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NEW METABOLITES OF *GIBBERELLA FUJIKUROI*—I

GIBBERELLIN A_7 AND GIBBERELLIN A_9

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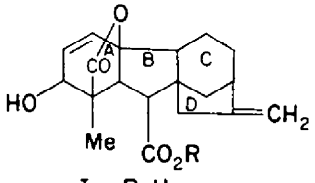
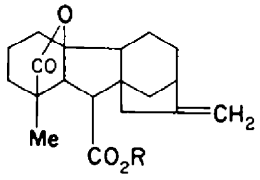
Abstract—Two new gibberellins, gibberellin A_7 and gibberellin A_9, have been isolated from culture filtrates of the fungus *Gibberella fujikuroi*. They are shown by degradation and interrelation with gibberellic acid via gibberellin A_4, to have structures I and III respectively.

THE isolation of two new plant growth-promoting acids which have been named* gibberellin A_7 and gibberellin A_9 and evidence for assigning them structure I and III



I; R=H
II; R=Me



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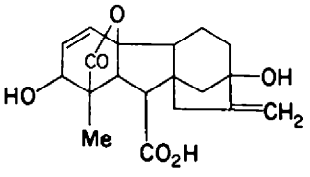
IV; R=Me



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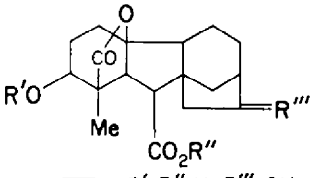
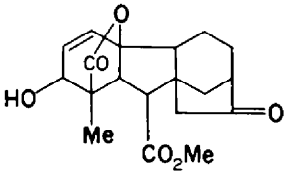
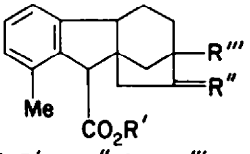


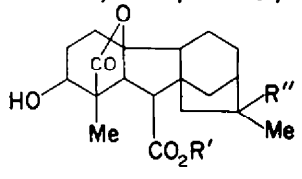
VI; R′=R″=H; R‴=CH_2
VII; R′=Ts; R″=Me; R‴=O
VIII; R′=H; R″=Me; R‴=O



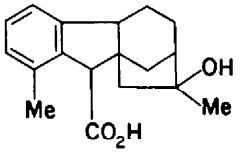
IX



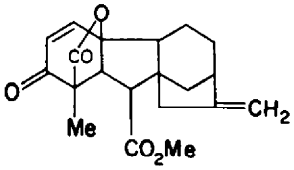
X; R′=H; R″=CH_2; R‴=OH
XI; R′=Me; R″=O; R‴=H



XII; R′=H; R″=OH
XIII; R′=R″=H
XIV; R′=Me; R″=H



XV



XVI

* Gibberellins A_8 and A_9 have been isolated from the seed of *Phaseolus multiflorus* by J. MacMillan, J. C. Seaton and P. J. Suter (*Tetrahedron* in the press). For reviews on the gibberellins see P. W. Brian, J. F. Grove and J. MacMillan, *The Gibberellins* in Zechmeister, *Prog. Chem. Org. Nat. Prod.* **18**, 350 (1960), and J. F. Grove, *Quart Rev.* **15**, 56 (1961).

respectively has been briefly reported elsewhere.[1,2] The present paper describes this work in full.

When the fungus *Gibberella fujikuroi* (Saw.) Wr. strain ACC. 917[3] is grown in stirred culture at the natural pH on a Raulin Thom or a glucose–ammonium nitrate medium it produces gibberellic acid[3–5] (V). However, by modification of the fermentation conditions it is possible to produce a mixture of gibberellins although gibberellic acid remains the major constituent. In particular when the fungus is cultured in the normal manner[5] until the inorganic nitrogen is exhausted from the medium and then potassium hydroxide solution is added to raise and maintain the pH of the broth at about 7 for the remainder of the fermentation, gibberellin A_7 and gibberellin A_9 are produced in yields of 25 and 3–4 mg/l. of culture filtrate respectively. Gibberellin A_4[6,7] (VI) has also been isolated from one of these fermentations in very low yield. The gibberellins were extracted from the culture filtrate by a modification of the method previously described[4,5] for the isolation of gibberellic acid. Sodium bicarbonate solution was used in place of the phosphate buffer to separate the acidic from the non-acidic metabolites. The acidified sodium bicarbonate extracts were extracted with ethyl acetate. Concentration of these extracts *in vacuo* gave crude crystalline gibberellic acid whilst the mother liquors afforded a gum containing the new gibberellins. The gum was chromatographed on a celite–charcoal column.[8,9] Stepwise elution with water containing increasing concentrations of acetone gave in order of elution, succinic acid, 5-hydroxymethylfuran-2-carboxylic acid,[10] fumaric acid, gibberellic acid, gibberellins A_4 and A_7, and gibberellin A_9. The gibberellin containing fractions were usually gums and were purified by chromatography on celite–silica gel followed by crystallization.

