Mechanism and Stereochemistry of Conjugate Reduction of Enones from Gibberellins A_3 and A_7

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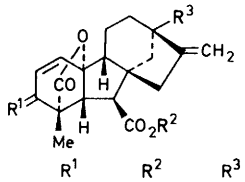
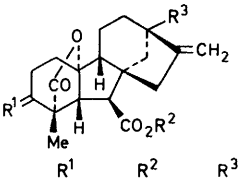
Conjugate reduction of the methyl esters of 3-didehydrogibberellin A_3 13-acetate and of 3-didehydrogibberellin A_7, in aprotic solvents by borohydride (or borodeuteride), is shown to introduce hydrogen (or deuterium) at the 1β- and 3β-positions in the products, 3-*epi*-gibberellin A_1 13-acetate and 3-*epi*-gibberellin A_4 methyl esters. The third hydrogen (or deuterium) comes from the proton (or deuteron) source used in the work-up. A mechanism for conjugate reduction of enones is proposed. The products from the borodeuteride reduction of 3-didehydrogibberellin A_7 methyl ester with proton and deuteron work-up were chemically converted into [1β-$^2H_1$]-, [1β,2-$^2H_2$]-, and [1β,3α-$^2H_2$]-gibberellin A_4 and the stereochemistries of the deuterium atoms were determined from the deuterium content of the metabolites, formed from these labelled gibberellins in cultures of *Gibberella fujikuroi*, mutant B1-41a.

GIBBERELLIN A_3 (1) and mixtures of gibberellins A_7 (2) and A_4 (32) are available in reasonable amounts from cultures of the fungus, *Gibberella fujikuroi*. They are therefore convenient starting materials for the partial syntheses of less accessible gibberellins occurring in higher plants. Gurvich *et al.*[1] and Voigt *et al.*[2] have shown that conjugate reduction of the enone (3), derived from gibberellin A_3 (GA_3) (1), gives the saturated alcohol



	R^1	R^2	R^3
(1)	H,β-OH	H	OH
(2)	H,β-OH	H	H
(3)	=O	Me	OH
(4)	=O	Me	OAc
(5)	=O	Me	H
(6)	H,α-OH	Me	OH
(7)	H,α-OH	Me	OAc
(8)	H,α-OH	Me	H
(9)	D,α-OH	Me	OAc
(10)	D,α-OH	Me	H



	R^1	R^2	R^3
(11)	H,α-OH	Me	OH
(12)	H,β-OH	Me	OAc
(13)	H,β-OH	H	OH
(14)	H,β-OH	Me	H

(11) which, in principle, could be transformed into many natural gibberellins. Furthermore, conjugate reduction introduces hydrogen at carbons-1, -2, and -3 and thereby offers a method of labelling gibberellins with deuterium and tritium at these positions. We have therefore investigated the conditions, and mechanism, of conjugate reduction of the enones (4) and (5), derived from GA_3 (1) and GA_7 (2) respectively.

RESULTS AND DISCUSSION

Reduction Conditions.—Gurvich *et al.*[1] and Voigt *et al.*[2] found that reduction of the enone (3) with lithium borohydride in tetrahydrofuran, and with sodium borohydride in methanol, gave mainly the saturated 3α-alcohol (11), while reduction with sodium borohydride in aqueous dioxan yielded mainly the allylic 3α-alcohol (6). In all cases the corresponding 3β-alcohols were detected chromatographically but could not be isolated.

In the present work, which was designed to determine the origin of deuterium and tritium atoms in the products of conjugate reduction, only aprotic solvents were considered to avoid exchange of label [3] when borodeuteride and tritiide were used. Since lithium borotritiide was not commercially available, sodium borohydride (deuteride or tritiide), in the presence of lithium bromide, was used from the outset.

