

PATENT SPECIFICATION

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COMPLETE SPECIFICATION

Gibberellic Acid and Derivatives thereof

We, IMPERIAL CHEMICAL INDUSTRIES LIMITED, of Imperial Chemical House, Millbank, London, S.W.1, a British Company, and PERCY WRAGG BRIAN and MARGARET EDITH RADLEY and PHILIP JOHN CURTIS and GEORGE WYNN ELSON, all British subjects, of The Frythe, Welwyn, Hertfordshire, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to a new organic acid having plant growth promoting properties, and to active derivatives thereof, in particular salts and esters. The invention further relates to a method of producing the acid by a metabolic process. The invention further relates to compositions for agricultural use containing the new acid or an active derivative thereof.

We have found that from a culture of *Gibberella fujikuroi* (Sawada) Wollenweber (= *Fusarium moniliforme* Sheldon) there may be extracted a new organic acid having the property of promoting plant growth. This acid is hereinafter termed gibberellic acid.

The present invention therefore provides a new chemical compound, gibberellic acid, such as is obtainable from a culture of *Gibberella fujikuroi*, the acid being characterised by the following properties:—

Melting point 233—235° C. (vigorous gas evolution).

Optical Rotation $[\alpha]_D^{25}$, 83° (c, 0.5 in methanol).

Monomethyl ester—melting point 209—210° C.

—optical rotation $[\alpha]_D^{25}$, 75° (c, 0.5 in ethanol).

p-Bromophenacyl ester—melting point 218—219° C.

Hydrolysis with boiling mineral acid—1 mole carbon dioxide evolved.

Acetyl derivative—melting point 233—234° C. (dec.).

—optical rotation $[\alpha]_D^{25}$, 152° (c, 0.5 in ethanol).

Methyl acetyl gibberellate—melting point 180—181° C.

—optical rotation $[\alpha]_D^{25}$, 150° (c, 0.4 in ethanol).

Infra-red absorption curve (Nujol "mull"—Nujol is a Registered Trade Mark) of material crystallised from ethyl acetate—light petroleum (b.p. 60—80° C.) as shown in the drawing accompanying Provisional Specification No. 19238/54. X-ray powder diffraction lines as shown in Table I. The intensities "I" of the lines on the powder photographs are estimated by reference to the strongest line which is assigned an intensity of 10.

TABLE I.
X-RAY POWDER DIFFRACTION LINES OF
GIBBERELIC ACID
(ex ethyl acetate)

Spacing "d"	Intensity "I"	Spacing "d"	Intensity "I"
10.20	5 b	2.786	1
8.73	2 b	2.687	1
7.33	5 b	2.549	2
6.69	w	2.485	2
6.01	5 b	2.418	w
5.31	10 b	2.350	1
5.06	$\frac{1}{2}$	2.305	1
4.84	$3\frac{1}{2}$	2.249	w
4.60	7	2.172	1
4.33	3	2.120	1
4.03	3	2.040	w
3.85	w	2.003	w
3.714	3	1.971	w
3.591	3	1.936	w
3.389	$1\frac{1}{2}$ b	1.920	w
3.214	1	1.863	w
3.085	w	1.853	w
2.993	$1\frac{1}{2}$	1.780	w
2.882	$1\frac{1}{2}$	1.698	w

w denotes a weak reflection.

b denotes a broad diffuse reflection.

The third figure beyond the decimal point is barely significant.

[Price 3s. 6d.]

The invention also provides functional derivatives of gibberellic acid, which are salts and esters thereof, having plant growth-promoting properties. Examples of the esters include alkyl esters and, specifically, the methyl, ethyl, isopropyl, butyl and octyl esters. Compounds in which the alcoholic hydroxyl groups of gibberellic acid are esterified with aliphatic or aromatic acids, specifically acetic, butyric and benzoic acids, comprise an additional type of ester of gibberellic acid. The salts preferably utilized are the alkali metal salts and other salts such as the alkaline earth metal salts, e.g., calcium.

The present invention also provides mixtures having plant growth-promoting properties comprising gibberellic acid, or an active derivative thereof, and a diluent. The diluent may be liquid or solid and may consist of or contain plant nutrients, particularly sources of nitrogen.

Further, the present invention provides a process of producing gibberellic acid, or mixtures containing said acid, comprising cultivating an active strain of *Gibberella fujikuroi* in an aqueous medium and continuing the cultivation after active growth has ceased.

Gibberellic acid may be produced by *Gibberella fujikuroi* when grown as a surface culture or as a deep, stirred and aerated culture in a suitable aqueous medium. A suitable medium must contain a carbon source (e.g., sucrose, glucose or glycerol, at 2—30 w/v), an ammonium salt or a nitrate or a digest of protein (e.g., peptone as nitrogen source) to give a concentration of N in the medium of 0.01—0.5, a concentration of 0.08 being generally suitable, a magnesium salt (e.g., magnesium sulphate heptahydrate at 0.05 or thereabouts, which also conveniently adds sulphate which is also necessary but which may be added in some other form if another salt of magnesium is used), a phosphate (e.g., potassium dihydrogen phosphate at 0.05—0.5, 0.1 being a suitable concentration), a potassium salt (which may be conveniently the phosphate, see above) and traces (ca. 1—10 p.p.m. of metal) of salts of iron, copper, zinc, manganese and a trace (1—10 p.p.m. of metal) of a molybdate.

Fermentations can be conveniently carried out at a temperature in the range 25—33 °C.

Not all strains of the fungus produce gibberellic acid. Results of tests carried out on a variety of strains do indicate however that strains isolated from rice seedlings infected with the "bakanae" disease are more likely to produce gibberellic acid than strains isolated from other host plants such as cotton, maize and pineapple. Of strains found on sugar cane some are active in producing gibberellic acid, others are not. Strains of the fungus which produce the acid are morphologically indistinguishable from strains which produce

none, and whether or not a strain is active can be determined only by growing it and testing the culture.

Strains of *Gibberella fujikuroi* which do produce gibberellic acid are the so-called "Sawada" and "Miyake" strains obtainable from the Centraalbureau voor Schimmelcultures at Baarn, Holland. Four strains numbered 1001, 1004, 1135 and 1139 in our collection of moulds are also productive of gibberellic acid. Samples of these strains have been deposited with the Centraalbureau and with the Commonwealth Mycological Institute, Key, Surrey. Cultures of these strains when grown as described herein will contain gibberellic acid.

A method of obtaining gibberellic acid comprises generally the following steps:—

i) growth of a mould culture in an aqueous medium;

ii) filtration and extraction of the medium with an adsorbent;

iii) elution of the adsorbent;

iv) purification of the acid obtained by the elution.

A preferred adsorbent is activated charcoal.

Gibberellic acid has been produced in still and in stirred culture using Raulin-Thom medium containing sucrose as the carbon source.

To obtain maximum yields in stirred cultures it is necessary to carry fermentation on for what is a long time by usual standards. This is shown by the following yield/time data for 100 litre batch of Raulin-Thom medium containing 4% sucrose, initially adjusted to pH 3.5 and at 25 °C.

TABLE II.

Hrs. fermentation	Yield (mg./l.)	
93	18	105
117	33	
141	39	
164	82	
187	105	
212	92	110
236	138	
262	160	
284	189	
331	193	
402	249	115

The time at which gibberellic acid begins to accumulate in a culture of a suitable strain of *Gibberella fujikuroi* and the final yield of gibberellic acid are influenced by the concentrations of the carbon source and nitrogen source and by the ratio of these two concentrations in the medium used.

The following Examples are given in illustration.

