Partial Synthesis of Gibberellin A_9 and [3α- and 3β-$^2H_1$]Gibberellin A_9; Gibberellin A_5 and [1β,3-$^2H_2$ and -$^3H_2$]Gibberellin A_5; and Gibberellin A_{20} and [1β,3α-$^2H_2$ and -$^3H_2$]Gibberellin A_{20}

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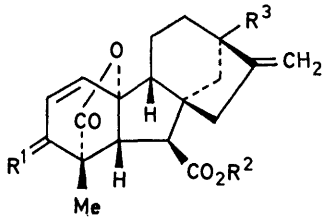
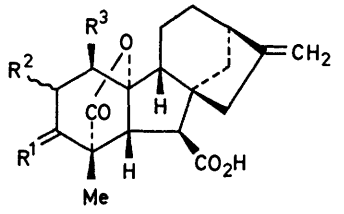
The 3α-alcohols, obtained by reduction of 3-didehydrogibberellin A_3 methyl ester 13-acetate with lithium borohydride, borodeuteride, and borotritiide, have been converted into the 3β-chloro-derivatives and, hence, by reduction with tri-n-butylstannane followed by hydrolysis, into gibberellin A_{20}, [1β,3α-$^2H_2$]gibberellin A_{20}, and [1β,3α-$^3H_2$]gibberellin A_{20}. [3ξ-$^2H_1$]Gibberellin A_{20} has been prepared from the product of the reduction of the 3-thiobenzoate of the 3α-alcohol with tri-n-butyl[^{2}H]stannane. The 3β-alcohols, minor products of these reductions, have been dehydrated and hydrolysed to give gibberellin A_5 and [1β,3-$^2H_2$ and -$^3H_2$]gibberellin A_5. The 3α-alcohol, from the lithium borohydride reduction of 3-didehydrogibberellin A_7 has been transformed into the 3β-chloro-derivative and the 3-thiobenzoate which, with tri-n-butylstannane, or with tri-n-butyl[$^2H_1$]stannane, followed by hydrolysis, yielded gibberellin A_9, [3β-$^2H_1$]gibberellin A_9, or [3ξ-$^2H_1$]gibberellin A_9. In an analogous way, the product of reduction of 3-didehydrogibberellin A_4 with lithium borodeuteride was converted into [3α-$^2H_1$]-gibberellin A_9. The mass spectral fragmentations of the methyl esters and methyl ester trimethylsilyl ethers of [^{2}H]gibberellins are also discussed.

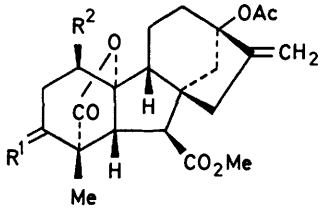
CONJUGATE reduction of the enones (1) and (2) from gibberellins A_7 (GA_7) (4) and A_4 (GA_4) (7) with borohydride and with borodeuteride, was described in the previous paper.[1] In the course of this mechanistic study, the products from the reduction of the enone (2) by borodeuteride were chemically transformed into [1β-$^2H_1$]GA_4 (8), [1β,2ξ-$^2H_2$]GA_4 (9), and [1β,3α-$^2H_2$]GA_4 (10). This paper gives further examples whereby the available gibberellin A_3 (3), and mixtures of gibberellins A_7 (4) and A_4 (7), are converted into unlabelled, deuteriated, and tritiated gibberellins by conjugate reduction of the enones (1) and (2), followed by 3-deoxygenation of the reduction products.



	R^1	R^2	R^3
(1)	O	Me	OAc
(2)	O	Me	H
(3)	H,β-OH	H	OH
(4)	H,β-OH	H	H
(5)	H,α-OH	Me	OAc
(6)	H,α-OH	Me	H



	R^1	R^2	R^3
(7)	H,β-OH	H	H
(8)	H,β-OH	H	D
(9)	H,β-OH	D	D
(10)	D,β-OH	H	D
(11)	H_2	H	H
(12)	H,α-D	H	H
(13)	D,β-OH	H	H
(14)	H,β-D	H	H

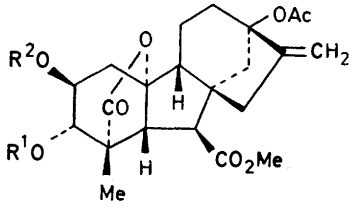


	R^1	R^2
(15)	H,α-OH	H
(16)	H,β-OH	H
(17)	H_2	H
(18)	H,α-PhCSO	H
(19)	H,β-Cl	H
(20)	D,α-OH	D
(21)	D,β-OH	D
(22)	T,α-OH	T
(23)	T,β-OH	T

RESULTS AND DISCUSSION

In all reductions, sodium borohydride in the presence of lithium bromide was used although, for the reasons given in the preceding paper,[1] sodium borohydride alone is probably preferable. As shown in the preceding paper, the best yields of conjugate reduction products were obtained in di-(2-methoxyethyl) ether and this solvent was used for the preparation of unlabelled compounds from GA_3 (3). Under these conditions the yields of isolated products from the enone (1) were 3-*epi*-GA_1 methyl ester 13-acetate (15) (39%), GA_1 methyl ester 13-acetate (16) (8%), 3-*epi*-GA_3 methyl ester 13-acetate (5) (14%), and a new product (7%), identified as the 2β-hydroxy-compound (24) as follows. In addition to signals for C-Me, OCOMe, CO_2Me, and C=CH_2, expected in a reduction product of the enone (1), the ^{1}H n.m.r. spectrum of the new compound contained three two-proton singlets at δ 2.72, 3.58, and 4.19. On adding [Eu(fod)$_3$] the singlet at δ 2.72 was resolved into an AB system with J 10 Hz, and is assigned to the 5- and 6-protons. The signal at δ 4.19 disappeared on addition of deuterium oxide and is assigned to two hydroxy-groups. From the chemical shift the two-proton signal at δ 3.58 is assigned to two hydroxymethine protons but, although they were resolved by adding [Eu(fod)$_3$], the signals were too broad to obtain J values. However, in the ^{1}H

n.m.r. spectrum of the triacetate (25) two acetoxymethine protons, at δ 5.3 and *ca.* 5.07, were assigned to the 3- and 2-protons in structure (25) from the following coupling constants (Hz) which were obtained from double irradiation experiments: $J_{2,3}$ 9.5; $J_{2,1\beta}$ 8.0; $J_{2\,1\alpha}$ 2.5; and $J_{1\alpha,1\beta}$ 13.5. From these data structure (24) is deduced for the new reduction product. The way in



(24) $R^1 = R^2 = H$
(25) $R^1 = R^2 = Ac$

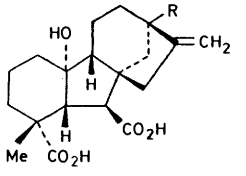
which 2-hydroxylation occurs during reduction of the enone (1) is not known.