In exploratory experiments small-scale reductions of the enone (5) were conducted with sodium borohydride–lithium bromide in three solvents (Table 1). The products were identified by g.l.c.–mass spectrometry and the relative yields of products were estimated by triangulation of the g.l.c. peaks. Typical results are given in Table 1; the highest ratio of 1,4- to 1,2-reduction was obtained in di-(2-methoxyethyl) ether. The alcohol (19) predominated over the 3β-alcohol (14).

Gurvich *et al.*[1] also investigated the reduction of the enone (3), and the corresponding acid, with lithium tri-t-butoxyaluminium hydride and reported that, although 1,2-reduction predominated, the 3β-alcohols were the major products of 1,4-reduction. These results were

TABLE 1

Reduction of enone (5) with $NaBH_4$–LiBr

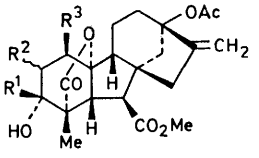
Solvent	Products (relative % yield) * (19)	(14)	(8)	Ratio 1,4 : 1,2 reduction
Tetrahydrofuran	58	11	31	69 : 31
$(MeOCH_2CH_2)_2O$	76	14	10	90 : 10
Pyridine	65	8	27	73 : 27

* G.l.c. analysis.

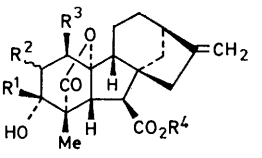
confirmed in small-scale experiments in tetrahydrofuran, pyridine, and di-(2-methoxyethyl) ether. However the relative yields (by g.l.c.) of the 3β-alcohol (12) were low (*ca.* 25%) compared to those (60—65%) of 1,2-reduction and this method of preparing GA_1 (13) and GA_4 (32) was not investigated further.

Mechanism of Conjugate Reduction.—Although conjugate reduction of enones to saturated alcohols by metal hydride has long been known, few explicit discussions of the mechanism have been published. Dilling and Plepys [4] investigated the conjugate reduction of the enone (28) (Scheme 1) by deuterium labelling. After

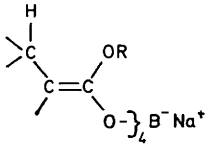
reduction with lithium aluminium deuteride, and addition of water or deuterium oxide, they obtained the saturated alcohol (29) with two or three deuterium atoms. They located only one of the incorporated deuterium



	R^1	R^2	R^3
(15)	H	H	H
(16)	D	H	D
(17)	D	D	D
(18)	H	D	H



	R^1	R^2	R^3	R^4
(19)	H	H	H	Me
(20)	D	H	D	Me
(21)	D	D	D	Me
(22)	H	D	H	Me
(23)	H	H	D	H
(24)	D	H	D	H
(25)	H	H	H	H
(26)	D	D	D	H
(27)	H	D	D	H



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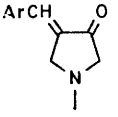

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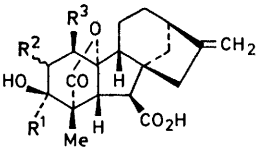
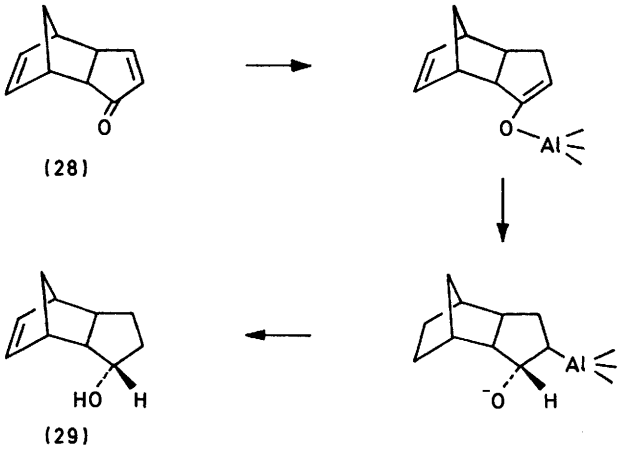


