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Plant Growth Regulators in the Pea Plant (*Pisum sativum* L.)

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A pea inhibitor was isolated as natural free acid and as its methyl ester from the garden peas (*Pisum sativum* L.) and they were identified with (+)-abscisic acid (Ia) and its methyl ester (Ib), respectively. On the other hand, sayanedine, mp 165—166°, $C_{17}H_{14}O_5$, which is a new isoflavone and which had kinetin-like activity in the tabacco pith callus bioassay, was also isolated from the same plant, and its chemical structure was confirmed to be 4′-hydroxy-7,3′-dimethoxyisoflavone (VI) on the basis of spectral and synthetic evidences.

There have been many papers reporting the presence of plant-growth regulators in the pea plant (*Pisum sativum* L.), and five growth promotors and one inhibitor were isolated in natural and pure states.

The present authors[2] reported the isolation of one of five promotors from the pea pod, which had gibberellin-like activity in the rice seedling growth test and which was identified with gibberellin A_{20}. Other four promotors, which had auxin-like activity, were isolated from immature seeds of peas and their chemical structures were confirmed to be 4-chloroindole-3-acetic acid, its methyl ester, α-(N-methoxycarbonylacetyl)-, and α-(N-ethoxycarbonylacetyl)-D-4-chlorotryptophan by Marumo, *et al.*[3] In addition to the above five natural growth promotors, two kinetin-like active substances were isolated by Hall, *et al*[4] after hydrolysis of s-RNA obtained from the garden peas and their chemical structures were found to be N^6-(isopent-2-enyl)adenosine and 6-(*cis*-4-hydroxy-3-methylbut-2-enyl)amino-9-β-D-ribofuranosylpurine.

On the other hand, the present authors reported, in a preliminary communication,[5] that a pea growth inhibitor was isolated from the pea pod and it was identified with (+)-abscisic acid.[6]

Recently, (+)-abscisic acid as its methyl ester and sayanedine, which was a new isoflavone and had kinetin-like activity in the tabacco pith callus bioassay, were isolated from the same pea pod as described above.

The subjects of this paper are the isolation and identification of (+)-abscisic acid and its methyl ester, and the isolation of sayanedine and its structural elucidation on the basis of spectral and synthetic evidences.

1) Location: *a) Komaba, Meguro-ku, Tokyo; b) Hongo, Bunkyo-ku, Tokyo.*
2) Y. Komoda, Y. Isogai and T. Okamoto, *Sci. Papers Coll. Gen. Educ. Univ. Tokyo*, **18**, 221 (1968). This paper constitutes Part VI of a series entiled "Studies on Plant-growth Regulators," by Toshihiko Okamoto and Yo Isogai. Part V: T. Okamoto, Y. Isogai, and Koizumi, *Chem. Pharm. Bull.* (Tokyo), **18**, 1815 (1970).
3) S. Marumo, H. Abe, H. Hattori, and K. Munakata, *Agr. Biol. Chem.* (Tokyo), **32**, 117 (1968); idem, *Nature*, **219**, 959 (1968); Abstr. Papers, 4th Ann. Meeting Soc. Chem. Regulation of Plants, Japan, 1969.
4) R.H. Hall, L. Csonka, H. David, and B. McLennan, *Science*, **156**, 69 (1967).
5) Y. Isogai, T. Okamoto, and Y. Komoda, *Chem. Pharm. Bull.* (Tokyo), **15**, 1256 (1967).
6) The name of abscisin II and dormin was unified to abscisic acid at the 6th International Conference of the Plant Growth Regulators in Canada in 1967.

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Isolation of a Pea-Growth Inhibitor[2)]

The acidic fraction (OSA,[7)] 22.10 g) obtained from 1274 kg of pea pods in July, 1966, by the extraction methods as shown in the Experimental part was treated with active charcoal column chromatography as shown in Fig. 1 (CMC-I). OSA was loaded on a column containing 90 g of active charcoal mixed with 180 g of Celite 545 and eluted with a mixture of acetone and water. Dried residue of each fraction was tested by bioassay, whose method is mentioned in the Experimental part. The dried residue (6.50 g) of fraction Nos. 12—15 from CMC-I had

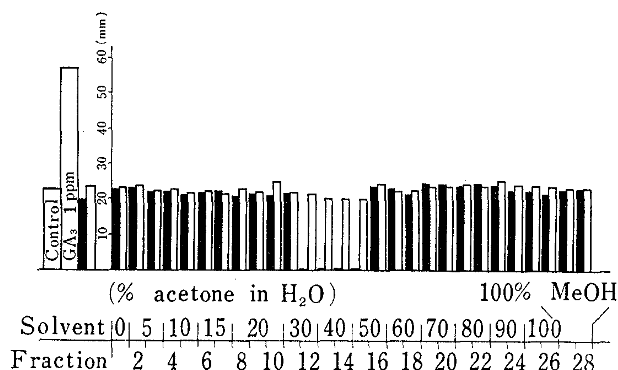

Fig. 1. Active Charcoal Column Chromatography of the Acidic Fraction (OSA) obtained in 1966: CMC-I

sample: 22.10 g of OSA (1966)
column: 90 g of active charcoal and 180 g of Cellite 545 (10 cm in diameter and 11 cm in height)
Each fraction was eluted with 1500 ml of the solvent.
ordinate: Average length of 15 of second leaf sheath in the rice seedling growth test.
■: 100 ppm, □: 10 ppm