A gibberellic acid producing strain of the mould was grown in 30 litre batches of a medium of the following composition

magnesium sulphate	30 gm.	MnSO ₄ ·7H ₂ O	0.01 gm.
potassium dihydrogen phosphate	150 gm.	K ₂ MoO ₄	0.01 gm.
minor element concentrate*	60 ml.	water	100 ml.
glucose monohydrate			
5 ammonium nitrate	see below		
water	30 l.		

The medium was stirred, aerated at a rate of 10 litres/minute and maintained at a temperature of 25 °C. The pH value of the medium was adjusted to 4.5 before sterilizing.

*The composition of the minor element concentrate was:—

10 FeSO ₄ ·7H ₂ O	0.1 gm.
CuSO ₄ ·5H ₂ O	0.015 gm.
ZnSO ₄ ·7H ₂ O	0.1 gm.

Yields of gibberellic acid (mg./l.) at various times during fermentation on media containing different concentrations of glucose and ammonium nitrate are given in the following tables.

TABLE III.
Yield of Gibberellic acid (mg./l.)
Concentration of NH₄NO₃ (g./l.)

	Glucose Monohydrate	Hours of fermentation	Concentration of NH ₄ NO ₃ (g./l.)			
			1.2	2.4	4.8	9.6
30	16	120	—	—	—	—
	..	161	19	—	—	—
	..	195	—	—	—	—
	..	212	—	59	—	—
	..	219	19	—	—	—
35	..	258	61	146	44	49
	..	330	102	114	204	5
	..	402	139	312	204	4
	..	..	..	..	..	..
40	..	138	—	—	—	—
	..	168	—	—	—	—
	..	185	65	34	—	—
	..	210	—	—	4	5
	..	282	100	71	8	8
45	..	305	107	71	5	7
	..	..	..	..	..	..
	2	100	—	—	—	—
	..	114	—	—	—	—
	..	137	—	5	—	—
50	..	161	41	5	2	—
	..	185	42	7	—	—
	..	..	..	..	..	..
55	20	139	—	3	—	—
	..	163	—	20	—	—
	..	235	—	123	—	—
	..	259	—	134	—	—
	..	283	—	162	—	—
	..	307	—	189	—	—
	..	331	—	244	—	—
60	..	402	—	364	—	—
	..	426	—	368	—	—
	..	474	—	408	—	—
	..	618	—	544	—	—
	..	666	—	424	—	—
	..	738	—	465	—	—
65	..	785	—	501	—	—
	..	..	..	..	..	..

These results show (a) the yield of gibberellic acid increases with increasing ratio of glucose/NH₄NO₃ (b) the yield of gibberellic acid increases with increasing glucose.

TABLE IV
 Yield of Gibberellic acid - mg./l.

NH ₄ NO ₃		0.96	0.48	0.24	0.12
Dextrose					
5	16	94	211	310	148
	8	8	60	185	200
	4	13	—	24	118
	2	5	5	8	50

(c) in media of given glucose concentration production of gibberellic acid commences soonest in media of low NH₄NO₃ concentration.

(d) in media of given NH₄NO₃ concentration production of gibberellic acid commences soonest in media of low glucose concentration.

(e) Gibberellic acid is produced by the fungus only during times when it is accumulating or subsisting upon internal reserves of fat and carbohydrate, that is to say when net protein synthesis or active growth of the fungus has been checked by exhaustion of either the nitrogen or carbon in the nutrient. The best conditions for production of the acid appear to be those in which nitrogen is the factor which limits active growth and carbon is in excess though, of course, it is necessary that sufficient nitrogen be originally present in the nutrient to permit adequate growth of the fungus before acid production commences.

In general, high yields of gibberellic acid are obtained by growing the mould in a nutrient containing from 0.2—0.3% ammonium nitrate (or equivalent source of nitrogen) and from 10—30% glucose (or equivalent source of carbon), i.e., from 0.07—0.1% N and from 3.5—10% C.

EXAMPLE 1.

For still culture the Raulin Thom medium was dispensed in one litre portions in earthenware vessels and the cultures incubated for about ten days at 25°. Incubation of stirred cultures lasted three to five days at 25°. The culture filtrate, of pH 4 or less (adjusted with N-hydrochloric acid if necessary), was extracted with 10 g./l. of B.D.H. activated charcoal. The air dried charcoal (containing 20 to 30% moisture) was continuously eluted with acetone, and the eluate evaporated under reduced pressure to give an aqueous concentrate which was extracted twice with at least an equal volume of ethyl acetate. The combined ethyl acetate solutions were extracted twice with 25% of their volume of phosphate buffer pH 6.3 (36 g. KH₂PO₄ and 24 g. KOH in 1 l. of water). The buffer extracts were combined, adjusted to pH 3.8, and extracted twice with an equal volume of ethyl acetate. On concentrating these extracts, crude gibberellic acid crystallised in yields of about 40 mg./l. The crude acid was purified by

adding an equal volume of light petroleum (60—80° C.) to a solution in boiling ethyl acetate. Repetition of this process gave pure gibberellic acid as jagged lathes decomposing at 233—235° C., optical rotation $[\alpha]_D^{25} + 86$ (c, 2.12 in ethanol). (Found: C, 65.9, 65.85; H, 6.6, 6.55. Eq. wt., 342. C₂₁H₃₆O₉ requires C, 65.9; H, 6.4. Eq. wt. (monobasic) 346). The molecular weight has been confirmed by the X-ray method. The unit cell was tetragonal and had *a* *b* 10.905, *c* 28.74 Å. The density was 1.34 ± 0.01, whence the molecular weight (8 molecules per unit cell) is 345.

Direct extraction of the culture filtrate with organic solvent will remove gibberellic acid but not so effectively as when charcoal is used as in the above Example I.

The empirical formula for the acid and derivatives would be $[C_{21}H_{36}O_9]_{COOX}$
 $[C_{21}H_{36}O_9]_{OY}$

where X may be hydrogen, alkyl or aryl radical or metal and Y may be hydrogen or acyl radical.

Gibberellic acid gives a methyl ester, m.p. 209—210° C., optical rotation $[\alpha]_D^{25} + 75$ (c, 0.5 in ethanol). (Found: C, 66.65; H, 6.85; OMe, 8.5. C₂₂H₃₈O₉ requires C, 66.65; H, 6.7; OMe, 8.6). with either, diazomethane or methyl iodide and potassium carbonate in acetone. The *p*-bromophenacyl ester has m.p. 218—219° C. (Found: C, 57.65; H, 5.0; Br, 14.35. C₂₂H₃₅O₉Br requires C, 57.75; H, 5.2; Br, 14.2). Acetyl-gibberellic acid, m.p. 233—234° C. (dec.) optical rotation $[\alpha]_D^{25} + 152$ (c, 0.5 in ethanol). (Found: C, 65.1; H, 6.35. C₂₃H₃₈O₁₁ requires C, 64.9; H, 6.2). prepared with acetic anhydride in pyridine at room temperature, yields a methyl ester, m.p. 180—181° C., optical rotation $[\alpha]_D^{25} + 150$ (c, 0.4 in ethanol). (Found: C, 65.3; H, 6.7. C₂₄H₄₀O₁₁ requires C, 65.65; H, 6.5). also obtained by acetylating methyl gibberellate.

