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PLANT HORMONES—I

ISOLATION OF GIBBERELLIN A_1 AND GIBBERELLIN A_5 FROM *PHASEOLUS MULTIFLORUS*

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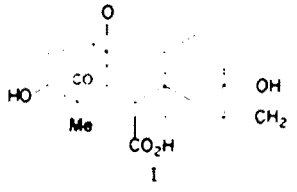
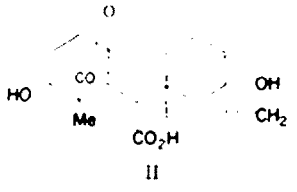
Abstract—The isolation of the growth-promoting acids, gibberellin A_1 and gibberellin A_5, from immature seed of *Phaseolus multiflorus* is described. The structure of gibberellin A_5 is established as V by degradation and by its preparation from gibberellin A_1.

FOUR gibberellins have been isolated from the fungus, *Gibberella fujikuroi*: gibberellic acid (I), gibberellin A_1 (II), gibberellin A_2 (III), and gibberellin A_4 (IV).[1]



I



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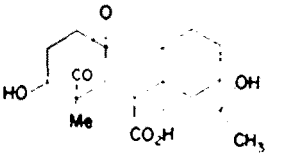

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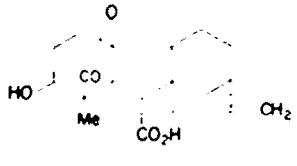


IV

The growth responses induced in plants by these fungal metabolites are essentially normal responses. This fact and other more specific arguments[2-4] led to the suggestion that the gibberellins or physiologically similar substances are growth-regulating hormones occurring in higher plants. This suggestion, supported by the isolation[1] from many plant sources of crude extracts with gibberellin-like biological properties, has now been confirmed by the isolation of two pure hormones, gibberellin A_1 (II) and a new gibberellin, gibberellin A_5 (V).

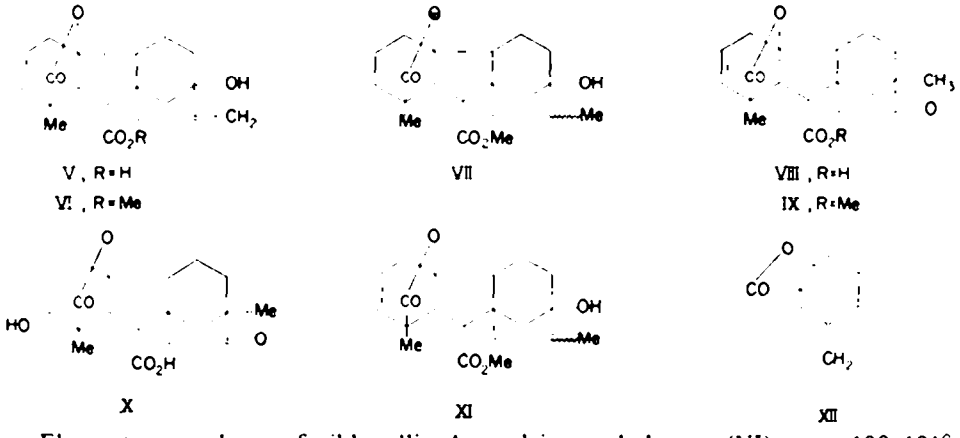
Since our brief note[5] on the isolation of gibberellin A_1 from immature seeds of *Phaseolus multiflorus*, this gibberellin has been isolated from seed of *P. vulgaris* by

[1] For review see P. W. Brian, J. F. Grove and J. MacMillan, *The Gibberellins*, in Zechmeister, *Prog. Chem. Org. Nat. Prod.* **18**, 350 (1960).
[2] M. Radley, *Nature, Lond.* **178**, 1070 (1956).
[3] P. W. Brian, *Symp. Soc. Exp. Biol.* **11**, 166 (1957).
[4] F. Lona, *Nuovo. G. Bot. Ital.* **63**, 61 (1956).
[5] J. MacMillan and P. J. Suter, *Naturwissenschaften* **45**, 46 (1958).