	R^1	R^2	R^3
(32)	H	H	H
(33)	H	H	D
(34)	H	D	D
(35)	D	H	D

atoms at the carbinyl carbon and proposed the mechanism shown in Scheme 1. Conjugate reduction of α,β-unsaturated esters with sodium borohydride has been investigated by Schauble *et al.*;[5] using deuterium



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SCHEME 1 Mechanism of reduction of enones (Dilling and Plepys [4])

labelling they showed that the β- and α-hydrogens in the reduced ester came respectively from the hydride and aqueous acid used in the work-up and concluded that an enol boronate (30) was the intermediate.

Our approach was similar to that of Schauble *et al.*[5] The origin of the hydrogens was determined by reduction of the enones (4) and (5) with sodium borohydride or borodeuteride, in the presence of lithium bromide, and working up the reaction with protons or deuterons. The deuterium content in the products was determined by g.l.c.–mass spectrometry of the trimethylsilyl (TMSi) ethers. Representative results are shown in Table 2. In all three solvents reduction by borodeuteride and work-up with protons gave saturated alcohols with two deuterium atoms and allylic alcohols with one deuterium atom. In tetrahydrofuran and pyridine, borodeuteride reduction and work-up with deuterons gave saturated alcohols containing three deuterium atoms; in di-(2-methoxyethyl) ether under these conditions a third deuterium atom was not incorporated. Similarly, reduction with borohydride and work-up with deuterons led to the incorporation of one deuterium atom in

TABLE 2

Incorporation of deuterium in borohydride (deuteride) reduction of the enones (4) and (5)

Experiment	Enone	Solvent [a]	Reductant	Work-up	Deuterium atoms per molecule [b] in 3α-Saturated alcohol (15) or (19)	Deuterium atoms per molecule [b] in Allylic alcohol (7) or (8)
1	(5)	A	^2H	^1H	1.83	0.91
2	(5)	A	^2H	^2H	2.43	0.89
3	(5)	A	^1H	^2H	0.77	0.00
4	(5)	B	^2H	^1H	1.72	0.91
5	(5)	B	^2H	^2H	2.72	
6	(5)	B	^1H	^2H	0.95	0.00
7	(4)	C	^2H	^1H	1.78	0.76
8	(4)	C	^2H	^2B	1.83	
9	(4)	C	^1H	^2H	0.00	

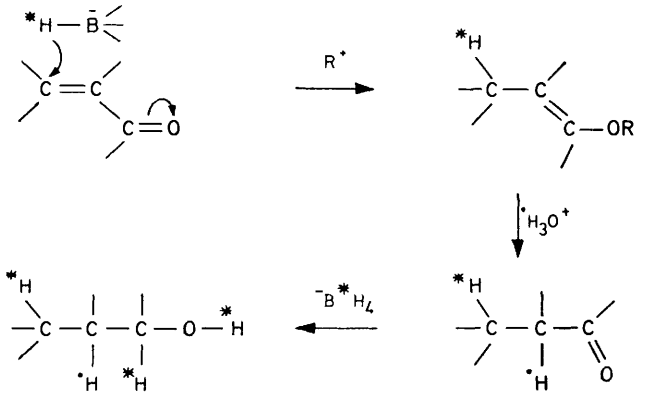
[a] A = tetrahydrofuran, B = pyridine, C = di-(2-methoxyethyl) ether. [b] Calculated by Method A (see text).

tetrahydrofuran and in pyridine but not in di-(2-methoxyethyl) ether. The failure to incorporate deuterium from deuteron work-up in di-(2-methoxyethyl) ether was confirmed; the reason is unknown (*cf.* Jackson and Zurquiyah [6]). Evidence is presented later to show that the two deuteriums which come from the borodeuteride are located in the 1β- and 3β-positions in the saturated alcohols, *e.g.* (20) and (21), and that the deuterium from work-up is at carbon-2, *e.g.* (21) and (22).