3-*epi*-GA_1 Methyl ester 13-acetate (15) was converted into GA_{20} methyl ester 13-acetate (17) in two ways. One method proceeded *via* the thiobenzoate (18), prepared by the method of Barton and McCombie [2] and reduced by tri-n-butylstannane in the presence or absence of 2,2'-azobis(2-methylpropionitrile); in addition to the required GA_{20} methyl ester 13-acetate (17), some starting alcohol (15) was also formed (*cf.* ref. 2). Alternatively 3-*epi*-GA_1 methyl ester 13-acetate (15) was treated with phosphoryl chloride in pyridine to give the 3β-chloro-compound (19) together with minor amounts of GA_5 methyl ester 13-acetate (31) which was also prepared directly from GA_1 methyl ester 13-acetate (16) and phosphoryl chloride. Dechlorination of the chloro-compound (19) by tri-n-butylstannane and initiator gave GA_{20} methyl ester 13-acetate (17). Alkaline hydrolysis of the last mentioned compound (17) was accompanied by some opening of the lactone, the bis-TMSi-ester TMSi-ether of the hydroxy-diacid (26) being detected by g.l.c.–mass spectrometry of the derivatised crude hydrolysis product. The total hydrolysis product was therefore briefly heated at 80 °C to re-close the lactone and provide GA_{20} (28). Similar treatment of GA_5 methyl ester 13-acetate (31) afforded GA_5 (32). The overall yields of GA_{20} (28) and GA_5 (32) from GA_3 (3) were 15–20% and *ca.* 2%, respectively.

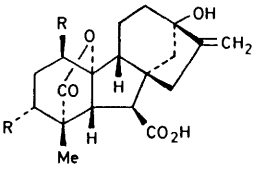
Using the same methods as for the unlabelled compounds, [1β,3α-$^2H_2$]GA_{20} (29) and [1β,3-$^2H_2$]GA_5 (33), containing 1.86 and 1.70 atoms deuterium per molecule, respectively, were prepared from the products (20) and (21) of the reduction of the enone (1) by borodeuteride.[1] Similarly [1β,3α-$^3H_2$]GA_{20} (30) and [1β,3-$^3H_2$]GA_5 (34), each with a specific activity of 25.8 mCi mmol^{-1}, were prepared from the products (22) and (23) of reduction of the enone (1) with tritiated borohydride. The 3α-stereochemistry in [1β,3α-$^2H_2$ and -$^3H_2$]GA_{20} was assigned by analogy with 3α-[^{2}H]GA_9 (12) (see later). [3ξ-$^2H_1$]-GA_{20}, containing 0.89 atoms deuterium per molecule, was prepared by hydrolysis of the product of reduction of the thiobenzoate (18) with tri-n-butyl[^{2}H]stannane.

To prepare GA_9 (11) from mixtures of GA_4 (7) and GA_7 (4) the initial oxidation step depended upon the percentage composition of the mixture. As in the preceding paper,[1] when the content of GA_7 (4) was high (85%), the mixture was methylated, then oxidised with manganese dioxide, to yield the enone (2). Reduction of the enone (2) with sodium borohydride–lithium bromide in tetrahydrofuran gave 3-*epi*-GA_4 methyl ester (35) in 45% yield and 3-*epi*-GA_7 methyl ester (6) in 8% yield. Deoxygenation of 3-*epi*-GA_4 methyl ester (35) formed by tri-n-butylstannane reduction of the thiobenzoate (36), or of the 3β-chloro-compound (37), gave GA_9 methyl ester (38) which was hydrolysed to GA_9 (11); again some hydroxy-diacid (27) was formed and re-lactonised by heating. The overall yield of GA_9 (11) from the GA_4–GA_7 mixture was 15—20%.

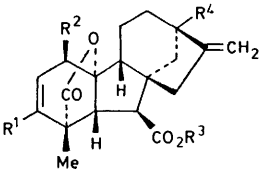
When the mixture of GA_4 (7) and GA_7 (4) contained a higher proportion (65%) of GA_4 (7) it was methylated and oxidised with Jones reagent. The resultant mixture of 3-ketones (2) and (39) was then reduced by sodium



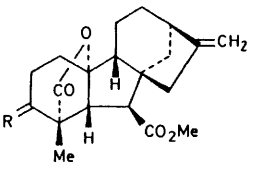
(26) R = OH
(27) R = H



(28) R = H
(29) R = D
(30) R = T



	R^1	R^2	R^3	R^4
(31)	H	H	Me	OAc
(32)	H	H	H	OH
(33)	D	D	H	OH
(34)	T	T	H	OH



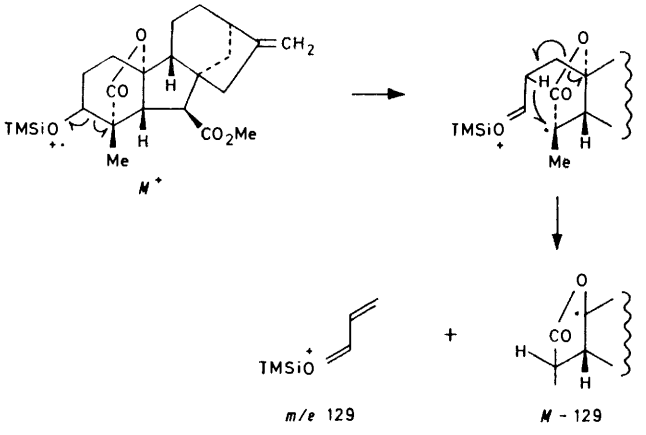
(35) R = H, α-OH
(36) R = H, α-PhCSO
(37) R = H, β-Cl
(38) R = H_2
(39) R = =O
(40) R = D, α-OH
(41) R = D, β-Cl
(42) R = H, α-D
(43) R = H, β-D