West *et al.*[6-8] and from water sprouts of *Citrus unshui* by Karawada and Sumiki[9]. In addition to gibberellin A_1, West *et al.* isolated a second active acid, bean factor II, which they were unable to characterize fully due to lack of material. Bean factor II is now known to be identical (West[8] and below) with gibberellin A_5 (V), a growth-promoting acid whose isolation from the seed of *P. multiflorus* has been briefly described[10] by us. We now wish to report our work in full.

The extraction and purification procedures were followed by bio-assay using dwarf (Meteor) pea seedlings as described by Radley[11]. The crude acidic extract from immature seed of *P. multiflorus* was chromatographed on a column of celite-charcoal (cf. ref. 7) and was eluted stepwise with water containing increasing proportions of acetone. Elution with 55 per cent acetone in water gave gibberellin A_1 which was separated, by further chromatography on celite-silicic acid, from traces of an unknown acid, m.p. 207–209°. Gibberellin A_1 was identified by elementary analyses, infra-red spectrum, optical rotation and conversion to the methyl ester which was identified by mixed m.p. and infra-red spectrum. The melting point (Kofler) of gibberellin A_1 is variable, usually 256–260°, and is not a good criterion of purity; a higher melting modification, m.p. 285–287°, was obtained on one occasion. Gibberellin A_1 was isolated in a yield of about 2·0 mg per kilogram of fresh weight of seed.

Gibberellin A_5, m.p. 260–261°, $[\alpha]_D^{22}$ −77°, was eluted from the celite-charcoal column with 65–70 per cent acetone in water and was further purified by chromatography on celite-silicic acid; it was isolated in a total of 37 mg (ca 1·0 mg/kg of fresh weight of seed).



Elementary analyses of gibberellin A_5 and its methyl ester (VI), m.p. 190–191°, $[\alpha]_D^{25}$ 75°, indicated the molecular formula, $C_{19}H_{22}O_5$, for the acid. The infra-red spectrum of the acid showed absorption attributable to hydroxyl (3436 cm^{-1}), γ-lactone (1765 cm^{-1}), carboxylic acid (1734 cm^{-1}), exocyclic methylene (1659, 893 cm^{-1}) and *cis*-disubstituted olefinic (1624, 694 cm^{-1}) groupings. These assignments were confirmed by the infra-red spectrum of the methyl ester and account for all the oxygen functions.

[6] C. A. West and K. Murashige, *Plant. Physiol.* (Supplement) **33**, xxviii (1958).
[7] C. A. West and B. O. Phinney, *J. Amer. Chem. Soc.* **81**, 2424 (1959).
[8] C. A. West. Personal communication.
[9] A. Karawada and Y. Sumiki, *Bull. Agric. Chem. Soc. Japan* **23**, 343 (1959).
[10] J. MacMillan, J. C. Seaton and P. J. Suter, *Proc. Chem. Soc.* 325 (1959).
[11] M. Radley, *Ann. Bot.* **22**, 297 (1958).

The presence of two olefinic double bonds was confirmed by hydrogenation of the methyl ester which absorbed two molecules of hydrogen and gave a tetrahydro-derivative (VII), presumably (see ref.[1] and below) a mixture of C-8 epimers. Hydrogenation caused the disappearance of bands 1659, 1624 and 697 cm $^{-1}$ and a marked decrease in intensity of the band at 893 cm^{-1}; these changes are consistent with the above olefinic assignments.

Treatment of gibberellin A_5 with dilute hydrochloric acid gave a keto-acid (VIII) showing absorption at 1740 cm $^{-1}$, ascribed to a saturated five-ring carbonyl group, in addition to bands at 1770 (γ-lactone) and 1695 (carboxylic acid) cm^{-1}; the methyl ester (IX) showed carbonyl absorption at 1775, 1734 and 1721 cm $^{-1}$. The acid VIII and methyl ester IX showed absorption bands at 674 and 678 cm^{-1} respectively, ascribed to a *cis*-disubstituted double bond. The rearrangement of gibberellin A_5 to a keto-acid (VIII) appears to be analogous to the Wagner–Meerwein rearrangement[12] of gibberellin A_1 (II) to the keto-acid (X).[13,14]

