

## Partial Synthesis of *ent*-13-Hydroxy-2-oxo-20-norgibberella-1(10),16-diene-7,19-dioic Acid, a Catabolite of Gibberellin A<sub>29</sub>, and of Related Compounds

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Gibberellin A<sub>3</sub> has been converted into the title compound, both unlabelled and labelled with oxygen-18, and the structure of a catabolite of gibberellin A<sub>29</sub> in developing seed of *Pisum sativum* has thereby been established. The methyl esters of four related compounds, occurring in seed of *P. sativum* have also been prepared including the 13-deoxy-analogue of the title compound.

By g.l.c.-mass spectrometry of methylated and trimethylsilylated extracts of seed of *Pisum sativum* cv. Progress No. 9, Sponsel and MacMillan<sup>1</sup> detected a compound of unknown structure as the trimethylsilyl (TMSi) derivative. Durley *et al.*<sup>2</sup> also detected this compound by g.l.c.-mass spectrometry and radio-g.l.c. of the derivatised products from a feed of tritiated gibberellin A<sub>20</sub> to seed of *P. sativum* cv. Meteor. Recently<sup>3</sup> this compound has been shown to be a catabolite of gibberellin A<sub>29</sub> (1) by feeding [17-<sup>13</sup>C]gibberellin A<sub>29</sub> to seed of *P. sativum* cv. Progress No. 9.

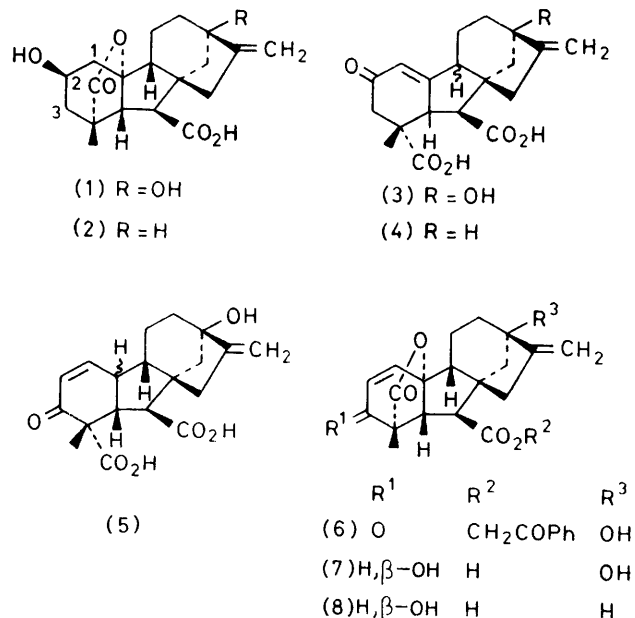
A tentative structure (3) for the GA<sub>29</sub>-catabolite was deduced from the following information. First, Sponsel and MacMillan<sup>4</sup> showed that a sample of the partially purified compound, isolated from seed of *P. sativum*, had strong u.v. absorption at λ<sub>max</sub>. 231 nm and was reduced by sodium borohydride to products expected from the

seed of *P. sativum* cv. Progress No. 9, it was metabolised at a slower rate than the endogenous, unlabelled gibberellin A<sub>29</sub>, thus suggesting that the metabolism of gibberellin A<sub>29</sub> (1) proceeded by oxidation at C-2.

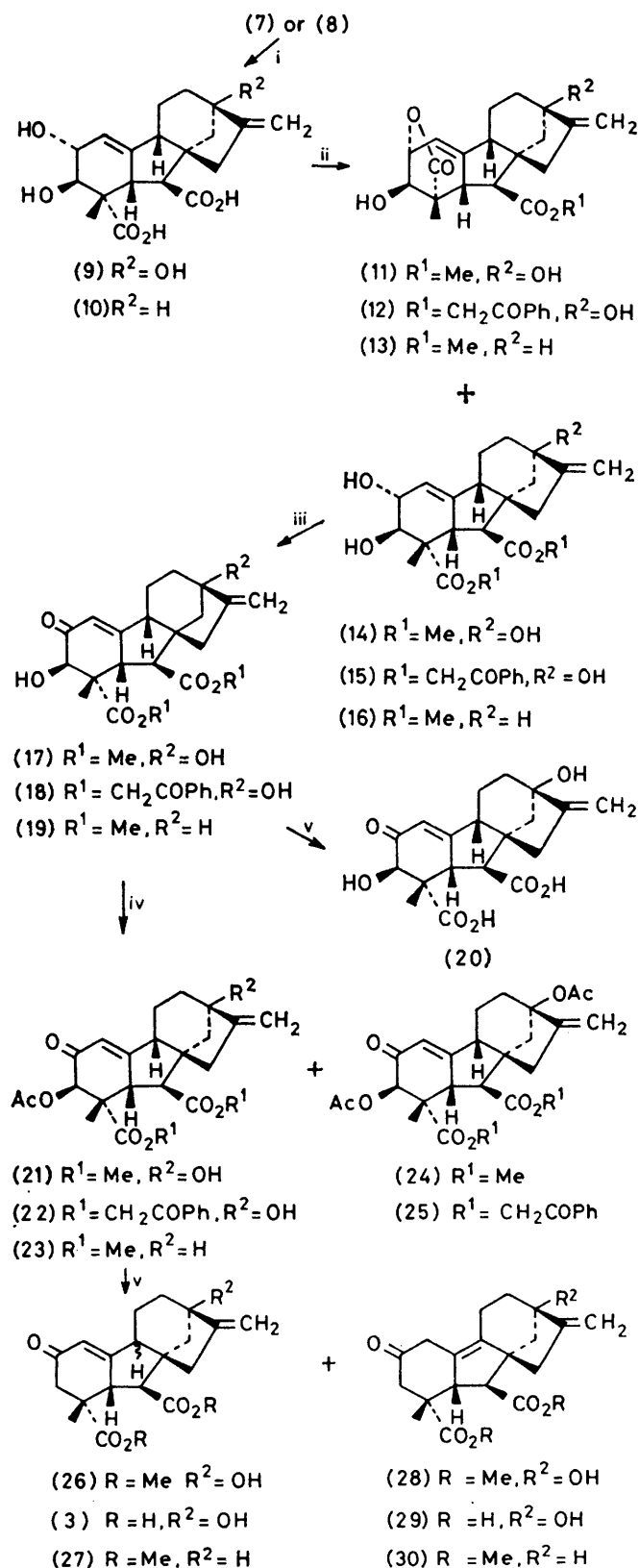
This paper describes the synthesis of the di-acid (3), unlabelled and [19-<sup>18</sup>O]-labelled, and of the dimethyl ester (26, Scheme 1), thereby establishing the structure (3) for the compound, present in seed of *P. sativum* and formed from GA<sub>29</sub>. The preparation of the methyl esters of other related naturally occurring compounds, including the 13-deoxy-analogue (27), is also reported.

The starting materials from these syntheses (Scheme 1) were gibberellins A<sub>3</sub> (7) and A<sub>7</sub> (8). The simplest objective, the preparation of the dimethyl ester (26) was undertaken first. The route (Scheme 1A) from gibberellin A<sub>3</sub> (7) followed that described by Cross<sup>6</sup> as far as the mono-acetate (21). The triol di-acid (9), obtained from the treatment of gibberellin A<sub>3</sub> (7) with aqueous alkali, has appreciable water-solubility; in order to obtain a high recovery it was necessary to use the minimum volume of aqueous alkali and to extract the acidified reaction mixture at low temperature (5 °C). Also, to minimise re-lactonisation, solutions of the di-acid (9) must not be evaporated to dryness or heated above 40 °C. Despite these precautions the dimethyl ester (14), obtained with diazomethane, contained appreciable amounts of the 19,2-lactone (11) which was removed by p.l.c. The pure trihydroxy-dimethyl ester (14) was characterised by its <sup>1</sup>H n.m.r. spectrum and oxidised by manganese dioxide to the ketol (17) which showed the expected i.r., u.v., and <sup>1</sup>H n.m.r. spectra. The mass spectra of this ketol (17) and of its bis-TMSi ether were identical to those of the corresponding derivatives of a compound, detected in extracts of seed of *Phaseolus coccineus*.<sup>7</sup>

Acetylation of the ketol (17) gave the mono-acetate (21), separated by p.l.c. from a minor amount of the accompanying diacetate (24), and characterised by its <sup>1</sup>H n.m.r. spectrum. Reductive de-acetoxylation of the mono-acetate (21) with activated zinc<sup>8</sup> and acetic acid at room temperature gave the required dimethyl ester (26) which showed the expected i.r., u.v., and <sup>1</sup>H n.m.r. spectra. However, g.l.c. and g.l.c.-mass spectrometry of the product showed the presence of 10% of an isomer (28) which was identical, by g.l.c.-mass spectrometry of it and its TMSi ether, with the corresponding derivatives

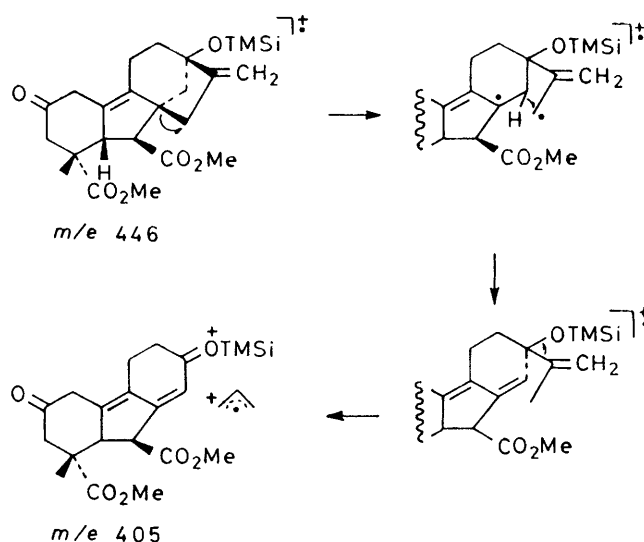


reduction of one carbonyl group. Secondly, the mass spectrum of the MeTMSi derivative of the compound from pea seed showed fragmentations reminiscent of those of the compound (5), obtained by reduction of 3-didehydrogibberellin A<sub>3</sub> phenacyl ester (6) with zinc and acetic acid.<sup>5</sup> Thirdly,<sup>4</sup> when [2α-<sup>2</sup>H<sub>1</sub>]GA<sub>29</sub> was fed to