As further examples of alkyl esters of gibberellic acid the following were prepared in each case by interaction of the carboxyl group of gibberellic acid and the appropriate diazo-hydrocarbon in a suitable solvent: ethyl gibberellate forms a hemi-methanolate, prismatic needles, m.p. 150—151° C. (Found: C, 66.6; H, 7.6. C₂₃H₃₈O₉·1/2CH₃O requires

- C, 66.2; H, 7.2 which on being heated to 140° C. *in vacuo* yields the solvent-free ester, m.p. 155—156° C. (Found: C, 67.3; H, 7.2. $C_{22}H_{36}O_6$ requires C, 67.4; H, 7.0). iso-Propyl gibberellate forms a hemi-methanolate, needles, m.p. 151—152° C. (Found: C, 66.5; H, 7.7. $C_{22}H_{36}O_6 \cdot \frac{1}{2}CH_3O$ requires C, 66.8; H, 7.5). Butyl gibberellate hemi-methanolate forms prismatic needles, m.p. 152—153° C. (Found: C, 67.6; H, 7.7. $C_{22}H_{36}O_6 \cdot \frac{1}{2}CH_3O$ requires C, 67.4; H, 7.7). Octyl gibberellate forms prismatic needles, m.p. 157—159° C. (Found: C, 70.2; H, 8.3. $C_{22}H_{36}O_6$ requires C, 70.7; H, 8.4).

- 15 Further examples of esters formed by acylation of an hydroxyl group in gibberellic acid are:—butyryl gibberellic acid, m.p. 192° C. (Found: C, 66.1; H, 7.0. $C_{22}H_{36}O_6$ requires C, 66.2; H, 6.8) prepared with butyric anhydride in pyridine at room temperature, and benzoyl gibberellic acid, m.p. 204—205° C. (Found: C, 69.2; H, 5.9. $C_{22}H_{36}O_6$ requires C, 69.3; H, 5.8) prepared with benzoyl chloride in pyridine at room temperature.

- 25 Examples of derivatives of gibberellic acid in which the carboxyl group is esterified and an hydroxyl group is acylated are as follows:—ethyl acetyl gibberellate, m.p. 202—204° C. (Found: C, 66.0; H, 6.9. $C_{22}H_{36}O_6$ requires C, 66.3; H, 6.8), isopropyl acetyl gibberellate, m.p. 202—203° C. (Found: C, 66.7; H, 7.1. $C_{22}H_{36}O_6$ requires C, 67.0; H, 7.0), butyl acetyl gibberellate, m.p. 147—149° C. (Found: C, 67.1; H, 7.2. $C_{22}H_{36}O_6$ requires C, 67.5; H, 7.3), ethyl butyryl gibberellate, m.p. 175—176° C. (Found: C, 67.2; H, 7.5. $C_{22}H_{36}O_6$ requires C, 67.5; H, 7.3) and ethyl benzoyl gibberellate, m.p. 192° C. (Found: C, 70.5; H, 6.8. $C_{22}H_{36}O_6$ requires C, 70.3; H, 6.3), and octyl acetyl gibberellate which consists of a colourless syrup, b.p. 195—202° C. (bath temperature)/5—10 mm. (Found: C, 69.6; H, 8.0. $C_{22}H_{36}O_6$ requires C, 69.6; H, 8.1).

- 45 Diacetyl gibberellic acid prepared by allowing gibberellic acid to react with acetic anhydride in pyridine at room temperature for 5 days formed prisms, m.p. 186.5—187° C. (Found: C, 64.25; H, 6.15. $C_{22}H_{36}O_6$ requires C, 64.2; H, 6.1). Methyl diacetyl gibberellate was prepared by the action of excess ethereal diazomethane on a methanolic solution of diacetyl gibberellic acid. It crystallised from methylene chloride-light petroleum (b.p. 60—80° C.) and from acetone-light petroleum (b.p. 60—80° C.) in massive prisms, m.p. 166.5—168.5° C. (Found: C, 65.1; H, 6.45. $C_{22}H_{36}O_6$ requires C, 64.85; H, 6.35).

- 60 An example of the procedure utilized to prepare the methyl ester of gibberellic acid is shown in the following example

EXAMPLE II

- 65 A: A solution of 207 mg of gibberellic

acid in 7 ml. of methanol, was cooled to 0° C. and an excess of diazomethane in ether was added with stirring. Five hours after the completion of the addition, the solution was placed on a water-bath and rapidly evaporated to dryness. The residue was crystallised from a benzene-methanol mixture to give 159 mg. of methyl gibberellate melting at 208—210° C. After a second crystallisation, the ester melted at 209—210° C. and had an optical rotation of $[\alpha]_D^{25} = 75$, when determined in a 0.5 percent solution in ethanol.

(B: A mixture of 125 mg. of gibberellic acid, 0.3 ml. of methyl iodide, 0.7 g. of anhydrous potassium carbonate and 7 ml. of dry acetone was heated under reflux for 8 hours. The acetone was distilled off and the residue washed with water and dried. Repeated crystallisation from a mixture of ethyl acetate and benzene gave methyl gibberellate melting at 204—205° C.

Of course, the other esters, as generally noted heretofore, can similarly be prepared.

The preparation of an ester from reaction of the gibberellic acid with an acyl-group-containing compound is illustrated by the following example, which shows the general method of preparation of such esters.

EXAMPLE III.

To a solution of 113 mg. of gibberellic acid in 1.8 ml. of pure dry pyridine was added 1 ml. of acetic anhydride. The mixture was left for 45 hours at room temperature and then the solution was placed on a water-bath and the solvents were rapidly distilled off *in vacuo*. The residue was dissolved in sodium bicarbonate solution, the solution filtered, and the filtrate acidified with hydrochloric acid. The resulting precipitate was collected by filtration and crystallised from a mixture of ethyl acetate and light petroleum (b.p. 60—80° C.) giving acetyl gibberellic acid as rhombs melting at 233—234° C. (decomposition) and having an optical rotation of $[\alpha]_D^{25} = 152$, when determined in a 0.5 percent solution in ethanol.

It should be stressed that other esters of gibberellic acid are within the scope of this invention, as, for example, the glycol and phenyl esters. The foregoing are merely by way of illustration.

In addition, it should be noted that various salts of gibberellic acid have been prepared. Particularly the alkali metal, alkaline earth metal and ammonium salts are of interest, as for example: sodium gibberellate sesquihydrate, indefinite m.p. at 230—238° C. (decomposition) (Found: C, 57.4; H, 6.15. $2C_{22}H_{36}O_6 \cdot Na \cdot 3H_2O$ requires C, 57.7; H, 6.12), potassium gibberellate sesquihydrate, indefinite m.p. at 225—229° C. (decomposition) (Found: C, 55.6; H, 5.7. $2C_{22}H_{36}O_6 \cdot K \cdot 3H_2O$ requires C, 55.5; H, 5.9), calcium gibberellate trihydrate, decomposition at 230—270° C. (Found: C, 58.7; H, 6.0. $C_{22}H_{36}O_6 \cdot Ca \cdot 3H_2O$ requires C, 58.2; H, 6.0).

6.2 and ammonium gibberellate monohydrate, m.p. 167—170° C. with decomposition. (Found: C, 59.8; H, 7.0. $C_{21}H_{34}O_8N$. H_2O requires C, 59.8; H, 7.1%.)

The following example illustrates a method for preparing the sodium salt of gibberellic acid. It can be appreciated that the other salts generally noted heretofore can be similarly prepared.

EXAMPLE IV.

