This research was supported in part by Grant-in-Aid for Developmental Scientific Research from the Ministry of Education for which the authors wish to express their gratitude. They are also grateful to the members of Central Analysis Room of the Faculty for elemental analysis and spectral data.

[Chem. Pharm. Bull. 15(2) 163～168 (1967)]

UDC 581.19 : 581.143 : 582.736 : 547.757

22. Toshihiko Okamoto,*1 Yo Isogai,*2 Tôru Koizumi,*1 Hisako Fujishiro,*1 and Yaeko Sato*2 : Studies on Plant Growth Regulators. III.*3 Isolation of Indole-3-acetonitrile and Methyl Indole-3-acetate from the Neutral Fraction of the Moyashi Extract.

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Methyl indole-3-acetate and indole-3-acetonitrile were identified in the neutral fraction of Moyashi (F-1 AcOEt fraction) by thin-layer chromatography and vapor phase chromatography.

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In the previous paper[1] it was reported that indole-3-acetamide and phenylacetamide were isolated from the neutral fraction (F-1) of Moyashi by alumina column chromatography using a mixture of ethylacetate and methanol as the eluting solvent. It was also reported that the growth promoting activity was found in the ethyl acetate eluting fraction by the alumina chromatography.[1] (It was tentatively called as F-1 AcOEt fraction.)

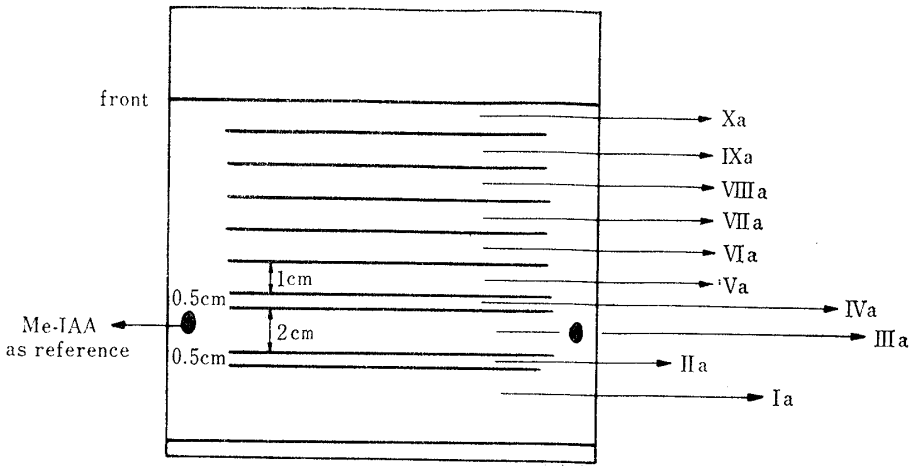

Fig. 1. Preliminary Thin-layer Chromatography
adsorbent : Camag Kieselgel
solvent : Ether-Hexane (2:1)

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*3 Part II. T. Okamoto, Y. Isogai, T. Koizumi : This Bulletin, **15**, 159 (1967).
1) Y. Isogai, T. Okamoto, T. Koizumi : This Bulletin, **15**, 151 (1967).

This paper deals with the active substances in this F-1 AcOEt fraction. In order to separate the fraction, the preparative thin-layer chromatography was applied. 24 mg. of the F-1 AcOEt fraction was submitted to preparative thin-layer chromatography (TLC) using a silica gel plate (300 μ) and *n*-hexane-ether (1:2) as solvent. The chromatogram was divided into ten bands as Fig. 1 and the adsorbents corresponding to each band were eluted with ether to give ten fractions.

The biological activity of each fraction in Avena straight growth test was shown in Table I.