borohydride–lithium bromide to give 3-*epi*-GA_4 methyl ester (35) and 3-*epi*-GA_7 methyl ester (6) in 80 and 4% yield. Re-oxidation of 3-*epi*-GA_4 methyl ester gave pure GA_4 methyl ester 3-ketone (36) in 65% overall yield from the GA_4–GA_7 mixture. Reduction of this ketone (39) with sodium borodeuteride–lithium bromide gave [3β-^{2}H]-3-*epi*-GA_4 methyl ester (40) which was converted into the 3β-chloro-compound (41) and, hence by reduction with tri-n-butylstannane, into [3α-^{2}H]GA_9 methyl ester (42). The [3α-^{2}H]GA_9 (12), obtained by

alkaline hydrolysis of the methyl ester, was converted into [3α-^{2}H]GA_4 (13) by a cell-free enzyme preparation from the seeds of *Cucurbita maxima*.[3] The [3α-^{2}H]-configuration was assigned to the GA_9 (12) on the assumption for which there are precedents [4] that the enzymatic hydroxylation occurs with retention of configuration. It would then follow that the reduction of the chloro-compound (41) with tri-n-butylstannane also takes place with retention of configuration. The compound, obtained by the similar reduction of the chloro-compound (37), by tri-n-butyl[^{2}H]stannane, was therefore assigned the [3β-^{2}H]-configuration (43) and, on hydrolysis, gave [3β-^{2}H]GA_9 (14). [3ξ-$^2H_1$]GA_9, containing 0.92 atoms deuterium per molecule, was obtained by alkaline hydrolysis of the product from the reduction of the thiobenzoate (36) with tri-n-butyl[^{2}H]stannane.

The mass spectra of the methyl ester TMSi-ethers of the deuteriated gibberellins, prepared in this and the preceding paper,[1] were compared with the mass spectra [5] of the unlabelled compounds. This comparison provided the following information on fragmentation processes.

A prominent ion at *m/e* 129 is characteristic of the spectra of the methyl ester TMSi-ether of 3-hydroxy-gibberellins. In the case of GA_4 methyl ester TMSi-ether (Scheme) this ion, which is accompanied by an



SCHEME Fragmentation of GA_4 methyl ester TMSi-ether

$M^+ - 129$ ion, has [5] the composition $C_6H_{13}OSi$ and the fragmentation process, shown in the Scheme, has been proposed.[5] For both [1β-^{2}H]- and [3α-^{2}H]-GA_4 derivatives (8) and (13), the *m/e* 129 ion was weak but an intense ion at *m/e* 130, accompanied by an $M^+ - 130$ ion was present. In the spectrum of the methyl ester TMSi-ether of [1β,3α-$^2H_2$]GA_4 (10) the corresponding ions were at *m/e* 131 and $M^+ - 131$. The spectrum of the methyl ester TMSi-ether of [1β,2ξ-$^2H_2$]GA_4 (9) contained both *m/e* 130 and *m/e* 131 ions; the former ion was the more intense indicating the loss of some deuterium. This loss must be from carbon-2 since it was shown earlier that the 1β-deuterium was retained in the fragmentation of the [1β-^{2}H]GA_4 derivative. This was confirmed by the presence of an ion at *m/e* 290 ($M^+ - 130$) in the spectrum of the [1β,2ξ-$^2H_2$]GA_4 derivative, corresponding to the ion at *m/e* 289 ($M^+ - 129$) in the spectrum of the unlabelled GA_4 derivative. These data establish the fragmentation process shown in the Scheme.

The ions at *m/e* 261 ($M^+ - 157$) and 233 ($M^+ - 185$) in the mass spectrum of the methyl ester TMSi-ether of GA_4 (7) are also present in the spectra of the deuteriated compounds. These ions, therefore, arise by successive losses of 28 Daltons from the $M^+ - 129$ ion. The presence of additional ions at *m/e* 262 and 234 in the spectrum of [1β,2ξ-$^2H_2$]GA_4 methyl ester TMSi-ether again demonstrated that deuterium is transferred from carbon-2.

The mass spectrum of GA_4 methyl ester TMSi-ether contains intense ions at *m/e* 284 ($M^+ - 134$) and 225/4 ($M^+ - 193/4$) which were previously reported to have the composition $C_{19}H_{24}O_2$ and $C_{13}H_{25/24}$ OSi. However re-measurement of the *m/e* 225/4 ion has now shown it to have the composition $C_{17}H_{21/20}$. The mass spectra of the methyl ester TMSi-derivatives of deuteriated GA_4 derivatives show that these ions have retained the ring A and are probably formed by the losses $M^+ -$ TMSiOH $-$ CO_2 (*m/e* 284) and *m/e* 284 $- CO_2Me/HCO_2Me$ (*m/e* 225/4).

As discussed in the preceding paper,[1] the base peak in the mass spectrum of GA_{16} methyl ester bis(TMSi)-ether (Scheme 3 of preceding paper) occurs at $M^+ - 116$ (*m/e* 390). It occurs at *m/e* 391 in the spectra of the [1β-^{2}H]-, [1β,2ξ,$^2H_2$]-, and [1β,3α-$^2H_2$]-derivatives thus confirming that the fragmentation involves loss of carbons-2 and -3, as shown in the Scheme, rather than the loss of carbons-1 and -2. Nevertheless an analogous, but minor, fragmentation route with the loss of carbon-1 and -2 does occur, followed by the loss of 90 Daltons (TMSiOH). Thus the ion at *m/e* 300 ($M^+ - 206$) which is present in the spectra of the methyl ester bis(TMSi)-ethers of GA_{16}, [1β-^{2}H]GA_{16}, and [1β,2ξ-$^2H_2$]GA_{16} occurs at *m/e* 301 in the spectrum of the [1β,3α-$^2H_2$]-derivatives.

The spectra of the methyl ester and methyl ester TMSi-ether of GA_{20} (28) contain an ion at $M^+ - 43$. This ion has been attributed,[6] in the case of the methyl ester, to the loss of carbons-1, -2, and -3 from ring A. This conclusion is confirmed by the absence of an $M^+ - 43$ ion, and the presence of $M^+ - 45$ ions in the spectrum of the [1β,3α-$^2H_2$]-derivatives. The ions at *m/e* 207 and 207/8 present in the spectrum of GA_{20} and GA_5 methyl ester TMSi-ethers respectively, are unchanged in the spectra of the [1,3-$^2H_2$]-derivatives, thus confirming that these ions do not contain ring A.

