Biochemical Studies on "Bakanae" Fungus

Part XXXIV. Isolation of Gibberellins and Their Properties

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It has been confirmed that gibberellin A is a mixture of three components, gibberellin A1, gibberellin A2 and gibberellic acid (namely, gibberellin X), by treating their methyl ester through the chromatography on Al2O3 column. Attempts to separate them in free acid were made. The physical and chemical properties of each gibberellin as well as its physiological properties are described.

Gibberellin A and B1,2) the metabolic products of Gibberella fujikuroi which cause abnormal growth of young tissues of higher plants, were isolated and further investigation to elucidate the chemical structure have been published already3,4,5,6,7). Gibberellin A, isolated through the process shown in Fig. 1, was chromatographed on paper using various solvents, butanol-NH3aq, ethanol-NH3aq, benzene-acetic acid-water, ethanol-ethyl acetate-ligroin, ethanol-ethyl acetate-bezene, butanol saturated with water, but in every case only one spot was found. Further, as the results of the thirty plates countercurrent distribution method using the solvent system of ethyl acetate and one mole phosphate buffer adjusted to pH 5.3, we found only one peak. From these facts, we had to arrive at the conclusion, that our gibberellin is a homogeneous compound. However, in contrast to these results, it was found that degradation process3) of gibberellin A to gibberellin C and gibberic acid at the same time, seen in previous reports, could not be explained from the fact that no moiety corresponding to the latter was to be found, and moreover the same results could hardly be obtained by repetition of the experiments. From these contradictory facts, we got to have some doubts on the purity and homogeneity of crystal gibberellin A. At that time, the private communication of April 7, 1954 from Stodola informed us that he had isolated gibberellin X, C_{18}H_{20}O_7 which yields gibberic acid, C_{18}H_{20}O_5 liberating one mole of CO₂ in acid hydrolysis. Fortunately, we succeeded in separating the gibberellin A methyl ester into three different methyl esters on chromatography of Al₂O₃, and it was proved that gibberellin A is a mixture of three components, gibberellin A₁, A₂ and A₃. The empirical formula and physical constants of these three esters are shown in Table I.

It should be emphasized that the an-
Fig. 1. Process of Separation of Gibberellin A.

Filtered culture
 \[ \text{adsorption with carbon} \]

\[ \text{Carbon} \]
\[ \text{eluted with MeOH containing 3% NH}_3 \text{ and}
\text{concentrated in vacuo}
\text{dissol. in NaHCO}_3 \]
\[ \text{extracted with butyl acetate} \]

\[ \text{Aq. layer} \]
\[ \text{acidified with HCl (pH 3.5)} \]
\[ \text{extracted with butyl acetate} \]

\[ \text{Solvent layer} \]
\[ \text{concentrated in vacuo} \]

\[ \text{Filtrate} \]
\[ \text{concentrated} \]
\[ \text{dissolved in 50% aq. alcohol} \]
\[ \text{added with basic lead acetate} \]

\[ \text{Filtrate} \]
\[ \text{treated with H}_2\text{S} \]
\[ \text{concentrated in vacuo} \]
\[ \text{7 plates countercurrent}
\text{(one mole phosphate buffer of pH 5.0)}
\text{ethyl acetate solvent system)} \]

\[ \text{recrystallization} \]

\[ \text{Mother Liquor} \]
\[ \text{Crystal} \]

Analytical data of the methyl ester did not give a satisfactory result in previous reports\(^2\). Gibberellin A\(_3\) methyl ester was confirmed to be identical with that of gibberellic acid, which was kindly sent to us by Curtis and Cross\(^8, 9\) who have isolated this from Gibberella fujikuroi, after comparing both infra-red spectra and mixed melting point. Stodola et al\(^10\) also isolated the same substance and named it gibberellin X.

As the publication of gibberellin A\(_3\) has been first reported by Curtis et al\(^8, 9\) under the name of gibberellic acid, we propose to adapt the name of gibberellic acid.

It was observed that there was a great difference in the recovery of the three components by different strains and composition of culture media. (Table II). Although we were able to separate each component of gibberellin as its methyl ester, it was necessary to separate the three components in the state of free acid, in order to examine their chemical and physical properties as well as their physiological activities which their esters failed to have shown.