Boiling collidine converted the monomesylate of the C-8 epimeric mixture of methyl tetrahydrogibberellates into an unsaturated ester (XI) which showed two pairs of carbonyl bands in the infra-red and is presumably a C-8 epimeric mixture. Hydrogenation of XI afforded the methyl ester (VII) of tetrahydrogibberellin A_5; a slight difference in melting points and insignificant differences in the infra-red spectrum are ascribed to different proportions of C-8 epimers in the two samples. Gibberellin A_5 is thus a 2-deoxygibberellin A_1 containing a *cis*-disubstituted double bond.

The complete absence of hydrogenolysis products in the catalytic reduction of gibberellin A_5 methyl ester indicated the absence of a double bond allylic to the lactone hydroxyl function (cf. gibberellic acid[14]). The olefinic double bond was located as in V by dehydration of the methyl ester of the rearrangement product X of gibberellin A_1, both directly with phosphoric oxychloride and indirectly via the *p*-toluene-sulphonyl derivative. The methyl ester IX obtained was identical with the methyl ester of the rearrangement product VIII of gibberellin A_5; acidic hydrolysis of IX gave the free acid VIII. In addition to IX, treatment of the methyl ester of X with phosphorus oxychloride gave the corresponding 2-chloro-derivative.

Structure V for gibberellin A_5 was confirmed by dehydration of gibberellin A_1 via the methyl ester of the mono-*p*-toluene sulphonyl derivative. The product, gibberellin A_5 methyl ester, yielded gibberellin A_5 on alkaline hydrolysis.

The formation of the anhydro-derivatives IX and V from the appropriate sulphonic esters supports the revised structure I for ring A of gibberellic acid.[14,15] Kitamura *et al.*[16] have briefly reported ring A anhydro-derivatives of gibberellin A_2 and of methyl tetrahydrogibberellate and have assigned the structure XII to them. Our results show that these derivatives should be regarded as Δ^2-enes.

All compounds with a Δ^2-double bond show absorption maxima at 220–225 mμ (ϵ, 1400) —due possibly to trans-annular interaction between the double bond and

[12] J. F. Grove, J. MacMillan, T. P. C. Mulholland and W. B. Turner, *J. Chem. Soc.* 3049 (1960).
[13] N. Takahashi, Y. Seta, H. Kitamura, A. Karawada and Y. Sumiki, *Bull. Agric. Chem. Soc. Japan* **21**, 75 (1957).
[14] B. E. Cross, *J. Chem. Soc.* 3022 (1960).
[15] B. E. Cross, J. F. Grove, J. MacMillan, J. S. Moffatt, T. P. C. Mulholland, J. C. Seaton and N. Sheppard, *Proc. Chem. Soc.* 302 (1959).
[16] H. Kitamura, N. Takahashi, Y. Seta and Y. Sumiki, *Bull. Agric. Chem. Soc. Japan* **22**, 434 (1958).

the lactone carbonyl group—and show a large ΔM_D (−380) compared with the corresponding saturated compounds.

Gibberellin A_5 and the bean factor II, described by West and Phinney[7], have been shown to be identical by direct comparison of their infra-red spectra. Gibberellin A_5 also shows the same differential growth-promoting activity as bean factor II on the dwarf mutants, *d*-1, *d*-3, and *d*-5 of *Zea mais* (see Table 1).*

TABLE 1. RELATIVE ACTIVITIES OF GIBBERELLIC ACID, GIBBERELLIN A_5, AND BEAN FACTOR II FOR THE MUTANTS, *d*-1, *d*-3, AND *d*-5 OF *Zea mais*

Mutant of *Zea mais*	Gibberellic acid	Gibberellin A_5	Bean factor II
d-1	100	10	5
d-3	100	500	300
d-5	100	100	90

EXPERIMENTAL

The following chromatographic materials were used: activated charcoal (B.D.H.), silica gel M.F.C. (Hopkins and Williams), and celite 545 (Johns-Mandeville). Unless otherwise stated, ultraviolet spectra were determined in ethanol, optical rotations in methanol, and infra-red spectra as "Nujol" mulls using a Grubb Parsons G.S.2 spectrophotometer. "Light petroleum" refers to the fraction of b.p. 60–80°.