Finally, comparison of the mass spectra of [3α-^{2}H]GA_9 methyl ester (42) and of [3β-^{2}H]GA_9 methyl ester (43) with the spectrum of GA_9 methyl ester showed that all the major ions were mono-deuteriated. Thus no fragmentation of ring A was observed and the $M^+ - 43$ ion, reported by Takahashi *et al.*,[6] was absent.

EXPERIMENTAL

General experimental details are described in the preceding paper.[1] For tritiated compounds, chemical and radiochemical purity were established by t.l.c.–radio-counting and g.l.c.–radio-counting.

Reduction of ent-13-*Acetoxy*-10β-*hydroxy*-3-*oxo*-20-*nor*-*gibberela*-1,16-*diene*-7,17-*dioic Acid* 7-*Methyl Ester* 19,10-*Lactone* (1).—The enone (1) (200 mg), in di-(2-methoxyethyl) ether (2 ml) was reduced with sodium borohydride (23 mg) and lithium bromide (52 mg) as described in the preceding paper.[1] The crude product (210 mg), after p.l.c. with ethyl acetate–light petroleum (7 : 3) gave: (*a*) GA_1 methyl ester 13-acetate (16) (19 mg, R_F 0.60) as a gum: (*b*) 3-*epi*-GA_3 *methyl ester* 13-*acetate* (5) (30 mg) R_F 0.50, m.p. 163—164 °C (from ethyl acetate–light petroleum) (Found: C, 66.0; H, 6.7. $C_{22}H_{26}O_7$ requires C, 65.8; H, 6.5%); δ 1.29 (s, 18-H_3), 2.03 (s, OCOMe), 2.83 and 2.97 (both d, *J* 10 Hz, 6- and 5-H), 3.77 (s, CO_2Me), 4.29 (m, 3-H) 5.02 and 5.19 (both nr, 17-H_2), 5.89 (dd, *J* 2.5 and 10 Hz, 2-H), and 6.24 (dd, *J* 1.5 and 10 Hz, 1-H); *m/e* (MeTMSi-derivative) 474 (M^+, 5%), 280 (44), 221 (33), 157 (30), 75 (39), 73 (100), and 43 (52): (*c*) 3-*epi*-GA_1 methyl ester 13-acetate (15) (83 mg), m.p. 143—145 °C, R_F 0.40: and (*d*) 3-epi-GA_8 *methyl ester* 13-*acetate* (24) (15 mg), R_F 0.20, m.p. 209—212 °C (from acetone–light petroleum) (Found: C, 63.15; H, 7.1%; M^+ 420.174. $C_{22}H_{29}O_8$ requires C, 62.9; H, 6.7%; *M* 420.178); $\nu_{max.}$ 3 425, 3 365, and 1 740(br) cm^{-1}; δ 1.16 (s, 18-H_3), 2.02 (s, OCOMe), 2.72 (s, 5- and 6-H), 3.58 (br s, 2- and 3-H), 3.74 (s, CO_2Me), 4.19 (br s, 2- and 3-OH), removed by addition of D_2O), 5.00 (br s, 17-H), and 5.15 (br s, 17-H); on adding [Eu(fod)$_3$], the 2- and 3-proton signals were resolved into two broad singlets and the 5- and 6-proton signals became an AB system with *J* 10 Hz; *m/e* [bis(TMSi)-ether] 564 (M^+, 100%), 286 (20), 217 (36), 147 (26), and 43 (13). The triacetate (25), prepared from the diol (47 mg) (24), acetic anhydride (2 ml), and toluene-*p*-sulphonic acid (2 mg), crystallised from acetone–light petroleum in needles (55 mg), m.p. 207–209 °C (Found: C, 61.2; H, 6.4%; M^+, 504.202. $C_{26}H_{32}O_{10}$ requires C, 61.9; H, 6.35%; *M*, 504.200); δ 1.07 (s, 18-Me), 2.03 (s, 2 × OCOMe), 2.12 (s, OCOMe), 2.75 (d, *J* 10 Hz, 6-H), 2.82 (dd, *J* 8 and 13.5 Hz, 1β-H), 2.94 (d, *J* 10 Hz, 5-H), 3.78 (s, CO_2Me), 5.03 (m, 17-H), 5.09 (dq, 2-H), 5.18 (m, 17-H), and 5.31 (d, *J* 9.5 Hz, 3-H); irradiation at δ 2.8 clarified the δ 5.2—5.0 region from which the following *J* values (0.5 ± Hz) were directly measured: $J_{2,3}$ 9.5, $J_{2,1\beta}$ 8, $J_{2.1\alpha}$ 2.5, and $J_{1\alpha,1\beta}$ 13.5; $\nu_{max.}$ 1 797, 1 732, 1 743(sh), 1 661, 887, and 873 cm^{-1}; *m/e* 504 (M^+, 35%), 473 (11), 463 (26), 462 (100), 444 (27), 412 (24), 384 (72), and 43 (>100).

This reduction was repeated with essentially the same results on a larger scale. In this case the products from the enone (1) (10.2 g) were isolated by column chromatography on silica gel (340 g, 40 × 4 cm), eluted with increasing concentrations of ethyl acetate in light petroleum. In addition to the compounds (5), (15), and (16), a fraction (670 mg) was eluted with 90—100% ethyl acetate and was separated by p.l.c. with ethyl acetate–light petroleum (7 : 3) into 3-*epi*-GA_8 methyl ester (24) (505 mg, R_F 0.20—0.25) and 3-*epi*-GA_1 methyl ester 13-acetate (15) (118 mg, R_F 0.45—0.50).