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At first, in the purpose to separate gibberellin A\(_1\) and A\(_2\), the crystal gibberellin A from strain No.-4 was used in the following experiments which was fortunately ascertained to be a mixture of gibberellin A\(_1\) and A\(_2\) containing no gibberellic acid, by the chromatography of its methyl ester.

As a direct method of separation, partition chromatography of silica gel was applied. After the preliminary micro test using silica gel column adjusted to pH 4.8, 5.0, 5.2, 5.4, and with the solvent system of CHCl\(_3\)-ethyl acetate, CHCl\(_3\)-butanol, benzene-butanol, benzene-ethyl acetate, a system of CHCl\(_3\)-ethyl acetate and buffer of pH 5.2 was chosen for a large scale. The relation between the fraction number and the recovery curve showed two peaks. But it was proved that every fraction of these two peaks contained gibberellin A\(_1\) and A\(_2\) from the result obtained by the method of ester chromatography. Adsorption chromatography and ion exchange resin were tried without success.

As indirect methods of separation, attempts were made to prepare their derivatives and separate them taking advantage of the changed physical and chemical properties.

Gibberellin A\(_1\) methyl ester absorbs one mole of hydrogen in catalytic reduction resulting the dihydrogibberellin A\(_1\) methyl ester but gibberellia A\(_1\) methyl ester absorbs no hydrogen. A mixture gibberellin A was hydrogenated catalytically using Adams' catalyser and attempts to separate dihydrogibberellin A\(_1\) and gibberellin A\(_2\) were made by partition chromatography of silica gel resulting in failure in consequence of their similarity of solubility to the various solvents.

In the same manner as the catalytic reduction, gibberellin A\(_1\) methyl ester absorbs bromine but gibberellin A\(_2\) methyl
ester none. The syrup obtained by bromination of gibberellin A mixture at 0°—8° was subjected to chromatography of silica gel. Two kinds of crystal were separated, one of them, monobromo derivative, gives a positive test with the Beilstein reaction and has the formula C_{19}H_{23}O_{6}Br, dp. 215-7°.

Investigation for the purpose of clarifying this peculiar phenomenon, in other words, that we could not obtain the dibromo derivative which should be produced on the addition of one mole of bromine to a double bond, is now under way. The other is the free acid of gibberellin A, giving a negative Beilstein test, the formula C_{20}H_{28}O_{6} being assigned. Its methyl ester is identical with gibberellin A, methyl ester in mp. and infra-red spectra.

As the separation of methyl ester was successful, hydrolysis of esters by acid and base to the original acid was tried. When it was treated with N/10—N/100 NaOH at room temperature, two kinds of acids were obtained, one of them had the formula C_{15}H_{26}O_{6}, dp. 232-5°, the ester of which is identical with gibberellin A, methyl ester in mp. and infra-red spectra. The other is thought to be an epimer of gibberellin A, having the same formula as gibberellin A, and should be called pseudogibberellin A. The gibberellin C was obtained by hydrolysis of gibberellin A, methyl ester with 20% H_{2}SO_{4}.

It took ten days to hydrolyse gibberellin A, methyl ester with N/10 NaOH at 30° and the product was assigned to be C_{19}H_{22}O_{6} which failed to regenerate the original ester in esterification, resulting an ester of mp. 167-9°. Various concentrations of H_{2}SO_{4} were used to hy-

<table>
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</thead>
<tbody>
<tr>
<td>cone. of gibb. %</td>
<td>136</td>
<td>125</td>
<td>122 (0.0005%)</td>
<td>82 (0.0005%)</td>
<td>121</td>
</tr>
<tr>
<td>0.0016</td>
<td>124</td>
<td>118</td>
<td></td>
<td></td>
<td>130</td>
</tr>
<tr>
<td>0.0008</td>
<td>109</td>
<td>102</td>
<td></td>
<td></td>
<td>118</td>
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<tr>
<td>0.0004</td>
<td>99</td>
<td>93</td>
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<td>92</td>
</tr>
<tr>
<td>0.0002</td>
<td>83</td>
<td>79</td>
<td></td>
<td></td>
<td>84</td>
</tr>
<tr>
<td>control (dist. water)</td>
<td>82</td>
<td></td>
<td></td>
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</tr>
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</table>

Table IV
Melting Points and Mixed Melting Points of the Three Components.