By the term "worked up as usual" is meant washed successively with dil hydrochloric acid, sodium hydrogen carbonate solution, and water, dried with sodium sulphate and evaporated.

Extraction

Immature seed (87·3 kg) of *Phaseolus multiflorus* were deep-frozen and then extracted with 70% aqueous ethanol (2 × 40 l.) for 24 hr at room temp. The filtered extract was concentrated at 40–50° in a climbing film evaporator and the aqueous concentrate (3·0 l.; pH 5·4), after adjustment to pH 3 with 2 N HCl, was extracted with ethyl acetate (12 × 1 l.). Concentration of the ethyl acetate extract to ca. 500 ml gave fumaric acid (8·0 g), identified by m.p. and infra-red spectrum after sublimation at $150^\circ/10^{-3}$ mm and crystallization from water. The ethyl acetate mother liquors were then extracted with phosphate buffer (20 × 100 ml, pH 6·2), washed with water, dried, and evaporated giving a biologically inactive gum (37·5 g). The phosphate buffer extract, after adjustment to pH 3 with 3 N HCl (360 ml), was extracted with ethyl acetate (14 × 250 ml) which, on recovery, gave a biologically-active acidic gum (14·6 g). Attempted preparation of a crystalline cyclohexylamine salt, by addition of cyclohexylamine to the acidic gum in acetone, produced a gum; dissolution of the latter in water, acidification with 3 N HCl, and recovery in ethyl acetate gave a gum (12·0 g) which is referred to below as the *crude acidic extract*.

Chromatography

A. *Celite:charcoal.* The crude acidic extract (1·0–2·0 g) was adsorbed on silicic acid (5·0 g) by evaporation of an acetone solution and placed on top of a column (40 × 3 cm) of celite (60 g): charcoal (30 g). The column was eluted in 200 ml fractions, first with water then with water containing acetone increasing in 5% steps. Bio-assay showed that fraction 12, eluted with 55% acetone, possessed most of the biological activity.

These fractions were worked up by evaporation of the acetone and extraction of the aqueous residue with ethyl acetate (4 × 50 ml); recovery gave gums which were further chromatographed as follows.

B. *Celite: silicic acid.* The fractions from (A), adsorbed on silicic acid (2·0 g), were placed on

* We are grateful to Dr. C. A. West and Professor B. O. Phinney, University of California, Los Angeles, for making the comparisons of gibberellin A_5 and bean factor II and for their permission to quote these results.

top of a column (18 × 1·5 cm) of celite (10 g):silicic acid (5 g) and eluted in 50 ml fractions of chloroform containing ethyl acetate increasing in 5% steps.

Isolation of gibberellin A_1. Fraction 12 (40–50 mg), obtained as in A from the crude acid extract (1·0 g), crystallized from ethyl acetate–light petroleum in ill-defined plates or prisms (15–18 mg) m.p. 256–260° (decomp), of gibberellin A_1. (Found: C, 65·3; H, 6·9%; equiv. (potentiometric), 354. Calc. for $C_{19}H_{24}O_6$: C, 65·5; H, 6·9%; M, 348); $[\alpha]_D^{21}$ + 35° (*c*, 1·02 in ethanol). The infra-red spectrum was identical with that of an authentic specimen. The methyl ester, prepared with diazomethane, had m.p. and mixed m.p. 232–234° and showed infra-red absorption identical with that of an authentic specimen.

Fractions 12, from 3·5 g crude acidic extract, and from which gibberellin A_1 had been removed by crystallization were combined with the total fractions 12 from 3·5 g of crude acidic extract, and were chromatographed on celite:silicic acid as in B. The following fractions were collected:

(1) 10% ethyl acetate eluted azelaic acid (5·0 mg) m.p. and mixed m.p. 100–103°.