The results in Table 2 indicate that conjugate reduction occurs in two stages as shown in Scheme 2. Thus initial β-attack of hydride occurs in the reaction mixture giving an enolate (the nature of R is discussed later) and this enolate is protonated during work-up to give the saturated ketone which is then reduced by an excess of hydride. This mechanism differs from that of Dilling and Plepys (Scheme 1)[4] with respect to the stage at which the carbonyl group is reduced; it is, however, analogous to that proposed by Schauble *et al.*[5] for conjugate reduction of α,β-unsaturated esters. The proposed mechanism (Scheme 2) is in accord with two recent publications. First, Fortunato and Ganem [7] have shown that reduction of α,β-unsaturated ketones and esters with one molar equivalent of lithium tri-s-butylborohydride, followed by the addition of an alkyl

halide, gave α-alkylated saturated ketones and esters. The first reduction step in Scheme 2 is in accord with this result. Secondly, Barton *et al.*[8] have described the use of lithium enolates to protect saturated ketones during hydride reduction of other functional groups. They found that the unmasked carbonyl group formed on work-up was reduced by excess of hydride. This reduction is analogous to the second reduction step in Scheme 2.

In the present work, lithium bromide was added because it is known [9] that cations are necessary for the sodium borohydride reduction of saturated carbonyl groups in aprotic solvents. However, Handel and Pierre have reported [10] that sodium borohydride gave superior yields to lithium borohydride in the conjugate reduction of cyclohexenone in ether. In the light of the mechanism (Scheme 2), the reduction of enones in aprotic solvents by sodium borohydride in the absence of lithium bromide was re-examined. I.r. absorption, with naphthalene as internal standard, was used to determine if conjugate reduction occurred before work-up. In tetrahydrofuran and in pyridine both cyclohexenone and the enone (5) were reduced by sodium borohydride, before work-up and in the absence of lithium bromide.



SCHEME 2 Mechanism of hydride reduction of enones

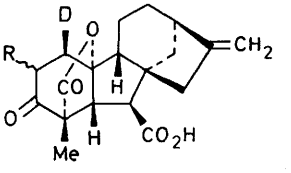
Lithium ions are therefore not necessary for the initial conjugate attack by hydride (Scheme 1) and, consequently, for the reductions listed in Table 1; Handel and Pierre [10] also noted an initial reaction of lithium and sodium borohydrides with cyclohexenone and cyclopentenone using warming, evolution of gas, and visual changes as criteria. The use of sodium borodeuteride in the absence of lithium bromide for the reductions, listed in Table 2, would have the merit that dilution of deuterium by exchange [3] with protons used in the work-up would not occur. Since sodium borohydride effects reduction in the absence of lithium ions, the group R in the intermediate enolate (Scheme 2) is unlikely to be the alkali metal cation but may be boronate as suggested by Schauble *et al.*[5]

An alternative mechanism for the reduction of enones to the saturated alcohols by metal hydrides has been advanced. Southwick *et al.*[11] suggested that reduction of enones of the type (31) by sodium borohydride in di-(2-methoxyethyl) ether occurs by rearrangement of the corresponding allylic alcohols. Similarly, Iqbal and Jackson [12] concluded that reduction of compounds of the type RCH=CHCOPh by sodium borohydride, in the presence of pyridine, also proceeded by rearrangement of the corresponding allylic alcohols, not by the pyridine, but by alkoxy-anions formed in the reduction. However, this mechanism may operate only where the methine hydrogen of the alcohol is activated by an adjacent group, such as a carbonyl or phenyl. For example, Iqbal and Jackson [12] found that the allylic alcohols PhCH=CHCH(OH)Me and 3-phenylcyclohex-2-enol were not reduced to the saturated alcohols by sodium borohydride in pyridine; the corresponding enones were reduced to the saturated alcohols under the same conditions, but no mechanism was suggested.