Gibberellin A_7 (I), $[\alpha]_D^{24}$ +20°, crystallized in two polymorphic forms (see Experimental) giving melting points between 169–172° and 202° (decomp). It titrated as a monobasic acid, gave a monomethyl ester (II), m.p. 152–153° or 168–170°, and a mono-acetate, m.p. 190–192°. On hydrogenation the methyl ester took up 2·3 moles of hydrogen. Analyses of these compounds indicate that gibberellin A_7 has the molecular formula $C_{19}H_{22}O_5$ and this together with the activity of the acid in the dwarf pea seedling tests[11] for gibberellic acid suggested that it was a new gibberellin.

In chloroform solution the infra-red spectrum of gibberellin A_7 methyl ester showed absorption attributed to hydroxyl (3578 cm^{-1}), γ-lactone (1767 cm^{-1}), ester (1732 cm^{-1}), olefin (1657 cm^{-1}) and terminal methylene (887 cm^{-1}) groupings. These

[1] B. E. Cross, R. H. B. Galt and J. R. Hanson, *Tetrahedron Letters* No. 15, 18 (1960).
[2] B. E. Cross, R. H. B. Galt and J. R. Hanson, *Tetrahedron Letters* No. 23, 22 (1960).
[3] A. Borrow, E. G. Jefferys, R. H. J. Kessell, E. C. Lloyd, P. B. Lloyd and I. S. Nixon, *Canad. J. Microbiol.* **7**, 227 (1961).
[4] P. J. Curtis and B. E. Cross, *Chem. & Ind.* 1066 (1954).
[5] Brit. Pat., 783611.
[6] N. Takahashi, Y. Seta, H. Kitamura and Y. Sumiki, *Bull. Agric. Chem. Soc. Japan* **21**, 396 (1957).
[7] J. F. Grove, J. MacMillan, T. P. C. Mulholland and W. B. Turner, *J. Chem. Soc.* 3049 (1960).
[8] C. A. West and B. O. Phinney, *J. Amer. Chem. Soc.* **81**, 2424 (1959).
[9] J. MacMillan, J. C. Seaton and P. J. Suter, *Tetrahedron* **11**, 60 (1960).
[10] J. F. Grove, P. W. Jeffs and T. P. C. Mulholland, *J. Chem. Soc.* 1236 (1958).
[11] P. W. Brian, H. G. Hemming and D. Lowe, *Ann. Bot.* **22**, 539 (1958).

assignments account for all the oxygen functions. The terminal methylene group* was shown to be exocyclic to a 5-membered ring by ozonolysis of the methyl ester (II) which gave formaldehyde (0·43 mole) and a nor-ketone $C_{19}H_{22}O_6$ (IX) showing absorption at 1755 cm^{-1} (5-ring ketone) in addition to bands at 3440 (hydroxyl), 1784 (γ-lactone) and 1738 (ester) cm^{-1}. Although the nor-ketone gave a mono-acetate it was stable to periodate (cf. methyl gibberellate nor-ketone[13]) and hence gibberellin A_7 is a 7-deoxygibberellin.

The structure of ring A in gibberellin A_7 has been shown to be the same as in gibberellic acid. Thus, treatment of gibberellin A_7 with dilute mineral acid at 20° gave an acid, $C_{18}H_{22}O_3$, shown by its infra-red and ultra-violet spectra to be an aromatic hydroxy-acid. Under similar conditions gibberellic acid undergoes aromatization of ring A to give allogibberic acid[14] (X) whilst the 7-deoxygibberellin, gibberellin A_4 (VI), is converted to gibberellin A_2 (XII) by addition of water to the terminal methylene group.[15] The acid $C_{18}H_{22}O_3$ was therefore assigned structure XV and this was supported by the aromatization of gibberellin A_7 methyl ester nor-ketone with boiling dilute acid to an ester $C_{18}H_{20}O_3$ (XI) which showed carbonyl absorption at 1744 (5-ring ketone) and 1714 (ester) cm^{-1}. The ring A structure was confirmed by oxidation of gibberellin A_7 methyl ester with active manganese dioxide which afforded an $\alpha\beta$-unsaturated ketone $C_{20}H_{22}O_5$ (XVI, λ_{max} 228 mμ ε 6,900). Chromatographic purification of this unsaturated ketone revealed the presence of a small amount of gibberellin A_4 methyl ester which must have been derived from gibberellin A_4 present in the gibberellin A_7 used in this preparation.†

