STUDIES ON AUXINS AND INHIBITORS IN *Pinus taeda* and *Pinus Pinaster*

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Introduction

Our knowledge of auxin and inhibitor of pine tree is limited.

CZAJA (1934) found that auxin occurs in shooting buds of *Pinus silvestris* and *Pinus Hedreichii*.

ZIMMERMANN (1937) investigated the quantity of buds of *Pinus strobus*.

MIROV (1941) in Ponderosa pine and Torrey Pine, ONAKA (1950) in *Pinus Thunbergii*, investigated on the distribution of auxin in shoots.

These investigators have not described the chemical nature of auxin.

FRANSSON (1953) reported that auxin in shoots of *Pinus silvestris* is different from β – indoleacetic acid.

MIROV et al. (1959) stated that the recent discovery of many auxin substances or preqursors such as indoleacetonitrile and indoleacetaldehyde indicates the need for re-evaluating these older studies of growth substances in pine.

Recently OGASAWARA (1961a) found several auxins and inhiditors in buds and leaves of *Pinus Thunbergii*. One of the auxins is identfied as IAA. However, the other auxins and inhibitors were not identified.

In this report, authors chromatographycally investigated on auxins and inhibitors occuring in *Pinus taeda* and *Pinus Pinaster*.

Materials and methods

Buds and leaves of *Pinus taeda* and *Pinus Pinaster* were used as experimental materials.

P. taeda ..... 5 years old

(This pine grafted on *Pinus Thunbergii* 5 years ago)

P. Pinaster ..... 10~15 years old

10 grams of samples were taken from the buds (or leaves), cut into slices and extracted at 2 degrees C° with 100 ml of ether for 20 hours.

This extract was shaken repeatedly with 2 per cent of sodium bicarbonated solution.

The ether fraction is the concentrated neutral fraction.

The aqueous fraction was adjusted to PH 3.0 with 15 per cent tartaric acid and then solution was shaken with ether fraction.

This is the concentrated acid fraction. Acid and neutral fraction (1/10 degrees samples)

were spotted on the starting line of chromatogram papers. The chromatograms were developed in the solvnts for about 20 cm depending on the season.

The solvents are as follows;

isopropanol—ammonia—water (8 : 1 : 1 v/v/v)

butanol—ethanol—water (4:1:1 v/v/v)

butanol—ethanol—ammonia (1 : 1 : 2 v/v/v)

70 % ethanol

The paper developed in isopropanol—ammonia—water, was then removed from grasscylinder and dried. Then, it was cut transversally into 10 segments. Each chromatogram segment was immersed in 2 ml of 2 per cent sucrose solution in a small Petri dish.

10 sections of 2.3 mm long from Avena coleoptiles were placed in a thermostat at 25 degrees C°.

The length of the Avena sections was measured after incubation for 20 hours. As control only the length of these sections was measured which were floated on the solution with the control chromatogram paper without spotting.

Color reaction

Reagents are as follows;

1. EHRLICH reagent (p-dimethylaminobenzaldehyde 2g — 20 ml HCl — 80 ml abs. ethanol)
2. GORDON & WEBER reagent (0.05 M $FeCl_3$ — 5 % $HClO_4$ 1 : 50 v/v)
3. MITCHELL & BRUNSTETTER reagent (KNO_2 — HNO_3 1 g — 200ml)
4. TANG & BONNER reagent (0.5 M $FeCl_3$ — H_2SO_4 — H_2O, 3 : 60 : 100 v/v/v)
5. 2 % $FeCl_3$

Paper sprayed by reagent is heated for a few minutes at 60 — 70 C° in a thermostat for color development.

The treatment of Tryptophane (TTP)

Samples were treated with 1000 ppm solution of DL — Tryptophane at 25 C° for 48 hours in the dark.

After the treatment, auxins and inhibitors in samples were measured with the above methods.

Results

Chromatograms of ether extracts from the buds of *Pinus taeda* are presented in Fig. 1.

A growth promoting zone (Rf 0.00 — 0.50) and a growth inhibiting zone (Rf 0.50 —1.00) were detected in the acid fraction and a inhibiting zone (0.30—1.00) was found in the neutral fraction.