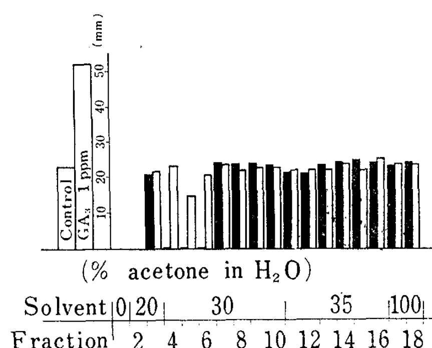

Fig. 2. Active Charcoal Column Chromatography of the Dried Residue of Fraction Nos. 12—15 of CMC-I: CMC-II

sample: 6.50 g of fraction Nos. 12—15 of CMC-I
column: 30 g of active charcoal and 60 g of Celite 545 (6 cm in diameter and 11 cm in height)
Each fraction was eluted with 500 ml of the solvent.
ordinate: Average length of 15 of second leaf sheath in the rice seedling growth test.
■: 100 ppm, □: 10 ppm

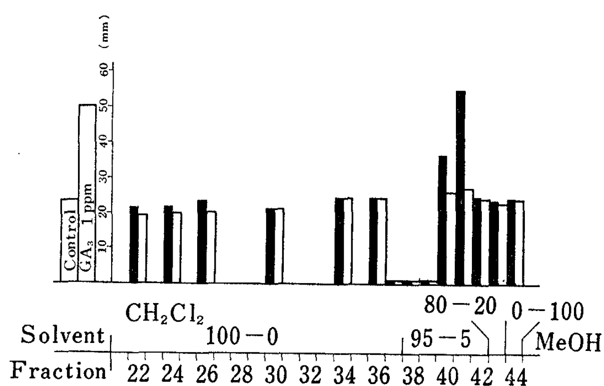

Fig. 3. Adsorption Column Chromatography of the Dried Residue of Fraction No. 4—6 of CMC-II: CMC-III

sample: 1.321 g of fraction No. 4—6 of CMC-II
column: 140 g of silica gel (4.6 cm in diameter and 16 cm in height)
Each fraction was eluted with 300 ml of the solvent.
ordinate: Average length of 15 of second leaf sheath in the rice seedling growth test.
■: 100 ppm, □: 10 ppm

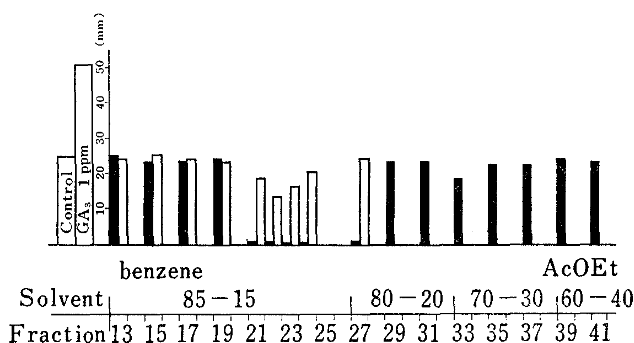

Fig. 4. Adsorption Column Chromatography of the Dried Residue of Fraction No. 39 of CMC-III: CMC-IV

sample: 95 mg of fraction No. 39 of CMC-III
column: 20 g of silica gel (treated with oxalic acid) and 20 g of Celite 545 (3 cm in diameter and 15 cm in height)
Each fraction was eluted with 100 ml of the solvent.
ordinate: Average length of 15 of second leaf sheath in the rice seedling growth test.
■: 10 ppm, □: 1 ppm

7) Abbreviations used in this paper are as follows: ORD (optical rotatory dispersion), UV (ultraviolet), IR (infrared), NMR (nuclear magnetic resonance), s (singlet), d (doublet), q (quartet), m (multiplet), OSA (organic solvent-soluble acidic fraction), OSN (organic solvent-soluble neutral fraction), CMC (column chromatography), TLC (thin-layer chromatography).