Structure I for gibberellin A_7 was finally established by the selective catalytic reduction in ethyl acetate solution of the ring A double bond in gibberellin A_7 using a 2 per cent palladium on barium carbonate catalyst partially poisoned with pyridine.‡ The product was separated by chromatography into gibberellin A_4 and a gummy mixture of hydrogenolysis acids. Treatment of the latter with boiling dilute mineral acid afforded gibberellin A_2 (XII) and dihydrogibberellin A_4 (XIII), presumably by lactonization of unsaturated acids of the type XVII.§ Catalytic reduction of gibberellin A_7 methyl ester over 25 per cent palladized charcoal gave 60 per cent of acidic products and dihydrogibberellin A_4 methyl ester[16] (presumably a mixture of C.8

* Oxidation of the terminal methylene groupings in gibberellic acid and gibberellin A_7 with sodium periodate–potassium permanganate[12] gave in each case 0·3 mole of formaldehyde which was estimated spectrophotometrically.

† Gibberellin A_4 and gibberellin A_7 show the same R_f values in four solvent systems [J. MacMillan, J. C. Seaton and P. J. Suter, *Advances in Chemistry* No. 28, 18 (1961)] and are not readily separable by chromatography on celite–silica gel. However, it is possible to separate gibberellin A_4 methyl ester from a mixture of the esters by careful fractional crystallization.

‡ This catalyst was first used in the gibberellin series for the reduction of gibberellic acid to gibberellin A_1, D. F. Jones and J. MacMillan, personal communication.

§ Analogous results have been obtained with unsaturated acids derived by hydrogenolysis of methyl gibberellate and gibberellic acid, P. McCloskey and T. P. C. Mulholland, personal communication.

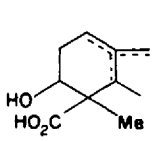
[12] R. U. Lemieux and E. von Rudloff, *Canad. J. Chem.* **33**, 1710 (1955).

[13] B. E. Cross, *J. Chem. Soc.* 3022 (1960).

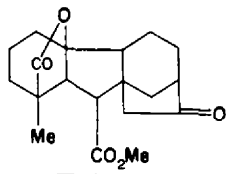
[14] P. W. Brian, J. F. Grove, H. G. Hemming, T. P. C. Mulholland and M. E. Radley, *Plant Physiol.* 329 (1958).

[15] J. F. Grove, *J. Chem. Soc.* 3545 (1961).

[16] N. Takahashi, Y. Seta, H. Kitamura and Y. Sumiki, *Bull. Agric. Chem. Soc. Japan* **23**, 405 (1959).



XVII



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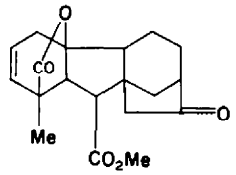
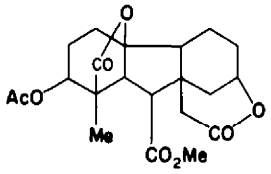
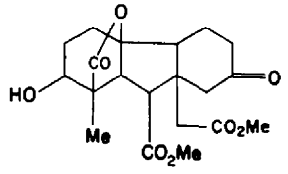


XIX



XX



XXI

epimers; XIV), m.p. 148–151°. The high yield of hydrogenolysis acids (cf. methyl gibberellate[13]) provides further support for the Δ^3 position of the ring A double bond.

Gibberellin A_9 (III), m.p. 208–211°, $[\alpha]_D^{22}$ −22°, and its methyl ester (IV), m.p. 136°, gave analyses consistent with the formula $C_{19}H_{24}O_4$ for the acid. Microhydrogenation revealed the presence of one double bond. In the infra-red the ester showed bands at 1777 (γ-lactone), 1738 (ester), 1659 and 873 (=CH_2) cm^{-1}. These assignments account for all the functional groups in gibberellin A_9. The presence of a terminal methylene group was confirmed by ozonolysis of gibberellin A_9 methyl ester which yielded formaldehyde (0·6 mole) and a nor-ketone $C_{19}H_{24}O_5$ (XVIII), which showed absorption at 1738 cm^{-1} ascribed to ester and a 5-ring ketone carbonyl in addition to a band at 1772 (γ-lactone) cm^{-1}. Structure III for gibberellin A_9, suggested by the above results, was established by degradation of gibberellin A_4 to the nor-ketone (XVIII) in the following manner. Treatment of the tosylate (VII) of gibberellin A_4 methyl ester nor-ketone (see below; cf. refs. 7, 16) VIII with boiling collidine afforded the Δ^2-olefin (XIX), $C_{19}H_{22}O_5$, which on catalytic hydrogenation gave gibberellin A_9 methyl ester nor-ketone (XVIII).