In the acid fraction, six substances showing positive reactions by EHRLICH reagent or 2 % $FeCl_3$ were found. Among three substances (Rf around 0.25, Rf around 0.28

and Rf around 0.32) are auxin and one substance (Rf around 0.53 – 0.93) is inhibitor.

In the neutral fraction, there are no substance showing positive reaction with reagent.

Chromatography reveals growth promoter which corresponds in Rf with IAA, while no color was upon the application of EHRLICH reagent etc. to the chromatogram.

Authors could not confirm that this promoter was IAA. Chromatograms of ether extracts from the buds were with TTP treatment shown in Fig. 2.

Auxin which corresponds in Rf with IAA increased and showed the positive reaction by Ehrlich reagent etc. .

Also, a new substance showing positive reaction by EHRLICH reagent was found in a inhibiting zone of acid fraction.

Color reactions and Rf values of substances in the color reaction and Rf values of substances in the acid fraction are presented in theTable 1 - 2.

Color reaction and Rf value of substance F are quite the same as that of synthesized IAA.

From these results, substance F is identified as IAA. However,other substances could not be identified.

Auxins and inhibitors of leaves are the same as those of buds. (Fig. 3 – 4)

The experimental results upon auxins and inhibitors of *Pinus Pinaster* are presented in Fig. 5 – 8 and Table 3.

These results are similar to that of *Pinus taeda*

Discussion

FRANSSON (1953) reported that activity and diffusion coefficient of auxin from *Pinus silvestris* differ from these of pure IAA.

He considered that pine auxin is not IAA but is an other auxin.

OGASAWARA (1961a) found three auxins and two inhibitors in acid fraction of ether extracts from buds and leaves of *Pinus Thunbergii* and considered that one of these auxins is IAA. Also, OGASAWARA (1961b) found four auxins in *Pinus strobus* and considered that one of those auxins is probably IAA.

In *Pinus taeda* and *Pinus Pinaster*, three auxins, showing positive reaction by EHRLICH reagent, were found in the acid fraction. Moreover, auxin which corresponds in Rf with IAA is detected, but no reaction by Ehrlich reagent etc. . Therefore, authors could not confirm that this auxin is IAA.

It is considered that IAA is produced from TTP by the action of an enzyme.

WILDMANN et al. (1948) have shown that an enzyme is distributed through the Avena coleoptile in a manner strikingly parallel to the distribution of auxin itself and the greatest concentration of enzymatic activity as measured by the production of auxin from TTP was found at the tip of the coleoptile.

If IAA is produced in these pines, by means of the addition of TTP to pines, these

pines must produce IAA from TTP by TTP-IAA converting enzyme.

After the treatment with TTP, auxins and inhibitors of pines were measured.

Auxins which corresponds in Rf with IAA increased and showed positive reaction by Ehrlich reagent etc. .

Color reaction with five reagents and Rf value in four solvents of this auxin are quite the same as that of synthesized IAA.

This auxin is identified as IAA. From these results, it may be considered that TTP -IAA converting enzyme exists in these pines and these pines produce IAA from TTP even without the addition of TTP.

It may be suggested that the quantity of IAA obtained from the untreated samples might be too small to show the positive reaction by EHRLICH reagent etc. .

The presence of auxins moving more slowly than IAA in isopropanol – ammonia– water, in plant extract has been found in the other plant.

For example, two auxins more slowly than IAA have been reported by NITSCH (1955) in strawberry, by STOWE et al. (1954) in corn kernels, by STOWE et al. (1956) in corn endosperm, by OGASAWARA (1961a) in *Pinus Thuubergii*, by OGASAWARA (1961b) in *Pinus strobus*.

STOWE et al. (1954) reported that spot of lower Rf value than IAA was identified as IPA in corn kernels. NITSCH (1955) described that substance (Rf around 0.32) in strawberry is probably IPA.

Two auxins (Rf around 0.28, Rf around 0.32) in acid fraction of ether extracts from these pines are probably indole compound, judging from their positive reaction by indole indicating reagent.