Table I. Results of Bioassay of Each Fraction in Preliminary Thin-layer Chromatography

	Concn. (p.p.m.)	Length (mm.)		Concn. (p.p.m.)	Length (mm.)
Control		6.8, 6.4	IAA	1	8.3, 8.1
Ia	1	6.6	IIa	1	8.4
	10	6.5		10	7.8
	100	6.3		100	6.3
IIIa	1	7.6	IVa	1	6.7
	10	8.1		10	6.6
	100	6.7		100	7.1
Va	1	6.8	VIa	1	6.8
	10	6.9		10	6.3
	100	6.6		100	6.8
VIIa	1	6.9	VIIIa	1	6.6
	10	6.7		10	6.5
	100	6.7		100	6.5
IXa	1	6.5	Xa	1	6.2
	10	6.7		10	7.2
	100	7.1		100	6.5
			NF-1	1	7.2
			AcOEt	10	8.5
			fraction	100	7.7

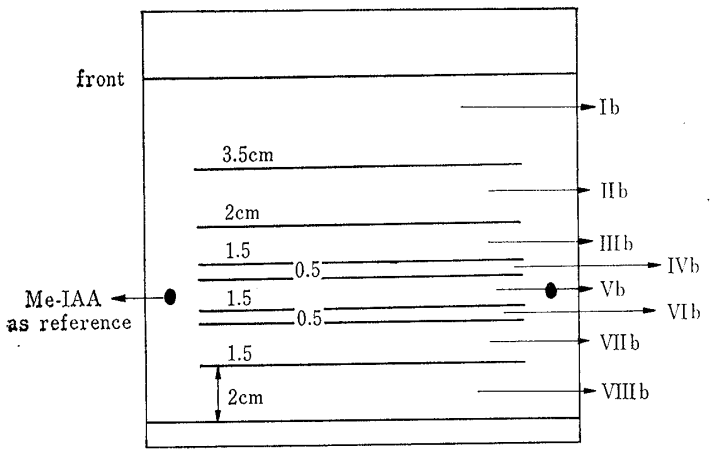


Fig. 2. Preparative Thin-layer Chromatography

adsorbent: Camag Kieselgel
solvent: Ether -Hexane (2:1)

The promoting action was observed in the fraction IIa and IIIa. The bluish purple spot corresponding to Me-IAA was observed in thin-layer chromatogram (silica gel, ether-*n*-hexane (2:1)) of the fraction IIIa. But the corresponding spot could not be observed in the chromatogram of the fraction IIa. From this result it was concluded the F-1 AcOEt fraction contained two growth promoting substances whose Rf values were similar to that of Me-IAA in thin-layer chromatography. In order to isolate the two growth promoting substances in the F-1 AcOEt fraction, considerable amount of this fraction was submitted to preparative thin-layer chromatography. 165.4 mg. of this fraction (which was obtained from 779.5 mg. of F-1 fraction) was applied to eight TLC plates (300 μ) and developed with ether-*n*-hexane (2:1). The chromatograms

were divided into eight bands as shown in Fig. 2 and the adsorbents corresponding to each band were collected and eluted with methylene chloride to give eight fractions.

The weight of each fraction and the results of Avena straight growth test were shown in Table II.

Table II. Results of Bioassay of Each Fraction (IV, V, VI) in Preparative Thin-layer Chromatography

	Weight (mg.)	Concn. (p.p.m.)	Length (mm.)
Control			6.3
IAA		1	7.9
Ib	24.6		
IIb	10.6		
IIIb	5.2		
IVb	2.1	100	7.3
		10	6.5
		1	6.0
Vb	5.4	100	7.5
		10	7.8
		1	6.5
VIb	2.6	100	7.2
		10	7.7
		1	6.5
VIIb	3.3		
VIIIb	6.0		

The promoting action was observed in the fractions obtained from the band Vb and VIb. In order to identify the active substances, vapor phase chromatography and thin-layer chromatography were applied to these two fractions.

The fraction Vb was submitted to thin-layer chromatography and the presence of the spot corresponding to authentic Me-IAA was confirmed under the following conditions.

Aluminium Oxyd (Camag)	CH_2Cl_2	Rf 0.7
	AcOEt-*n*-hexane (1:1)	0.3
	acetone-*n*-hexane (1:3)	0.4
Kieselgel (Camag)	CH_2Cl_2-MeOH (99.9 : 0.1)	0.8
	Et_2O-*n*-hexane (1:1)	0.6

As ethyl indole-3-acetate (Et-IAA) has the same Rf value as that of Me-IAA, Et-IAA might be hold in this fraction. In order to settle this problem and further to confirm the presence of Me-IAA, the vapor phase chromatography was employed.