A solution of gibberellic acid (94.03 mg.) in methanol which had been freshly distilled over sodium hydroxide (3 ml.) was treated dropwise with the calculated volume (10.93 ml.) of approximately 0.02N carbonate-free, aqueous sodium hydroxide, the volume being calculated on the basis of a parallel titration, to phenolphthalein, of an aliquot portion (58.20 mg.) of gibberellic acid which required 6.76 ml. of the alkali solution for neutralisation. The first mentioned solution of sodium gibberellate was rapidly evaporated under reduced pressure at room temperature. The residual sodium salt was dried for 7 days in a vacuum desiccator over phosphorus pentoxide; it consisted of transparent flakes, which disintegrate at ca. 160° C. to a white powder becoming yellow-brown at 210—215° C. and melting at 230—238° C. with decomposition, of sodium gibberellate sesquihydrate (Found: C, 57.4; H, 6.15. $2C_{21}H_{34}O_8Na$, $3H_2O$ requires C, 57.7; H, 6.12%).

Examples of other metallic salts of gibberellic acid are as follows: — rubidium gibberellate, m.p. 213—216° (Found: C, 53.1; H, 5.5. $C_{21}H_{34}O_8Rb$ requires C, 53.0; H, 5.0%), silver gibberellate dihydrate, m.p. 199° with previous decomposition (Found: C, 46.7; H, 5.1; Ag, 21.7. $C_{21}H_{34}O_8Ag$, $2H_2O$ requires C, 46.6; H, 5.2; Ag, 22.1%), manganese gibberellate trihydrate, m.p. 261—266° with previous decomposition (Found: C, 57.5; H, 5.8. $C_{21}H_{34}O_8Mn$, $3H_2O$ requires C, 57.1; H, 6.0%), copper gibberellate trihydrate, m.p. 245—248° with previous decomposition (Found: C, 56.0; H, 5.7. $C_{21}H_{34}O_8Cu$, $3H_2O$ requires C, 56.4; H, 6.0%) and lead gibberellate monohydrate, m.p. 215—218° (decomposition) (Found: C, 49.8; H, 5.0. $C_{21}H_{34}O_8Pb$, H_2O requires C, 49.8; H, 4.8%).

Gibberellic acid, and its functional derivatives which are salts and esters, particularly acetyl gibberellic acid, diacetyl gibberellic acid, butyryl gibberellic acid and monobenzoyl gibberellic acid have the property of promoting the growth of plants, as is shown by the following description of experiments on wheat and pea seedlings and grass.

When wheat seedlings are grown in water

culture and gibberellic acid is added to the culture solution in proportion of from 1—10 parts per million of solution, the seedlings grow taller than untreated plants. Increases in height of the order of 50% are common, the increase being due to (a) a great increase in length of the mesocotyl and (b) a smaller increase in the length of later internodes and of leaves. The leaves, although larger, are narrower than those of untreated plants. The enlarged plants are paler in colour than untreated plants in solutions of low nutrient status but this effect can be offset by increasing the concentration of nutrients in the culture solution.

Results from four experiments, where gibberellic acid was supplied in the culture solution at 5 p.p.m., are summarised in Table V. It will be seen that (a) fresh weight and dry weight of shoots are fairly consistently increased; (b) fresh weight and dry weight of roots are reduced, particularly in the low-level nutrient. The original data from which Table V was compiled also showed that the weight increases in the shoot are usually far greater than the losses in weight of the roots, so that the net effect of gibberellic acid on the whole plant is to increase fresh weight and dry weight. If the shoots alone are considered, the dry weight increases are due partly to redistribution of dry matter between root and shoot, and partly due to greater dry matter synthesis by the plant. The carbon, nitrogen, phosphorus and ash contents of equal weights of material from treated and untreated plants do not differ greatly, though there appears possibly to be an increase in the percentage carbon content and a slight fall in ash, nitrogen and phosphorus. In any case, there is an undoubted great increase in the carbon assimilated per plant. This is a point of some interest; it could be due to the treated plants having a greater leaf area in which photosynthesis could take place or it could be due to an increase in the rate of carbon assimilation per unit area of leaf. In experiments where the necessary estimates of leaf area have been made it appears that the increased carbon assimilation is due to increased leaf area. There are qualitative differences in the carbon components of the treated plants. For example the total carbohydrate soluble in 80% ethanol is increased by about 75% over controls, and by chromatography of sap expressed from cytolyzed plants, it has been found that the sucrose content is increased by about 25% and fructose and glucose concentration by about 100%. It appears, therefore, that, as a result of the treatment, glucose replaces sucrose as the main sugar constituent of the plants.

TABLE V.
WHEAT mean percentage increase or decrease per seedling after 3 weeks growth resulting from gibberellic acid treatment.

5	Expt.	Nutrient level	Height	FW shoot	DW shoot	FW root	DW root
	G0	low	44	—	5	—	24**
		high	80	—	39**	—	3
	G1	low	86**	9	24**	24**	15**
		high	108**	37*	33**	13**	0
10	G2	low	74**	21	26**	12	17**
		high	76**	11	14**	25	14**
	G3	low	93**	17	16**	35	34**
		high	69**	5	2	2	16
		** statistically significant (P = .01)					
15		* statistically significant (P = .05)					
		not statistically analysed					
		FW fresh weight					
		DW dry weight					

20 The effect of gibberellic acid on pea seedlings growing in water culture is even more striking than the effect on wheat seedlings. The height of the plant is greatly increased—in some experiments the seedlings produced have been five times the height of control plants.

25 This is due to a great increase in the length of internodes. The plants are much paler in colour than control plants.

The effects on the chemical composition of pea seedlings is similar to the effects on wheat seedlings. There is a great increase in fresh weight of shoots and a smaller, but still considerable increase in dry weight. Fresh weight increased carbon assimilation is due to in- and dry weight of roots falls as a result of gibberellic acid treatment. There is an increase in the amount of carbon, nitrogen, ash, phos-

phorus and potassium per plant in the shoots, though in terms of percentage of dry weight the content of all these except carbon fell slightly. In the roots all these fall in amount.

The amount of carbohydrate soluble in 80% ethanol increased, as a result of gibberellic acid treatment, by about 30% taking the plant as a whole, by 40% if the shoots only were considered. As in the case of wheat, the glucose content of expressed sap was markedly increased.

Reduced root growth is not necessarily obtained under all conditions. For example when gibberellic acid is applied to pea shoots, in a lanoline paste, increased weight of both roots and shoots is obtained.

The effect on pea seedlings of some derivatives of gibberellic acid is shown in Table VI.

55	Compound	Applied through roots	Applied through leaves
	Acetyl gibberellic acid	high activity (equal to GA)	high activity (equal to GA)
	Diacetyl gibberellic acid	high activity	high activity
	Methyl acetyl gibberellate	moderate activity	slight activity
60	Monobenzoyl gibberellic acid	high activity	high activity (equal to GA)
	Ethyl gibberellate	moderate activity	inactive
	Butyl gibberellate	moderate activity	inactive
	Butyl acetylgibberellate	high activity	inactive
	iso-propyl acetylgibberellate	moderate activity	inactive
65	iso-propyl gibberellate	high activity	inactive
	Butyryl gibberellic acid	high activity	high activity (equal to GA)
	Octyl gibberellate	moderate activity	inactive
	Ethyl acetylgibberellate	high activity	inactive
	Phenyl acetylgibberellate	high activity	not tested
70	Sodium gibberellate sesquihydrate	high activity	high activity (equal to GA)
	Potassium gibberellate sesquihydrate	high activity (equal to GA)	high activity (equal to GA)
	Calcium gibberellate sesquihydrate	high activity (equal to GA)	high activity (equal to GA)

Applied through roots in culture solution containing the compound at a concentration of 10 p.p.m.