These auxins are in the region of IPA. However, direct comparison with synthesized IPA cannot be shown at this time, and precise identification of these auxins must be deferred.

Inhibitor (Rf around 0.53-0.93) in acid fraction is similar to inhibitor-β of BONNET -CLARK et al. (1953).

SHIBAOKA et al. (1957) found three growth inhibitors which inhibited the IAA induced growth of Avena coleoptile sections in *Heleanthus* leaves, and two of them were identified with chlorogenic acid and isochlorogenic acid.

HENDERSHOTT et al. (1959) reported that growth inhibitor in dormant buds of a peach was identified with Naringenin.

Authors could not confirm the chemical nature of pine inhibitor.

However, pine inhibitor is probably different from above known inhibitor, judging from the color reaction.

Fig. 1 Buds

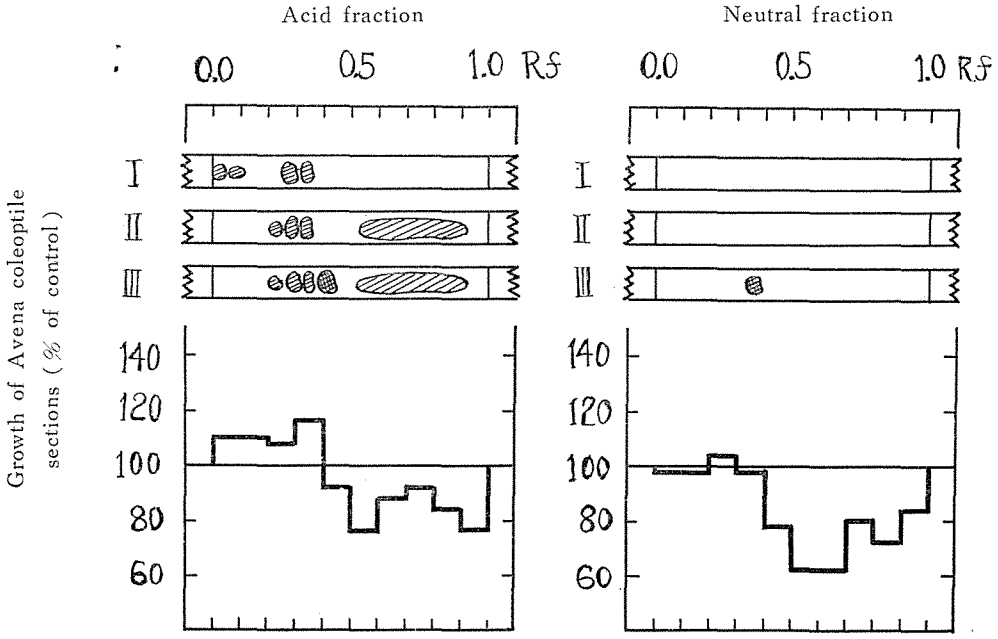

Fig 2 Leaves

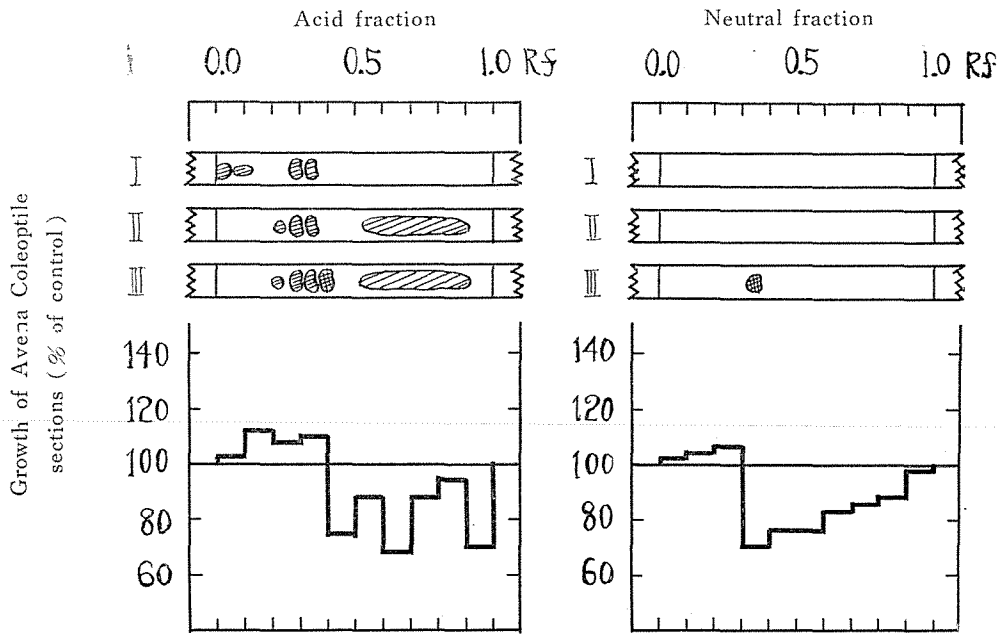

Fig 1—2 Chromatograms of ether extracts obtained from *Pinus taeda*, developed in isopropanol – ammonia – water (8:1:1), assayed by Avena straight growth test.

I : Reactions of chromatograms of ether extracts by 2% $FeCl_3$

II : Reactions of chromatograms of ether extracts by EHRLICH reagent

III : Reactions of guide chromatograms of adding synthesized IAA to ether extracts by EHRLICH reagent