SCHEME 1 (A) Synthesis of the dimethyl ester (26) where  $R = R^1 = Me, R^2 = OH$ . Reagents: i, 0.8M KOH; ii,  $CH_3N_3$ ; iii,  $MnO_2$  or pyridinium chlorochromate; iv,  $Ac_2O$ -pyridine; v,

of a compound, also present<sup>3,4</sup> in extracts of seed of *P. sativum*. Attempts to separate the isomers (26) and (28) by p.l.c. and by h.p.l.c. were not successful. Structure (28) for the minor component was deduced by comparing the relative intensities of the ions at  $m/e$  405 ( $M^+ - 41$ ) and  $m/e$  238 in the mass spectra of the TMSi ethers of both isomers (26) and (28). The ion at  $m/e$  405 was stronger (21 vs. 1%) and the  $m/e$  238 ion was weaker (3 vs. 19%) in the spectrum from the minor isomer (28). The  $m/e$  238 ion is characteristic<sup>5</sup> of the ring B cleavage (31) with methoxy transfer, and the ion at  $m/e$  405 involves the loss of C-15, -16, and -17.<sup>9</sup> The observed differences in the relative intensities of these two ions in the spectra of the TMSi ethers of the two isomers (26) and (28) suggest that the minor isomer has a 9,10-double bond which favours the loss of ring D as rationalised in Scheme 2 and which disfavors the fragmentation (31).

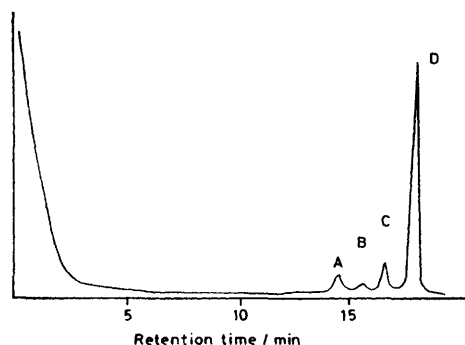


SCHEME 2

Trimethylsilylation of the mixture of dimethyl esters (26) and (28) was complicated by the formation of TMSi enol ethers. G.l.c.-mass spectrometry of the product, obtained with hexamethyldisilazane and trimethylchlorosilane (HMDS-TMCS), gave four peaks (Figure). Peaks D and A were respectively the TMSi ethers of the dimethyl esters (26) and (28). Peaks B and C had  $M^+$  518, corresponding to enol TMSi ethers. Using the more powerful silylating reagents *NO*-bistrimethylsilylacetamide (BSA) or *NO*-bistrimethylsilyltrifluoroacetamide (BSTFA), the TMSi ether (peak D) of the dimethyl ester (26) was converted into the TMSi enol ether (peak C). Of the three possible structures for the two

Zn-AcOH. (B) Synthesis of the di-acid (3) where  $R = H, R^1 = CH_2COPh, R^2 = OH$ . Reagents: i, 0.8M KOH; ii,  $H^+$  then  $PhCOCH_2Br$ , 18-crown-6-ether; iii, pyridinium chlorochromate; iv, and v as in (A). (C) Synthesis of the [19-<sup>18</sup>O] di-acid (3) [ $R, R^1, R^2$  as in (B) except for <sup>18</sup>O in 19-carboxylate]. Reagents: i, K,  $Bu^tOH, H_2^{18}O$ , then  $PhCOCH_2Br$ , 18-crown-6-ether, benzene; ii-v as in (B). (D) Synthesis of the dimethyl ester (27) where  $R = R^1 = Me, R^2 = H$ . Reagents as in (A)

TMSi enol ethers, structure (32) for the enol ether (C) was indicated by the  $^1\text{H}$  n.m.r. spectrum of a solution of the dimethyl ester (26) in BSTFA. In addition to the two 17-vinyl protons, a one-proton doublet was observed at  $\delta$  5.80 and was assignable to the 1-proton in structure (32), coupled to the 5-proton only; also, the 9-H signal had disappeared. Structure (32) was also supported by the mass spectra of peak C and the corresponding peak from the  $[17-^{13}\text{C}]$  derivative.<sup>9</sup> Both spectra contained strong ions (65%) at  $m/e$  477 (i.e.  $M^+ - 41$  and  $M^+ - 42$  respectively), which can be



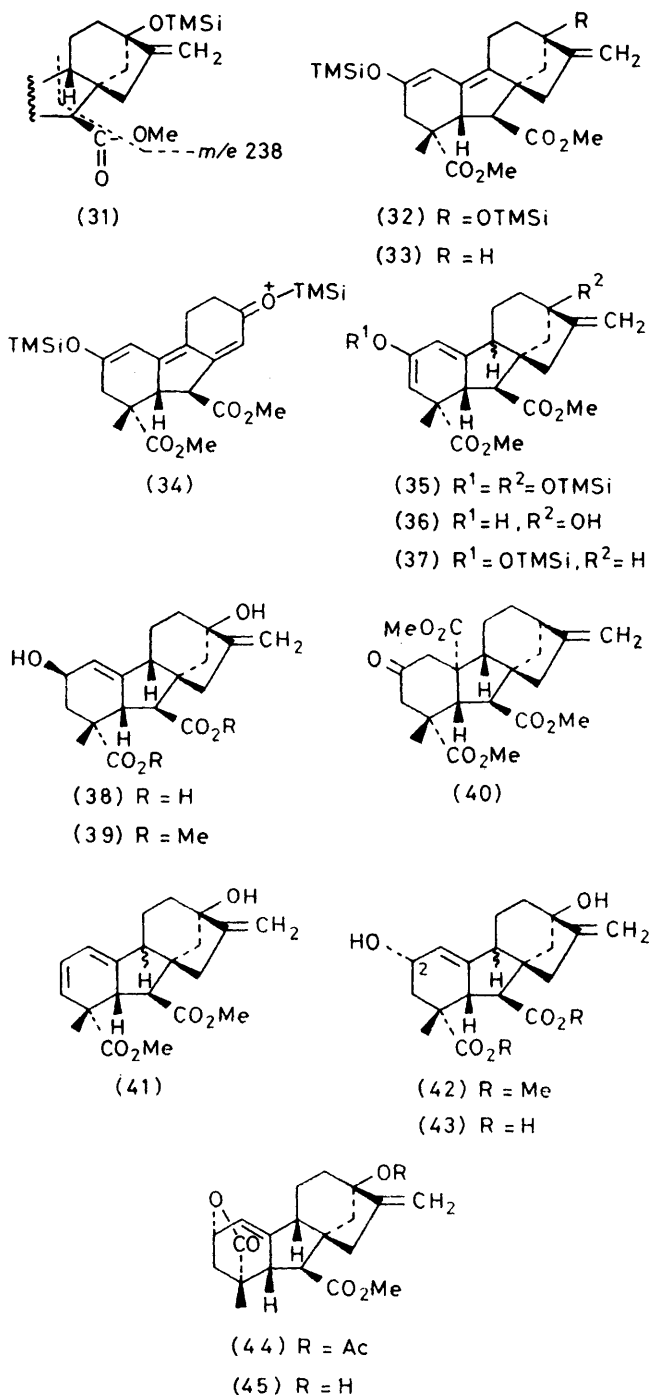
G.L.C. of synthetic mixture of (26) and (28), TMSi ethers, SE-33, 200 °C and temperature programmed at 2 °C min<sup>-1</sup>

assigned the highly conjugated structure (34) and formed by a fragmentation analogous to the process in Scheme 2.

Structure (35) for the second TMSi enol ether (peak B) was deduced as follows. The  $[3\text{E}-^2\text{H}_1]$ -derivative of the dimethyl ester (26), containing *ca.* 0.5 atoms deuterium per molecule, was prepared by reaction of the monoacetate (21) (Scheme 1) with zinc and deuteriated acetic acid. The  $[3\text{E}-^2\text{H}_1]$ -labelled dimethyl ester (26) showed reduced intensity of the AB-quartet of the 3-protons in the  $^1\text{H}$  n.m.r. spectrum and, on trimethylsilylation with HMDS-TMCS, gave the four g.l.c. peaks A–D (Figure). By mass spectrometry, the 13-TMSi ether of the dimethyl ester (26) (peak D) contained 0.43 atoms deuterium per molecule; the enol TMSi ether (32) (peak C) contained 0.40 atoms deuterium per molecule; and the enol TMSi ether (peak B) contained 0.12 atoms deuterium per molecule; this loss of deuterium on forming the enol TMSi ether (peak B) suggests structure (35).

Having established the structure (3) for the catabolite of gibberellin A<sub>29</sub> the free acid (3) was prepared both unlabelled to determine its biological activity and labelled with a stable isotope for use as an internal standard in conjunction with metabolic studies.<sup>3</sup> Attempts to demethylate the dimethyl ester (26) with aqueous alkali or with lithium iodide and collidine gave complex mixtures. In the route adopted (Scheme 1B) the phenacyl esters were used in place of the methyl esters since they could be reductively removed together with the 3 $\beta$ -acetoxy-group in the final step. Oxidation of the triol-diphenacyl ester (15) with manganese dioxide gave only 30% of the ketol (18). However pyridinium chlorochromate oxidation<sup>10</sup> gave the ketol (18) in 80% yield; oxidation of the

triol dimethyl ester (14) (Scheme 1A) with this reagent gave as high yields of the ketol (17) as with manganese dioxide and was more convenient. The 3-hydroxy-acid



(20), earlier noted as a constituent<sup>7</sup> of seed of *P. coccineus*, was prepared by reduction of the diphenacyl ester (18). The biological activities of the acids (3) and (20) will be presented elsewhere.