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growth inhibition, and was further treated by the method shown in Fig. 2 (CMC-II). The dried residue (1.321 g) of fraction No. 4—6 from CMC-II had growth-inhibiting activity and it was subjected to adsorption column chromatography as shown in Fig. 3 (CMC-III). The amorphous residue of fraction No. 37—39 from CMC-III showed strong growth inhibition and 95 mg of fraction No. 39 was further purified as shown in Fig. 4 (CMC-IV) by the adsorption column chromatography on silica gel pretreated with oxalic acid for the purpose of weakening adsorptivity.[8] The fraction No. 21—27 from CMC-IV completely inhibited the growth in a concentration of 1 ppm and the crystalline residue (9 mg) of fraction No. 21 and 22 was recrystallized twice from a mixture of hexane and ether to give 1.2 mg of minute plates. This. pure substance showed the strongest growth inhibition and so it was tentatively designated as pea inhibitor. The total yield of this pea inhibitor was 4.2 mg by the addition of a pure substance obtained from fraction No. 37—39 from CMC-III and No. 23—27 from CMC-IV.

Identification of Pea Inhibitor with (+)-Abscisic Acid[2]

The pea inhibitor has the following data:[7] mp 167—168°. $[\alpha]_D^{11}$ +488° (c=0.123, EtOH). ORD (c=0.0176, EtOH) $[\alpha]^{14}$ (mμ): +341° (700), +488° (D), +30700° (289), −85200° (245), +14200° (215), 0° (200). UV λ_{max}^{EtOH} mμ (ε): 240 (shoulder, 18800), 258 (21000). NMR (60 Mc, $CDCl_3$) τ: 8.96 (3H, s), 8.88 (3H, s), 8.06 (3H, d, J=1.5 cps), 7.94 (3H, d, J=1.5), 7.90, 7.63, 7.58, 7.31 (2H, AB-type q), 4.22 (1H, broad s), 4.01 (1H, broad s), 3.84 (1H, d, J=16), 2.18 (1H, d, J=16).

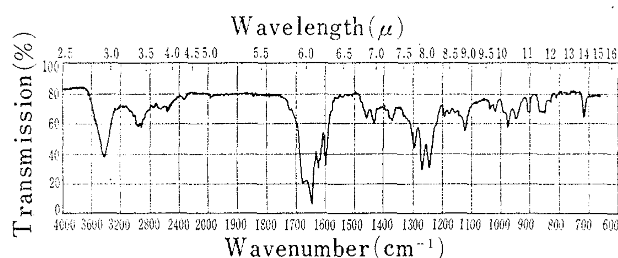

Fig. 5. Infrared Spectrum of (+)-Abscisic Acid (Ia) (KBr)

In TLC, in which silica gel treated with oxalic acid was used as adsorbent, the chromatogram was developed with a mixture of methanol and methylene chloride (1/9), and the plate was sprayed with dilute sulfuric acid and heated. The pea inhibitor (*Rf* 0.45) gave a light yellow spot with no tailing and a characteristic yellowish green fluorescence under ultraviolet ray.

The physical data of the pea inhibitor described above were identical with those of (+)-abscisic acid which was isolated from the young fruit of cotton (*Gossypium hirsutum* L.),[9a] the leaves of sycamore (*Acer pseudoplatanus* L.),[9b] and the seeds with the pods of lupin (*Lupinus luteus* L.)[9c] as a plant-growth inhibitory substance and whose chemical structure was determined to be Ia by physicochemical methods and confirmed by synthesis. Moreover, the pea inhibitor was directly identified with (+)-abscisic acid by Dr. Cornforth by mixed fusion and by comparison of its IR spectrum and ORD curve with those of natural and synthetic dextrorotatory enantiomorphs.

8) A column was prepared by the following method: Silica gel was soaked in 0.5N oxalic acid solution for 2 days and then washed with water after filtration. A mixture of this silica gel and Celite 545, which was used for increasing the elution speed, was placed in a glass tube and washed consecutively with water, acetone, and benzene.

9) *a*) K. Ohkuma, J.L. Lyon, F.T. Addicott, and O.E. Smith, *Science*, **142**, 1592 (1963); K. Ohkuma, F.T. Addicott, O.E. Smith, and W.E. Thiessen, *Tetrahedron Letters*, **1965**, 2529; *b*) J.W. Cornforth, B.V. Milborrow, G. Ryback, and P.F. Wareing, *Nature*, **205**, 1269 (1965); J.W. Cornforth, B.V. Milborrow, and G. Ryback, *ibid*., **206**, 715 (1965); J.W. Cornforth, B.V. Milborrow, G. Ryback, and W. Draber, *Chem. Commun*., **1967**, 114; *c*) K. Koshimizu, H. Fukui, T. Kusaki, T. Mitsui, and Y. Ogawa, *Agr. Biol. Chem*. (Tokyo), **30**, 941 (1966); *d*) T. Hashimoto, T. Ikai, and S. Tamura, *Planta*, **78**, 89 (1968); *e*) M.J. Chrispeels and J.E. Varner, *Plant Physiol*., **42**, 1008 (1967); K. Dörffling, *Naturwissenscaften*, **54**, 23 (1967); J.W. Cornforth, B.V. Milborrow, G. Ryback, K. Rothwell, and R.L. Wain, *Nature*, **211**, 742 (1966).