<table>
<thead>
<tr>
<th>Gibb. A,</th>
<th>Free acid (dp.)</th>
<th>Me-ester</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gibb. A,</td>
<td>232-5°</td>
<td>232-4°</td>
</tr>
<tr>
<td>Gibb. A,</td>
<td>235-7°</td>
<td>190-2° (anhydrous)</td>
</tr>
<tr>
<td>Gibberellic acid</td>
<td>230-2°</td>
<td>183-5° (anhydrous)</td>
</tr>
<tr>
<td>Gibb. A, + Gibberellic acid</td>
<td>232-5°</td>
<td>203-4°</td>
</tr>
<tr>
<td>Gibb. A, + Gibberellic acid</td>
<td>222-4°</td>
<td>211-7°</td>
</tr>
<tr>
<td>Gibb. A, + Gibb. A,</td>
<td>234-6°</td>
<td>180-4°</td>
</tr>
</tbody>
</table>
Table V
Analytical Data and Physical Constants of the Three Components.

<table>
<thead>
<tr>
<th>Component</th>
<th>Formula</th>
<th>mp.</th>
<th>Specific Rotation</th>
<th>Analytical data (Found)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A₁-free</td>
<td>C₁₉H₂₆O₆, C₂₂H₃₅O₇</td>
<td>232-5°</td>
<td>+42.3</td>
<td>C: 65.37, H: 7.13</td>
</tr>
<tr>
<td>A₁-methyl ester</td>
<td>C₂₀H₃₀O₆</td>
<td>232-4°</td>
<td>+35.1</td>
<td>C: 66.26, H: 6.92</td>
</tr>
<tr>
<td>A₂-free</td>
<td>C₂₀H₂₅O₅</td>
<td>235-7°</td>
<td>+11.7</td>
<td>C: 66.19, H: 8.01</td>
</tr>
<tr>
<td>A₂-methyl ester</td>
<td>C₂₅H₃₀O₆ ·½H₂O</td>
<td>190-2°</td>
<td>+28.1</td>
<td>C: 62.11, H: 7.88</td>
</tr>
<tr>
<td>Gibberellic acid methyl ester</td>
<td>C₂₁H₃₀O₆</td>
<td>183-5°</td>
<td>+29.1</td>
<td>C: 65.47, H: 7.77</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>C: 66.70, H: 7.72</td>
</tr>
</tbody>
</table>

(Required) Molecular Weight

<table>
<thead>
<tr>
<th>Component</th>
<th>C: 65.50, H: 6.94</th>
<th>(Found)</th>
<th>370</th>
<th>348.3</th>
<th>4.9</th>
</tr>
</thead>
<tbody>
<tr>
<td>A₁-methyl ester</td>
<td>C: 66.28, H: 7.23</td>
<td>(Found)</td>
<td>382</td>
<td>360</td>
<td>8.10</td>
</tr>
<tr>
<td></td>
<td>(Found)</td>
<td>(tiration)</td>
<td>414</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A₂-free</td>
<td>C: 65.91, H: 7.74</td>
<td>360</td>
<td>364.4</td>
<td>5.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(tiration)</td>
<td>(OCH₃)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A₂-methyl ester</td>
<td>C: 62.22, H: 8.15</td>
<td>372</td>
<td>405</td>
<td>8.32</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Found)</td>
<td>(OCH₃)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C: 65.12, H: 8.01</td>
<td>413</td>
<td>387</td>
<td>7.51</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(OCH₃)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C: 66.64, H: 7.99</td>
<td>370</td>
<td>378</td>
<td>8.38</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(OCH₃)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gibberellic acid methyl ester</td>
<td>C: 66.65, H: 6.71</td>
<td>360.4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Drolyse the ester of gibberellin A₂. Two kinds of crystals were obtained, dp. 255° and 290° as the acidic fraction. The amounts of the former crystal were too small to be analysed. The empirical formula C₁₉H₂₆O₆, was assigned to the latter.

Various attempts to separate free gibberellic acid from a mixture of gibberellin A₁, A₂ and gibberellic acid were made but so far without success.

The physiological activities of gibberellin A₁, A₂, pseudogibberellin A₁ isolated in the process described above and gibberellic acid kindly sent to us by Cross were examined. The results are shown in Table III. It was confirmed that the three components of gibberellin A mixture, gibberellin A₁, A₂ and gibberellic acid have strong physiological activities.