The  $[19-^{18}\text{O}]$ -labelled diacid was prepared (Scheme 1C)

as for the unlabelled diacid (3) except that the [ $^{18}\text{O}$ ]-label was introduced in the step from gibberellin  $\text{A}_3$  (7) to the triol diacid (9). The following procedure was adopted after much experimentation. To a solution of potassium metal in *t*-butyl alcohol was added [ $^{18}\text{O}$ ]-enriched water (0.70 atoms  $^{18}\text{O}$  per molecule) and gibberellin  $\text{A}_3$  (7) and the residue, obtained on evaporation, was directly treated with phenacyl bromide and 18-crown-6-ether in benzene. Provided the ratio of *t*-butyl alcohol to water is kept low and an excess of phenacyl bromide, corresponding to the alkalinity of the esterifying reaction mixture, is used, the [ $^{19-18}\text{O}$ ]-labelled triol diphenacyl ester (15) is obtained in acceptable yield (ca. 25%). Completion of the synthesis, as shown in Scheme 1C, gave the required [ $^{19-18}\text{O}$ ]-labelled diacid (3) with 0.69 atoms  $^{18}\text{O}$  per molecule.

A deoxy analogue of the gibberellin  $\text{A}_{29}$ -catabolite (3) has recently been detected in small quantities in developing pea seed.<sup>3</sup> Since gibberellin  $\text{A}_{51}$  (2) is known to be present in pea seeds,<sup>11</sup> and by analogy with the gibberellin  $\text{A}_{29}$ -catabolite (3), a likely structure for this new compound was (4) as a possible gibberellin  $\text{A}_{51}$ -catabolite. This structure was confirmed by partial synthesis of the dimethyl ester (27) from gibberellin  $\text{A}_7$  (8) by the same sequence (Scheme 1D) as for the dimethyl ester (26). The synthetic sample was shown by g.l.c.-mass spectrometry to be a mixture (10 : 1 : 3) of the postulated gibberellin  $\text{A}_{51}$ -catabolite (4) as its dimethyl ester, an unknown isomer, and its 9,10-double bond isomer (30). The latter compound was identified by its mass spectrum which showed an ion at  $M^+ - 41$  due to loss of C-15, -16, and -17 as described earlier (cf. Scheme 2). The dimethyl ester (27) also formed an enol trimethylsilyl ether on treatment with HMDS-TMCS although its formation did not occur as readily as was observed for the gibberellin  $\text{A}_{29}$ -catabolite dimethyl ester (26). By analogy with (32), structure (33) is assigned to this enol ether. The n.m.r. signals for the 17-protons of the diacetate (24) and the intermediates (13), (16), (19), (23), and (27) in the synthesis of the dimethyl ester (27) (Scheme 1D) resonated as a singlet at ca.  $\delta$  4.9. This is in agreement with the reported generalisation<sup>12</sup> that  $\text{C}_{19}$ -gibberellin derivatives containing a 1,10-double bond and a 13-acetate (or 13-hydrogen atom) have coincident 17-proton signals. These observations suggest that the generalisation may apply for  $\text{C}_{19}$ -gibberellin derivatives with an opened or an intact  $\gamma$ -lactone system in ring A.

In view of the ready formation of the enol-TMSi ethers (32) and (33), enolisation of the dimethyl ester (26) was investigated. Deuteration of the dimethyl ester in deuteriated trifluoroacetic acid gave a product which contained 14% deuterium by mass spectrometry. The  $^1\text{H}$  n.m.r. spectrum indicated that no rearrangement had occurred and the  $^2\text{H}$  n.m.r. spectrum showed a broad signal at  $\delta$  2.50. Since the 3-protons of the dimethyl ester occur at  $\delta$  2.25 and  $\delta$  2.79, it would appear that the deuterium had been incorporated at carbon-3 *via* the enol (36). These observations may explain the observed loss of label from the 3-position when [ $1\beta,3\alpha\text{-}^2\text{H}_2$ ]- and

[ $1\beta,3\beta\text{-}^3\text{H}_2$ ]-gibberellin  $\text{A}_{29}$  was metabolised in immature pea seed.<sup>4</sup>

In extracts of the seedlings of *P. sativum*, Sponsel and MacMillan<sup>4</sup> detected a hydroxy-diacid, as its dimethyl ester bis-TMSi ether, by mass spectrometry. They tentatively assigned the structure (38) to this hydroxy-diacid. Since a compound of this structure is a possible intermediate in the enzymatic conversion of gibberellin  $\text{A}_{29}$  (1) into the diacid (3) in seed of *P. sativum*, attempts were made to confirm structure (38) by reduction of the synthetic dimethyl ester (26).

Beeley and MacMillan<sup>13</sup> reported that Meerwein-Pondorff-Verley (MPV) reduction of the 2-ketone (40) gave the corresponding 2 $\beta$ -alcohol in 80% yield. MPV reduction of the dimethyl ester (26) gave a product the n.m.r. spectrum of which contained an olefinic proton signal at  $\delta$  5.42 indicating the presence of a 1,10-double bond. Although the reduction product appeared to be homogeneous by n.m.r. spectroscopy, g.l.c.-mass spectrometry of the trimethylsilylated product gave three peaks in the ratio 3(variable) : 2 : 18.

The component of variable intensity gave a mass spectrum consistent with the structure (41), and is considered to be an artefact of g.l.c. since its presence was not indicated by the n.m.r. spectrum of the reduction product. The major reduction product was shown to possess structure (42) by the identity of the mass spectrum of the TMSi ether with that of the compound (42), obtained by alkaline hydrolysis of the lactone (44).<sup>12</sup> The mass spectrum of the TMSi derivative of the minor component contained fragment ions at  $m/e$  473 ( $M^+ - 47$ ) and 445 ( $M^+ - 75$ ), indicative of a 1 $\alpha,2\alpha$ , or 3 $\alpha$ -TMSi ether;<sup>5</sup> the minor reduction product must therefore be the 2 $\alpha$ -hydroxy-9,10-diene (46), formed from the minor component (28), present in the synthetic dimethyl ester (26). The mass spectra and g.l.c. retention times of the TMSi derivative of these reduction products were different from the mass spectrum and g.l.c. retention time of the MeTMSi derivative of the hydroxy-acid detected in pea seeds.

Small-scale reduction of the dimethyl ester (26) with sodium borohydride gave a mixture which, by g.l.c.-mass spectrometry of the TMSi derivatives, contained the 2 $\alpha$ -hydroxy-1,10-ene (42) (5%) and the 2 $\alpha$ -hydroxy-9,10-ene (46) (27%), identical to the MPV reduction products, and the corresponding 19,2-lactones (45) (3%) and (49) (24%) which were distinguished by comparison with an authentic sample of (45). However, the main product (41%) was assigned the 2 $\beta$ -hydroxy-9,10-ene structure (47) since the mass spectrum of the TMSi derivative was very similar to that of the 2 $\alpha$ -hydroxy-9,10-ene (46) except for the absence of the ions due to  $M^+ - 47$  and  $M^+ - 75$ , characteristic of a 2 $\alpha$ -TMSi ether. The g.l.c. retention time and the mass spectrum of the TMSi derivative of the main reduction product (47) was identical to those of the MeTMSi derivative of the compound present in pea seed.

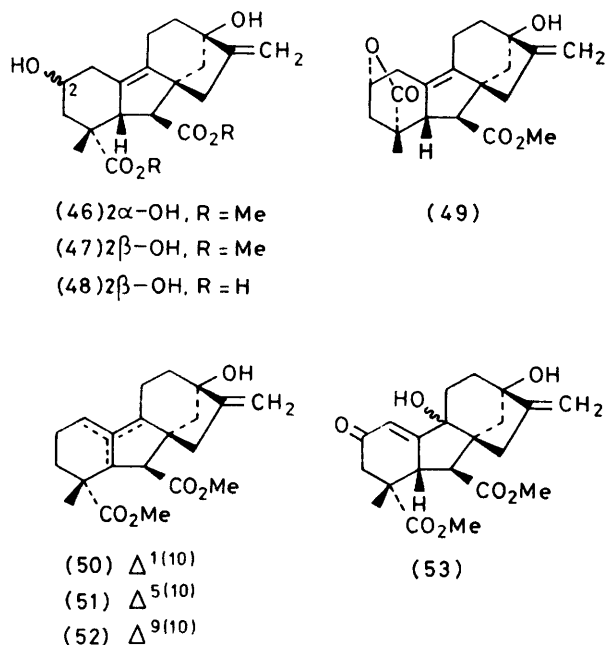
Reduction of the synthetic diacid (3) with sodium borohydride did not proceed to completion but gave a



mixture (4:1) of the 2 $\alpha$ -hydroxy-1,10-ene (43) and a product assigned the structure (38), since the mass spectrum of the MeTMSi derivative was very similar to that of the TMSi ether of the 2 $\alpha$ -hydroxy-1,10-ene (42) but it had different g.l.c. retention times.

Empirically, it was found that the bis-TMSi ethers of the 9,10-olefins (46) and (47) showed a mass spectral ion at  $M^+ - 118$ , whereas those of the 1,10-olefins (39) and (42) gave an ion at  $M^+ - 119$ .

G.l.c.-mass spectrometric analyses of extracts of *P. sativum* seedlings,<sup>4,5</sup> as the MeTMSi derivatives, show that only  $\Delta^{9,10}$ -2 $\beta$ -alcohol (48) is ever detected; the  $\Delta^{1,10}$ -2 $\beta$ -alcohol (38) has never been observed. This suggests that the  $\Delta^{9,10}$ -2 $\beta$ -alcohol (48) might be the genuine endogenous hydroxy-acid in *Pisum*. However,



the results of the reduction experiments on compound (26) indicate that 1,10  $\rightarrow$  9,10-double bond isomerisation occurs readily, during work up or g.l.c.-mass spectrometric analysis. It is therefore uncertain whether the 2 $\beta$ -hydroxy-9,10-ene (48) is an enzymatic product in pea seed or an artefact, formed non-enzymatically from the corresponding 1,10-ene (38) during extraction or g.l.c.-mass spectrometric analysis. Similarly it is uncertain if the di-acids (3) and (29, Scheme 1) are enzymatic products or whether one is formed non-enzymatically from the other. For this reason the stereochemistry of the 9-hydrogen in the di-acid (3) and di-ester (26) is not assigned.