ent-10β,13-*Dihydroxy*-20-*norgibberell*-16-*ene*-7,19-*dioic Acid* 19,10-*Lactone* (*Gibberellin* A_{20}) (28).—(*a*) 3-*epi*-Gibberellin A_1 methyl ester 13-acetate (15) (121 mg), phosphoryl chloride (0.4 ml), and pyridine (10 ml) were refluxed for 3 h. The reaction mixture was then poured into water which was acidified with 10M-hydrochloric acid and extracted with ethyl acetate. P.l.c. of the resultant brown oil with ethyl acetate–light petroleum (1 : 1) gave at R_F 0.7, 3β-chloro-GA_{20} methyl ester 13-acetate (19) (80 mg), m.p. 125—127 °C (from acetone–light petroleum) (Found: M^+, 422.150. $C_{22}H_{27}{}^{35}ClO_6$ requires *M*, 422.150); $\nu_{max.}$ 1 775, 1 740, and 1 670 cm^{-1}; δ 1.19 (s, 18-H_3), 2.02 (s, OCOMe), 2.7 and 3.32 (AB, *J* 10 Hz, 6- and 5-H), 3.74 (s, CO_2Me), 4.13 (m, 3-H), and 5.00 and 5.17 (each br s, 17-H_2); *m/e* 424 (M^+ +2, 1%), 422 (M^+, 3%), 382 (23), 380 (57), and 43 (100). A band at R_F 0.6 yielded a mixture (14 mg) of 3β-chloro-GA_{20} methyl ester 13-acetate (19) and GA_5 methyl ester 13-acetate (31) in the ratio of 1 : 2 (g.c. and g.c.–m.s.).

A solution of the 3β-chloro-compound (19) (79 mg), tri-n-butylstannane (0.2 ml) and a crystal of 2,2′-azobis(2-methylpropionitrile) in benzene (10 ml) was refluxed for 1.5 h. After removal of the solvent *in vacuo* the product was purified by p.l.c. with ethyl acetate–light petroleum (1 : 1). The band at R_F 0.5 gave GA_{20} methyl ester 13-acetate (17) (71 mg) m.p. 111—113 °C (from ethyl acetate–light petroleum) (Found: M^+, 388.190. $C_{22}H_{28}O_6$ requires *M*, 388.189); $\nu_{max.}$ 1 775, 1 734, and 1 665 cm^{-1}; δ 1.09 (s, 18-H_3), 2.03 (s, OCOMe), 2.56 and 2.74 [AB, *J* 10 Hz, 5 or (6)- and 6 or (5)-H], 3.74 (s, CO_2Me), and 4.99 and 5.15 (each br s, 17-H_2).

Gibberellin A_{20} methyl ester 13-acetate (17) (70 mg), methanol (60 ml), and 2M-sodium hydroxide (60 ml) were refluxed for 16 h. The usual work-up gave a gum which, by g.l.c.–mass spectrometry of the TMSi-derivative, was a 5 : 2 mixture of GA_{20} (28) and the lactone-opened diacid (26). After 3 days at 18 °C, or heating at 80 °C for 30 min, re-analysis by g.l.c.–mass spectrometry showed the gum to contain no diacid (26) but to contain 90% GA_{20} (28) and 10% of its methyl ester. P.l.c. yielded from the band at R_F 0.4, pure GA_{20} (28) (47 mg).

(*b*) 3-*epi*-Gibberellin A_1 methyl ester 13-acetate (15) (100 mg), a solution (2 ml)[2] of 0.5M $PhCCl{=}\overset{+}{N}Me_2Cl^-$ in dichloromethane, and tetrahydrofuran (2 ml) were left at 18° C for 50 h. Pyridine (1.3 ml) was added and, after 5 min, dry hydrogen sulphide was bubbled through the solution for 5 min. The mixture was poured into water which was acidified and extracted with ethyl acetate. The yellow oil, recovered from the extract, was purified by p.l.c. using ethyl acetate–light petroleum (7 : 3) to give, from the yellow band at R_F 0.7—0.8, the *thiobenzoate* (18) (98 mg), crystallised from ethanol as yellow needles, m.p. 166—168 °C (Found: C, 66.4; H, 6.3%; M^+, 524.182. $C_{29}H_{32}O_7S$ requires C, 66.4; H, 6.1%; *M*, 524.187); $\nu_{max.}$ 1 773, 1 736, 1 236, 799, and 693 cm^{-1}; $\lambda_{max.}$ (EtOH) 253 and 293 nm (ε 9 300 and 11 600); δ 1.16 (s, 18-H_3), 2.07 (s, OCOMe), 2.85 (s, 5- and 6-H), 3.77 (s, CO_2Me), 5.04 and 5.20 (each br s, 17-H_2), 5.91 (dd, *J* 6 and 10 Hz, 3-H), 7.52 (m, 3 × Ar-H), and 8.08 (m, 2 × Ar-H); *m/e* 524 (M^+, 3%), 386 (10), 342 (37), 301 (35), 282 (100), 241 (23), 223 (34), 121 (56), 105 (61), 77 (28), and 43 (58).

Elution of the p.l.c. pale yellow band at R_F 0.6—0.7 gave *NN*-dimethylthiobenzamide as a yellow oil (52 mg); $\nu_{max.}$ 3 058, 3 028, 2 932, 2 870, 1 515, 1 390, 1 292, 1 138, 764, and 702 cm^{-1}; δ 3.15 (s, NMe), 3.61 (s, NMe), and 7.34 (s, 5 × Ar-H); *m/e* 165 (M^+, 77%), 164 (96), 131 (40), 121 (100), and 77 (51).

The thiobenzoate (18) (25 mg) in toluene (3 ml) was added over 2 h to a solution of tri-n-butylstannane (0.02 ml), with or without 2,2′-azobis(2-methylpropionitrile) (1 mg) in toluene (5 ml) refluxing under nitrogen. After 1.5—4 h the solution was colourless and was evaporated in a stream of nitrogen; the product was purified by p.l.c. using ethyl acetate–light petroleum (7 : 3). Elution of the band at R_F 0.65—0.75 yielded GA_{20} methyl ester 13-acetate (17) (16 mg). Elution of the band at R_F 0.45—0.65 gave 3-*epi*-GA_1 methyl ester 13-acetate (15) (2 mg).

GA_{20} Methyl ester 13-acetate (17) (16 mg) in methanol

(6 ml) was heated under reflux for 18 h with 2.5M potassium hydroxide (6 ml). The usual work-up gave GA_{20} (28) (10 mg.)

Reduction of the thiobenzoate (18) (75 mg) in toluene (5 ml) with tri-n-butyl [^{2}H]stannane (80 μl) gave [3ξ-$^2H_1$]-GA_{20} methyl ester-13-acetate (50 mg) which was hydrolysed to [3ξ-$^2H_1$]GA_{20} (16 mg) containing 0.89 atoms deuterium per molecule.