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It was also reported that (+)-abscisic acid was isolated from the dormant aerial tubers of *Dioscorea batatas* by Tamura, *et al.*[9d] And its presence in many plants was also reported.[9e]

It has become clear that (+)-abscisic acid is very active against auxins, gibberellins, and kinetin. (+)-Abscisic acid will be expected to become highly useful for the elucidation of the mechanism of plant-growth regulation.

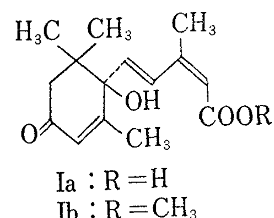

Chart 1

Isolation and Identification of (+)-Abscisic Acid as Its Methyl Ester

In 1967, another lot of 910 kg of the same variety of pea pods was treated by the extraction method mentioned in the Experimental part and 33.5 g of OSA was obtained, which was submitted to active charcoal column chromatography as shown in Fig. 8 (CMC-V). The dried residue of fractions from CMC-V, which had growth-inhibitory activity, was treated with diazomethane, and the methylated material obtained was then submitted to adsorption column chromatography on silica gel. The fractions, which indicated the same characteristic yellowish green fluorescence as abscisic acid in TLC, were further submitted to four preparative TLCs and 15.0 mg of crystalline matter obtained was recrystallized from hexane to 8.3 mg of granules, mp 109—110°. This substance was identified with methyl (+)-abscisate (Ib), which was obtained by the methylation of (+)-abscisic acid with diazomethane, through IR spectrum, mixed fusion, and TLC. Methyl (+)-abscisate was more active than its free acid in growth inhibition by the rice seedling growth test. This fact was also reported by Koshimizu, *et al.*[9c]

Isolation of Sayanedine

The neutral fraction (OSN) which also contained the weak acidic substances as shown in the extraction method in the Experimental part, was treated three times by column chromatography on silica gel. The solid material obtained was further purified by means of its insolubility in ether and the active charcoal treatment in methylene chloride. The crystalline product obtained was recrystallized from hexane to colorless needles.

It was found that this substance accelerated plant cell division in the tabacco pith callus bioassay and root growth in the rice seedling growth test, and that it was a new isoflavone, as will be described below. This substance was named sayanedine. The isolation of sayanedine suggests that flavonoids may have an important role in the plant-growth regulation. Biological activities of sayanedine will be reported elsewhere in the near future.

During the course of the isolation of sayanedine, no bioactive substances such as maackiain,[10] 7-O-methylmedicagol,[11] unhydropisatin,[12] hentriacontane, β-sitosterol, or methyl palmitate, were isolated.

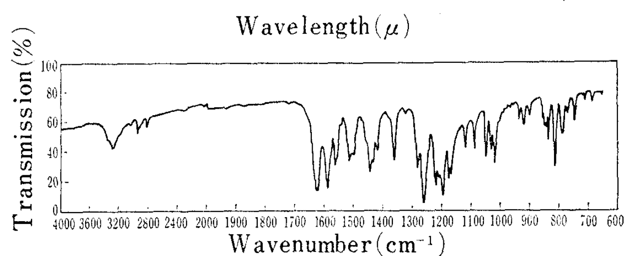

Fig. 6. Infrared Spectrum of Sayanedine (KBr)

Structure and Synthesis of Sayanedine

Sayanedine, mp 165—166°, $C_{17}H_{14}O_5$, afforded O-methylsayanedine (II) by the reaction with diazomethane, and O-acetylsayanedine (III) by acetylation with acetic anhydride and pyridine, and II was reduced to dihydro-O-methylsayanedine (IV) by catalytic reduction over palladium on charcoal.

10) H. Suginome, *Experientia*, **18**, 161 (1962).
11) A.L. Livingston, S.C. Witt, R.E. Lundir, and E.M. Bickoff, *J. Org. Chem.*, **30**, 2353 (1965).
12) D.R. Perrin and W. Bottomley, *J. Am. Chem. Soc.*, **84**, 1919 (1962).

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The IR spectra show that sayanedine, II, and III have aromatic rings and α,β-unsaturated ketones with another double bond in addition to an aromatic ring, but IV has no such α,β-unsaturated ketone group. In NMR spectra, sayanedine, II, and III have one singlet signals corresponding to one proton at τ 2.04—2.08 but, instead of this one singlet, IV has two singlets at τ 5.33 and 5.40, each being equivalent to one proton. Therefore, an isoflavone constitution of sayanedine was indicated by these data and also by the UV data in which sayanedine, II, and III show absorption spectra similar to those of isoflavones and IV, to isoflavanones. The NMR spectrum of sayanedine shows the presence of two methoxyl groups and one phenolic proton whose signal disappears on addition of deuterium oxide, and that of II has three methoxyl signals.