The melting point of gibberellin A₁, A₂, gibberellic acid and their esters and also mixed melting point of each two components are shown in Table IV. It is noteworthy that the mixed melting point of each two of the free acids which have a similar melting point do not depress, but although the methyl esters of these acids melt at a somewhat different temperature, their mixed melting point of each two of the three esters show no depression.

Analytical data and physical constants of the three components are summarized.
Fig. 2. Infra-Red Spectra of Free Acids and Their Esters.

Gibberellin A₁

Gibberellin A₂

Gibberellin A₂ methyl ester (containing water of crystallization) $\text{C}_{21}\text{H}_{20}\text{O}_{4} \cdot \frac{3}{2} \text{H}_{2}\text{O}$
Gibberellin A₄ methyl ester C₂₁H₃₂O₆ • ÷²H₂O

Gibberellin A₅ methyl ester (anhydrous) C₂₁H₃₂O₆

Gibberellic acid methyl ester

Gibberellin A₆ methyl ester
Ultra violet spectra of gibberellin A₁, A₃, gibberellic acid and their esters showed no maximum in the region of 225 mμ to 300 mμ at the concentration of 5 × 10⁻⁸ mol/cc.

Infra-red spectra of free acids and their esters are shown in Fig. 2.

**Experimental**

1) **Paper chromatography of gibberellin A.**

On the paper chromatography of gibberellin A using the following solvents at room temperature, only one spot was found.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Indicator</th>
<th>Rf</th>
</tr>
</thead>
<tbody>
<tr>
<td>BuOH and 1.5 N NH₃ (1:1)</td>
<td>B.C.P. 0.43</td>
<td></td>
</tr>
<tr>
<td>a mixture of EtOH and 1.5 N NH₃ (5:1)</td>
<td>B.C.P. 0.71</td>
<td></td>
</tr>
</tbody>
</table>

organic layer of a mixture of benzene acetic acid and water

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Indicator</th>
<th>Rf</th>
</tr>
</thead>
<tbody>
<tr>
<td>EtOH, ethyl acetate, ligroin and water (1:1:1:1)</td>
<td>B.P.B. 0.22</td>
<td></td>
</tr>
</tbody>
</table>

organic layer of a mixture of EtOH, ethyl acetate, benzene and water (1:1:1:1) | B.P.B. 0.96 |

BuOH saturated with water | B.P.B. 0.92 |

a mixture of benzene and EtOH

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Indicator</th>
<th>Rf</th>
</tr>
</thead>
<tbody>
<tr>
<td>EtOH, ethyl acetate, benzene and water (1:1:1:1)</td>
<td>B.P.B. 0.22</td>
<td></td>
</tr>
</tbody>
</table>

organic layer of a mixture of


<table>
<thead>
<tr>
<th>Solvent</th>
<th>Indicator</th>
<th>Rf</th>
</tr>
</thead>
<tbody>
<tr>
<td>EtOH, ethyl acetate, benzene and water (1:1:1:1)</td>
<td>B.P.B. 0.96</td>
<td></td>
</tr>
</tbody>
</table>

2) **Countercurrent distribution of gibberellin A.**

Gibberellin A (50.5 mg) of dp. 240–2° was dissolved in ethyl acetate (10 ml.) and subjected to thirty plates countercurrent distribution with a solvent system of ethyl acetate and one mole phosphate buffer (pH. 5.34). After completion, each buffer layer was extracted with ethyl acetate in the acidic side and each of extract was added to the corresponding upper layer and dried in vacuo. The plate number curve and melting point of each fraction are shown in Fig. 3.

![Fig. 3. Relation between Amount of Dried Substance and Plate No. on the Countercurrent Distribution Method.](image)

3) **Separation of gibberellin A₁, A₃ and gibberellic acid methyl esters.**

Gibberellin A (150 mg) from strain G–4 was dissolved in an alcohol ether mixture and treated with an excess of diazomethane in ether solution. On evaporation of the solvent, the residual crystal was dissolved in 20 ml. of ethyl acetate-benzene (1:5), and eluted with the solvent of the same composition, the effluent being collected in every 10 ml.

Gibberellin A, methyl ester: Fractions No. 10–14 were combined and recrystallized from ethyl acetate-ligroin into prisms, mp. 226–8°, [α]D +35.1° (C=5.04% in methanol).

Gibberellic acid methyl ester: Fractions No. 16–20 were combined (15 mg) and after repeated recrystallization, a crystal of mp. 200–2° in fine needles, was obtained, [α]D +67.0° (C=5.38% in methanol).