Gibberellin A_5 (32).—Gibberellin A_1 methyl ester 13-acetate (16) (74 mg) in pyridine (5 ml) was refluxed for 2 h with phosphoryl chloride (0.25 ml). The usual work-up gave an oil which, by p.l.c. with ethyl acetate–light petroleum (1 : 1), gave (R_F 0.6) GA_5 methyl ester 13-acetate (31) (34 mg). This methyl ester in methanol (30 ml) and 2M-sodium hydroxide (30 ml) was refluxed for 18 h. The usual work-up gave a gum which, after heating at 80 °C for 0.5 h under nitrogen, was purified by p.l.c. with ethyl acetate–light petroleum (1 : 1) and gave (R_F 0.4) GA_5 (32) (22 mg).

ent-1α,3β-*Dideuterio*-10β,13-*dihydroxy*-20-*norgibberell*-16-*ene*-7,19-*dioic Acid* ([1β,3α-$^2H_2$]*GA*$_{20}$) (29).—The procedures were the same as those described for the preparation of GA_{20} (28). Thus the [1β,3β-$^2H_2$]-3α-alcohol (20) [1] (225 mg, 1.78 atoms deuterium per molecule), pyridine (10 ml), and phosphoryl chloride (0.6 ml) gave the [1β,3α-$^2H_2$]-derivative of the chloro-compound (19) (105 mg, 1.83 atoms deuterium per molecule) and the [1β,3-$^2H_2$]-derivative of GA_5 methyl ester 13-acetate (31) (12 mg, 1.73 atoms deuterium per molecule).

The chloro-compound (104 mg), benzene (15 ml), tri-n-butylstannane (0.5 ml), and 2,2′-azobis(2-methylpropionitrile) (20 mg) gave [1β,3α-$^2H_2$]GA_{20} methyl ester 13-acetate (92 mg, 1.75 atoms deuterium per molecule) which was hydrolysed in methanol (90 ml) with 2M-sodium hydroxide (90 ml) to give [1β,3α-$^2H_2$]GA_{20} (29) (48 mg, 1.86 atoms deuterium per molecule).

ent-1α,3-*Dideuterio*-10β,13-*dihydroxygibberella*-2,16-*diene*-7,19-*dioic Acid* 19,10-*Lactone* ([1β,3-$^2H_2$]*GA*$_5$) (33).—As described for the unlabelled compound, the [1β,3-$^2H_2$]GA_5 methyl ester 13-acetate (12 mg) from the previous experiment was hydrolysed in methanol (10 ml) with 2M-sodium hydroxide (10 ml) to give [1β,3-$^2H_2$]GA_5 (33) (5 mg, 1.70 atoms deuterium per molecule).

ent-10β,13-*Dihydroxy*-1α,3β-*ditritio*-20-*norgibberell*-16-*ene*-7,19-*dioic Acid* 19,10-*Lactone* ([1β,3α-$^3H_2$]*GA*$_{20}$) (30).—Using the same conditions and quantities as for the borodeuteride reduction, reduction of the enone (1) with tritiated sodium borohydride (40 mg, 100 mCi) gave the 3α-[1β,3β-$^3H_2$]-3α-alcohol (22) (191 mg) and [1β,3α-$^3H_2$]-3β-alcohol (23) (49 mg). By g.l.c.–radio-counting of aliquots (as the MeTMSi-derivatives), both compounds gave single radio-active peaks with 5.3×10^2 and 4.6×10^2 disint. min^{-1} mm^{-2} respectively.

Using the conditions previously described [1] for the unlabelled compounds, the [1β,3β-$^3H_2$]-3α-alcohol (22) (190 mg), pyridine (10 ml), and phosphoryl chloride (0.4 ml) yielded the [1β,3α-$^3H_2$]-3β-derivative of the chloro-compound (19) (99 mg, 5.53×10^2 disint. min^{-1} mm^{-2}), and a mixture (17 mg) of the chloro-compound and the [1β,3-$^3H_2$]-derivative of the olefin (34) (see later) in the ratio 1.75 : 1. Dechlorination of the chloro-compound in benzene (15 ml) with tri-n-butylstannane (0.3 ml) and 2,2′-azobis(2-methylpropionitrile) (2 mg) gave [1β,3α-$^3H_2$]GA_{20} methyl ester 13-acetate (66 mg, 5.5×10^2 disint. min^{-1} mm^{-2}) which was hydrolysed in methanol (60 ml) with 2M-sodium hydroxide (60 ml) to yield [1β,3α-$^3H_2$]GA_{20} (30) (32 mg, 5.3×10^2 disint. min^{-1} mm^{-2}, 25.8 mCi $mmol^{-1}$).

ent-10β,13-*Dihydroxy*-1α,3-*ditritio*-20-*norgibberella*-2,16-*diene*-7,19-*dioic Acid* 19,10-*Lactone* ([1β,3-$^3H_2$]*GA*$_5$) (34).—Using the conditions described for the unlabelled compounds, the [1β,3α-$^3H_2$]-3β-alcohol (23) (49 mg), from the previous experiment, pyridine (5 ml), and phosphoryl chloride (0.12 ml) yielded [1β,3-$^3H_2$]GA_5 methyl ester 13-acetate (5.0 disint. min^{-1} mm^{-2}). This crude olefin, and the mixture (17 mg) of the [$^3H_2$]-derivative of the chloro-compound (19) and the [$^3H_2$]-olefin obtained in the previous experiment, were combined and purified by p.l.c. to give the pure [1β,3-$^3H_2$]GA_5 methyl ester 13-acetate (30 mg) which, in methanol (30 ml), was hydrolysed with 2M-sodium hydroxide (30 ml). The resultant [1β,3-$^3H_2$]GA_5 (34) (14 mg) had specific activity 25.8 mCi $mmol^{-1}$.

ent-3β,10β-*Dihydroxy*-20-*norgibberell*-16-*ene*-7,19-*dioic Acid* 7-*Methyl Ester* 19,10-*Lactone* (35).—(a) *From a* GA_7-*rich* (85%) *mixture of* GA_4 *and* GA_7. As described in the preceding paper [1] the mixture was methylated, then oxidised by MnO_2 and the resultant enone (2) was reduced to give the required 3α-alcohol (35) in 45% overall yield.