Fig. 3 Buds

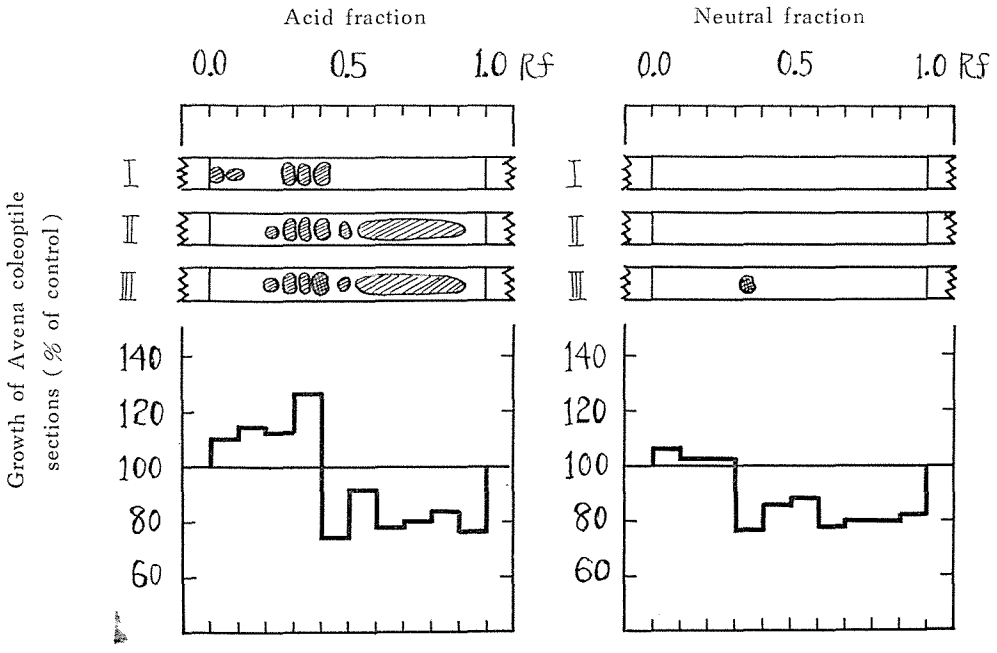

Fig. 4 Leaves

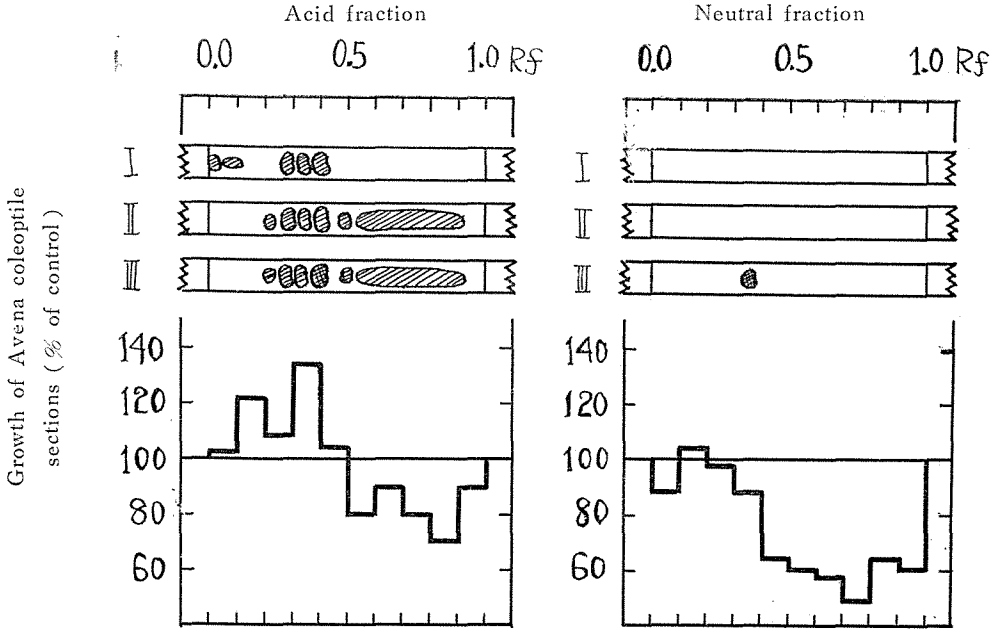

Fig 3—4 Chromatograms of ether extracts obtained from *Pinus taeda* were treated with 1000 ppm solution of Tryptophane for 48 hours at 25 C°, assayed by Avena straight test

I : Reactions of chromatograms of ether extracts by 2 % $FeCl_3$

II : Reactions of chromatograms of ether extracts by EHRLICH reagent

III : Reactions of guide chromatograms of adding synthesized IAA to ether extracts by EHRLICH reagent