Oxidation with alkaline hydrogen peroxide solution of O-ethylsayanedine (V), obtained by the reaction of sayanedine with ethyl iodide and potasium carbonate in dry acetone, followed by methylation with diazomethane afforded methyl 2-hydroxy-4-methoxybenzoate and methyl 3-methoxy-4-ethoxybenzoate. The result of this experiment indicated that C-7 and C-3′ bear methoxyl groups and C-4′ bears an ethoxyl in V.

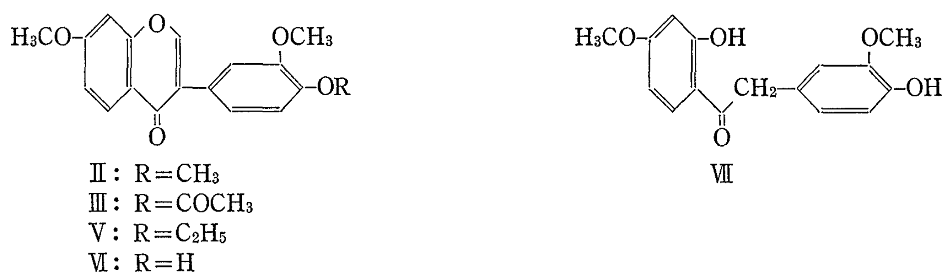

Chart 2

Therefore, a structure of 4′-hydroxy-7,3′-dimethoxyisoflavone (VI) may be proposed for sayanedine.

The synthesis of VI was accomplished as described below and sayanedine was found to be identical with VI in all respects (mixed fusion, TLC, IR, and UV spectra, and bioassay in the tabacco pith callus).

The Hoesch reaction of 3-methoxy-4-hydroxybenzylnitrile[13] and *m*-methoxyphenol gave VII, which was condensed with ethyl formate to afford VI.

Experimental[14]

Bioassay——As bioassay, the rice seedling growth test was always employed by the following procedures. Seeds of rice (*Oryza sativa*, var. Norin No. 29) were soaked in EtOH for 10 min and then in saturated solution of bleaching powder for 1 hr. The sterilized seeds were transferred into a large petri dish containing sterilized water of 1 cm depth. The petri dish was kept at 30° for 48 hr under white fluorescent and incandescent lamp (about 5,000 lux). The germinating seeds having 3—5 mm of coleoptiles were employed for further use. These seeds were placed in test tubes (3×10 cm), 15 to one test tube containing 1.35 ml of aqueous test solution. After covering the tubes with polyethylene sheet, they were kept for 5 days under white fluorescent and incandescent lamp (about 5000 lux). The second leaf sheaths of seedlings were then measured and compared with those of the control which grew in water alone. This test is insensitive to auxins and kinetin.

Starting Material——Pods (about 7 cm in length and 1.5 cm in width) of garden peas (*Pisum sativum* L. var. Kinuzaya, a tall variety) which contained five or six small seeds ($4\times3\times1$ mm) were obtained from a greengrocer's and used as the raw material.

Extraction Method——Extraction was carried out as shown in Fig. 7. MeOH was added to the raw material and macerated in a Waring blender. The macerate was filtered, the filtrate was concentrated

13) H.E. Fisher and H. Hibbert, *J. Am. Chem. Soc.*, **69**, 1208 (1947); K. Kratzl and E. Meisert, *Monatsh. Chem.*, **88**, 1056 (1957).

14) All melting points in this paper were taken on a micro-hotstage apparatus and are uncorrected. The mixing ratio of solvents were volume/volume in column and thin-layer chromatography.

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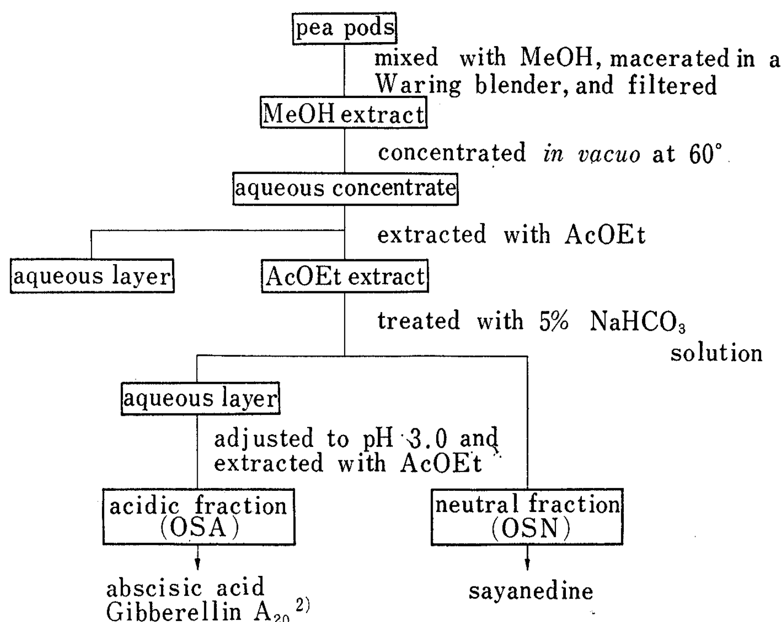