75 Applied through leaves in doses of 10 μ /plant in .002 ml alcohol
GA, gibberellic acid.

The degree of activity of gibberellic acid derivatives appears to fall off with increasing substitution. For example, when gibberellic acid is esterified with higher alcohols the degree of activity falls off as may be seen in Table VI, though the octyl ester still retains some activity. On the other hand, although acetyl gibberellic acid is as active as gibberellic acid itself, as this substituted acid is esterified with alcohols of higher molecular weight so the activity of the ester falls off, as will be noted in Table VI, until it is negligible, in this particular case, in octyl acetyl gibberellate. Again, although diacetyl gibberellic acid is quite active, the methyl ester is substantially inactive.

Where a derivative is active when applied through the roots but not when applied through the leaves this may in fact be due to hydrolysis of the derivative in the soil yielding free gibberellic acid.

The fact that great increases in yield of dry matter may be obtained by treatment of wheat and pea seedlings with very small quantities of gibberellic acid is of considerable practical importance. Experiments in which grass crops were treated with gibberellic acid have confirmed that similar increases in dry matter yield can be obtained under field conditions.

In a pasture trial it was found that applications of gibberellic acid (1—4 oz./acre) as an aqueous spray brought about an increase in the dry matter yield of herbage at the first cut. (See following Table VII). 0, 1, 2, 3 and 4 oz. gibberellic acid/acre were applied along with 0, 3 and 6 cwt/s fertiliser* per acre to 1/600th acre plots on 13th April 1954 and the plots harvested on 11 May, 1954.

TABLE VII.

Mean Dry Matter Yields of Herbage (cwt./acre)
Gibberellic Acid (oz./acre)

Fertiliser (cwt./acre)	0	1	2	3	4
0	10.03	16.41	18.39	19.16	18.72
3	14.75	19.87	22.16	21.65	21.85
6	18.29	21.72	21.81	22.42	23.27

* The fertiliser was one containing 12% N, 12% total P₂O₅ (11.9% water soluble) and 15% K₂O.

Although substantial increases in dry matter yield were obtained without application of fertiliser, the grass plants were yellow and mechanically weak. However the plants on the gibberellic acid plus fertiliser (6 cwt./acre) plots were normal in appearance.

In general, it has been found that, in order to obtain the most beneficial results from the growth-promoting properties of gibberellic acid, treatment with the acid should be accompanied by treatment with additional quantities

of plant nutrients, particularly nitrogen, which may be derived from a mixed fertiliser as above or from a source of nitrogen such as urea, sulphate of ammonia and nitrate of ammonia. A suitable rate of application of nitrogen which will substantially reduce or prevent the yellowing of the foliage on stimulation of growth is from 40—80 lb. N/acre. A preferred combination is one in which the ratio of active compound to nitrogen is from 1:320 to 1:640.

One of the most important aspects of the present invention in relation to increased production of grass is that applications of gibberellic acid or an active derivative to grass land in the early spring, autumn or winter will cause the grass to grow under conditions of low light intensity and low temperature, i.e., conditions under which grass could not be stimulated into growth merely by application of a fertiliser. As a result, it is possible to extend the grass growing season and so obtain an earlier "bite" for livestock or an earlier cut for hay or silage. The simultaneous application of fertiliser leads of course to earlier use of the fertiliser in the earlier growth.

The gibberellic acid or an active derivative thereof may be applied in a dry form, i.e., as a powder, in admixture with a diluent which may be inert, e.g., china clay, fuller's earth, kieselguhr or lime; or which may contain a plant nutrient, e.g., mixed fertiliser or sulphate or nitrate of ammonia. Alternatively it may be applied as a spray, the active constituent then being dispersed in water. The aqueous dispersion may additionally contain plant nutrients such as urea or an ammonium salt.

Preferably the active constituent is so applied that its main effect is achieved by absorption through the foliage rather than through the roots since in the latter circumstances it may depress the rate of root growth. Preferably therefore it is applied as a spray, more preferably as a low volume spray, say at a rate of 10 or 20 gallons of spray to the acre to give a distribution of 2 oz./acre of gibberellic acid or equivalent of active derivative.

A convenient method of preparing an aqueous dispersion of gibberellic acid or an active derivative is to dissolve the acid or derivative in a water-miscible solvent and pour the solution with stirring into water.

Although we have described processes for isolating pure gibberellic acid from cultures of *Gibberella fujikuroi*, the pure material being required for chemical analysis and general identification of the acid, it will be obvious that for agricultural purposes it will not be necessary to isolate the pure acid, a crude concentrate being adequate for treatment of plants or of ground in which plants are growing or are to grow.

What we claim is—

1. Gibberellic acid
2. Functional derivatives which are salts and



* C L M S *

The degree of activity of gibberellic acid derivatives appears to fall off with increasing substitution. For example, when gibberellic acid is esterified with higher alcohols the degree of activity falls off as may be seen in Table VI, though the octyl ester still retains some activity. On the other hand, although acetyl gibberellic acid is as active as gibberellic acid itself, as this substituted acid is esterified with alcohols of higher molecular weight so the activity of the ester falls off, as will be noted in Table VI, until it is negligible, in this particular case, in octyl acetyl gibberellate. Again, although diacetyl gibberellic acid is quite active, the methyl ester is substantially inactive.

Where a derivative is active when applied through the roots but not when applied through the leaves this may in fact be due to hydrolysis of the derivative in the soil yielding free gibberellic acid.

The fact that great increases in yield of dry matter may be obtained by treatment of wheat and pea seedlings with very small quantities of gibberellic acid is of considerable practical importance. Experiments in which grass crops were treated with gibberellic acid have confirmed that similar increases in dry matter yield can be obtained under field conditions.

In a pasture trial it was found that applications of gibberellic acid (1—4 oz/acre) as an aqueous spray brought about an increase in the dry matter yield of herbage at the first cut. (See following Table VII). 0, 1, 2, 3 and 4 oz. gibberellic acid/acre were applied along with 0, 3 and 6 cwt/s fertiliser* per acre to 1/600th acre plots on 13th April 1954 and the plots harvested on 11 May, 1954.

TABLE VII.

Mean Dry Matter Yields of Herbage (cwt/s/acre)
Gibberellic Acid (oz/acre)

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* The fertiliser was one containing 12% N, 12% total P.O. (11.9% water-soluble) and 15% K.O.

Although substantial increases in dry matter yield were obtained without application of fertiliser, the grass plants were yellow and mechanically weak. However the plants on the gibberellic acid plus fertiliser (6 cwt/acre) plots were normal in appearance.

In general, it has been found that, in order to obtain the most beneficial results from the growth-promoting properties of gibberellic acid, treatment with the acid should be accompanied by treatment with additional quantities

of plant nutrients, particularly nitrogen, which may be derived from a mixed fertiliser as above or from a source of nitrogen such as urea, sulphate of ammonia and nitrate of ammonia. A suitable rate of application of nitrogen which will substantially reduce or prevent the yellowing of the foliage on stimulation of growth is from 40—80 lb. N/acre. A preferred combination is one in which the ratio of active compound to nitrogen is from 1:320 to 1:640.

One of the most important aspects of the present invention in relation to increased production of grass is that applications of gibberellic acid or an active derivative to grass land in the early spring, autumn or winter will cause the grass to grow under conditions of low light intensity and low temperature, i.e., conditions under which grass could not be stimulated into growth merely by application of a fertiliser. As a result, it is possible to extend the grass growing season and so obtain an earlier "bite" for livestock or an earlier cut for hay or silage. The simultaneous application of fertiliser leads of course to earlier use of the fertiliser in the earlier growth.