(2) 20–30% ethyl acetate gave a solid which crystallized from ether in prisms (2·0 mg) m.p. 207–209° of an unidentified acid; ν_{max} 3480, ca. 2700, 1718, 1684, ca. 1670, 1637, 1610 cm^{-1}. (Perkin-Elmer Infracord 137); λ_{max} 259 ($E_{1cm}^{1\%}$ 964).

(3) 40–50% ethyl acetate eluted gibberellin A_1, crystallizing from acetone–light petroleum in prisms (33 mg) m.p. 285–287°; recrystallization from the same solvent, and seeding with a specimen m.p. 235–240°, gave prisms m.p. 250–255°; seeding the crystallization of the latter with the form m.p. 285–287° did not raise the m.p.; all forms had the same infra-red spectrum (Perkin Elmer Infracord 137).

Isolation of gibberellin A_4. The combined fractions 14 (195 mg), from the chromatography of 4·0 g crude acidic extract as in A, were rechromatographed as in B. After elution with 50 ml chloroform and 50 ml 5% ethyl acetate, elution with 10% ethyl acetate (4 × 50 ml) afforded *gibberellin* A_4, crystallizing from acetone–light petroleum in prisms (27 mg) m.p. 260–261° (for characterization and degradation see below).

The combined fractions 15 (301 mg) from 7·0 g crude acidic extract were re-chromatographed as in B. Elution with 50 ml of 5 and 10% ethyl acetate and crystallization of the recovered gum from ether gave gibberellin A_6 as prisms (10 mg) m.p. 256–260°.

Gibberellin A_4: derivatives and degradation

Gibberellin A_4 (V). Gibberellin A_4 crystallized from ether or acetone–light petroleum in colourless prisms m.p. 260–261°, $[\alpha]_D^{27}$ −77° (*c*, 0·5) (Found: C, 68·7; H, 7·1. $C_{19}H_{22}O_5$ requires: C, 69·1; H, 6·7%): ν_{max} 3436, 2700 (broad), 1765, 1734, 1659, 1624, 893 and 694 cm^{-1}: λ (shoulder) 225 mμ, (log ε 3·15).

Gibberellin A_4 methyl ester (VI). Excess ethereal diazomethane was added to a solution of gibberellin A_4 in methanol. The *methyl ester*, obtained by evaporation, crystallized from ether or acetone–light petroleum in prisms m.p. 191–193°, $[\alpha]_{16}^{D}$ −75° (*c*, 0·5) (Found: C, 69·8; H, 7·2. $C_{20}H_{24}O_5$ requires: C, 69·75; H, 7·0%); ν_{max} 3475, 1754, 1730, 1659, 1624, 893 and 697 cm^{-1}, λ (shoulder) 225 mμ (log ε 3·28).

Hydrogenation of gibberellin A_4 methyl ester (VII). The methyl ester (4·23 mg) in glacial acetic acid (5 ml) with Adams' catalyst absorbed 0·57 ml hydrogen at 25° and 740 mm Hg (1·8 double bonds). After filtration, the solvent was evaporated and the *tetrahydro-derivative* (VII; probably a C-8 epimeric mixture) crystallized from ether–light petroleum in rods (4·0 mg) m.p. 205–207°, $[\alpha]_D^{25}$ −30° (*c*, 0·35); ν_{max} 3571, 1774, and 1706 cm^{-1}. For analyses see following section.

Treatment of gibberellin A_4 with dilute hydrochloric acid. Gibberellin A_4 (9·5 mg) in ethanolic hydrochloric acid (1·0 ml) (ethanol:water:concentrated hydrochloric acid, 3:2:1) was heated at 100° for 2·5 hr. After evaporation of the solvent *in vacuo*, the residue was crystallized from acetone–light petroleum giving the *keto-acid* (VIII) as prisms (2 mg) m.p. 263–265°; ν_{max} 1770, 1740, 1695, 674 cm^{-1}; λ_{max} 221, 275 mμ (log ε 3·17 and 2·0). For analyses see following section. *Esterification* with diazomethane gave the *methyl ester* (IX) which crystallized from ether–light petroleum in prisms m.p. 158–160°; ν_{max} 1775, 1734, 1721, 678 cm^{-1}. For analyses see following section.