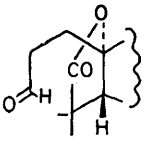
Location and Stereochemistry of the Deuterium Atoms.— The presence of a 3β-deuterium in the borodeuteride reduction products of the enones (4) and (5) was shown by n.m.r. For example, in the spectra of the saturated alcohols (16), (17), (20), and (21) the 3-H signal present at δ 3.63 and 3.70, respectively, in the 3α-alcohols (15) and (19) were absent.

The position and stereochemistry of the 1- and 2-deuterium atoms in the 3α-saturated alcohols (20) and (21), from experiments 1 and 2 (Table 2), were determined by converting them into [1β-^{2}H]GA_4 (33), [1β,2-$^2H_2$]GA_4 (34), and [1β,3α-$^2H_2$]GA_4 (35) and incubating these gibberellins with the mutant B1-41a of *Gibberella fujikuroi*. The labelled samples of GA_4 were prepared as follows.

The [$^2H_2$]alcohol (20), from experiment 1 (Table 2), was hydrolysed with aqueous sodium hydroxide. The crude acidic product, after heating to reform the 19,10-lactone, was oxidised by Jones reagent to yield the keto-acid (36), containing 0.74 deuterium atoms per molecule. Reduction of the keto-acid (36) with tri-isopropoxy-



(36) R = H
(37) R = D



(38)

aluminium, prepared *in situ*, gave [1β-^{2}H]GA_4 (33) and the 3α-epimer (23) in the ratio of 1 : 2; they were separated by p.l.c. and shown to contain 0.81 deuterium atoms per molecule. In a separate experiment the alkaline hydrolysis product of the [1β,3α-$^2H_2$]alcohol (20), containing 1.72 deuterium atoms per molecule, was separated by p.l.c. into [1β,3α-$^2H_2$]GA_4 (35) and the 3α-epimer (24) in the ratio of 1 : 12, both products containing 1.70 deuterium atoms per molecule. The retention of the [3-^{2}H] label is to be expected in this known [13] alkali-induced epimerisation which proceeds [14] *via* the intermediate (38). Further use was made of this epimerisation to show that the label in the 3α-alcohol

(22), from experiment 3 (Table 2), was at the 2-position. When this alcohol (22) was refluxed with aqueous sodium hydroxide, the products were GA_4 (32) and 3-*epi*-GA_4 (25), the deuterium at C-2 having been lost by base-catalysed exchange in the intermediate aldehyde (38). Since the preparation of the [1β-^{2}H]GA_4 (33) and the [1β,3α-$^2H_2$]GA_4 (35) involved alkaline treatment, any deuterium introduced at the 2-position during work-up by exchange of protons for deuterons in the boro-deuteride would have been removed by this epimerisation.

[1β,2ξ-$^2H_2$]GA_4 (34) was prepared from the [$^2H_3$]-alcohol (21) from experiment 2 (Table 2) *via* the 3α-tetrahydropyranyloxy-derivative which was successively hydrolysed by alkali to the free acid, and then by acid to the hydroxy-acid (26). The last mentioned compound (26) was then oxidised by Jones reagent to the ketone (37), which was reduced by tri-isopropoxy-aluminium, prepared *in situ*, to the required [1β,2*-$^2H_2$]-GA_4 (34) containing 1.42 deuterium atoms per molecule and the 3-epimer (27) containing 1.46 deuterium atoms per molecule. In the sequel it is shown that the minor loss of deuterium in these reactions occurred from position 2.