Structures I and III for gibberellin A_7 and gibberellin A_9 respectively are both dependent upon that of gibberellin A_4 (VI), hence an unambiguous interrelationship between gibberellin A_4 and a gibberellin of established structure such as gibberellic acid or gibberellin A_1[13,17] is essential. Although the structure of gibberellin A_4 has been related to that of gibberellin A_1 by Sumiki *et al.*,[18] it seemed desirable to provide an independent link. This has been achieved by the following series of reactions. Catalytic reduction of gibberellin A_7 methyl ester nor-ketone (IX) over palladized charcoal afforded an acidic gum and gibberellin A_4 methyl ester nor-ketone[7,16] (VIII). Acetylation of the nor-ketone followed by Baeyer–Villiger oxidation with perbenzoic acid afforded the δ-lactone (XX) which in bromoform solution showed carbonyl absorption at 1774 (γ-lactone) and 1738 (broad) (esters and δ-lactone) cm^{-1}. Acetylation of the keto-ester (XXI), derived[13] from gibberellic acid followed by hydrogenation

[17] B. E. Cross, J. F. Grove, J. MacMillan, J. S. Moffat, T. P. C. Mulholland, J. C. Seaton and N. Sheppard, *Proc. Chem. Soc.* 302 (1959).

[18] H. Kitamura, N. Takahashi, Y. Seta, A. Kawarada and Y. Sumiki, *Bull. Agric. Chem. Soc. Japan* **23**, 344 (1959).

using Adams' catalyst in acetic acid containing a trace of perchloric acid gave the same δ-lactone (XX).

Gibberellin A_7 is less and gibberellin A_9 much less active than gibberellic acid in promoting dwarf pea stem growth but both gibberellin A_7 and gibberellin A_9 are much more active than gibberellic acid in the cucumber hypocotyl growth test.[19]

EXPERIMENTAL

M.p's were determined on a Kofler block and were corrected. The following chromatographic materials were used: silica gel M.F.C. (Hopkin and Williams) activated charcoal (B.D.H.), celite 545 (Johns Mandeville), and alumina (Woelm neutral alumina, Grade II). Unless otherwise stated I.R. and U.V. spectra were determined on Nujol mulls and in ethanol solutions respectively. Optical rotations were measured in ethanol. Microhydrogenations were carried out in acetic acid with a palladium black catalyst. "Light petroleum" refers to the fraction of b.p. 60–80°. Ethyl acetate extracts were dried over anhydrous sodium sulphate.

Fermentation and extraction

Gibberella fujikuroi (Saw.) Wr. strain ACC. 917[3] was grown in stirred culture on a glucose–ammonium nitrate medium (see ref. 5) until the inorganic nitrogen was exhausted (ca. 90 hr). The pH of the broth was then adjusted to 6·75 by the addition of 5 N KOH; further additions of alkali were made periodically during 200 hr to maintain the pH in the range 6·7–6·8 until the fermentation was harvested. The mycelium was removed by filtration, the filtrate (38 l.) and washings (19 l.) were combined, adjusted to pH 3·2 by addition of dil HCl, and treated with activated charcoal (12 g/l.).* The charcoal was collected by filtration and the filtrate was treated again with activated charcoal (5 g/l.). The combined charcoal residues were air dried to 20–30% moisture content and then packed into a column and eluted with acetone (7·5 l.). The eluate was evaporated under red press leaving an aqueous concentrate which was extracted with ethyl acetate (3 × equal vol). The combined ethyl acetate solutions were extracted several times with sodium bicarbonate solution. The bicarbonate extracts were combined, acidified with dil HCl, and extracted with ethyl acetate. Concentration of these ethyl acetate extracts afforded crude gibberellic acid (4·15 g), m.p. 213–216° (decomp), whilst evaporation of the mother liquors *in vacuo* gave a dark-red acidic gum (27 g).

Isolation of Gibberellin A_7 *and Gibberellin* A_9

Part of the above acidic gum (11 g) was adsorbed on silica gel by evaporation of an acetone solution and placed on the top of a column (67·5 × 7 cm) of celite (600 g) and charcoal (300 g) which had been prepared in water. The column was eluted with water containing increasing concentrations of acetone.