When the monoacetate (21) (Scheme 1) was refluxed in acetic acid with zinc, a complex mixture was obtained. G.l.c.-mass spectrometry of the trimethylsilylated less polar compounds, separated by p.l.c., indicated the presence of three isomers, possibly (50), (51), and (52) arising from a Clemmensen-type reduction. The mass

spectrum of one of these TMSi derivatives was identical to that of the MeTMSi derivative of a compound, found in extracts of seedlings of *P. sativum*<sup>4,5</sup> and seeds of *Vicia faba*<sup>7</sup> and tentatively assigned<sup>4,7</sup> the structure (50). However the mass spectrum of the MeTMSi derivative was similar to that of the MeTMSi derivative of the 2 $\beta$ -hydroxy-9,10-ene (48) and it may be the 9,10-ene (52).

From the same deacetoxylation reaction, there was isolated in addition to the dimethyl ester (26, Scheme 1) a compound detected in derivatised extracts from pea seedlings.<sup>5</sup> Structure (53) for this compound was deduced from the following information. The molecular formula, containing one more oxygen atom than the dimethyl ester (26), was established by high-resolution mass spectroscopy. The i.r. spectrum in chloroform solution contained two  $\nu_{OH}$  bands at 3 600 and 3 420  $\text{cm}^{-1}$  and a  $\nu_{C=O}$  at 1 682  $\text{cm}^{-1}$ ; the u.v. spectrum had  $\lambda_{max}$  239 nm ( $\epsilon$ , 9 800). The n.m.r. spectrum was similar to that of the dimethyl ester (26) except that a signal in the latter assigned to the 9-proton at  $\delta$  2.8 was absent and that the 1-proton signal at  $\delta$  6.02 was a doublet instead of a triplet. Furthermore the compound did not form an enol TMSi ether with BSA and only formed a mono-TMSi ether, consistent with a sterically hindered hydroxy-group at C-9. This compound (53) has also been detected in trace amounts in the deacetoxylation of the mono-acetate (21) at room temperature but the mechanism of its formation is not known.

#### EXPERIMENTAL

For general experimental details see ref. 14. Mass spectra were obtained by g.l.c.-mass spectrometry except for those marked  $m/e^*$  which were obtained by direct probe insertion. The  $^2\text{H}$  n.m.r. spectra were obtained with a Jeol FX90Q instrument for chloroform solutions and a 2 000 Hz sweep width.

*Dimethyl ent-2 $\beta$ ,3 $\alpha$ ,13-Trihydroxy-20-norgibberella-1(10),-16-diene-7,19-dioate* (14).—Gibberellin A<sub>3</sub> (1.0 g) (7) was dissolved in 0.8M-aqueous potassium hydroxide solution (14 ml, 4 equiv.). After 20 h, concentrated hydrochloric acid was added to pH 3.0 and the products were extracted into ethyl acetate (6  $\times$  35 ml) at 5  $^{\circ}\text{C}$ . The combined extracts were washed with water (*ca.* 10 ml), the volume was reduced *in vacuo* at *ca.* 35  $^{\circ}\text{C}$  to approximately 10 ml, and ethereal diazomethane was added until the yellow colour persisted. Evaporation and preparative layer chromatography (p.l.c.), using ethyl acetate–light petroleum–acetic acid (90:10:1), gave, at  $R_F$  0.2–0.3, the triol dimethyl ester (14). Crystallisation from methanol gave the solvated triol (702 mg) as prisms, m.p. 95–100  $^{\circ}\text{C}$ ; (Found: C, 62.5; H, 7.9. Calc. for  $\text{C}_{21}\text{H}_{28}\text{O}_7 \cdot \text{CH}_3\text{OH}$ : C, 62.3; H, 7.6%;  $\nu_{max}$  3 400br., 3 310br., 3 250br., 1 733, 1 692, 1 660w, and 890  $\text{cm}^{-1}$ . The n.m.r. spectrum showed a singlet at  $\delta$  3.45 corresponding to one molecule of methanol per molecule. Drying at 100  $^{\circ}\text{C}$  *in vacuo* for several hours afforded the solvent-free triol (14), m.p. 138–140  $^{\circ}\text{C}$  (Found: C, 64.5; H, 7.1. Calc. for  $\text{C}_{21}\text{H}_{26}\text{O}_7$ : C, 64.3; H, 7.1%);  $\delta$  1.34 (s, 18-H<sub>3</sub>), 2.49br. (s, 9-H), 2.57, (d,  $J$  7 Hz, 6-H), 3.09br. (s,  $W_{\frac{1}{2}}$  12 Hz, 5-H; reduced to d,  $J$  7 Hz, by irradiation at  $\delta$  5.42), 3.65 (s, CO<sub>2</sub>Me), 3.71 (s, CO<sub>2</sub>Me), 3.85 (d,

$J$  5 Hz, 3-H), 4.15br. (s,  $W_{\frac{1}{2}}$  10 Hz, 2-H), 4.97br. (s,  $W_{\frac{1}{2}}$  6 Hz, 17-H), 5.10br. (s,  $W_{\frac{1}{2}}$  7 Hz, 17-H), and 5.42br. (s,  $W_{\frac{1}{2}}$  7 Hz, 1-H); reduced to t,  $J$  2 Hz by irradiation at  $\delta$  2.49;  $m/e$  ( $\text{Me}_3\text{Si}$  ether): 608( $M^+$ , 10%), 593(4), 518(11), 459(14), 421(34), 420(94), 399(25), 279(13), 269(25), 261(36), 204(12), 75(26), and 73(100).

**Dimethyl ent-3 $\alpha$ ,13-Dihydroxy-2-oxo-20-norgibberella-1(10),16-diene-7,19-dioate (17).**—The triol (14) (609 mg) and activated manganese dioxide<sup>15</sup> (6 g) in chloroform (30 ml) were stirred for 4 days. Filtration through a pad of 'Celite', and evaporation of the filtrate and washings, gave a gum (577 mg) which was purified by p.l.c. using ethyl acetate–light petroleum (9:1). Elution of the band at  $R_F$  0.35–0.45 gave the ketol (17) (363 mg) which was crystallised from methyl ethyl ketone–light petroleum as prisms, m.p. 98–101 °C (Found: C, 64.0; H, 6.7. Calc. for  $\text{C}_{21}\text{H}_{26}\text{O}_7$ : C, 64.6; H, 6.7%).  $\nu_{\text{max}}$ , 3 580, 3 390sh., 3 320, 3 225br., 1 715, 1 667, 1 648sh., 895, and 890  $\text{cm}^{-1}$ ;  $\lambda_{\text{max}}$  (EtOH) 239 nm ( $\epsilon$  13 400);  $\delta$  1.37 (s, 18- $\text{H}_3$ ), 2.82br. (s,  $W_{\frac{1}{2}}$  14 Hz, 9-H; sharpened by irradiation at  $\delta$  5.83), 3.06 (d,  $J$  5 Hz, 6-H), 3.39br. (s,  $W_{\frac{1}{2}}$  10 Hz, 5-H), 3.64 (s,  $\text{CO}_2\text{Me}$ ), 3.75 (s,  $\text{CO}_2\text{Me}$ ), 4.01 (s, 3-H), 5.00br. (s,  $W_{\frac{1}{2}}$  5 Hz, 17-H), 5.12br. (s,  $W_{\frac{1}{2}}$  6 Hz, 17-H), and 5.83 (t,  $J$  2 Hz, 1-H);  $m/e$  ( $\text{Me}_3\text{Si}$  ether) 534( $M^+$ , 48%), 519(11), 505(6), 475(10), 459(11), 415(9), 385(9), 346(8), 318(8), 287(10), 239(24), 239(100), 207(6), 75(29), and 73(68);  $m/e$  (enol  $\text{Me}_3\text{Si}$  ether) 606( $M^+$ , 100%), 591(9), 565(49), 487(33), 457(86), 397(44), 325(13), 307(25), 243(13), 207(4), 167(16), 75(82), and 73(93).

**Dimethyl ent-3 $\alpha$ -Acetoxy-13-hydroxy-1-oxo-20-norgibberella-1(10),16-diene-7,19-dioate (21).**—The ketol (17) (212 mg) and acetic anhydride (1.7 ml) in pyridine (3 ml) were left for 20 h. Water was added and the products were extracted into ethyl acetate at pH 3.0. Evaporation of the solvent and p.l.c., using ethyl acetate–light petroleum (9:1), gave, at  $R_F$  0.45–0.55, the monoacetate (21) (165 mg) which was crystallised from ethyl acetate–light petroleum as needles, m.p. 150–153 °C (Found: C, 63.6; H, 6.8. Calc. for  $\text{C}_{23}\text{H}_{28}\text{O}_8$ : C, 63.9; H, 6.5%).  $\nu_{\text{max}}$ , 3 520, 1 759, 1 745sh., 1 721, 1 681, 1 650sh., 887, and 880  $\text{cm}^{-1}$ ;  $\lambda_{\text{max}}$  (EtOH) 240 nm ( $\epsilon$  14 100);  $\delta$  1.23 (s, 18- $\text{H}_3$ ), 2.10 (s, OAc), 2.81br. (s,  $W_{\frac{1}{2}}$  11 Hz, 9-H; sharpened by irradiation at  $\delta$  5.91), 3.10 (d,  $J$  5 Hz, 6-H), 3.54 (dd,  $J$  2 and 5 Hz, 5-H), 3.66 (s,  $\text{CO}_2\text{Me}$ ), 3.77 (s,  $\text{CO}_2\text{Me}$ ), 5.02br. (s,  $W_{\frac{1}{2}}$  6 Hz, 17-H), 5.14br. (s,  $W_{\frac{1}{2}}$  7 Hz, 17-H), 5.47 (s, 3-H), and 5.91 (t,  $J$  2 Hz, 1-H);  $m/e$  ( $\text{Me}_3\text{Si}$  ether) 504( $M^+$ , 30%), 489(5), 475(17), 444(19), 385(31), 317(15), 259(9), 238(72), 207(6), 141(7), 75(16), 73(100), and 43(28);  $m/e$  (enol  $\text{Me}_3\text{Si}$  ether) 576( $M^+$ , 14%), 535(9), 517(4), 457(30), 444(22), 397(36), 385(18), 309(13), 117(13), 75(25), 73(100), and 43(20).

