54 8, 67 9, 68 3, 75 8, 127 2, 128 2, 130 2, 134 8, 171 0, 174 2

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DISTRIBUTION OF PIPERINE IN VEGETATIVE PARTS OF *PIPER NIGRUM*

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Key Word Index—*Piper nigrum*, Piperaceae, pepper; piperine, high performance liquid chromatography

Abstract—Piperine contents of greenhouse-grown *Piper nigrum* plants were analysed Only traces of alkaloid were detected in leaves and young shoots, more significant quantities (*ca* 0 03%) occurred in the root Remarkably high amounts of piperine (*ca* 0 2%) were found, however, in mature, fully differentiated shoots reaching the contents of commercially available white or black pepper of lower quality

Piperine (*E,E*-1-*N*-piperoylpiperidine), the pungent principle of pepper, occurs in the fruit of *Piper nigrum* and related species, e g *P longum* or *P clusii* [1] This alkaloid has also been found occasionally in other parts of piperaceous plants, e.g in stems of *P chaba* [2] and in the wood of *P novae-hollandiae* [3]. In general, however, the association of pepper seeds and piperine is so characteristic that it is widely believed that vegetative parts of pepper are devoid of this substance

Our interest in this question arose from plans to study the biogenesis of piperine. We soon became aware that it was extremely difficult, if not impossible, to achieve flowering and fruiting of *P nigrum* plants in the greenhouse at our northern geographical latitudes It was thus decided to analyse vegetative plant parts for the eventual accumulation of piperine. For this purpose, roots, young and mature shoot segments, and young and mature leaves, respectively, were extracted individually with $CHCl_3$ In addition, commercially available samples of black and white pepper kernels were worked up as references Qualitative analyses by reversed-phase HPLC, based on comparison of R_fs and spiking with authentic material, revealed that the extracts from root and shoot tissues contained significant amounts of piperine as the main or almost exclusive component Extracts from mature shoots in particular closely resembled those

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