The acetone was removed from the eluate by evaporation *in vacuo* to give either an aqueous concentrate or, from the later fractions, a dry residue. Recovery of the organic material by extraction with ethyl acetate followed by evaporation *in vacuo* gave the following results (% acetone, fraction volumes and weights in parentheses):— **A.** (35%; 2 l.; 0·013 g) gum, **B.** (40%; 1 l.; 0·019 g) and **C.** (45%; 1 l.; 0·035 g) were crude succinic acid, **D.** (50%; 1 l.; 0·074 g) afforded 5-hydroxymethylfuran-2-carboxylic acid, **E.** (55%; 1 l.; 0·165 g) yielded fumaric acid, **F.** (60%; 1 l.; 0·86 g) gum, **G.** (65%; 1 l.; 1·58 g) gave gibberellic acid, **H.** (65%; 1 l.; 0·90 g) gum, **I.** (70%; 2 l.; 2·638 g) and **J.** 75%; 1 l.; 0·39 g) were impure gibberellin A_7, **K.** (80%; 1 l.; 0·17 g) gum, **L.** (80%; 1 l.; 0·69 g) was a gum containing gibberellin A_9, **M.** (85%; 1 l.; 0·49 g) and **N.** (90%; 1 l.; 0·20 g) were gums.

For the preparation of larger quantities of gibberellin A_7 and gibberellin A_9 the crude acidic gum in ca. 60 g portions was chromatographed on celite (1200 g) and charcoal (600 g) in a column (95 × 7·5 cm). Gradient elution with a linearly increasing concentration of acetone in water over the range 35–90% acetone and collection of 2 l. fractions afforded the crude gibberellins. They were purified by the procedure described below.

Gibberellin A_7. Fraction 1 from above was absorbed on silica gel, placed on the top of a column (50 × 4·5 cm) of celite (190 g) and silica gel (95 g) and eluted with 500 ml fractions of chloroform containing ethyl acetate increasing in 5% steps. The first four fractions were intractable gums.

* cf. refs. 4, 5.

[19] P. W. Brian and H. G. Hemming, *Nature, Lond.* **189**, 74 (1961).

Crystallization of the next five fractions from acetone–light petroleum gave *gibberellin* A_7 (0·5 g) which formed needles m.p. 169–172° or prisms m.p. 202° (decomp) or a mixture of these two with intermediate m.p. They gave two distinct I.R. spectra, (i) ν_{max} 3450, 1742, 1722 and 1654 cm^{-1} and (ii) ν_{max} 3340, 1778, 1711, and 1674 cm^{-1}. Both forms gave identical I.R. spectra in chloroform solution, ν_{max} 1765 and 1708 cm^{-1} (Found: C, 69·05; H, 7·0%; equiv., 321. $C_{19}H_{22}O_5$ requires: C, 69·1; H, 6·7%; M, 330), $[\alpha]_D^{24}$ +20° (*c* 0·5). With cold conc H_2SO_4 it gave a brownish yellow colour with a green fluorescence.

The *methyl ester*, prepared with diazomethane, crystallized from acetone–light petroleum as needles, m.p. 152–153° or m.p. 168–170°, $[\alpha]_D^{23}$ +33° (*c* 0·7) (Found: C, 69·9; H, 7·2. $C_{20}H_{24}O_5$ requires: C, 69·75; H, 7·0%); ν_{max} 3460 (strong), 3320 (broad), 1774, 1733, 1718, 1659, 887 cm^{-1}; $\nu_{max}^{CHCl_3}$ 3578, 1767, 1732, 1657, 887 cm^{-1}.

The *acetate*, prepared with acetic anhydride–pyridine, crystallized from acetone–light petroleum as prisms, m.p. 190–192°. $[\alpha]_D^{24}$ +87° (*c* 0·9) (Found: C, 67·3; H, 6·7. $C_{21}H_{24}O_6$ requires C, 67·7; H, 6·5%).

Determination of exocyclic methylene (cf. ref. 12).

Gibberellin A_7 (1·5 mg) was dissolved in water (5 ml) in a volumetric flask (25 ml) with the addition of 0·1 N K_2CO_3 to bring the pH to 7–7·5. 0·02 M $NaIO_4$ (10 ml) and 0·005 M $KMnO_4$ (1 ml) were added, the solution made up to 25 ml with distilled water and left for 15 min. 1 ml was transferred to a test tube, the chromotropic acid reagent [chromotropic acid (1 g) dissolved in water (100 ml), filtered, and made up to 500 ml with 2:1 v/v sulphuric acid:water] (10 ml) added, and the solution heated on a water bath for 30 min.

The percentage transmission at 570 mμ in 1 cm cells was determined and compared to mesoerythritol and gibberellic acid standards. In 15 min the latter gave 30% available formaldehyde against mesoerythritol whilst gibberellin A_7 gave 30·5%.