Fig. 7. Method for Extraction of Starting Material

in vacuo at 60°, and the aqueous concentrate was extracted with AcOEt. The AcOEt solution was treated by the usual method using 5% $NaHCO_3$ solution and separated into acidic fraction (OSA) and neutral fraction (OSN) which also contained weakly acidic substances.

Isolation of (+)-Abscisic Acid as Its Methyl Ester——OSA (33.5 g) obtained from 910 kg of pea pods in 1967 was submitted to active charcoal column chromatography (CMC-V) as shown in Fig. 8. The dried residue (2.791 g) of fraction No. 28—34 from CMC-V, which had growth-inhibitory activity, was dissolved in 10 ml of MeOH and added with an excess of CH_2N_2 in ether at room temperature. The mixture was allowed to stand for 1 hr at room temperature. The product obtained was treated with 5% $NaHCO_3$ solution to afford an oily material (2.411 g) which was submitted to the adsorption column chromatography on silica gel (125 g) using $CHCl_3$ as a developing solvent. The fractions (233 mg) which gave the same characteristic yellowish green fluorescence as (+)-abscisic acid under ultraviolet ray in TLC after the plate was sprayed with dil. H_2SO_4 and heated were combined. This combined material was then

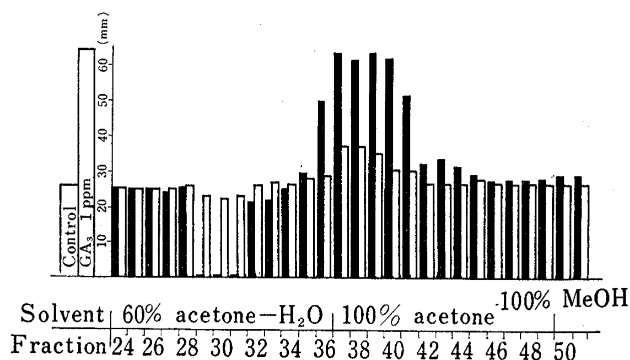

Fig. 8. Active Charcoal Column Chromatography of the Acidic Fraction (OSA) obtained in 1967: CMC-V

sample: 33.5 g of OSA (1967)
column: 1000 g of active charcoal and 3000 g of Celite 545 (15 cm in diameter and 69 cm in height)
Each fraction was eluted with 2000 ml of the solvent.
ordinate: Average length of 15 of second leaf sheath in the rice seedling growth test.
■: 100 ppm, □: 10 ppm

Table I. Preparative TLC in the Isolation of (+)-Abscisic Acid as Its Methyl Ester

Run of preparative TLC	Developping solvent (v/v)	Weight (mg) of objective fraction
1st	$CHCl_3$/AcOEt 4/1	128.1
2nd	$(C_2H_5)_2O$	62.3
3rd	$CHCl_3$/AcOEt 4/1	23.7
4th	$CHCl_3$/AcOEt 4/1	15.0

Silica gel was used as adsorbent.

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submitted to four consecutive preparative TLC. On each run, the plate of silica gel was used and the zones colored with vapor of I_2 were separated after the development with the solvent systems shown in Table I. The separated fractions were extracted with hot AcOEt and evaporation of AcOEt afforded the crude objective substance as shown in Table I.

The crystalline matter (15.0 mg) obtained from the fourth preparative TLC was recrystallized from hexane to 8.3 mg of granules, mp 109—110°. This substance was identified with methyl (+)-abscisate (Ib) which was prepared by the methylation of (+)-abscisic acid (Ia) with CH_2N_2 in ether, through IR spectrum, mixed fusion, and TLC.