Elution of the band at  $R_F$  0.7–0.8 gave the *diacetate* (24) as a gum (30 mg) (Found:  $M^+$ , 474.190.  $\text{C}_{25}\text{H}_{30}\text{O}_9$  requires  $M^+$ , 474.189);  $\nu_{\text{max}}$  ( $\text{CHCl}_3$ ) 1 755sh., 1 726, 1 687, and 1 656sh.  $\text{cm}^{-1}$ ;  $\delta$  1.24 (s, 18- $\text{H}_3$ ), 1.99 (s, 13-OAc), 2.12 (s, 3-OAc), 2.85br. (s,  $W_{\frac{1}{2}}$  9 Hz, 9-H), 3.12 (d,  $J$  5 Hz, 6-H), 3.73 (dd,  $J$  2 and 5 Hz, 5-H), 3.69 (s,  $\text{CO}_2\text{Me}$ ), 3.78 (s,  $\text{CO}_2\text{Me}$ ), 5.05br. (s,  $W_{\frac{1}{2}}$  6 Hz, 17- $\text{H}_2$ ), 5.49 (s, 3-H), and 5.94 (t,  $J$  2 Hz, 1-H);  $m/e^*$  474( $M^+$ , 12%), 432(21), 414(41), 373(28), 372(32), 355(48), 213(80), 295(32), 253(24), 235(23), and 43(100).

**Reduction of the Acetate (21) with Zinc and Acetic Acid.**—(a) *At room temperature.* The monoacetate (21) (140 mg) and freshly activated zinc dust<sup>8</sup> (350 mg) in glacial acetic acid (1 ml) were stirred for 2 h. Water was added and the product was extracted with ethyl acetate. Evaporation of the

solvent gave the  $\Delta^{1(10)}$ -dimethyl ester (26) as a gum (120 mg) (Found:  $M^+$  374.173.  $\text{C}_{21}\text{H}_{26}\text{O}_6$  requires  $M^+$  374.173);  $\nu_{\text{max}}$  ( $\text{CHCl}_3$ ) 3 595, 1 728, 1 668, and 895  $\text{cm}^{-1}$ ;  $\lambda_{\text{max}}$  (EtOH) 236 nm ( $\epsilon$  11 400);  $\delta$  1.33 (s, 18- $\text{H}_3$ ), 2.25 (d,  $J_{AB}$  16 Hz, 3-H), 2.78br. (s, 9-H), 2.79 (d,  $J_{AB}$  16 Hz, 3-H), 3.14 (d,  $J$  5 Hz, 6-H), 3.37 (dd,  $J$  2.5 and 5 Hz, 5-H), 3.63 (s,  $\text{CO}_2\text{Me}$ ), 3.76 (s,  $\text{CO}_2\text{Me}$ ), 5.00br. (s,  $W_{\frac{1}{2}}$  6 Hz, 17-H), 5.13br. (s,  $W_{\frac{1}{2}}$  6 Hz, 17-H), and 5.88br. (s,  $W_{\frac{1}{2}}$  6 Hz, 1-H; changed to d,  $J$  2.5 Hz on irradiation at  $\delta$  2.78);  $\delta$  (BSTFA) 1.27 (s, 18- $\text{H}_3$ ), 2.18 and 2.70 (each d,  $J_{AB}$  16 Hz, 3- $\text{H}_2$ ), 3.16 (d,  $J$  5 Hz, 6-H), 3.39br. (s,  $W_{\frac{1}{2}}$  9 Hz, 5-H), 3.62 (s,  $\text{CO}_2\text{Me}$ ), 3.70 (s,  $\text{CO}_2\text{Me}$ ), 4.95br. (s,  $W_{\frac{1}{2}}$  5 Hz, 17-H), 5.10br. (s,  $W_{\frac{1}{2}}$  6 Hz, 17-H), and 5.80br. (s,  $W_{\frac{1}{2}}$  7 Hz, 1-H);  $m/e$  374( $M^+$ , 42%), 372(4), 343(14), 333(10), 315(40), 256(18), 255(100), and 149(12);  $m/e$  ( $\text{Me}_3\text{Si}$  ether) 446( $M^+$ , 100%), 431(12), 429(8), 417(28), 414(7), 405(1), 387(32), 327(9), 259(8), 238(19), 75(8), and 73(58);  $m/e$  (enol  $\text{Me}_3\text{Si}$  ether) (32) 518( $M^+$ , 100%), 503(14), 478(31), 477(70), 459(7), 444(10), 429(10), 399(12), 369(10), 309(18), 307(13), 75(32), and 73(95);  $\Delta^8$ -dimethyl ester (28):  $m/e$  374( $M^+$ , 65%), 372(9), 359(11), 315(29), 314(26), 297(20), 255(43), 237(21), and 209(15);  $m/e$  ( $\text{Me}_3\text{Si}$  ether) 446( $M^+$ , 100%), 444(10), 431(10), 417(16), 405(21), 387(31), 327(21), 238(3), 141(20), 75(17), and 73(69).

(b) *Under reflux.* The acetate (21) (126 mg) and zinc dust (350 mg) in glacial acetic acid (12 ml) were refluxed for 3 h. Water was added and the products were extracted into ethyl acetate at pH 9.0. Evaporation of the solvent and p.l.c. using ethyl acetate–light petroleum (9:1), gave, at  $R_F$  0.45–0.55, the dimethyl ester (26) (54 mg) as in (a). Elution of the band at  $R_F$  0.70–0.75 gave a gum (15 mg) shown by g.l.c. to be mixture (1:2:3) of three compounds;  $m/e$  ( $\text{Me}_3\text{Si}$  ether) 432( $M^+$ , 75%), 417(11), 403(25), 400(10), 373(15), 372(15), 313(19), 261(23), 238(73), 208(100), 207(57), 75(13), and 73(100);  $m/e$  [tentatively assigned structure (52);  $\text{Me}_3\text{Si}$  ether] 432( $M^+$ , 100%), 417(9), 403(32), 400(10), 373(37), 372(41), 313(23), 273(16), 238(52), 135(31), 75(10), and 73(60);  $m/e$  ( $\text{Me}_3\text{Si}$  ether) 432( $M^+$ , 100%), 417(6), 403(28), 400(14), 373(43), 261(48), 260(63), 231(23), 208(74), 207(94), 93(23), 75(16), and 73(92).

Elution of the band at  $R_F$  0.55–0.60 gave the 9 $\xi$ -alcohol (53) (33 mg) which was crystallised from ethyl acetate–light petroleum, m.p. 183–184 °C (Found:  $M^+$  390.168.  $\text{C}_{21}\text{H}_{26}\text{O}_7$  requires  $M^+$  390.168);  $\nu_{\text{max}}$  ( $\text{CHCl}_3$ ) 3 600, 3 420, 1 729, and 1 682  $\text{cm}^{-1}$ ;  $\lambda_{\text{max}}$  (EtOH) 239 nm ( $\epsilon$  9 800);  $\delta$  1.28 (s, 18- $\text{H}_3$ ), 2.32 and 2.83 (each d,  $J_{AB}$  17 Hz, 3- $\text{H}_2$ ), 3.27 (d,  $J$  11 Hz, 6-H), 3.47 (dd,  $J$  3 and 11 Hz, 5-H; reduced to d,  $J$  11 Hz by irradiation at  $\delta$  6.02), 3.72 (s,  $\text{CO}_2\text{Me}$ ), 3.76 (s,  $\text{CO}_2\text{Me}$ ), 4.90 and 5.10 (each br. s, 17- $\text{H}_2$ ), 6.02 (d,  $J$  3 Hz, 1-H), and 7.95 (s, OH, disappeared on addition of  $\text{D}_2\text{O}$ );  $m/e$  ( $\text{Me}_3\text{Si}$  ether) 462( $M^+$ , 1%), 444(45), 430(3), 412(14), 385(16), 239(100), 239(36), 207(15), 167(11), 75(11), and 73(47).

**Dimethyl ent-3 $\xi$ -Deuterio-13-hydroxy-2-oxo-20-norgibberella-1(10),16-diene-7,19-dioate.**—(a) Acetic anhydride (1 ml, 10.6 mmol), pyridine (0.01 ml) and deuterium oxide (0.212 ml, 10.6 mmol) were warmed until a homogeneous solution was obtained. To this solution was added the mono-acetate (21) (30 mg) and activated zinc dust<sup>8</sup> (40 mg) with stirring for 2 h. Work-up as before gave the [ $3\xi$ - $^2\text{H}$ ]-labelled dimethyl ester (26) as a gum (21 mg) (*ca.* 0.50 atoms  $^2\text{H}$  per molecule).

(b) Deuteriotrifluoroacetic acid, prepared by the dropwise addition of trifluoroacetic anhydride (0.39 ml, 5.3 mmol) to deuterium oxide (0.21 ml, 10.5 mmol) at 0 °C, was added to

the dimethyl ester (26) (30 mg). After 4 h, anhydrous sodium carbonate (0.5 g) was added and the product extracted into ethyl acetate at pH 7.0. The organic solvent was removed *in vacuo* and the deuteriated product (30 mg) (14 atoms  $^2\text{H}$  per molecule) analysed by  $^2\text{H}$  n.m.r. spectroscopy;  $\delta$  ( $\text{CHCl}_3$ ) 2.50 (3- $\text{D}_2$ ), 4.70 (HOD), and 7.25 ( $\text{CDCl}_3$ ).