Gibberellin A₂ methyl ester: At this point, the solvent composition was changed to 1:1 and elution was continued. Fractions No. 23–28 (40 mg) were crystallized from the hot ethyl acetate into plates, mp. 190–2°, shrinking at 165–70°, [α]D +28.1° (C=3.33% in methanol).

4) **Ratio of the three components for different strains.**

The crystal and mother liquor after recrystallization of gibberellin A from the culture media of strains No.–4 and G–4 were esterified with
Vol. 19, No. 4, 1955] Biochemical Studies on “Bakanae” Fungus

diazomethane and three esters were separated by
the chromatography of Al₂O₃, and their ratio was
calculated. The result is shown in Table III.

5) Partition chromatography of
gibberellin A.

a) Preliminary test.
As the preliminary test, gibberellin A (15 mg)
and silicic acid (5 g) treated with pH. 4.8, 5.0,
5.2, 5.4 buffers (3.5 ml.) and solvent systems of
benzene-butanol (95 : 5), benzene-ethyl acetate
(50 : 50), CHCl₃-butanol (95 : 5), CHCl₃-ethyl
acetate (50 : 50) were used. Every 5 ml. of the
effluent was collected and titrated with N/100
alcoholic NaOH. The curve of ml. of effluent
and ml. of N/100 NaOH required, showed one
peak and one shoulder when the system of CHCl₃-
ethyl acetate and a pH. 5.2 phosphate buffer was
used.

b) Large scale experiments.
A sample of 175 mg was dissolved in CHCl₃-
ethyl acetate (50 : 50) 50 ml. The solution was
developed on the 30 g silicic acid treated with
25 ml. of pH. 5.2 phosphate buffer. Elution
was continued with the same solvent composition.
Every 20 ml. of effluent was collected and dried
in vacuo. Relation between the amount of dried
substance in each fraction and the fraction No.
are shown in Fig. 4.

![Fig. 4. Relation between Amount of Dried Substance and Fraction No. on the Partition Chromatography.](image)

Fractions No. 12–3, Fraction No. 14 and
Fractions No. 18–22 were methylated with
diazomethane and the esters thus obtained, were
chromatographed on Al₂O₃ to separate the gibberellin A₁ and A₂ methyl esters.

Ratio of A₁ and A₂ obtained:

\[
\begin{align*}
\text{Fracts.} & : \\
12–3 & : 7 : 3 \\
14 & : 6 : 4 \\
18–22 & : 6 : 4
\end{align*}
\]

6) Separation of Gibberellin A₂ by Bromination of a Gibberellin A Mixture.

A gibberellin A mixture was dissolved in 20
ml. of dioxan-ether (1 : 1). Ether solution of
bromine (0.034 g bromine/ml.) was added at
−3°−8° for thirty minutes. Additional shaking
for two hours was continued at 0°−5°. The
colour of the solution was light brown. About 30
ml. of ether was added and the ether solution was
extracted with aq. NaHCO₃ which was acidified
and reextracted with ethyl acetate. On evaporation
of the solvent, the syrup (320 mg) was
obtained. The partition chromatography through
silica gel, pretreated with a pH. 5.2 buffer was
used in preparing the crystal from the
syrup using a solvent of butanol-benzene (3 : 97).
A crystal (30 mg) positive to the Beilstein test
was obtained at this solvent composition. By-
pyramidal obtained on recrystallization from ethyl
acetate-ligroin melted at 215−7° with decom-
position, pKa 4.3.

**Anal.** Found: C, 53.36; H, 5.36; Br, 18.93.
Calcd. for C₁₉H₂₅O₅Br: C, 53.40; H, 5.39; Br, 18.74.
C₁₉H₂₃O₆Br: C, 53.12; H, 5.83; Br, 18.88.

When the butanol-benzene (10 : 90) was used
as the developing solvent, a crystal negative to
the Beilstein test was eluted. On recrystallization
from ethyl acetate-ligroin, a crystal of fine needles
was obtained, dp. 235−7°, pKa 5.37, [α]D\(^{15}\) +
11.7° (C = 3.16% in methanol).

This is the gibberellin A₂, the ester of which
is identical with gibberellin A₂ methyl ester
obtained from the chromatography of ester of
gibberellin A mixture, in all respects, the analytical
data, mp. and infra-red spectra.