Isolation of Sayanedine——The neutral fraction (OSN, 537 g) containing some weakly acidic substances, which was obtained from 778 kg of pea pods in 1964, was chromatographed on silica gel (3.5 kg) with increasing concentration of CH_2Cl_2 in hexane. A part (18.834 g) of the dried residue (39.056 g) eluted with CH_2Cl_2 alone was then purified by the same silica gel (1 kg) column chromatography. The fractions (11.153 g) obtained from a mixture of hexane and CH_2Cl_2 (1:2) was further chromatographed on silica gel (1.2 kg) with increasing concentration of ether in hexane. The solid material (1.616 g) eluted by a mixture of hexane and ether (2:1) was dissolved in ether, and insoluble substance (990 mg) obtained by filtration was submitted to active charcoal treatment in CH_2Cl_2. The filtrate was evaporated to give crystalline substance (346 mg), which was recrystallized from hexane to afford sayanedine as colorless needles, mp 165—166°. *Anal.* Calcd. for $C_{17}H_{14}O_5$: C, 68.45; H, 4.73. Found: C, 68.82; H, 4.80. UV λ_{max}^{EtOH} mμ (ε): 217 (25600), 247 (19800), 263 (23400), 286 (shoulder, 15300), 305 (shoulder, 9100). IR ν_{max}^{KBr} cm^{-1}: 3300 (OH), 1632 and 1625 (α,β-unsaturated ketone), 1590 (aromatic). NMR ($CDCl_3$) τ: 6.09 and 6.06 (each 3H, s, OCH_3), 4.28 (1H, s, disappeared with D_2O, OH), 3.13 (1H, d, J=2 cps, 8-H), 3.02 (2H, d, J=2, side aromatic H), 2.99 (1H, double d, J=2 and 9, 6-H), 2.74 (1H, side aromatic H), 2.07 (1H, s, 2-H), 1.77 (1H, d, J=9, 5-H). Mass Spectrum *m/e*: 298 (M^+).

O-Methylsayanedine (II)——To a solution of 65 mg of sayanedine in MeOH was added an excess of CH_2N_2 in ether, and the mixture was allowed to stand for 3 hr at 0°. The reaction mixture was treated in the usual manner and the product obtained was recrystallized from a mixture of hexane and CH_2Cl_2 to 22 mg of II, colorless needles, mp 163—164°. *Anal.* Calcd. for $C_{18}H_{16}O_5$: C, 69.22; H, 5.16. Found: C, 68.97; H, 5.09. UV λ_{max}^{EtOH} mμ (ε): 221 (28200), 248 (23200), 261 (24800), 282 (shoulder, 16400), 306 (shoulder, 10800). IR ν_{max}^{KBr} cm^{-1}: 1634 and 1620 (α,β-unsaturated ketone), 1608 and 1603 (aromatic). NMR ($CDCl_3$) τ: 6.14 (6H, s, $2\times OCH_3$), 6.11 (3H, s, OCH_3), 3.18 and 3.08 (each 1H, d, J=2, 8-H, and side aromatic H), 3.01 (1H, d, J=2, side aromatic H), 2.96 (1H, double d, J=2 and 9, 6-H), 2.80 (1H, d, J=2, side aromatic H), 2.08 (1H, s, 2-H), 1.81 (1H, d, J=9, 5-H). Mass Spectrum *m/e*: 302 (M^+).

O-Acetylsayanedine (III)——A mixture of 84 mg of sayanedine, 2 ml of Ac_2O, and 2 ml of pyridine was heated on a water bath for 1 hr. The reaction mixture was treated in a usual manner and the product obtained was crystallized from MeOH to 40 mg of III, colorless needles, mp 171—172°. *Anal.* Calcd. for $C_{19}H_{16}O_6$: C, 67.05; H, 4.75. Found: C, 66.89; H, 4.57. UV λ_{max}^{EtOH} mμ (ε): 217 (20800), 248 (20000), 285 (9800), 304 (shoulder, 7700). IR ν_{max}^{KBr} cm^{-1}: 1770 (acetyl), 1636 (broad, α,β-unsaturated ketone), 1603 (aromatic). NMR ($CDCl_3$) τ: 7.68 (3H, s, $OCOCH_3$), 6.14 and 6.08 (each 3H, s, OCH_3), 3.14 (1H, d, J=2, 8-H), 2.99 (1H, double d, J=2 and 9, 6-H), 2.93 (2H, broad s, side aromatic H), 2.66 (1H, broad s, side aromatic H), 2.04 (1H, s, 2-H), 1.77 (1H, d, J=9, 5-H).

Dihydro-O-methylsayanedine (IV)——The catalytic reduction of 37 mg of II with 50 mg of PtO_2 in 5 ml of EtOH gave an oily product, which was chromatographed on silica gel and the eluate obtained with a mixture of benzene and CH_2Cl_2 (5:1) was crystallized from ether to 15 mg of IV as colorless needles, mp 138—139°. *Anal.* Calcd. for $C_{18}H_{18}O_5$: C, 68.78; H, 5.77. Found: C, 68.74; H, 5.53. UV λ_{max}^{EtOH} mμ (ε): 230 (10200), 276 (8500), 314 (4200). IR ν_{max}^{KBr} cm^{-1}: 1666 (Ph–CO–), 1612 (aromatic). NMR ($CDCl_3$) τ: 6.15 (9H, s, $3\times OCH_3$), 6.15 (1H, m, 3-H), 5.40 and 5.33 (each 1H, s, 2-H), 3.56 (1H, d, J=2, 8-H), 3.41 (1H, double d, J=2 and 9, 6-H), 3.17 (3H, m, side aromatic H), 2.09 (1H, d, J=9, 5-H). Mass Spectrum *m/e*: 314 (M^+).