The three labelled samples (33), (34), and (35) of GA_4 were incubated with the mutant B1-41a of *G. fujikuroi*. This mutant is efficiently blocked [15] for GA-biosynthesis but has been shown [16] to convert GA_4 (32), which occurs after the block, into GA_3 (1), GA_1 (13), and GA_{16} (39). These three metabolites were detected by g.l.c.–mass spectrometry from each of the deuteriated GA_4 samples and their deuterium content was calculated from the mass spectra of the methyl ester TMSi-ethers by two methods, described in the Experimental section. The results are shown in Table 3 which includes data for the

TABLE 3

Deuterium content in metabolites from [$^2H_1$]- and [$^2H_2$]-gibberellin A_4

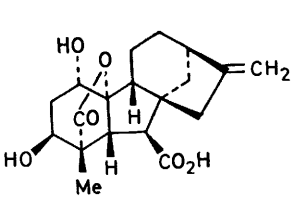
Compound	Deuterium atoms per molecule [a] from [1-^{2}H]-(33)	[1,3-$^2H_2$]-(35)	[1,2-$^2H_2$]-(34)
Substrate	0.81 (0.84)	1.70 (1.69)	1.42 (1.34)
Gibberellin A_3 (1)	0.72 (0.70)	1.59 (1.60)	1.09 (1.09)
19,2-Lactone (40)	0.77 (0.73)	1.58 (1.54)	0.97 (1.01)
Gibberellin A_1 (13)	0.81 (0.84)	*b*	1.45 (1.41)
Gibberellin A_{16} (39) [c]	0.79 (0.74)	0.80 (0.76)	0.91 (0.86)
Olefin (41) [d]	0.84 (0.78)		1.14 (1.14)

[a] Calculated from mass spectra by Method A. Figures in parentheses calculated by Method B (see text). [b] Spectrum too weak. [c] Calculations from base peak cluster ($M^+ - CH_2$-CHOTMSi). [d] Chemically derived by reaction with phosphoryl chloride.

methyl ester bis-TMSi-ether of 19,2-lactone (40), formed by rearrangement of GA_3 methyl ester bis-TMSi-ether during g.l.c., and for 2,3-didehydro-GA_9 methyl ester (41), obtained by dehydration of the appropriate GA_4 by phosphoryl chloride.

Evans *at al.*[17] have shown that the formation of the 1,2-double bond in the biosynthesis of GA_3 (1) in *G. fujikuroi* involves the loss of the 1α- and 2α-hydrogen atoms from its saturated precursors. Thus the retention of deuterium in the GA_3 derived from [1-^{2}H]- (33) and from [1,3-$^2H_2$]-GA_4 (35) establishes the 1β-stereochemistry of the label. The slightly lower deuterium content in the GA_3, derived from [1β-^{2}H]- (33) and [1β,3α-$^2H_2$]-GA_4 (35), is probably due to dilution of the GA_3 by unlabelled GA_3 formed from the slight leak [15] at the metabolic block in the mutant B1-41a.

The mass spectrum of the methyl ester bis-TMSi-ether of GA_{16} (39) contains a very weak molecular ion. The $M^+ - 116$ ion, however, is the base peak and it is evident



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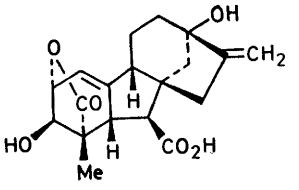

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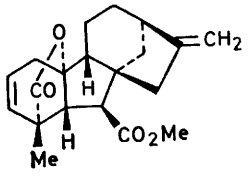
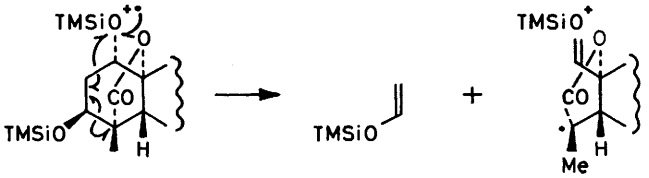


(41)

from the data in Table 3 that this ion is formed by loss of carbons-2 and -3 as shown in Scheme 3. Retention of deuterium in the $M^+ - 116$ ion of GA_{16} methyl ester bis-TMSi-ether also shows that 1α-hydroxylation of GA_4 (32) to form GA_{16} (39) occurs with retention of configuration.