**Diphenacyl ent-2 $\beta$ ,3 $\alpha$ ,13-Trihydroxy-20-norgibberella-1(10),16-diene-7,19-dioate (15).**—Gibberellin  $\text{A}_3$  (7) (1.08 g) was dissolved in aqueous potassium hydroxide solution (0.8M; 14 ml) and left for 20 h. The pH was reduced to 9.0 using concentrated hydrochloric acid and water was removed by azeotropic distillation with toluene. After drying in a vacuum desiccator, the residue, phenacyl bromide (1.56 g), and 18-crown-6-ether (104 mg) in dry cyanomethane (10 ml) were refluxed for 0.5 h. The solvent was removed *in vacuo*, water was added and the products were extracted into ethyl acetate. Evaporation gave a gum (2.60 g) which was chromatographed on a silica column (100 g;  $40 \times 2.5$  cm) made up in light petroleum and eluted with increasing proportions of ethyl acetate in light petroleum. Elution with 70–100% ethyl acetate, followed by evaporation, gave a gum (1.40 g) which was purified further by p.l.c. using ethyl acetate–light petroleum–acetic acid (90 : 10 : 1). Elution of the band at  $R_F$  0.15–0.25 gave the **diphenacyl ester (15)** as a gum (1.34 g) [Found (as a foam): C, 69.2; H, 6.1.  $\text{C}_{35}\text{H}_{36}\text{O}_9$  requires C, 70.0; H, 6.0%];  $\nu_{\text{max}}$  ( $\text{CHCl}_3$ ) 3 590, 3 450, 1 734, 1 702, 1 660, 1 602, 975, and 960  $\text{cm}^{-1}$ ;  $\lambda_{\text{max}}$  (EtOH) 243 nm ( $\epsilon$  18 000);  $\delta$  1.54 (s, 18- $\text{H}_3$ ), 2.75br. (s, 9-H), 3.08 (d,  $J$  7 Hz, 6-H), 3.22br. (d,  $J$  8 Hz, 5-H), 4.11 (d,  $J$  4 Hz, 3-H), 4.24br. (s,  $W_{\frac{1}{2}}$  5 Hz, 2-H), 5.00br. (s,  $W_{\frac{1}{2}}$  6 Hz, 17-H), 5.14br. (s,  $W_{\frac{1}{2}}$  4 Hz, 17-H), 5.29 (d,  $J_{AB}$  16 Hz,  $\text{PhCOCH}_2$ ), 5.45 (s,  $\text{PhCOCH}_2$ ), 5.47 (d,  $J_{AB}$  16 Hz,  $\text{PhCOCH}_2$ ), 7.56 (m,  $6 \times \text{Ar-H}$ ), and 7.93 (dd,  $J$  2 and 8 Hz,  $4 \times \text{Ar-H}$ );  $\delta$  ( $\text{C}_6\text{D}_6$ – $\text{CDCl}_3$ ; 1 : 1), 5.45br. (s,  $W_{\frac{1}{2}}$  6 Hz, 1-H);  $m/e^*$  600( $M^+$ , 0%), 464(1), 446(5), 401(55), 345(23), 328(28), 310(20), 300(40), 237(100), 209(33), 105(98), 91(51), and 77(61).

**Diphenacyl ent-3 $\alpha$ ,13-Dihydroxy-2-oxo-20-norgibberella-1(10),16-diene-7,19-dioate (18).**—The triol (15) (304 mg) in dichloromethane (1 ml) was added to a stirred suspension of pyridinium chlorochromate<sup>10</sup> (219 mg) and sodium acetate (21 mg) in dichloromethane (1 ml). After 40 min, diethyl ether (*ca.* 5 ml) was added and the mixture was passed through a short silica plug which was washed with ethyl acetate. Evaporation of the eluate and washings gave a gum (310 mg) which was fractionated by p.l.c. using ethyl acetate–light petroleum (9 : 1). Elution of the band at  $R_F$  0.45–0.55 gave the **ketol (18)** (238 mg), recrystallised from ethyl acetate–light petroleum, m.p. 219–221 °C (Found: C, 69.7; H, 5.6.  $\text{C}_{35}\text{H}_{34}\text{O}_9$  requires C, 70.2; H, 5.7%);  $\nu_{\text{max}}$  3 592, 3 480br., 3 390sh., 1 733, 1 698, 1 680, 1 653sh., 1 600, 980, and 967  $\text{cm}^{-1}$ ;  $\delta$  1.68 (s, 18- $\text{H}_3$ ), 3.02br. (s,  $W_{\frac{1}{2}}$  9 Hz, 9-H), 3.50 (d,  $J$  5 Hz, 6-H), 3.88 (dd,  $J$  2 and 5 Hz, 5-H), 4.28 (s, 3-H), 5.11 and 5.22 (each br. s,  $W_{\frac{1}{2}}$  6 Hz, 17- $\text{H}_2$ ), 5.31 (d,  $J_{AB}$  16 Hz,  $\text{PhCOCH}_2$ ), 5.47 (s,  $\text{PhCOCH}_2$ ), 5.49 (d,  $J_{AB}$  16 Hz,  $\text{PhCOCH}_2$ ), 5.96br. (s,  $W_{\frac{1}{2}}$  6 Hz, 1-H), 7.64 (m,  $6 \times \text{Ar-H}$ ), and 7.97 (m,  $4 \times \text{Ar-H}$ );  $m/e^*$  598( $M^+$ , 1%), 462(3), 444(1), 343(11), 326(30), 298(36), 253(28), 213(21), 185(31), 120(38), 105(100), 91(37), and 77(100).

**ent-3 $\alpha$ ,13-Dihydroxy-2-oxo-20-norgibberella-1(10),16-diene-7,19-dioic Acid (20).**—The ketol (18) (34 mg) and freshly activated zinc dust<sup>8</sup> (200 mg) in glacial acetic acid (1 ml) were stirred for 2 h. The insoluble material was deposited by centrifugation and the supernatant solution was removed; the residue was stirred ( $\times 2$ ) with acetone

(2 ml). The supernatant and acetone washings were combined, then evaporated, and the product purified by p.l.c. on pre-eluted silica plates (0.4 mm) using ethyl acetate–light chloroform–acetic acid (15 : 5 : 1). Elution of the band at  $R_F$  *ca.* 0.3 with water-saturated ethyl acetate and evaporation gave the **diacid (20)** as a gum (14 mg) (Found:  $M^+$  362.137.  $\text{C}_{19}\text{H}_{22}\text{O}_7$  requires  $M^+$  362.137);  $\lambda_{\text{max}}$  240 nm ( $\epsilon$  8 700);  $\delta$  ( $\text{CD}_3\text{COCD}_3$ ), 1.39 (s, 18- $\text{H}_3$ ), 3.17 (d,  $J$  5 Hz, 6-H), 3.66br. (s,  $W_{\frac{1}{2}}$  9 Hz), 3.90 (s, 3-H), 4.96br. (s,  $W_{\frac{1}{2}}$  7 Hz, 17-H), 5.10br. (s,  $W_{\frac{1}{2}}$  7 Hz, 17-H), and 5.74br. (s,  $W_{\frac{1}{2}}$  6 Hz, 1-H);  $m/e^*$  362( $M^+$ , <1%), 318(29), 301(23), 300(100), 272(19), 254(33), 171(37), 128(50), and 115(54);  $m/e$  ( $\text{Me}_3\text{Si}$  ester  $\text{Me}_3\text{Si}$  ester), 650( $M^+$ , 10%), 635(8), 560(3), 533(4), 517(6), 443(10), 415(16), 296(32), 147(16), 75(21), and 73(100).

**Diphenacyl ent-3 $\alpha$ -Acetoxy-13-hydroxy-2-oxo-20-norgibberella-1(10),16-diene-7,19-dioate (22).**—The ketol (18) (82 mg) and acetic anhydride (0.5 ml) in pyridine (0.9 ml) were left for 21 h. Water was added and the product was extracted into ethyl acetate at pH 3.0. Evaporation of the solvent and p.l.c. using ethyl acetate–light petroleum (7 : 3), gave, at  $R_F$  0.45–0.55, the **monoacetate (22)** as a gum (70 mg) (Found:  $M^+$  640.230.  $\text{C}_{37}\text{H}_{36}\text{O}_{10}$  requires  $M^+$  640.231);  $\nu_{\text{max}}$  ( $\text{CHCl}_3$ ) 3 590, 1 742, 1 703, 1 684, 1 654sh, 1 602, 986, and 971  $\text{cm}^{-1}$ ;  $\delta$  1.51 (s, 18- $\text{H}_3$ ), 2.15 (s, OAc), 2.95br. (s,  $W_{\frac{1}{2}}$  9 Hz, 9-H), 3.50 (d,  $J$  5 Hz, 6-H), 3.80br. (s,  $W_{\frac{1}{2}}$  8 Hz, 5-H), 5.02 and 5.14 (each br. s,  $W_{\frac{1}{2}}$  7 Hz, 17- $\text{H}_2$ ), 5.24 (d,  $J_{AB}$  16 Hz,  $\text{PhCOCH}_2$ ), 5.41br. (s,  $W_{\frac{1}{2}}$  4 Hz,  $\text{PhCOCH}_2$ ), 5.42 (d,  $J_{AB}$  16 Hz,  $\text{PhCOCH}_2$ ), 5.66 (s, 3-H), 5.94 (t,  $J$  3 Hz, 1-H), 7.50 (m,  $W_{\frac{1}{2}}$  12 Hz,  $6 \times \text{Ar-H}$ ), and 7.86 (m,  $W_{\frac{1}{2}}$  15 Hz,  $4 \times \text{Ar-H}$ );  $m/e^*$  640( $M^+$ , 1%), 598(1), 444(2), 401(3), 299(5), 253(12), 237(10), 131(8), 120(6), 105(100), 91(13), 77(60), and 43(85).