Fig. 5

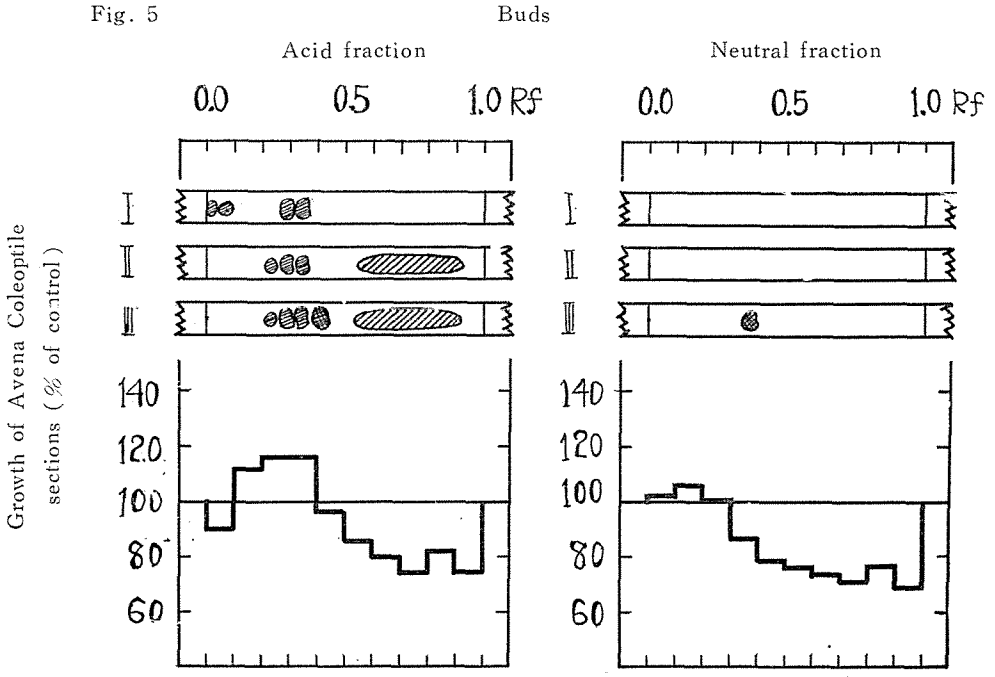

Fig. 6

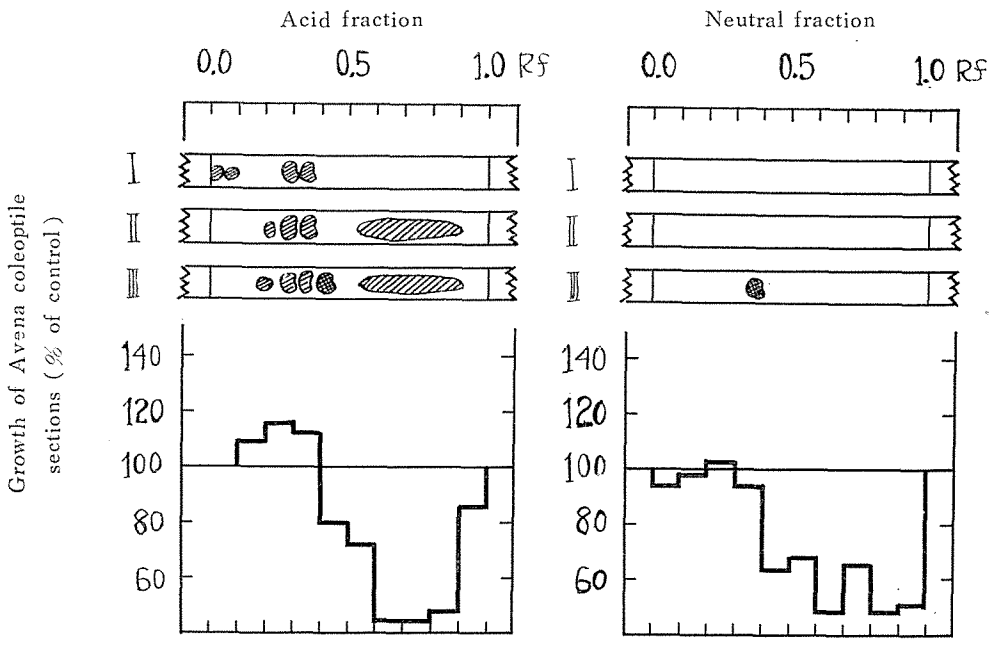

Fig 5–6 Chromatograms of ether obtained from *Pinus Pinaster*, developed in isopropanol – ammonia -- water (8:1:1), assayed by Avena straight growth test.

I : Reactions of chromatograms of ether extracts by 2% $FeCl_3$

II : Reactions of chromatograms of ether extacts by EHRLICH reagent

III : Reactions of guide chromatograms of adding sythesized IAA to ether extracts by EHRLICH reagent