Preparation of gibberellin A_4 and its derivatives from gibberellin A_1

The monomethane sulphonate of the methyl tetrahydrogibberellates (C-8 epimeric mixture). The mixture of C-8 epimers (200 mg) and methane sulphonyl chloride (0·1 ml) in pyridine (25 ml) were

allowed to stand at room temp for 65 hr. The solvent was evaporated under reduced pressure and the residue was dissolved in ethyl acetate which was worked up as usual. The residue was added to a column of alumina (16 × 1·5 cm) in benzene and eluted with benzene containing increasing proportions of chloroform giving (i) 1:1 benzene:chloroform; a colourless gum (80 mg); (ii) 45:55 benzene:chloroform; the *monomethane sulphonate* crystallizing from acetone-light petroleum in prisms (60 mg) m.p. 194–197° (Found: C, 56·9; H, 6·8. $C_{21}H_{30}O_8S$ requires: C, 57·1; H, 6·8%); ν_{max} 3559, 1778, 1724, 1351, 1176, 935 cm^{-1}.

The anhydro-ester (XI). The above monomethane sulphonate (20 mg) was refluxed in dry collidine for 6 hr. The solvent was removed under reduced pressure and the residue was dissolved in ethyl acetate and was worked up as usual. The crystalline residue when recrystallized from acetone-light petroleum gave the *anhydro-ester* (XI) as prisms (10 mg) m.p. 180–185° (Found: C, 69·1; H, 7·6. $C_{20}H_{24}O_8$ requires: C, 69·3; H, 7·6%), ν_{max} 3525, 3505, 1777, 1762, 1734, 1714 cm^{-1}; λ_{max} 221 mμ, (log ε 3·12).

Hydrogenation of the anhydro-ester (XI). The anhydro-ester (17·44 mg) in glacial acetic acid (5 ml) with Adams' catalyst absorbed 1·04 ml of hydrogen at N.T.P. (0·9 double bonds). The solution was filtered, the solvent was removed, and the residue was crystallized from ether-light petroleum giving rods m.p. 212–215°, $[\alpha]_D^{23}$ + 27 (*c*, 0·37) (Found: C, 68·65; H, 8·35. $C_{20}H_{28}O_8$ requires: C, 68·9; H, 8·1%), identical (mixed m.p. and infra-red spectrum) with tetrahydrogibberellin A_8 methyl ester.

The p-*toluene sulphonate of the keto-ester*. The keto-ester (X, 350 mg) and *p*-toluene sulphonyl chloride (250 mg) were allowed to stand in dry pyridine (10 ml) for 80 hr. The solvent was evaporated under reduced pressure and the residue was dissolved in ethyl acetate and worked up as usual. The crystalline residue was recrystallized from acetone light petroleum giving the *p-toluene sulphonate* as needles (245 mg) m.p. 208–210° (Found: C, 62·5; H, 6·4. $C_{27}H_{32}O_8S$ requires: C, 62·8; H, 6·2%); ν_{max} 1789, 1739, 1597, 1370, 935 cm^{-1} (Infracord).

The anhydro-keto-ester IX. (a) The *p*-toluene sulphonate (100 mg) was refluxed in collidine (10 ml) for 6 hr. The solvent was evaporated under reduced pressure and the residue dissolved in ethyl acetate and worked up as usual. Crystallization of the residue from acetone-light petroleum gave the *anhydro-keto-ester* (IX) as prisms (60 mg) m.p. 160–164° $[\alpha]_D^{19}$ − 55 (*c*, 0·8) (Found: C, 69·6; H, 7·1. $C_{20}H_{24}O_8$ requires: C, 69·75; H, 7·0%) identical (mixed m.p. and infra-red spectrum) with the methyl ester IX of the acid VIII, obtained by treatment of gibberellin A_8 with dilute hydrochloric acid.