Elution of the band at  $R_F$  0.65–0.75 gave the **diacetate (25)** as a gum (16 mg);  $\nu_{\text{max}}$  ( $\text{CHCl}_3$ ), 1 736, 1 706, 1 686, 1 653sh., and 1 603  $\text{cm}^{-1}$ ;  $\delta$  1.50 (s, 18- $\text{H}_3$ ), 1.96 (s, 13-OAc), 2.16 (s, 3-OAc), 2.95br. (s,  $W_{\frac{1}{2}}$  10 Hz, 9-H), 3.44 (d,  $J$  4 Hz, 6-H), 3.81 (dd,  $J$  3 and 4 Hz, 5-H), 5.07br. (s,  $W_{\frac{1}{2}}$  7 Hz, 17- $\text{H}_2$ ), 5.25, 5.30, 5.45, and 5.51 (each d,  $J_{AB}$  16 Hz,  $2 \times \text{PhOCH}_2$ ), 5.70 (s, 3-H), 5.98br. (s,  $W_{\frac{1}{2}}$  6 Hz, 1-H), 7.51 (m,  $6 \times \text{Ar-H}$ ), and 7.88 (dd,  $J$  2 and 7 Hz,  $4 \times \text{Ar-H}$ );  $m/e^*$  682( $M^+$ , <1%), 622(1), 486(1), 443(8), 401(12), 384(4), 325(2), 237(24), 105(100), 91(25), 77(66), and 43(>100).

**ent-13-Hydroxy-2-oxo-20-norgibberella-1(10),16-diene-7,19-dioic Acid (3).**—The monoacetate (22) (84 mg) and freshly activated zinc dust<sup>8</sup> (500 mg) in glacial acetic acid (1 ml) were stirred for 2 h. Work-up and p.l.c. as for the diacid (20) gave, at  $R_F$  *ca.* 0.3, the **diacid (3)** as a gum (19 mg) (Found:  $M^+$  364.141.  $\text{C}_{19}\text{H}_{22}\text{O}_6$  requires  $M^+$  364.142);  $\lambda_{\text{max}}$  (EtOH) 237.5 nm ( $\epsilon$  9 900);  $\delta$  ( $\text{CD}_3\text{COCD}_3$ ) 1.36 (s, 18- $\text{H}_3$ ), 2.19 and 2.70 (each d,  $J_{AB}$  16 Hz, 3- $\text{H}_2$ ), 3.19 (d,  $J$  4 Hz, 6-H), 3.45br. (s,  $W_{\frac{1}{2}}$  8 Hz, 5-H), 4.95br. (s,  $W_{\frac{1}{2}}$  6 Hz, 17-H), 5.10br. (s,  $W_{\frac{1}{2}}$  7 Hz, 17-H), and 5.80 (t,  $J$  3 Hz, 1-H);  $m/e^*$  346( $M^+$ , 100%), 328(95), 318(34), 310(10), 300(64), 289(31), 287(25), 272(18), 255(74), and 115(68);  $m/e$  ( $\text{Me}_3\text{Si}$  ester,  $\text{Me}_3\text{Si}$  ether) 562( $M^+$ , 71%), 547(37), 533(10), 472(60), 445(44), 355(22), 327(50), 318(20), 296(17), 147(18), 75(39), and 73(100);  $m/e$  ( $\Delta^9$ -isomer  $\text{Me}_3\text{Si}$  ester,  $\text{Me}_3\text{Si}$  ether) 562( $M^+$ , 23%), 547(13), 521(3), 472(8), 444(23), 355(8), 327(30), 75(42), and 73(100).

**ent-[19- $^{18}\text{O}$ ]-13-Hydroxy-2-oxo-20-norgibberella-1(10),16-diene-7,19-dioic Acid.**—(a) **Triol diphenacyl ester (15).** Freshly cut potassium metal (180 mg), washed with light petroleum, was dissolved in *t*-butyl alcohol (2.0 ml) and



[ $^{18}\text{O}$ ]-enriched water (0.6 ml, 0.70 atoms  $^{18}\text{O}$  per molecule) and gibberellin  $\text{A}_3$  (7) (550 mg) were added. The mixture was stirred for 20 h then the solvent was evaporated using toluene for azeotropic removal of water. The residue, dried in a vacuum desiccator, phenacyl bromide (2 g) and 18-crown-6-ether (40 mg) in benzene (8 ml) were refluxed for 1 h. Evaporation of the solvent gave a crude product which was chromatographed on a column (30  $\times$  3 cm) of silica gel (135 g), made up in light petroleum. The column was eluted with increasing proportions of ethyl acetate in light petroleum and then with increasing proportions of methanol in ethyl acetate. The fractions, collected with 80% ethyl acetate–20% methanol, gave a gum (310 mg) which was subjected to p.l.c. on silica gel, using ethyl acetate–light petroleum–acetic acid (90 : 10 : 1). Elution of the band at  $R_F$  0.25–0.35 gave the [ $^{19-^{18}\text{O}}$ ]-labelled triol diphenacyl ester (15) (257 mg).

(b) *Ketol diphenacyl ester* (18). Pyridinium chlorochromate  $^{10}$  (170 mg) and sodium acetate (20 mg) in dichloromethane (1 ml) were stirred for 10 min, the [ $^{18}\text{O}$ ]-labelled triol (15) (257 mg) in dichloromethane (1 ml) was added, and stirring was continued for 40 min. Diethyl ether (15 ml) was added and the insoluble material was deposited by centrifugation. The supernatant solution and washings were evaporated and fractionated by p.l.c. using ethyl acetate–light petroleum–acetic acid (90 : 10 : 1). Elution of the band at  $R_F$  0.5–0.7 gave the diphenacyl ester of the [ $^{19-^{18}\text{O}}$ ]-ketol (18) (114 mg).

(c) *Acetylation of the ketol diphenacyl ester*. The  $^{18}\text{O}$ -labelled ketol (18) (114 mg) and acetic anhydride (0.7 ml) in pyridine (1.3 ml) were left for 16 h. Water was added and the products extracted into ethyl acetate at pH 3.0. Evaporation and p.l.c., using ethyl acetate–light petroleum (7 : 3) gave, at  $R_F$  0.35–0.50, the [ $^{19-^{18}\text{O}}$ ]-monoacetate (22) (97 mg). Elution of the band at  $R_F$  0.65–0.75 gave the [ $^{19-^{18}\text{O}}$ ]-diacetate (25) (13 mg).

(d) *Diacid*. The  $^{18}\text{O}$ -labelled monoacetate (22) (97 mg) and freshly activated zinc dust  $^8$  (500 mg), in glacial acetic acid (1.5 ml), were stirred for 2 h. Work-up and p.l.c. as for the unlabelled diacid (3) gave, at  $R_F$  0.15–0.30, the [ $^{19-^{18}\text{O}}$ ]-diacid (3) (33 mg), shown by g.l.c.–mass spectrometry (as the methyl trimethylsilyl derivative) to have 0.69 atoms of  $^{18}\text{O}$  per molecule.

*Meerwein–Ponndorf–Verley Reduction of the Dimethyl Ester* (26).—(a) *Small scale*. Aluminium foil (25 mg), cut into strips, and mercury(II) chloride (2 mg) in dry propan-2-ol (1 ml) were brought to the boil then carbon tetrachloride (0.002 ml) was added and the mixture refluxed for 4 h. The  $\text{GA}_{29}$ -catabolite dimethyl ester (26) (5 mg) in propan-2-ol (1 ml) was added and the mixture refluxed for another 2 h. The solvent was evaporated off *in vacuo*, water added, and the products extracted into ethyl acetate at pH 3.0. Evaporation and p.l.c. using ethyl acetate–light petroleum (7 : 3) gave, at  $R_F$  0.3–0.5, the  $\Delta^9$ -19,2-lactone (49);  $m/e$  [ $\text{Me}_3\text{Si}$  ether] 416( $M^+$ , 100%), 401(13), 387(8), 384(10), 369(5), 357(49), 343(12), 313(13), 311(15), 255(10), 75(13), and 73(72). Elution of the band at  $R_F$  0.2–0.3 gave a mixture (5 : 1) of the  $\Delta^9(10)$ -2 $\alpha$ -alcohol (46) and  $\Delta^1(10)$ -2 $\alpha$ -alcohol (42);  $m/e$  [compound (46)] ( $\text{Me}_3\text{Si}$  ether) 520( $M^+$ , 12%), 505(4), 491(1), 479(1), 473(1), 460(9), 445(2), 430(78), 402(33), 398(49), 371(48), 221(17), 141(20), 75(59), and 73(110);  $m/e$  (42) ( $\text{Me}_3\text{Si}$  ether) 520( $M^+$ , 8%), 505(2), 491(5), 460(8), 430(15), 401(17), 398(7), 371(10), 239(4), 239(5), 223(16), 221(29), 208(5), 207(4), 193(12), 75(100), and 73(55).

(b) *Large scale*. Experiment (a) was repeated using aluminium foil (600 mg), mercury(II) chloride (17 mg), carbon tetrachloride (0.02 ml) and the  $\text{GA}_{29}$ -catabolite dimethyl ester (26) (180 mg) in propan-2-ol (20 ml). The crude product (170 mg) was purified by p.l.c. (twice) as in (a). Elution of the band at  $R_F$  0.35–0.45 gave a mixture (1 : 9) (51 mg) of the  $\Delta^9(10)$ -2 $\alpha$ -alcohol (46) and the  $\Delta^1(10)$ -2 $\alpha$ -alcohol (42) (Found:  $M^+$  376.188. Calc. for  $\text{C}_{21}\text{H}_{28}\text{O}_6$   $M^+$  376.189;  $\nu_{\text{max}}$  ( $\text{CHCl}_3$ ) 3 601, 3 405, 1 726, and 1 658  $\text{cm}^{-1}$ ;  $\delta$  1.24 (s, 18- $\text{H}_3$ ), 2.97 (m,  $W_{\frac{1}{2}}$  9 Hz, 5-H; reduced to br. d,  $J$  5 Hz by irradiation at  $\delta$  5.42), 3.07 (d,  $J$  5 Hz, 6-H), 3.64 (s,  $\text{CO}_2\text{Me}$ ), 3.72 (s,  $\text{CO}_2\text{Me}$ ), 4.73 (m,  $W_{\frac{1}{2}}$  12 Hz, 2-H; reduced to dd,  $J$  6 and 8 Hz by irradiation at  $\delta$  5.42 and to dd,  $J$  2 and 8 Hz by irradiation at  $\delta$  2.55, 3-H), 4.98br. (s,  $W_{\frac{1}{2}}$  6 Hz, 17-H), 5.10br. (s,  $W_{\frac{1}{2}}$  7 Hz, 17-H), and 5.42br. (s,  $W_{\frac{1}{2}}$  7 Hz, 1-H); g.l.c.–mass spectrometry gave three peaks corresponding to (41), (46), and (42) in the ratio of 3(variable) : 2 : 18,  $m/e$  (41) ( $\text{Me}_3\text{Si}$  ether) 430( $M^+$ , 75%), 415(9), 401(30), 398(18), 389(12), 371(33), 370(36), 311(48), 221(51), and 73(100).