Fig. 7 Buds

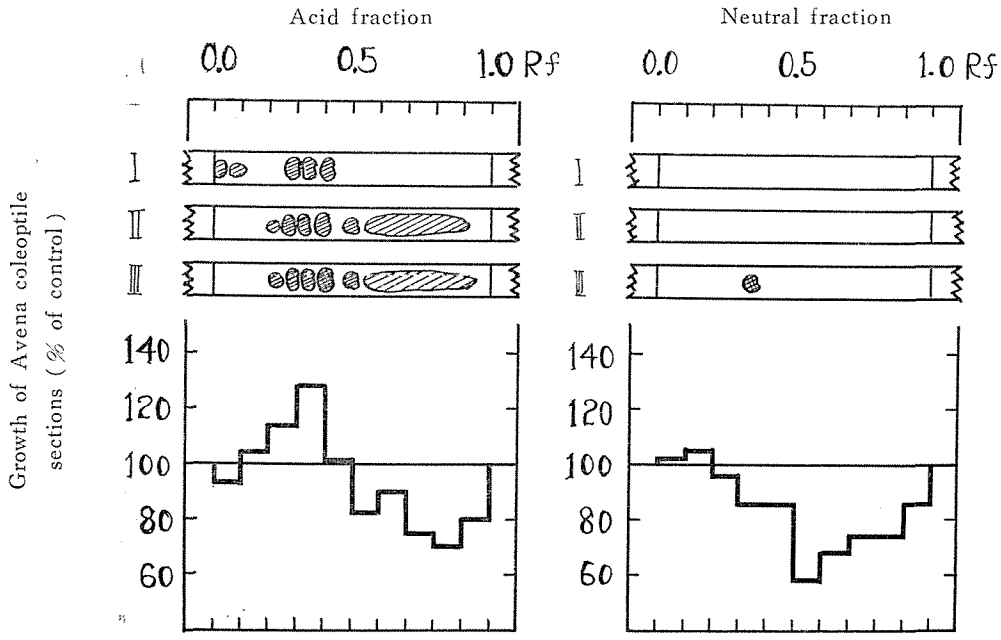

Fig. 8 Leaves

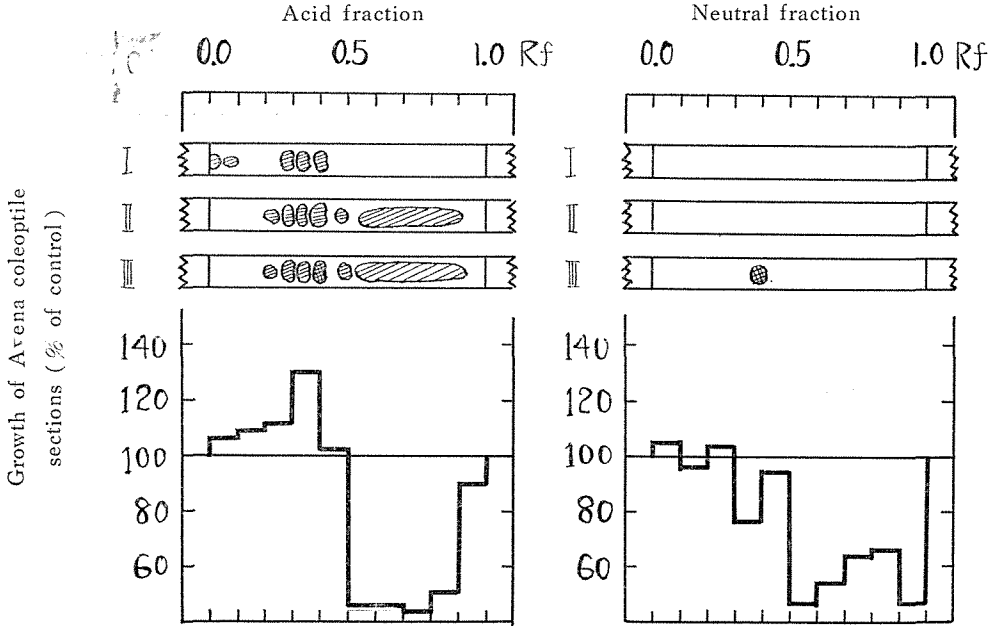

Fig 7–8 Chromatograms of ether extracts obtained from *Pinus Pinaster* were treated with 1000 ppm solution of Tryptophane for 48 hours at 25 C, assayed by Avena straight growth test

I : Reactions of chromatograms of ether extracts by 2 % $FeCl_3$

II ; Reactions of chromatograms of ether extracts by EHRLICH reagent

III ; Reactions of guide chromatograms of adding synthesized IAA to ether extracts by EHRLICH reagent.

Table 1. Rf values of substances showing positive reaction with EHRLICH reagent or $FeCl_3$ in acid fraction of ether extracts from the buds treated with Tryptophane of *Pinus taeda*

Substance		Rf Values in			
		isopropanol —ammonia —water 8 : 1 : 1	Butanol —ethanol —water 4 : 1 : 1	Butanol —ethanol —ammonia 1 : 1 : 2	70 % ethanol
Substance	A	0.03	0.02		
———	B	0.08	0.12		
———	C	0.25	0.18	0.44	
———	D	0.28	0.28	0.58	0.64
———	E	0.32	0.33	0.62	0.74
———	F	0.40	0.42	0.72	0.78
———	G	0.46	0.49	0.80	0.80
———	H	0.53~0.93	0.51~0.93	0.83~1.00	0.84~1.00
Synthesized	IAA	0.40	0.42	0.72	0.78