(b) The keto-ester (X, 180 mg) was dissolved in pyridine (10 ml) and refluxed with phosphorus oxychloride (3 ml) for 2 hr. After cooling the solution was poured over ice and extracted with ethyl acetate. The extract was worked up as usual. Fractional crystallization of the residue from acetone–light petroleum gave (i) fine needles (30 mg) m.p. 225° (Found: C, 62·9; H, 6·6; Cl, 9·35. $C_{20}H_{23}O_8Cl$ requires: C, 63·1; H, 6·6; Cl, 9·1%, (ii) prisms (90 mg) m.p. 160–164° identical (mixed m.p. and infra-red spectrum) with the anhydro-keto ester IX. *Hydrolysis* of the ester IX with boiling hydrochloric acid for 2 hr gave the keto-acid VIII which crystallized from acetone–light petroleum in prisms m.p. 264° $[\alpha]_D^{19}$ − 61° (*c*, 0·6) (Found: C, 68·55; H, 6·8. $C_{19}H_{22}O_8$ requires: C, 69·1; H, 6·7%) identical (mixed m.p. and infra-red spectrum) with the acid VIII, obtained by treatment of gibberellin A_8 with dilute hydrochloric acid. *Hydrogenation* of the ester IX (15·0 mg) in glacial acetic acid (5 ml) with a palladium charcoal catalyst gave the *dihydro*-derivative, crystallizing from acetone-light petroleum in prisms m.p. 190–191°, $[\alpha]_D^{19}$ + 50° (Found: C, 69·0; H, 7·6. $C_{20}H_{26}O_8$ requires: C, 69·3; H, 7·6%).

p-*Toluene sulphonation of gibberellin* A_1 *methyl ester*. Gibberellin A_1 methyl ester (500 mg) and *p*-toluene sulphonyl chloride (300 mg) were dissolved in pyridine (5 ml) and allowed to stand for 50 hr. Ethyl acetate was added and the solution was worked up as usual. The residue was added to an alumina column (16 × 1·5 cm) in benzene and eluted with benzene containing increasing proportions of chloroform giving (i) 9:1 benzene:chloroform, the *di*-p-*toluene sulphonate* as a glass (120 mg) (Found: C, 61·15; H, 5·6. $C_{34}H_{38}O_{10}S$ requires: C, 60·9; H, 5·8). (ii) 4:1 benzene:chloroform, the *mono*-p-*toluene sulphonate* as a glass (322 mg) (Found: C, 62·8; H, 6·3. $C_{27}H_{32}O_8S$ requires: C, 62·8; H, 6·2%) (iii) 1:1 benzene:chloroform, gibberellin A_1 methyl ester (70 mg) m.p. 234°.

Gibberellin A_1 *methyl ester*. The above mono-*p*-toluene sulphonate (300 mg) was heated under reflux in collidine (10 ml) for 5 hr. The neutral product was crystallized from acetone light petroleum

giving *gibberellin* A_8 *methyl ester* (VI) as prisms (98 mg) m.p. 191–192°, $[\alpha]_D^{21}$ −79° (*c*, 0·63) (Found: C, 69·8; H, 7·3%).

On hydrogenation in glacial acetic acid with Adams' catalyst, the ester absorbed 1·9 moles hydrogen giving tetrahydrogibberellin A_8 methyl ester (probably the C-8 epimeric mixture) which crystallized from acetone–light petroleum in prisms m.p. 206–208°.

Gibberellin A_8. Gibberellin A_8 methyl ester (70 mg) was dissolved in methanol (2 ml) and heated at 100° for 2 hr with aqueous potassium hydroxide (2 ml of 10%). Water was added and the methanol was removed under reduced pressure. The resulting solution was washed with ethyl acetate, acidified with dil. hydrochloric acid and extracted with ethyl acetate. After drying (sodium sulphate) the extract was evaporated and residue crystallized from ether giving gibberellin A_8 as prisms (20 mg) m.p. 260–261° $[\alpha]_D^{21}$ − 76° (*c*, 0·64) (Found: C, 69·0; H, 6·9%).

Acknowledgements—We thank Dr. P. W. Brian for his encouragement in initiating this work; Miss M. Radley for the bio-assays: Dr. J. R. Bartels-Keith and Mr. M. B. Lloyd for the infra-red spectra; and numerous colleagues for their help in obtaining the immature seed of *P. multiflorus*.