Ozonolysis of gibberellin A_7 *methyl ester*

Ozonized oxygen (1·1 moles) was passed through a solution of gibberellin A_7 methyl ester (41 mg) in acetic acid (15 ml). After being kept 0·5 hr, the solution was diluted with water to 100 ml and rapidly steam-distilled. The distillate (100 ml) was treated with aqueous dimedone and after 5 days formaldehyde-dimethon (m.p. 189–190°; 13 mg; 43%) was collected. The acetic acid solution was neutralized with aqueous sodium bicarbonate and the organic residue recovered with ethyl acetate. Evaporation of the solvent gave a gum which was chromatographed on silica gel. Elution with ethyl acetate–light petroleum (1:4) gave *gibberellin* A_7 *methyl ester nor-ketone* (IX; 23 mg) which crystallized from ethyl acetate–light petroleum a prisms, m.p. 185°, $[\alpha]_D^{22}$ −88° (*c* 0·8) (Found: C, 65·75; H, 6·5. $C_{19}H_{22}O_6$ requires: C, 65·9; H, 6·4%). Acetylation with acetic anhydride–pyridine gave the *acetate* as needles, m.p. 187–189°, from acetone–light petroleum (Found: C, 65·2; H, 6·45. $C_{21}H_{24}O_7$ requires: C, 64·9; H, 6·2%); $\nu_{max}^{CHBr_3}$ 1781 and 1738 cm^{-1}; ν_{max} 1779 and 1742 (broad) cm^{-1}.

Stability of the nor-ketone (IX) *to periodate*

The nor-ketone (15 mg) in methanol (1 ml) was treated with 0·1 N $NaIO_4$·2O (2 ml) at room temp for 24 hr. Dilution with water and recovery with ethyl acetate afforded the starting material (13 mg) as needles, m.p. 186°, identified by its I.R. spectrum.

Aromatization of gibberellin A_7

A suspension of gibberellin A_7 (95 mg) in dil HCl (25 ml, 10:1–water:conc HCl) was allowed to stand at room temp for 5 days. The solution was extracted with ethyl acetate and the gummy product chromatographed on silica gel. Elution with light petroleum–ethyl acetate (1:1 and 2:3) gave the *acid* (XV, 33 mg), m.p. 220–225° (needles from acetone–light petroleum) (Found: C, 75·5; H, 8·0. $C_{18}H_{22}O_3$ requires: C, 75·5 H, 7·7%); ν_{max} 3410 and 1684 cm^{-1}; λ_{max} 269, 272 mμ (ε 418, 377).

Aromatization of gibberellin A_7 *methyl ester nor-ketone* (IX)

The nor-ketone (49 mg) in acetone (2 ml) was added to 3 N HCl (20 ml) and heated under reflux for 1 hr. Recovery with ethyl acetate and chromatography on silica gel in ethyl acetate–light petroleum (1:4) gave the aromatic *keto-ester* (XI; 22 mg) which crystallized from ether–light petroleum as needles, m.p. 119–120° (Found: C, 76·2; H, 7·3. $C_{18}H_{20}O_3$ requires: C, 76·0; H, 7·1%); ν_{max} 1744, 1714 and 1590 cm^{-1}.

Oxidation of gibberellin A_7 *methyl ester* (II) *with manganese dioxide*

The ester (50 mg, m.p. 153–164°) and active manganese dioxide (0·5 g) were shaken in chloroform (3 ml) at room temp for 90 hr. The filtrate and washings were evaporated giving a gum (47 mg) which was chromatographed on alumina. Elution with light petroleum containing increasing concentrations of ethyl acetate gave (a) with 15% ethyl acetate—the *αβ-unsaturated ketone* (XVI; 24 mg), m.p. 139–140° (rhcmbs from acetone–light petroleum) (Found: C, 70·0; H, 6·7. $C_{20}H_{22}O_5$ requires: C, 70·2; H, 6·5%); λ_{max} 228 mμ (ε 6,900); $\nu_{max}^{CHCl_3}$ 1776, 1724 and 1691 cm^{-1}. (b) with 30% ethyl acetate—gibberellin A_4 methyl ester (3 mg) present as an impurity in this sample of gibberellin A_7 methyl ester.* (c) with 40% ethyl acetate—the starting ester (14 mg).

Hydrogenation of gibberellin A_7

(a) *In methanol.* Gibberellin A_7 (300 mg) in dry methanol (15 ml) and pyridine (1 ml) was hydrogenated over 2% palladium on barium carbonate (300 mg) until uptake ceased (19 ml at N.T.P.; 0·98 mole). The catalyst was removed by filtration, the solvent evaporated, and the crude gum chromatographed on celite–silica gel (2:1; 24 × 1·5 cm). Careful elution with ethyl acetate–chloroform (1:7) gave gibberellin A_4 (25 mg) which crystallized from acetone–light petroleum as prisms, m.p. 212–215° (decomp), identical (I.R. spectrum and mixed m.p.) with an authentic specimen.