The gibberellic acid or an active derivative thereof may be applied in a dry form, i.e., as a powder, in admixture with a diluent which may be inert, e.g., china clay, fuller's earth, kieselguhr or lime; or which may contain a plant nutrient, e.g., mixed fertiliser or sulphate or nitrate of ammonia. Alternatively it may be applied as a spray, the active constituent then being dispersed in water. The aqueous dispersion may additionally contain plant nutrients such as urea or an ammonium salt.

Preferably the active constituent is so applied that its main effect is achieved by absorption through the foliage rather than through the roots since in the latter circumstances it may depress the rate of root growth. Preferably therefore it is applied as a spray, more preferably as a low volume spray, say at a rate of 10 or 20 gallons of spray to the acre to give a distribution of 2 oz/acre of gibberellic acid or equivalent of active derivative.

A convenient method of preparing an aqueous dispersion of gibberellic acid or an active derivative is to dissolve the acid or derivative in a water-miscible solvent and pour the solution with stirring into water.

Although we have described processes for isolating pure gibberellic acid from cultures of *Gibberella fujikuroi*, the pure material being required for chemical analysis and general identification of the acid, it will be obvious that for agricultural purposes it will not be necessary to isolate the pure acid, a crude concentrate being adequate for treatment of plants or of ground in which plants are growing or are to grow.

What we claim is:—

1. Gibberellic acid
2. Functional derivatives which are salts and

esters of gibberellic acid having plant growth promoting activity.

3. Acylated gibberellic acid wherein the acyl group is acetyl, butyryl or benzoyl.

4. Alkyl esters of gibberellic acid wherein the alkyl group contains up to eight carbon atoms.

5. Salts of gibberellic acid.

6. Alkali metal and alkaline earth metal salts of gibberellic acid.

7. Derivatives of gibberellic acid as hereinbefore described with reference to Table VI.

8. A culture broth of *Gibberella fujikuroi* containing gibberellic acid.

9. A process of producing gibberellic acid comprising cultivating an active strain of *Gibberella fujikuroi* in an aqueous medium and continuing the cultivation after active growth has ceased.

10. A process as claimed in Claim 9 wherein the ratio of carbon source to nitrogen source in the aqueous medium is greater or less than that ratio in which they are taken up in active growth of the fungus so that one or other is in excess when active growth ceases.

11. A process as claimed in Claim 10 in which the carbon source is in excess.

12. A process as claimed in Claim 11 in which the nutrient contains from 0.2—0.3% ammonium nitrate (or equivalent source of nitrogen) and from 10—30% glucose monohydrate (or equivalent source of carbon).

13. A process as claimed in any one of Claims 9—12 wherein the culture is filtered and the filtrate is extracted with an adsorbent.

14. A process as claimed in Claim 13 in which the adsorbent is activated charcoal.

15. A process of producing gibberellic acid substantially as hereinbefore described with reference to Example I.

16. A process of producing an active ester of gibberellic acid substantially as hereinbefore described with reference to Example II.

17. A process of producing an active acylated derivative of gibberellic acid substantially as

hereinbefore described with reference to Example III.

18. A process of producing an active salt of gibberellic acid substantially as hereinbefore described with reference to Example IV.

19. Compounds produced by a process claimed in any one of Claims 15—18.

20. A composition for promoting plant growth comprising a compound as claimed in any one of Claims 1—7 and 19 and a diluent.

21. A composition as claimed in Claim 20 in which the diluent is inert.

22. A composition claimed in Claim 20 in which the compound is dispersed in water as diluent.

23. A process of producing a composition as claimed in Claim 22 in which the compound is first dissolved in a water-miscible solvent, the solution then being dispersed in water.

24. A solution in a water-miscible solvent of a compound as claimed in any one of Claims 1—7 and 19.

25. A composition as claimed in Claim 20 in which the diluent comprises a source of nitrogen for the plants to be treated.

26. A composition as claimed in Claim 25 in which the diluent comprises a mixed fertiliser.

27. A composition as claimed in Claim 25 in which the source of nitrogen is urea.

28. A composition as claimed in Claim 25 in which the source of nitrogen is an ammonium salt.

29. A composition as claimed in Claim 22 and in Claim 27 or 28 in which the source of nitrogen is dissolved in the water.

30. A composition for promoting plant growth as claimed in any one of Claims 25—29 in which the active compound and the source of nitrogen are present in such a proportion that the ratio of active compound to nitrogen is from 1:320 to 1:640.

ALFRED O. BALL.

Agent for the Applicants.

PROVISIONAL SPECIFICATION

No. 19238 A.D. 1954

Gibberellic Acid and Derivatives thereof

We, IMPERIAL CHEMICAL INDUSTRIES LIMITED, a company organised under the laws of Great Britain, of Imperial Chemical House, Millbank, London, S.W.1, and PERCY WRAGG BRIAN and MARGARET EDITH RADLEY and PHILIP JOHN CERTIS and GEORGE WYNNE ELSON, all British subjects of The Frythe, Welwyn, Hertfordshire, do hereby declare this invention to be described in the following statement:—

This invention relates to a new organic acid having plant growth promoting properties, and to derivatives thereof, in particular salts and esters. The invention further relates to a

method of producing the acid by a metabolic process.

We have found that from cultures of *Gibberella fujikuroi* (Sawada; Wollenweber; *Fusarium moniliforme* Sheldon) there may be extracted a new organic acid having the property of promoting plant growth. This acid is hereinafter termed gibberellic acid.

The present invention therefore provides a new chemical compound, gibberellic acid, such as is obtainable from a culture of *Gibberella fujikuroi*, the acid being characterised by the following properties:—



* C L M S *

Melting point	233-235	vigorous gas evolution
Optical Rotation	[α] _D +83	in methanol
5 Monomethyl ester	m.p. 207-209	
<i>p</i> -Bromophenacyl ester	m.p. 218-219	
Hydrolysis with boiling mineral acid	1 mole carbon dioxide evolved	
Acetyl derivative	m.p. 233-234	dec.
10 Methyl acetyl gibberellate	m.p. 180-181	
Infra-red absorption curve as shown in the accompanying drawing		
X-ray powder diffraction lines as shown in Table I.		
15		

TABLE I
X-ray Powder Diffraction Lines of Gibberellic Acid (ex ethyl acetate)
Spacing "d" Intensity "I"

20	10.20	5 b
	8.73	2 b
	7.75	5 b
	6.69	w
	6.01	5 b
25	5.31	10 b
	5.06	1/2
	4.84	3 1/2
	4.60	7
	4.33	3
30	4.03	3
	3.85	w
	3.714	3
	3.591	3
	3.389	1 1/2 b
35	3.214	1
	3.085	w
	2.993	1 1/2
	2.882	1 1/2
	2.786	1
40	2.687	1
	2.549	2
	2.485	2
	2.418	w
	2.350	1
45	2.304	1
	2.249	w
	2.172	1
	2.120	1
	2.040	w
50	2.003	w
	1.971	w
	1.936	w
	1.920	w
	1.863	w
55	1.853	w
	1.780	w
	1.698	w

w denotes a weak reflection

b denotes a broad diffuse reflection

The third figure beyond the decimal point is barely significant.

The invention also provides derivatives of gibberellic acid, in particular salts and esters thereof.

The present invention also provides mixtures having plant growth promoting properties comprising gibberellic acid, or an active derivative thereof, and a diluent.

Further, the present invention provides a process of producing gibberellic acid, or mixtures containing said acid, from a culture of *Gibberella fujikuroi*.