7) Hydrolysis of gibberellin A₁, methyl ester.

a) Acid hydrolysis.
Gibberellin A₁, methyl ester (150 mg) was boiled
with 10 ml. of H₂SO₄ (1 : 5) for one hours. The
reaction mixture was extracted with ether con-
tinuously and the ether layer was reextracted with
aq. NaHCO₃. The aq. layer was then acidified
and extracted with ether for two days. On evaporating the ether, the residual crystal amounted to 130 mg. By recrystallization, a large amount of plates, dp. 247-9°, and a small amount of needles, dp. 255-7°, were obtained. The substance of dp. 247-9° was found to be gibberellin C from the mixed melting point and analytical data.


The amount of the crystal of dp. 255-7° was too small to be analysed.

**b) Alkali hydrolysis.**

i) N/500 NaOH.

Gibberrellin A₁ methyl ester (150mg) was shaken with N/500 NaOH (200ml.) for 4 hours at 25°. All crystals were gradually dissolved. The solution was extracted with ethyl acetate at pH 7.5-8.0. On evaporation of the solvent, a crystal (130mg) remained. This was an original substance, mp. 224-5°.

ii) N/100 NaOH.

Gibberrellin A₁ methyl ester (150mg) was added to the N/100 NaOH (100ml). For two days, at a temperature of 30°, all crystals were dissolved, and the solution was extracted with ethyl acetate at pH 8.0 and next pH 2.0. The acidic fraction was a syrup (130mg) which was subjected to the partition chromatography of silica gel using the solvent of butanol-benzene (5:95). In this solvent composition, a small amount of yellow syrup was eluted. Elution was continued with the solvent of butanol-benzene (10:90). At this stage, 35 mg of crystal was eluted and recrystallized from alcohol-ethyl acetate-ligroin gave prisms, dp. 232-5°, [α]D+42.3° (C=3.26 in methanol), pKa 4.9.

The ester of this crystal had dp. 232-4° and its infra-red spectrum was quite similar to that of gibberellin A₁ methyl ester. The mixed melting point of these two crystals indicated no depression. After this crystal was eluted, the solvent was changed to CHCl₃-butanol (80:20). Another crystal (60mg) was eluted and recrystallized from ethyl acetate-alcohol-ligroin into prisms, dp. 225-7°; [α]D+33.7° (C=3.86 in methanol).


This acid was named pseudogibberellin A₁ which was considered to be an epimer of gibberellin A₁ having the same formula.

Pseudogibberellin A₁ methyl ester was obtained by the reaction of diazomethane and melted at 182-3°.


On the catalytic reduction of pseudogibberellin A₁ with PtO₂, one mole of hydrogen was absorbed, resulting dihydropseudogibberellin A₁, dp. 290°.


The same result was obtained in the hydrolysis of gibberrellin A₁ methyl ester by N/10 and N/50 NaOH.

8) **Hydrolysis of gibberrellin A₂ methyl ester.**

a) **Acid Hydrolysis.**

Gibberellin A₂ methyl ester (300mg) and HCl (1:3, 70 ml.) were refluxed for two hours. The reaction mixture was extracted with ethyl acetate. This extract was treated with aq. NaHCO₃ and the aq. layer was acidified and reextracted with ethyl acetate. The residue obtained by evaporation of the solvent weighed was 70mg. This acidic fraction was subjected to the partition chromatography of silica gel treated with a pH 5.2 buffer and solvent of butanol-benzene (5:95), and elution was continued. At this stage, 5 mg of crystal was eluted and recrystallized in needles, dp. 254-5°. The amount of this crystal was too small to be analysed. When the elution was continued with the solvent of butanol-benzene (10:90), 20 mg of crystal was obtained. It was recrystallized from ethyl acetate-ligroin in prisms, dp. 292-4°, mol. wt. 376 by Rast.


b) **Alkali Hydrolysis.**

Gibberellin A₂ methyl ester (110mg) was shaken with N/10 NaOH (20 ml) for ten days at 30°. After the extraction with ether at pH 7.5-8.0 and then 3.0, the acidic fraction (95 mg) recrystallized from alcohol-ligroin in needles, dp. 238-40°.

**Anal.** Found: C, 64.40; H, 7.33. Calcd. for C₁₉H₂₆O₅: C, 64.64; H, 6.63.

Methyl ester of this acid was different from gibberellin A₂ methyl ester in respects of solubility to ethyl acetate and its melting point.
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