O-Ethylsayanedine (V)——A mixture of 80 mg of sayanedine, 1 g of EtI, 1 g of Na_2CO_3, and 10 ml of dry CH_3COCH_3 was refluxed for 24 hr. The reaction mixture was filtered and the residue obtained by distillation of solvent from the filtrate was dissolved in $CHCl_3$, washed with 2N NaOH solution and H_2O, and dried over Na_2SO_4. Evaporation of the solvent afforded 77 mg of V, which was recrystallized from a mixture of hexane and CH_2Cl_2 to colorless prisms, mp 167—169°. *Anal.* Calcd. for $C_{20}H_{22}O_5$: C, 69.92; H, 5.56. Found: C, 69.99; H, 5.55. IR ν_{max}^{KBr} cm^{-1}: 1637 and 1626 (α,β-unsaturated ketone), 1605 (aromatic). NMR ($CDCl_3$) τ: 8.55 (3H, t) and 5.88 (2H, q) corresponding to OCH_2CH_3, 6.12 (6H, s, $2\times OCH_3$).

Oxidation of O-Ethylsayanedine (V)——A mixture of 29 mg of V, 1 ml of 30% H_2O_2 solution, and 5% KOH solution in 5 ml of 80% EtOH was heated for 2 hr at 44—46°. The reaction mixture was cooled, added with H_2O and conc. HCl, and extracted with ether. The ether extract was washed with H_2O, dried over Na_2SO_4, and distilled off to afford 25 mg of crystalline material, which was reacted with CH_2N_2 in ether for 1 min at room temperature. The methylated product was chromatographed on silica gel with CH_2Cl_2 to give methyl 2-hydroxy-4-methoxybenzoate, mp 48—49°, and methyl 3-methoxy-4-ethoxybenzoate, mp 78—80°, which were completely identical with their authentic samples in IR spectra, TLC, and mixed fusion.

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2′-Hydroxy-4′-methoxy-2-(4-hydroxy-3-methoxyphenyl)acetophenone (VII)——A mixture of 5.415 g of 3-methoxy-4-hydroxybenzylnitrile,[13] 4.112 g of *m*-methoxyphenol, and *ca.* 14 g of powdered anhyd. $ZnCl_2$ in 100 ml of anhyd. ether was cooled in ice–salt mixture, and a rapid stream of HCl was passed through for 45 min. A flask containing the reaction mixture was stoppered, allowed to stand for 9 days at 4°, and added with ice and 2N HCl. The ether was decanted, the residue was heated on a water bath for 2 hr, and extracted with a mixture of $CHCl_3$ and MeOH. The extract was washed with 5% $NaHCO_3$ solution and then extracted with 10% NaOH solution. The aqueous layer was acidified with 2N HCl and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with H_2O, dried over Na_2SO_4 and distilled off to give 2.156 g of an oily material, which was chromatographed on silica gel with CH_2Cl_2. The eluate afforded 947 mg of VII which was recrystallized from MeOH to colorless granules, mp 136—137°. *Anal.* Calcd. for $C_{16}H_{16}O_5$: C, 66.66; H, 5.59. Found: C, 66.95; H, 5.88. IR ν_{max}^{KBr} cm^{-1}: 3415 (OH), 1610 (Ph–CO–), 1580 (aromatic). NMR ($CDCl_3$) τ: 6.18 and 6.15 (each 3H, s, 2×OCH_3), 5.88 (2H, s, –CH_2–), 4.39 (1H, broad s, 4-OH), 3.58 (1H, d, *J*=2, 3′-H), 3.57 (1H, double d, *J*=2 and 10, 5′-H), 3.15—3.27 (3H, m, 2-, 5-, 6-H), 2.26 (1H, d, *J*=10, 6′-H), −2.69 (1H, s, 2′-OH).

4′-Hydroxy-7,3′-dimethoxyisoflavone (VI, Sayanedine)——To *ca.* 900 mg of Na dispersions was dropped a solution of 421 mg of VII and 50 ml of HCOOEt during 30 min with ice cooling and stirring. The mixture was allowed to stand for 39 hr at 4° and HCOOEt was evaporated *in vacuo* after the addition of 2N HCl. The residue was heated on a water bath for 1 hr and extracted with CH_2Cl_2. The extract was washed with H_2O, dried over Na_2SO_4, and evaporated to give 425 mg of a crude crystalline material, which was recrystallized from MeOH and then from hexane affording VI as colorless needles, mp 162—163°. *Anal.* Calcd. for $C_{17}H_{14}O_5$: C, 68.45; H, 4.73. Found: C, 68.55; H, 4.74. VI was found to be identical with sayanedine by comparison of their TLC behavior, IR and UV spectra, and bioactivity in the tabacco pith callus bioassay, and by mixed fusion.

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