(b) *From a* GA_4-*rich* (65%) *mixture of* GA_4 *and* GA_7. The mixture (2.0 g) in acetone (150 ml) was oxidised with an excess of Jones reagent at room temperature for 1 h. Work-up in the normal way gave a mixture (2.0 g) of 3-didehydro-GA_4 and 3-didehydro-GA_7 which, without purification, was dissolved in di-(2-methoxyethyl) ether (20 ml) and treated at 0 °C for 1.5 h with sodium borohydride (400 mg) and lithium bromide (870 mg). The reaction mixture was poured into water which was acidified and extracted with ethyl acetate. P.l.c. of the gum, recovered from the ethyl acetate, gave, from the band at R_F 0.30—0.40, the 3-*epi*-GA_4 (1.6 g), cubes from ethyl acetate–light petroleum, m.p. 220—225 °C (lit.,[7] 215—220) which, with diazomethane, gave the required 3-*epi*-GA_4 methyl ester (35), m.p. 170—171 °C (lit.,[7] 166—167).

Methylation of the material, recovered from the band at R_F 0.4—0.45, and p.l.c. gave a trace of 3-*epi*-GA_7 methyl ester (6) and the pure methyl ester (90 mg) of GA_4 (7).

ent-10β-*Hydroxy*-3-*oxo*-20-*norgibberell*-16-*ene*-7,19-*dioic Acid* 7-*Methyl Ester* 19,10-*Lactone* (39).—As in the preceding experiment a mixture (5 g) of GA_4 (70%) and GA_7 was methylated, then oxidised with Jones reagent and the crude mixture (5.1 g) of ketones was reduced with sodium borohydride–lithium bromide. The crude reduction product was chromatographed on a column (250 g, 40 × 3.5 cm) of silica gel which was eluted with increasing amounts of ethyl acetate in light petroleum. Elution with 30—40% ethyl acetate gave the methyl ester (115 mg) of GA_4 (7). Further elution with 40—100% ethyl acetate gave a mixture (3.64 g), shown, by g.l.c.–mass spectrometry of the TMSi-ethers, to contain mainly 3-*epi*-GA_4 methyl ester (35). This mixture, in acetone (100 ml), was oxidised in Jones reagent (4.5 ml) in the usual way to give the required ketone (39) (3.38 g), m.p. 142—144 °C (lit.,[8] 122—124 °C); $\nu_{max.}$ 1 773, 1 740, 1 725, and 1 660 cm^{-1} (lit.,[8] 1 790, 1 770, 1 725, and 1 654 cm^{-1}).

ent-10β-*Hydroxy*-20-*norgibberell*-16-*ene*-7,19-*dioic Acid* 7-*Methyl Ester* 19,10-*Lactone* (*GA*$_9$ *Methyl Ester*) (38).—(*a*) 3-*epi*-GA_4 methyl ester (35) (96 mg) and phosphoryl chloride (0.2 ml) in pyridine (0.5 ml) were refluxed for 3 h under nitrogen. The reaction mixture was poured into water, which was acidified and then extracted with ethyl acetate. The recovered gum was fractionated by p.l.c.

with acetone–light petroleum (1 : 5) to give, at R_F 0.35, 2,3-didehydro-GA_9 methyl ester (8 mg).[7] The band at R_F 0.45 gave 3β-chloro-GA_9 methyl ester (37) as a gum (80 mg) (Found: M^+ 364.143. $C_{20}H_{25}O_4{}^{35}Cl$ requires M 364.144); δ 1.2 (s, 18-H_3), 2.71 (d, J 11 Hz, 6-H), 3.28 (d, J 11 Hz, 5-H), 3.73 (s, CO_2Me), 4.14 (m, 3-H), 4.88 (br, 17-H), and 5.0 (br, 17-H); *m/e* 366 (M^+ +2, 2%), 364 (M^+, 5), 334 (32), 332 (83), 306 (38), 304 (100), 284 (59), 225 (50), and 224 (40).

A solution of the 3β-chloro-compound (121 mg), tri-n-butylstannane (0.25 ml) and 2,2′-azobis(2-methylpropionitrile) (6 mg) in benzene (13 ml) was refluxed for 1.5 h. Removal of the solvent and p.l.c. of the residue with ethyl acetate–light petroleum (6 : 4) gave, at R_F 0.9, GA_9 methyl ester (38) as a crystalline solid (122 mg), admixed with a little tri-n-butylstannane. Recrystallisation gave the methyl ester as platelets, m.p. 138—140 °C (lit.,[8] 136 °C).

(*b*) 3-*epi*-GA_4 methyl ester (35) (286 mg) in tetrahydrofuran (6 ml) was treated with a solution (6 ml) [2] of 0.5M $PhCCl{=}NMe_2Cl$ in dichloromethane. After 40 h at room temperature, pyridine (4 ml) was added and, after a further 5 min, hydrogen sulphide gas was passed through the mixture for 5 min. The reaction mixture was poured into water which was adjusted to pH3 and extracted with ethyl acetate. Recovery from the extract gave a yellow oil which was fractionated by p.l.c. with ethyl acetate–light petroleum (6 : 4). Elution of the band at R_F 0.90—0.95 gave the *thiobenzoate* (36) (198 mg) which was recrystallised from ethanol–chloroform, m.p. 230—232 °C (Found: C, 69.7; H, 6.3; S, 5.5; M^+ 466.182. $C_{27}H_{30}O_5S$ requires C, 69.5; H, 6.4; S, 6.8%; M 466.181); δ 1.17 (s, 18-H_3), 2.85 (s, 5- and 6-H), 4.76 (s, CO_2Me), 4.91 and 5.03 (both br, 17-H_2), 5.93 (dd, J 7 and 10 Hz, 3-H), 7.48 (m, 3 × Ar-H), and 8.29 (m, 2 × Ar-H); $\nu_{max.}$ 1 769, 1 733, 1 454, 782, and 701 cm^{-1}.