(b) *In ethyl acetate.* Gibberellin A_7 (500 mg) in ethyl acetate (30 ml) and pyridine (2 ml) was hydrogenated over 2% palladium on barium carbonate (500 mg) until uptake ceased (30 min; 43 ml at N.T.P.; 1·2 moles). The catalyst was removed by filtration, the solution diluted with ethyl acetate, extracted with dil HCl and washed with water. Recovery of the product and chromatography on celite–silica gel (2:1, 45 g) gave on elution with ethyl acetate–chloroform (15:85) gibberellin A_4 as prisms (200 mg), m.p. 211–214° (decomp), $[\alpha]_D^{22}$ −16° (*c* 0·9). Further elution with ethyl acetate–chloroform (1:3) and (3:10) gave a gummy mixture of hydrogenolysis acids (250 mg).

Relactonization of the hydrogenolysis acids

The acids (250 mg) from the preceding experiment in acetone (10 ml) were refluxed with dil HCl (15 ml) for 1 hr. Dilution with water and recovery with ethyl acetate afforded a gum which was chromatographed on celite–silica gel (2:1; 25 g). Elution with ethyl acetate–chloroform (1:9) gave *dihydrogibberellin* A_4 (XIII; 80 mg) which crystallized from acetone–light petroleum as needles, m.p. 252–254° (decomp) (Found: C, 68·3; H, 7·5. $C_{19}H_{26}O_5$ requires: C, 68·2; H, 7·8%); ν_{max} 3530, 1733 and 1712 cm^{-1}. Further elution with ethyl acetate–chloroform (1:3) gave gibberellin A_3[15] (XII; 41 mg) which crystallized from acetone–light petroleum as prisms, m.p. 238–242° (with a polymorphic change to needles at 230°), identified by its I.R. spectrum.

Hydrogenation of gibberellin A_7 *methyl ester*

The ester (18 mg) in ethyl acetate (4 ml) was hydrogenated over 25% palladized charcoal (10 mg, previously saturated with hydrogen; uptake 2·3 moles). After filtration, the solution was diluted with ethyl acetate and separated into acidic and neutral fractions with aqueous sodium bicarbonate. The acid fraction (8 mg) was intractable but the neutral fraction (7 mg) gave dihydrogibberellin A_4 methyl ester[16] (XIV) as needles from acetone–light petroleum m.p. 148–151°, identified by its I.R. spectrum.

Reduction of gibberellin A_7 *methyl ester nor-ketone*

The nor-ketone (IX; 75 mg) in ethyl acetate (10 ml) was hydrogenated over 25% palladized charcoal (60 mg; uptake 4·7 ml at N.T.P.; 1.1 moles). After filtration the solution was diluted with ethyl acetate and separated with aqueous sodium bicarbonate into acidic and neutral fractions. The acid fraction (30 mg) was an intractable gum. The neutral fraction (45 mg) gave gibberellin A_4 methyl ester nor-ketone[7] as prisms, m.p. 206–208° (from acetone–light petroleum), identified by its I.R. spectrum.

Acetylation of gibberellin A_4 methyl ester nor-ketone with acetic anhydride–pyridine gave the *acetyl derivative* which crystallized from ethyl acetate–light petroleum as needles, m.p. 189° (Found: C, 64·6; H, 7·0. $C_{21}H_{26}O_7$ requires: C, 64·6; H, 6·7%); ν_{max} 1767 and 1732 cm^{-1}.

* See footnote p. 453.

Baeyer-Villiger oxidation of acetylgibberellin A_4 methyl ester nor-ketone

The nor-ketone (60 mg) in chloroform (2 ml) was treated with 0·43 N perbenzoic acid (2 ml) and *p*-toluenesulphonic acid (10 mg) at 0° for 16 hr. The solution was diluted with ethyl acetate (100 ml), extracted with aqueous sodium bicarbonate, and washed with water, Evaporation of the solvent and chromatography of the residual gum on silica gel gave after crystallization from ethyl acetate–light petroleum, needles, m.p. 190–192° (36 mg), identical (I.R. and mixed mp.) with the δ-lactone obtained below.

Preparation of the δ lactone (XX) *from gibberellic acid*

The seco-ester (XXI)[20], was prepared[13] from methyl gibberellate by ozonolysis, reduction and methylation. Acetylation with acetic anhydride–pyridine at room temp gave the *2-acetyl derivative* which crystallized from ethyl acetate–light petroleum as needles, m.p. 193–195° (Found: C, 60·8; H, 6·55. $C_{23}H_{32}O_9$ requires: C, 60·5; H, 6·5%).