Table 2. Color reactions of substances in acid fraction of ether extract from the buds of *Pinus taeda*

Substance		Color reactions with				
		EHRLICH reagent	GORDON & WEBER reagent	TANG & BONNER reagent	MITCHELL & BRUNST -ETTER reagent	2 % $FeCl_3$
Substance	A					Yellow
———	B					Bluc
———	C	Pink	Pink	Pink		Pink
———	D	Green	Pink	Pink	Yellow	Purpurish brown
———	E	Blue	Bluish red	Pink	Yellow	Bitter orange
———	F	Bluish red	Pink	Red	Red	Pink
———	G	Yellow				
———	H	Pink				
Synthesized	IAA	Bluish red	Pink	Red	Red	Pink

Table 3. Color reactions of substances in acid fraction of ether extract from the buds of *Pinus Pinaster*

Substance		Color reactions with				
		EHRLICH reagent	GORDON & WEBER reagent	TANG & BONNER reagent	MITCHELL & BRUNST -ETTER reagent	2 % $FeCl_3$
Substance	A					Yellow
———	B					Blue
———	C	Pink	Pink	Pink		Pink
———	D	Green	Pink	Pink	Yellow	Purpurish brown
———	E	Blue	Bluish red	Pink	Yellow	Bitter orange
———	F	Bluish red	Pink	Red	Red	Pink
———	G	Yellow				
———	H	Pink				
Synthesized	IAA	Bluish red	Pink	Red	Red	Pink

Summary

Auxins and inhibitors in *Pinus taeda* and *Pinus Pinaster* were investigated by means of paper chromatography followed by bioassay with straight growth of Avena coleoptile sections.

On chromatographying in isopropanol – ammonia – water (8: 1: 1), three auxins (Rf around 0.25, Rf around 0.28, Rf around 0.32) and one inhibitor (Rf around 0.53 - 0.93) showing positive reactions by EHRLICH reagent found in acid fraction of ether extracts were obtained from buds and leaves of two pines. Moreover, two substances (Rf around 0.03, Rf around 0.08) showing positive reaction 2% $FeCl_3$ were found in acid fraction.

Chromatography reveals a growth promoting substance which corresponds in Rf with IAA, while no color was observed upon the application of reagent to the chromatogram.

After the treatment with Tryptophane, auxins and inhibitors of samples were measured.

Auxin which corresponds in Rf with IAA increased and showed positive reaction by EHRLICH reagent etc. .

This auxin was identified as IAA comparing it with Rf value and color reaction of synthesized IAA controls.

It may be considered that a Tryptophane – IAA converting enzyme system exists in these pines and these pines produce IAA from Tryptophane.

The identification of other auxins and inhibitors must be deferred.

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和 文 要 約

テーダマツ，フランス海岸松の生長物質および抑制物質

小笠原隆三・近藤芳五郎

テーダマツ，フランス海岸松の生長物質および抑制物質をエーテルで抽出し，ペーパー，クロマトグラフイーとアベナ伸長試験で調べた結果，両者とも同じ生長物質と抑制物質の存在することが認められた。

即ち，展開溶媒としてイソプロパノールーアンモニアー水（8：1：1）を用いた場合酸性区分に Ehrlich 試薬で陽性を示す3つの生長物質（Rf0.25, Rf 0.28, Rf 0.32）と1つの抑制物質（Rf 0.53～0.93）が認められた。その他 $FeCl_3$ で陽性を示す2つの物質（Rf 0.03, Rf 0.08）も認められた。これらの物質の化学的性質は明らかではないが，2つの生長物質（Rf 0.28, Rf 0.32）については呈色反応からみて明らかにインドール化合物と思われる。

IAA 位置にも生長促進がみられることから IAA が存在するものと考えられるが，呈色反応ではこれを確認できなかつた。

しかし，人為的に与えた Thyptophane から IAA を生成する能力を持つていることから，これらのマツは IAA を生成しているものと推定される。

尚中性区分では Rf 0.40～1.00 附近に抑制物質がみられた。