The thiobenzoate (55 mg), in toluene (5 ml), was added during 1 h to a refluxing solution of tri-n-butylstannane (0.05 ml) and 2,2′-azobis(2-methylpropionitrile) (1 mg) in toluene (5 ml) under nitrogen gas. The solution was evaporated in a stream of nitrogen gas and the product was purified by p.l.c. using ethyl acetate–light petroleum (1 : 1). Elution of the band at R_F 0.5—0.6 yielded GA_9 methyl ester (38) (35 mg). Elution of the band at R_F 0.25—0.35 gave starting material, 3-*epi*-GA_4 methyl ester (3 mg).

ent-10β-*Hydroxy*-20-*norgibberell*-16-*ene*-7,19-*dioic Acid* 19,10-*Lactone* (*GA_9*) (11).—(*a*) Gibberellin A_9 methyl ester (38) (124 mg) in dioxan (6 ml) and aqueous 2M-sodium hydroxide (6 ml) were refluxed for 15 h. The dioxan was removed *in vacuo* and the aqueous residue was diluted with water, adjusted to pH 2.5 and extracted with ethyl acetate. The gum (109 mg), recovered from the ethyl acetate, was heated on a steam-bath for 0.5 h to re-form the lactone, then purified by p.l.c. Elution of the band at R_F 0.55 gave unchanged methyl ester. Elution of the band at R_F 0.35 gave GA_9 (11), crystallising from ethyl acetate–light petroleum in cubes (61 mg), m.p. 210—214 °C (lit.,[8] 208—211 °C).

ent-3β-*Deuterio*-10β-*hydroxy*-20-*norgibberell*-16-*ene*-7,19-*dioic Acid* 19,10-*Lactone* ([3α-^{2}H]*GA_9*) (12).—To dry di-(2-methoxyethyl) ether (5 ml) at 0 °C was added sodium borodeuteride (40 mg) and lithium bromide (87 mg). After stirring at 0 °C for 10 min, GA_4 ketone methyl ester (39) (400 mg) was added and stirring was continued at 0 °C for 1 h. Addition to water, acidification, and extraction with ethyl acetate gave a gum which was separated by p.l.c. using ethyl acetate–light petroleum (3 : 2) into [3β-^{2}H]-3-*epi*-GA_4 methyl ester (40) (278 mg, R_F 0.45, 0.95 atoms deuterium per molecule) and [3α-^{2}H]GA_4 methyl ester (43 mg, R_F 0.55, 0.90 atoms deuterium per molecule).

The [3β-^{2}H]-3-*epi*-GA_4 methyl ester (268 mg) in pyridine (15 ml) was refluxed with phosphoryl chloride (600 μl) for 2 h and then poured into water. Addition of concentrated hydrochloric acid and p.l.c. of the product, recovered in ethyl acetate, with ethyl acetate–light petroleum (1 : 1) gave the [3α-^{2}H]-3β-chloro-compound (41) (130 mg, R_F 0.60, 0.96 atoms deuterium per molecule) and [3-^{2}H]-2,3-dehydro-GA_9 methyl ester (11 mg, R_F 0.50, 0.95 atoms deuterium per molecule).

The chloro-compound (41) (130 mg) in benzene (10 ml) was refluxed for 1 h with tri-n-butylstannane (0.6 ml) and 2,2′azobis(2-methylpropionitrile) (2 mg). Removal of the solvent *in vacuo* and p.l.c. with ethyl acetate–light petroleum (2 : 3) gave [3α-^{2}H]GA_9 methyl ester (42) (109 mg, R_F 0.60, 0.92 atoms deuterium per molecule).

A solution of this ester in methanol (130 ml) and 2M-sodium hydroxide (130 ml) was refluxed for 18 h. The normal work-up gave a product shown by g.l.c.–mass spectrometry to contain [^{2}H]GA_9 (12) and some deuteriated di-acid (27). It was heated at 70 °C for 20 min, then purified by p.l.c. with ethyl acetate–light petroleum–acetic acid (50 : 50 : 1) to give (R_F 0.40) [3α-^{2}H]GA_9 (12) (70 mg, 0.92 atoms deuterium per molecule).

ent-3β-*Deuterio*-3β,10β-*dihydroxy*-20-*norgibberell*-16-*ene*-7,19-*dioic Acid* 19,10-*Lactone* ([3α-^{2}H]*GA_4*) (13) (*by Professor J. E. Graebe*).—[3α-^{2}H]GA_9 (12) (1.0 mg, 0.92 atoms deuterium per molecule) was incubated with 20 ml of endosperm prepared [3] from immature seed of *Cucurbita maxima*. The incubation conditions and extraction procedure were as described by Graebe *et al.*[9]

One-tenth of the ethyl acetate extract was methylated and trimethylsilylated, and analysed by g.l.c.–mass spectrometry. The extract contained unchanged [3α-^{2}H]GA_9 (12) and [3α-^{2}H]GA_4 (13) (0.86 atoms deuterium per molecule).

ent-3α-*Deuterio*-10β-*hydroxy*-20-*norgibberell*-16-*ene*-7,19-*dioic Acid* 19,10-*Lactone* ([3β-^{2}H]*GA_9*) (14).—3β-Chloro-GA_9 methyl ester (37) (80 mg) in benzene (10 ml) was refluxed for 1 h in a stream of nitrogen gas with tri-n-butyl[^{2}H]stannane (0.15 ml) and 2,2′azobis(2-methylpropionitrile) (1 mg). The usual work-up, followed by p.l.c. with acetone–light petroleum (1 : 5) yielded (R_F 0.45) [3β-^{2}H]GA_9 methyl ester (43) (65 mg, 0.96 atoms deuterium per molecule).

A solution of this ester (64 mg) in methanol (20 ml) and 2M-sodium hydroxide was refluxed for 18 h. The usual work-up gave a gum which was heated at 85 °C for 30 min, then partitioned between ethyl acetate and aqueous saturated sodium hydrogencarbonate. From the ethyl acetate was recovered unchanged methyl ester (24 mg). Acidification of the sodium hydrogencarbonate solution, and extraction with ethyl acetate, gave the required [3β-^{2}H]GA_9 (14) (35 mg).

Reduction of the thiobenzoate (36) (70 mg) in toluene (4 ml) with tri-n-butyl[^{2}H]stannane (70 μl) in toluene (5 ml) gave [3ξ-$^2H_1$]HA_9 methyl ester (45 mg) which was hydrolysed to give [3ξ-$^2H_1$]GA_9 (27 mg), containing 0.92 atoms deuterium per molecule.

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