Gibberellic acid is produced by *Gibberella fujikuroi* when grown as a surface culture or as a deep stirred and aerated culture in a suitable aqueous medium. A suitable medium must contain a carbon source (e.g. sucrose, glucose or glycerol at 2-10% w/v), an ammonium salt or a nitrate or a digest of protein (e.g. peptone) as nitrogen source (to give a concentration of N in the medium of 0.01-0.5%), a concentration of 0.04% being generally suitable; a magnesium salt (e.g. magnesium sulphate heptahydrate at 0.05% or thereabouts, which also conveniently adds sulphate which is also necessary but which may be added in some other form if another salt of magnesium is used); a phosphate (e.g. potassium dihydrogen phosphate at 0.05-0.5%, 0.1% being a suitable concentration); a potassium salt (which may be conveniently the phosphate, see above) and traces (ca. 1-10 p.p.m. of metal) of salts of iron, copper, zinc, manganese and a trace (1-10 p.p.m. of metal) of a molybdate.

Fermentations can be conveniently carried out at a temperature in the range 25-33°C.

Gibberellic acid has been produced in still and in stirred culture using Raulin Thom medium containing sucrose as the carbon source. For still culture this medium was dispensed in one litre portions in earthenware vessels and the cultures incubated for about ten days at 25°C. Incubation of stirred cultures lasted three to five days at 25°C. The culture filtrate, of pH 4 or less (adjusted with N-hydrochloric acid if necessary), was extracted with 10 g./l. of B.D.H. activated charcoal. The air dried charcoal (containing 20 to 30% moisture) was continuously eluted with acetone, and the eluate evaporated under reduced pressure to give an aqueous concentrate which was extracted twice with at least an equal volume of ethyl acetate. The combined ethyl acetate solutions were extracted twice with 25% of their volume of phosphate buffer pH 6.3 (36 g. KH₂PO₄ and 24 g. KOH in 1 l. of water). The buffer extracts were combined, adjusted to pH 3.8, and extracted twice with an equal volume of ethyl acetate. On concentrating these extracts, crude gibberellic acid crystallised in yields of about 40 mg./l. The crude acid was purified by adding an equal volume of light petroleum (60-80°C) to a solution in boiling ethyl acetate.

Repetition of this process gave pure gibberellic acid as jagged lathes decomposing at 233—235 °C. [α]_D²⁰ +82° (c, 2.12 in ethanol) (Found: C, 65.9; H, 6.6; O, 6.55; Eq. wt., 335, 342. $C_{30}H_{48}O_7$ requires C, 65.9; H, 6.4%. Eq. wt. (monobasic, 346). The molecular weight has been confirmed by the x-ray method. The unit cell was tetragonal and had $a = b = 10.905$, $c = 28.74$ Å. The density was 1.34 ± 0.01 , whence the molecular weight (8 molecules per unit cell) is 345.

Gibberellic acid gives a methyl ester, m.p. 207—209 °C (Found: C, 66.65; H, 6.85; OMe, 8.5. $C_{31}H_{50}O_7$ requires C, 66.65; H, 6.7; OMe, 8.6%). with either diazomethane or methyl iodide and potassium carbonate in acetone. The *p*-bromophenacyl ester has m.p. 218—219 °C (Found: C, 57.65; H, 5.0; Br, 14.35. $C_{31}H_{47}O_7Br$ requires C, 57.75; H, 5.2; Br, 14.2%). Acetylgibberellic acid, m.p. 233—234 °C (dec.) (Found: C, 65.1; H, 6.35. $C_{32}H_{50}O_8$ requires C, 64.9; H, 6.2%), prepared with acetic anhydride in pyridine at room temperature, yields a methyl ester, m.p. 180—181 °C (Found: C, 65.3; H, 6.7. $C_{32}H_{52}O_8$ requires C, 65.65; H, 6.5%), also obtained by acetylating methyl gibberellate.

Direct extraction of the culture filtrate with organic solvent will remove gibberellic acid but not so effectively as when charcoal is used.

To obtain maximum yields in stirred cultures it is necessary to carry fermentation on for what is a long time by usual standards. This is shown by the following yield/time data for 100 litre batch of Raulin-Thom medium containing 4% sucrose, initially adjusted to pH 3.5 and at 25 °C.

	Hrs. fermentation	Yield (mg./l.)
	93	18
40	117	33
	141	39
	164	82
	187	105
	212	92
45	236	138
	262	160
	284	189
	331	193
	402	249

Gibberellic acid, and certain of its derivatives such as salts and esters particularly the methyl ester, has the property of promoting the growth of plants, as is shown by the following description of experiments on wheat and pea seedlings.

When wheat seedlings are grown in water culture and gibberellic acid is added to the culture solution in proportions of from 1—10

parts per million of solution, the seedlings grow taller than untreated plants. Increases in height of the order of 50% are common, the increase being due to (a) a great increase in length of the mesocotyl and (b) a smaller increase in the length of later internodes and of leaves. The leaves, although larger, are narrower than those of untreated plants. The enlarged plants are paler in colour than untreated plants in solutions of low nutrient status but this effect can be offset by increasing the concentration of nutrients in the culture solution.

Results from four experiments, where gibberellic acid was supplied in the culture solution at 5 p.p.m., are summarised in Table 2. It will be seen that (a) fresh weight and dry weight of shoots are fairly consistently increased; (b) fresh weight and dry weight of roots are reduced, particularly in the low-level nutrient. The original data from which Table 2 was compiled also showed that the weight increases in the shoot are usually far greater than the losses in weight of the roots, so that the net effect of gibberellic acid on the whole plant is to increase fresh weight and dry weight. If the shoots alone are considered, the dry weight increases are due partly to redistribution of dry matter between root and shoot, and partly due to greater dry matter synthesis by the plant. The carbon, nitrogen, phosphorus and ash contents of equal weights of material from treated and untreated plants do not differ greatly, though there appears possibly to be an increase in the percentage carbon content and a slight fall in ash, nitrogen and phosphorus. In any case, there is an undoubted great increase in the carbon assimilated *per plant*. This is a point of some interest: it could be due to the treated plants having a greater leaf area in which photosynthesis could take place or it could be due to an increase in the rate of carbon assimilation per unit area of leaf. In experiments where the necessary estimates of leaf area have been made it appears that the increased carbon assimilation is due to increased leaf-area. There are qualitative differences in the carbon components of the treated plants. For example the total carbohydrate soluble in 80% ethanol is increased by about 75% over controls, and by chromatography of sap expressed from cytolysed plants, it has been found that the sucrose content is increased by about 25%, and fructose and glucose concentration by about 100%. It appears therefore that, as a result of the treatment, glucose replaces sucrose as the main sugar constituent of the plants.

TABLE 2.

WHEAT: mean percentage increase or decrease per seedling after 3 weeks growth resulting from gibberellic acid treatment

5	Expt	Nutrient level	Height	FW shoot	DW shoot	FW root	DW root
	G0	low	44	—	5	—	24**
		high	80	—	39**	—	3
	G1	low	86**	9	24**	24**	15**
		high	108**	37*	33**	13**	0
10	G2	low	74**	21	26**	12	17**
		high	76**	11	14**	25	14**
	G3	low	93**	17	16**	35	34**
		high	69**	5	2	2	16

** statistically significant (P .01)

* statistically significant (P .05)

not statistically analysed

FW fresh weight

DW dry weight

The effect of gibberellic acid on pea seedlings growing in water culture is even more striking than the effect on wheat seedlings. The height of the plant is greatly increased—in some experiments the seedlings produced have been five times the height of control plants. This is due to a great increase in the length of internodes. The plants are much paler in colour than control plants.