The acetyl derivative (52 mg) in acetic acid (10 ml) was hydrogenated over Adams' catalyst (60 mg) in the presence of perchloric acid (5 drops) until the rapid uptake of hydrogen ceased (25 min). After filtration the solution was diluted with ethyl acetate and extracted with aqueous sodium bicarbonate and water. Evaporation of the solvent gave a gum (46 mg) which was chromatographed on silica gel. Elution with ethyl acetate–light petroleum (45:55) gave the δ-lactone (XX; 20 mg) which crystallized from ethyl acetate–light petroleum as needles (13 mg), m.p. 193–194° (Found: C, 62·3; H, 6·7. $C_{21}H_{26}O_8$ requires: C, 62·1; H, 6·45%); ν_{max} 1764, 1737 and 1722 cm^{-1}; $\nu_{max}^{CHBr_3}$ 1774 and 1738 cm^{-1}, identical with the product obtained by Baeyer–Villiger oxidation (*vide supra*).

Gibberellin A_9

Fraction (L, 0·69 g), eluted with 80% acetone in water (see above), was chromatographed (32·5 × 3 cm column) on celite–silica gel (2:1; 63 g) eluting with light petroleum containing increasing concentrations of ethyl acetate. Only the fraction eluted with 20% ethyl acetate gave *gibberellin* A_9 (0·11 g) which crystallized in needles from acetone–light petroleum, m.p. 208–211°, $[\alpha]_D^{17}$ −22° (*c* 0·25) (Found: C, 72·4; H, 7·65%; equiv., 298. $C_{19}H_{24}O_4$ requires: C, 72·1; H, 7·65% M, 316); ν_{max} 3098, 1740, 1723, 1659, and 893 cm^{-1}. It gave a weak yellow colour with cold conc. H_2SO_4.

The *methyl ester*, prepared with diazomethane, crystallized in rhombs from aqueous methanol, m.p. 136°, $[\alpha]_D^{17}$ −15° (*c* 0·25) (Found: C, 72·4; H, 8·1. $C_{20}H_{26}O_4$ requires C, 72·7; H, 7·9%); ν_{max} 1777, 1738, 1659 and 873 cm^{-1}. On microhydrogenation, the ester absorbed 1 mole of hydrogen giving a mixture of epimers one of which, m.p. 186–188°, crystallized readily from acetone–light petroleum.

Gibberellin A_9 *methyl ester nor-ketone* (XVIII)

Excess ozonized oxygen was bubbled through gibberellin A_9 methyl ester (25 mg) in glacial acetic acid (10 ml) at room temp. The ozonide was decomposed with water and the solution steam distilled. The distillate was added to a saturated solution of dimedone and after 1 day crystals of the dimedone derivative of formaldehyde (13 mg; 60%) separated. Extraction of the non-steam-volatile residue with ethyl acetate gave the *nor-ketone* (XVIII; 20 mg), m.p. 204–207° (plates from acetone–light petroleum) (Found: C, 68·9; H, 7·4. $C_{19}H_{24}O_5$ requires: C, 68·65; H, 7·3%); ν_{max} 1772 and 1738 (strong) cm^{-1}.

Preparation of the Δ^3*-olefin* (XIX)

A mixture of gibberellin A_4 methyl ester nor-ketone (VIII; 43 mg) and *p*-toluenesulphonyl chloride (200 mg) in dry pyridine (2 ml) was allowed to stand at room temp for 3 days. The solution was added to dil HCl, extracted with ethyl acetate and washed with more acid then water. The gummy product was chromatographed on alumina and elution with ethyl acetate–light petroleum (3:7 and 35:65) gave the tosylate (VII; 28 mg) as a gum, which was refluxed in pure collidine (2 ml) for 3·5 hr. The solution was worked up as in the tosylation above and the gummy product (18 mg) chromatographed on celite–silica gel (2:1). Elution with ethyl acetate–light petroleum (1:4) gave the Δ^3*-olefin* (XIX

[20] Y. Seta, N. Takahashi, A. Kawarada, H. Kitamura and Y. Sumiki, *Bull. Agric. Chem. Soc. Japan* **23**, 412 (1959).

10 mg), m.p. 160–161° (rods from acetone–light petroleum) (Found: C, 68·9; H, 7·0. $C_{19}H_{22}O_6$ requires: C, 69·1; H, 6·7%); ν_{max} 1778 and 1735 (strong) cm^{-1}.

Hydrogenation of the Δ^3*-olefin* (XIX)

On microhydrogenation the Δ^3-olefin (5 mg) took up 1·2 moles of hydrogen. Repeated crystallization of the product from acetone–light petroleum gave plates (3 mg), m.p. and mixed m.p. with gibberellin A_9 methyl ester nor-ketone, 203–206°. The I.R. spectra of the two compounds were identical.

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