The presence of Me-IAA was confirmed under the following conditions.

Condition 1 : Column : 5% QF-1 on Chromosorb W (60~90 mesh) 1 m. × 1/4 inch od.

Column Temp.	165°
Sample Heater Temp.	335°
N_2 : 0.95 kg./cm²	H_2 : 0.65 kg./cm²
Air : 0.75 kg./cm²	
Me-IAA	t_R 20.0 min.
Et-IAA	24.8
Vb fraction	20.4
Vb fraction+Me-IAA	19.4

Condition 2 : Column : 5% QF-1 on Chromosorb W (60~90 mesh) 1 m. × 1/4 inch od.

Column Temp.	180°
Sample Heater Temp.	335°

N_2 : 0.95 kg./cm²	H_2 : 0.65 kg./cm²
Air : 0.95 kg./cm²	
Me-IAA	t_R 9.3 min.
Et-IAA	11.3
Vb fraction	9.3
Vb fraction+Me-IAA	9.2

No peak corresponding to Et-IAA was found in the vapor phase chromatogram. One example of the chromatograms was shown in Fig. 3.

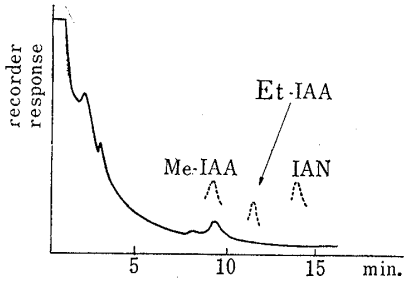


Fig. 3. Vapor Phase Chromatogram of Fraction Vb

Column : 5% QF-1 on Chromosorb W. 1 m. ×1/4 inch od.
Condition : Column temp. 180°
Sample heater temp. 335°
Carrier gas : N_2 0.95 kg./cm²

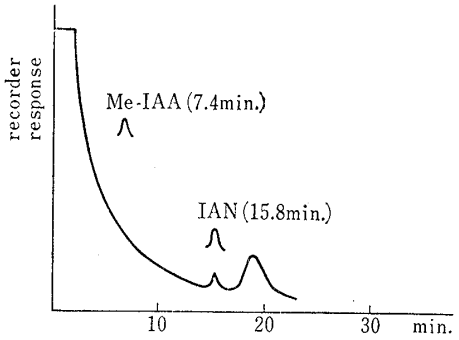


Fig. 4. Vapor Phase Chromatogram of Fraction VIb

Column : 10% Versamid 900 on Chromosorb W. 1 m. ×1/4 inch od.
Condition : Column temp. 230°
Sample heater temp. 330°
Carrier gas : N_2 0.83 kg./cm²

The fraction VIb was subjected to thin-layer chromatography under the following conditions.

Kieselgel (Camag)	*n*-hexane-ether (1:2)	Rf 0.2
	n-hexane-AcOEt (20:5)	0.3
	CH_2Cl_2	0.3
Aluminium Oxyd	*n*-hexane-ether (1:2)	0.3

The spot corresponding to authentic indole-3-acetonitrile (IAN) was confirmed in each condition, while Me-IAA could hardly be detected.

The vapor phase chromatography was applied to this fraction and the presence of IAN in the fraction was confirmed under the following conditions. One example of the chromatograms was shown in Fig. 4.

Condition 1 : 10% versamid 900 on Chromosorb W (60~90 mesh) 1 m. × 1/4 inch od.

ColumnTemp.	230°
Sample Heater Temp.	330°
N_2 : 0.83 kg./cm²	H_2 : 0.78 kg./cm²
Air : 0.93 kg./cm²	
IAN	t_R 15.8 min.
VIb fraction	16.0
VIb fraction+IAN	15.9

Condition 2 : 1% Versamid 900 on Chromosorb W (60~90 mesh) 1 m. × 1/4 inch od.

Column Temp.	215°
Sample Heater Temp.	320°
N_2 : 0.80 kg./cm²	H_2 : 0.88 kg./cm²
Air : 0.98 kg./cm²	
IAN	t_R 27.9 min.
VIb fraction	27.7
VIb fraction+IAN	27.6

Experimental

Material——The neutral fraction (F-1) was obtained by the large scale extraction method[1] starting from about 100 kg. of Moyashi each time.