The effects on the chemical composition of pea seedlings is similar to the effects on wheat seedlings. There is a great increase in fresh weight of shoots and a smaller, but still considerable increase in dry weight. Fresh weight and dry weight of roots falls as a result of gibberellic acid treatment. There is an increase in the amount of carbon, nitrogen, ash, phosphorus and potassium per plant in the shoots, though in terms of percentage of dry weight the content of all these except carbon fell slightly. In the roots all these fall in amount.

The amount of carbohydrate soluble in 80% ethanol increased as a result of gibberellic acid treatment, by about 30% taking the plant as a whole, by 40% if the shoots only were considered. As in the case of wheat, the glucose content of expressed sap was markedly increased.

Reduced root growth is not necessarily obtained under all conditions. For example when gibberellic acid is applied to pea shoots, in a lanoline paste, increased weight of both roots and shoots is obtained.

The fact that great increases in yield of dry matter may be obtained by treatment of wheat and pea seedlings with very small quantities of gibberellic acid is of considerable practical importance. Experiments in which grass crops were treated with gibberellic acid have confirmed that similar increases in dry matter yield can be obtained under field conditions.

In a pasture trial it was found that applications of gibberellic acid (1—4 oz/acre) as an aqueous spray brought about an increase in the dry matter yield of herbage at the first cut. (see following table 3). 0, 1, 2, 3 and 4 oz. gibberellic acid/acre were applied along with 0, 3 and 6 cwt/s* fertiliser per acre to 1/600th acre plots on 13th April 1954 and the plots harvested on 11th May, 1954.

TABLE 3.
Mean Dry Matter Yields of Herbage
(cwt/s/acre)
Gibberellic Acid (oz/acre)

Fertiliser (cwt/acre)	0	1	2	3	4
0	10.03	16.41	18.39	19.16	18.72
3	14.75	19.87	22.16	21.65	21.85
6	18.29	21.72	21.81	22.42	23.27

* The fertiliser was one containing 12% N, 12% total P₂O (11.9% water soluble) and 15% K₂O.

Although substantial increases in dry matter yield were obtained without application of fertiliser, the grass plants were yellow and mechanically weak. The plants on the gibberellic acid plus fertiliser (6 cwt/acre) plots were normal in appearance.

In general, it has been found that, in order to obtain the most beneficial results from the growth-promoting properties of gibberellic acid, treatment with the acid should be accompanied by treatment with additional quantities of plant nutrients such as fertilizer.

ALFRED O. BAEL
Agent for the Applicants.

PROVISIONAL SPECIFICATION

No. 34129 A.D. 1954

Gibberellic Acid and Derivatives thereof

We, IMPERIAL CHEMICAL INDUSTRIES LIMITED, a Company organised under the laws of Great Britain, of Imperial Chemical House, Millbank, London, S.W.1, and PERCY WRAGG BRIAN and MARGARET EDITH RADLEY and PHILIP JOHN CURTIS and GEORGE WYNNE ELSON, all British subjects, of The Frythe, Welwyn, Hertfordshire, do hereby declare this invention to be described in the following statement:—

This invention relates to a new organic acid having plant growth promoting properties and to a method of producing the acid by a metabolic process.

In our copending Application No. 19238/54 we have described a new organic acid, gibberellic acid, having the property of promoting plant growth. We have also described a process of producing the acid by cultivating a species of mould *Gibberella fujikuroi* in a suitable aqueous medium.

We have now found that not all strains of the fungus produce gibberellic acid. Results of tests carried out on a variety of strains do indicate however that strains isolated from rice seedlings infected with the "bakanae" disease are more likely to produce gibberellic acid than strains isolated from other host plants such as cotton, maize and pineapple. Of strains found on sugar cane some are active in producing gibberellic acid, others are not. Strains of the fungus which produce the acid are morphologically indistinguishable from strains which produce none, and whether or not a strain is active can be determined only by growing it and testing the culture.

The present invention therefore provides a culture of *Gibberella fujikuroi* containing gibberellic acid.

Strains of *Gibberella fujikuroi* which do produce gibberellic acid are the so-called "Sawada" and "Miyake" strains obtainable from the Centraalbureau voor Schimmelcultures at Baarn, Holland. Four strains numbered 1001, 1004, 1135 and 1139 in our collection of moulds are also productive of gibberellic acid. Samples of these strains

have been deposited with the Centraalbureau and with the Commonwealth Mycological Institute, Kew, Surrey. Cultures of these strains when grown as described in Application No. 19238/54 will contain gibberellic acid which may be extracted also as described in that application.

We have also found that the time at which gibberellic acid begins to accumulate in a culture of a suitable strain of *Gibberella fujikuroi* and the final yield of gibberellic acid are influenced by the concentrations of the carbon source and nitrogen source and by the ratio of these two concentrations in the medium used.

The following Examples are given in illustration.

A gibberellic acid producing strain of the mould was grown in 30 litre batches of a medium of the following composition:

magnesium sulphate	30 gm.	
potassium dihydrogen phosphate	150 gm.	
minor element concentrate*	60 ml.	70
glucose monohydrate	} see below	
ammonium nitrate		
water	30 l.	

*The composition of the minor element concentrate was

FeSO ₄ ·7H ₂ O	0.1 gm.	75
CuSO ₄ ·5H ₂ O	0.015 gm.	
ZnSO ₄ ·7H ₂ O	0.1 gm.	
MnSO ₄ ·7H ₂ O	0.01 gm.	
K ₂ MoO ₄	0.01 gm.	
water	100 ml.	80

The medium was stirred, aerated at a rate of 10 litres/minute and maintained at a temperature of 25° C. The pH value of the medium was adjusted to 4.5 before sterilizing.

Yields of gibberellic acid (mg./l.) at various times during fermentation on media containing different concentrations of glucose and ammonium nitrate are given in the following tables.

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		Yield of gibberellic acid (mg./l.) Concentration of NH_4NO_3 (g./l.)			
	Glucose Monohydrate	Hours or fermentation	1.2	2.4	4.8 9.6
5	16%	120	—	—	—
	"	161	19	—	—
	"	195	—	—	—
	"	212	—	59	—
	"	219	40	—	—
10	"	258	61	146	44 49
	"	330	102	114	204 5
	"	402	139	312	204 4
15	4%	138	—	—	—
	"	168	—	—	—
	"	185	65	34	—
	"	210	—	—	4 5
	"	282	100	71	8 8
	"	305	107	71	5 7
20	2%	100	—	—	—
	"	114	—	—	—
	"	137	—	5	—
	"	161	41	5	2 —
	"	185	42	7	—

25 These results show that (a) yield appears to increase with increasing glucose concentration in the medium; (b) the optimum nitrogen concentration increases with the increasing glucose concentration, 2.4 g. NH_4NO_3 /l. being optimal with 16% glucose, 1.2 g. NH_4NO_3 /l. being optimal for 4%, and 2% glucose (of course the actual optima may lie between the values tested); (c) in media of any given glucose concentration production of gibberellic acid commences soonest in media of low nitrogen concentration.

35 Similar results are obtained with a glucose monohydrate concentration as high as 20% w/v. Tests carried out on wheat and pea seedlings as described in our copending application No. 19238/54 show that the mono-

acetyl derivative of gibberellic acid referred to in that application is as effective as gibberellic acid itself in promoting plant growth.

45 In our copending application we have described processes for isolating pure gibberellic acid from cultures of *Gibberella fujikuroi*, the pure material being required for chemical analysis and general identification of the acid. It will be obvious however that for agricultural purposes it will not be necessary to isolate the pure acid, a crude concentrate being adequate for treatment of plants or of ground in which plants are growing or are to grow.

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ERRATUM

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