*Alkaline Hydrolysis of the Acetate* (44).—The acetate (44) (2 mg) and potassium hydroxide (34 mg) in methanol (3 ml) were refluxed for 1 h. Amberlite IR-120(H) (B.D.H.) cation-exchange resin (300 mg) was added and the mixture stirred for 10 min. The supernatant solution was evaporated and methylated with ethereal diazomethane. G.l.c.–mass spectrometric analysis revealed two main peaks corresponding to the  $\Delta^1(10)$ -2 $\alpha$ -alcohol (42) and the  $\Delta^1(10)$ -19,2-lactone (45);  $m/e$  [ $\text{Me}_3\text{Si}$  ether of compound (45)] 416( $M^+$ , 89%), 401(12), 387(13), 370(77), 357(12), 238(47), 208(15), 207(13), 193(12), and 73(100).

*Sodium Borohydride Reductions*.—(a) *Dimethyl ester* (26). The dimethyl ester (26) (0.5 mg) and sodium borohydride (5 mg) in ethanol (0.3 ml) were stirred for 2 h. The solvent was evaporated off, water was added, and the products were extracted into ethyl acetate at pH 3.0. G.l.c.–mass spectrometric analysis of the trimethylsilylated product indicated a mixture of compounds (46), (42), (49), (45), and the  $\Delta^9(10)$ -2 $\beta$ -alcohol (47) in the ratio of 27 : 5 : 24 : 3 : 41;  $m/e$  [ $\text{Me}_3\text{Si}$  ether of compound (47)] 520( $M^+$ , 17%), 505(14), 491(3), 488(8), 479(1), 460(12), 430(71), 402(39), 398(42), 371(61), 311(18), 221(14), 75(59), and 73(100).

(b) *The diacid* (3). The previous experiment was repeated using the  $\text{GA}_{29}$ -catabolite (3) (0.5 mg) and the product was methylated using ethereal diazomethane. The reaction did not proceed to completion but two products, the  $\Delta^1(10)$ -2 $\alpha$ -alcohol (42) and the  $\Delta^1(10)$ -2 $\beta$ -alcohol (38), in the ratio of 4 : 1, were identified by g.l.c.–mass spectrometry;  $m/e$  [ $\text{Me}_3\text{Si}$  ether of compound (38)] 520( $M^+$ , 23%), 505(8), 491(26), 460(18), 430(21), 401(34), 370(16), 355(16), 311(21), 238(13), 223(26), 221(21), 207(8), 193(16), 75(100), and 73(61).

*Dimethyl ent-2 $\beta$ ,3 $\alpha$ -Dihydroxy-20-norgibberella-1(10),16-diene-7,19-dioate* (16).—A mixture (15 : 85; 500 mg) of gibberellin  $\text{A}_4$  and gibberellin  $\text{A}_7$  (8) was dissolved in 0.8M potassium hydroxide solution (10 ml). After 64 h, the pH was reduced to 3 with concentrated hydrochloric acid and the solution was extracted with ethyl acetate (6  $\times$  30 ml) at ca. 5  $^{\circ}\text{C}$ . The organic solvent was washed with a little water (10 ml) and was then evaporated *in vacuo* at 20  $^{\circ}\text{C}$ . The residue, dissolved in methanol, was methylated with ethereal diazomethane. After evaporation of the solvents, the residue was subjected to p.l.c. using ethyl acetate–light petroleum (4 : 1). Elution of the band at  $R_F$  0.70–0.85 yielded the *dimethyl ester* (16) as a gum (430 mg), which was



recrystallised from acetone–light petroleum, m.p. 104–106 °C (Found: C, 66.9; H, 7.6.  $C_{21}H_{28}O_6$  requires C, 67.0; H, 7.5%);  $\nu_{\max}$  3 492, 3 422, 1 742, 1 722, 1 658, and 873  $cm^{-1}$ ;  $\delta$  1.33 (s, 18- $H_3$ ), 2.62 (d,  $J$  7 Hz, 6-H), 3.07 (m, 5-H), 3.64 (s,  $CO_2Me$ ), 3.70 (s,  $CO_2Me$ ), 3.88 (d,  $J$  5 Hz, 3-H), 4.16 (m, 2-H), 4.88br. (s, 17- $H_2$ ), and 5.39 (q,  $J$  2.5 Hz, 1-H);  $m/e^+$  376( $M^+$ , 0%), 358(3), 326(15), 312(28), 298(28), 281(100), 267(22), 239(72), 221(71), 155(31), and 91(35);  $m/e$  (bis- $Me_3Si$  ether) 520( $M^+$ , 3%), 502(2), 461(2), 371(7), 332(100), 311(31), 281(20), 273(86), 75(29), and 73(64).

*Dimethyl ent-3 $\alpha$ -Hydroxy-2-oxo-20-norgibberella-1(10),16-diene-7,19-dioate* (19).—Pyridinium chlorochromate<sup>10</sup> (500 mg) and sodium acetate (50 mg) in dichloromethane (3 ml) were stirred for 5 min, and the diol (16) (420 mg) in dichloromethane (3 ml) was then added. After 40 min, diethyl ether was added and the supernatant solution was removed after centrifugation. Evaporation of the solution and p.l.c. of the residue, using ethyl acetate–light petroleum (7:3), yielded, from the band at  $R_F$  0.5–0.65, the ketol (19) (210 mg) which was recrystallised from acetone–light petroleum, m.p. 180–182 °C (Found: C, 67.2; H, 7.4.  $C_{21}H_{26}O_6$  requires C, 67.4; H, 7.0%);  $\nu_{\max}$  3 488, 1 734, 1 714, 1 682, 1 671, and 884  $cm^{-1}$ ;  $\lambda_{\max}$  (EtOH) 239 nm ( $\epsilon$  8 700);  $\delta$  1.38 (s, 18- $H_3$ ), 2.90 (m, 9-H), 3.02 (d,  $J$  5 Hz, 6-H), 3.60 (m, 5-H), 3.64 (s,  $CO_2Me$ ), 3.74 (s,  $CO_2Me$ ), 4.05 (d,  $J$  4 Hz, 3-H), 4.91br. (s, 17- $H_2$ ), and 5.84br. (t,  $J$  2 Hz, 1-H);  $m/e$  ( $Me_3Si$  ether) 446( $M^+$ , 36%), 431(12), 414(5), 399(6), 387(25), 371(69), 327(99), 258(99), 199(100), and 73(62).

*Dimethyl ent-3 $\alpha$ -Acetoxy-2-oxo-20-norgibberella-1(10),16-diene-7,19-dioate* (23).—The ketol (19) (194 mg) was dissolved in acetic anhydride (1.6 ml) and pyridine (2.9 ml). After 18 h, water was added and the mixture was acidified to pH 3 using concentrated hydrochloric acid. The solution was extracted with ethyl acetate and evaporation of the extract yielded the acetate (23) as a gum (188 mg) (Found:  $M^+$  416.185.  $C_{23}H_{28}O_7$  requires  $M^+$  416.185);  $\nu_{\max}$  ( $CDCl_3$ ) 1 728, 1 681, 1 658, and 888  $cm^{-1}$ ;  $\lambda_{\max}$  (EtOH) 239 nm ( $\epsilon$  8 000);  $\delta$  1.24 (s, 18- $H_3$ ), 2.11 (s, OAc), 2.90 (m, 9-H), 3.05 (d,  $J$  5 Hz, 6-H), 3.55 (m, 5-H), 3.68 (s,  $CO_2Me$ ), 3.77 (s,  $CO_2Me$ ), 4.92 (s, 17- $H_2$ ), 5.45 (s, 3-H), and 5.89br. (s, 1-H);  $m/e^+$  416( $M^+$ , 56%), 374(59), 324(16), 315(42), 297(100), 255(50), 237(60), and 43(41).

*Dimethyl ent-2-Oxo-20-norgibberella-1(10),16-diene-7,19-dioate* (27) (*Proposed GA<sub>51</sub>-Catabolite Dimethyl Ester*).—The

acetate (23) (120 mg) in glacial acetic acid (2 ml) was stirred with freshly activated zinc dust<sup>8</sup> (150 mg) for 1 h. The mixture was diluted with ethyl acetate and the insoluble material was deposited by centrifugation. The supernatant solution on evaporation yielded the proposed *GA<sub>51</sub>-catabolite dimethyl ester* (27) as a gum (88 mg) (Found:  $M^+$  358.178.  $C_{21}H_{26}O_5$  requires  $M^+$  358.178);  $\nu_{\max}$  ( $CHCl_3$ ) 1 727, 1 670, and 889  $cm^{-1}$ ;  $\lambda_{\max}$  (EtOH) 235 nm ( $\epsilon$  11 000);  $\delta$  1.32 (s, 18- $H_3$ ), 2.84 (m, 9-H), 3.08 (d,  $J$  4.5 Hz, 6-H), 3.38 (dd,  $J$  4.5 and 3 Hz, 5-H), 3.60 (s,  $CO_2Me$ ), 3.77 (s,  $CO_2Me$ ), 4.91 (s, 17- $H_2$ ), and 5.87 (dd,  $J$  3 and 2 Hz, 1-H);  $m/e$  358 ( $M^+$ , 29%), 326(10), 299(25), 298(17), 240(20), 239(100), 211(9), 171(9), and 44(23);  $m/e$  [ $\Delta^{9(10)}$ -isomer (30)], 358( $M^+$ , 97%), 330(10), 326(9), 317(3), 299(38), 298(48), 239(100), 171(31), 129(21), and 44(35);  $m/e$  (enol  $Me_3Si$  ether) 430( $M^+$ , 100%), 415(4), 401(2), 399(2), 389(5), 387(3), 371(6), 311(36), and 73(43).

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