	Moyashi (kg.)	Date	F-1 (g.)	F-2 (g.)
IX	104	1962 Oct. 29~30	3.0	9.6
X	104	Nov. 12~13	1.1	9.8
XI	120	Nov. 23~24	2.8	12.7
XII	120	Dec. 5~6	1.6	11.4
XIII	120	Dec. 15~16	1.6	11.2
XIV	80	1963 Jan. 12~13	1.5	5.0

The all F-1 fractions which were obtained from IX~XIV large scale extractions were combined. This was chromatographed with Woelm neutral alumina (3%, H_2O). The ethyl acetate eluate was evaporated to dryness and the F-1 AcOEt fraction was obtained.

Preparative Thin-layer Chromatography——A thin-layer of 300 μ was made on a glass plate (20×20 cm.) with Kieselgel (Camag Chemie Erzeugnisse und Adsorption Technik AG Muthenz/Schweiz) using an applicator (Camag) and thin-layer plates were heated on 110~120° for 1.5 hr. The F-1 AcOEt fraction was applied to the thin-layer plate as a narrow band. Then at both sides of this band Me-IAA was spotted as the reference. The development was carried out with the mixture of Et_2O-*n*-hexane (2:1). After development, the plates were dried at room temperature and the reference spot of Me-IAA was detected by spraying Ehrlich reagent. The obtained chromatogram was divided into the several bands by the aid of the reference spots of Me-IAA and each band was scraped off. These adsorbents corresponding to each band collected from the chromatoplates were extracted with ether or CH_2Cl_2 at room temperature. Each extracted fraction was filtered through a small glass funnel and the filtrate was evaporated. The residue was weighed and applied to the bioassay and thin-layer chromatography and vapor phase chromatography.

Bioassay——Avena straight growth test was employed for the detection of plant growth promoting activity.[1]

Thin-layer Chromatography and Vapor Phase Chromatography——Silicagel (Camag, Kieselgel for TLC) and alumina (Camag, Aluminium Oxyd for TLC) were used as TLC adsorbent, pure Me-IAA, Et-IAA and IAN as authentic specimens, Ehrlich reagent as a spray reagent. Me-IAA and Et-IAA were colored to bluish purple and IAN was colored to darkish pink with Ehrlich reagent. The coloration of IAN was rather weaker than that of Me-IAA.

The Ohkura Riken Gas Chromatograph Model 2100 (FID type detector) was used for VPC with the following three columns.

Column packed with 10% Versamid 900 on Chromosorb W (60~90 mesh) 1 m.×1/4 inch od. Column packed with 1% Versamid 900 on Chromosorb W (60~90 mesh) 1 m.×1/4 inch od. Column packed with 5% QF-1 on Chromosorb W (60~90 mesh) 1 m.×1/4 inch od.

Preliminary Preparative Thin-layer Chromatography——24 mg. of the F-1 AcOEt fraction in a small volume of CH_2Cl_2 was spotted to a thin-layer plate and the development was carried out with the mixture of Et_2O-*n*-hexane (2:1). After development, the plates were dried at room temperature and the reference spot of Me-IAA was detected with Ehrlich reagent. The chromatogram was divided into ten bands as shown in Fig. 1. The adsorbents corresponding to each band were collected and eluted with ether to give ten fractions. After the solvent was evaporated, each fraction was subjected to Avena straight growth test. The results were shown in Table I.

The promoting action was observed in the fraction IIa and IIIa. The thin-layer chromatogram of fraction IIIa (Kieselgel, ether-*n*-hexane (2:1)) gave a bluish purple spot corresponding to Me-IAA by spraying Ehrlich reagent. But the thin-layer chromatogram of the fraction IIa (Kieselgel, ether-*n*-hexane (2:1)) gave no distinct spot.

Preparative Thin-layer Chromatography——779.5 mg. of the F-1 fraction was chromatographed with 160 g. of Woelm neutral alumina (3% H_2O) using ethyl acetate as solvent. 165.4 mg. of the F-1 AcOEt fraction was obtained and developed with the mixture of ether-*n*-hexane (2:1). After development, the plates were dried at room temperature and the reference spot of Me-IAA was detected with Ehrlich reagent. The chromatograms were divided into the eight bands as shown in Fig. 2. The adsorbents corresponding to each band were collected and eluted with CH_2Cl_2 at room temperature to give eight fractions. After the solvent was evaporated, the fractions, IVb, Vb and VIb were subjected to Avena straight growth test. The results were shown in Table II. The promoting action was observed in the fractions, Vb and VIb.

Identification of Me-IAA in the Fraction Vb by TLC and VPC——The fraction Vb was submitted to thin-layer chromatography under the above-described conditions. The bluish purple spot (with Ehrlich reagent) corresponding to Me-IAA was detected in each condition. The fraction Vb was submitted to vapor phase chromatography (VPC) and the presence of Me-IAA in the fraction Vb was confirmed under the above-described conditions.

Identification of IAN in the Fraction VIb by TLC and VPC——The fraction VIb was submitted to thin-layer chromatography under the above-described conditions. The darkish pink spot (with Ehrlich reagent) corresponding to IAN was detected in each condition. The fraction VIb was submitted to vapor phase chromatography and the presence of IAN in the fraction VIb was confirmed under the above-described conditions.

The author's thanks are due to Shionogi & Co., Ltd. for financial supports. They are also grateful to the members of Central Analysis Room of the Faculty for elemental analysis and spectral data.

[Chem. Pharm. Bull. 15(2) 168～172 (1967)]

UDC 547.833.3.02 : 541.63

23. Masao Okamoto : Stereochemistry of Decahydroisoquinolines and Related Compounds. V.*1 Syntheses of 2-Methyl-decahydro-8-isoquinolinols.

(*Kyoto College of Pharmacy**2)

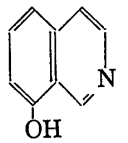
Three isomeric bases of 2-methyl-decahydro-8-isoquinolinol (XIVa, XIVb, XIVc) were prepared by catalytic hydrogenation of 2-methyl-5-chloro-1,2,3,4-tetrahydro-8-isoquinolinol (VIII) and also of 2-methyl-1,2,3,4-tetrahydro-8-isoquinolinol (XIII).

The configuration of the ring juncture of these alcoholic bases and the configuration of their hydroxyl groups were clarified on the basis of chemical evidences and NMR informations and the rates of chromic acid oxidation and so on.

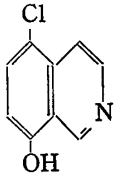
(Received April 28, 1966)

Previously, the authors reported preparation of 2-methyl-decahydroisoquinolinols possessing hydroxyl groups at C_5-, C_6- and C_7-position respectively, and the corresponding ketones and confirmation of steric configuration at ring juncture of these compounds by chemical evidences.[1~3,*1] In the present paper, it deals with the synthetic method of three isomers of 2-methyl-decahydro-8-isoquinolinol, starting from 2-methyl-5-chloro-1,2,3,4-tetrahydro-8-isoquinolinol (VIII), and steric investigations concerning ring junction of these alcohols, including configuration of hydroxyl group.

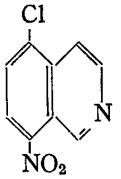
Preparative methods of 2-methyl-decahydro-8-isoquinolinols (XIV), hitherto, taken by us can be classified into two processes. Thus, the one is direct perhydrogenation of corresponding isoquinolinols by one step, followed by N-methylation and the other is partial hydrogenation of 1,2,3,4-tetrahydro derivatives. At first, 8-isoquinolinol (I) was considered to be the useful intermediate on the shortest process leading to the



I



II



III

Chart 1.

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*1 Part IV. S. Kimoto, M. Okamoto : Yakugaku Zasshi, **85**, 371 (1965).

*2 Nakauchi-cho, Yamashina-misasagi, Higashiyama-ku, Kyoto (岡本正夫).

1) S. Kimoto, M. Okamoto : This Bulletin, **9**, 480 (1961).

2) *Idem* : *Ibid.*, **10**, 362 (1962).

3) M. Okamoto, M. Yamada : *Ibid.*, **11**, 554 (1963).