The deuterium content in the metabolites from [1,2-$^2H_2$]GA_4 (34) indicates a mixture of 2α- and 2β-deuterium in the GA_4. From the deuterium content of the $M^+ - 116$ ion cluster in the spectrum of GA_{16} methyl ester bis-TMSi-ether, the [1,2-$^2H_2$]GA_4 contains 0.51 deuterium atoms per molecule at carbon-2, of which only 0.31 are lost from the 2α-position [17] in forming GA_3



SCHEME 3 Mechanism of formation of the $M^+ - 116$ ion from gibberellin A_{16} MeTMSi-ether

(1). Also chemical dehydration of [1,2-$^2H_2$]GA_4 (34) to the 2,3-didehydro-derivative (41) results only in the loss of 0.28 deuterium atoms per molecule. The close agreement between the two figures (0.31 and 0.28 deuterium atoms per molecule at the 2α-position) may be fortuitous since the dehydration of [1,2-$^2H_2$]GA_4 (34) is probably not completely stereoselective. Since the total deuterium content at carbon-2 in [1,2-$^2H_2$]GA_4 (34) is less than that expected from the deuterium content (2.43

atoms ^2H) of the starting material (21), some deuterium loss from C-2 has occurred during the preparation of [1,2-$^2H_2$]GA_4 (34) and some epimerisation at C-2 with retention of deuterium may also have occurred.

EXPERIMENTAL

General Procedures.—Light petroleum refers to the fraction with b.p. 60—80 °C. For t.l.c. and p.l.c., Merck Kieselgel HF was used and, unless otherwise stated, the solvent system was ethyl acetate–light petroleum–acetic acid (70 : 30 : 1). Analytical plates were visualised under u.v. light by spraying with 5% sulphuric acid in ethanol and heating at 100 °C for 10 min. M.p.s were determined on a Kofler hot stage. Unless stated otherwise, i.r. spectra are for Nujol mulls and n.m.r. spectra (100 MHz) are for $CDCl_3$ solutions with $SiMe_4$ as internal standard. Probe and high-resolution mass spectra were determined at 70 eV. G.l.c. was performed using silanised glass columns (127 × 0.4 cm), packed with either 2% SE-33 or 2% QF-1 on 80—100 mesh Gas Chrom Q at an N_2-flow of 60 ml min^{-1}. G.l.c.–mass spectra were obtained at 24 eV with a silicone membrane separator at 190 °C and a source temperature of 210 °C; the data were processed on-line by a DEC Linc 8 computer.

All deuteriated compounds were identified by mass spectrometry of the methyl esters and methyl ester TMSi-ethers and, in some cases, by their ^1H n.m.r. spectra. Their chemical purity was established by t.l.c., g.l.c., and g.l.c.–mass spectrometry.

Calculation of Deuterium Content.—The deuterium content was calculated from mass spectra, obtained by g.l.c.–mass spectroscopy routinely recorded at 3 s per mass decade. For the final products of the synthetic sequences and for the metabolites from microbiological transformations, the spectra were recorded at 6.5 s per decade. Calculations were made on the molecular-ion cluster of MeTMSi-derivatives except for GA_{16} methyl ester where the deuterium content of the M^+ 116 (base peak) ion cluster was determined. Two methods were used. (*a*) *Method A.* The deuteriated and undeuteriated spectra were compared using the formula (1).

No. deuterium atoms per molecule

$$= \frac{\Sigma(m/e \times I_D)}{\Sigma I_D} - \frac{\Sigma(m/e \times I)}{\Sigma I} \quad (1)$$

Here I_D and I are the ion intensities in the deuteriated and undeuteriated spectra respectively.

This method was used throughout. (*b*) *Method B.* The percentage of molecules containing 0, 1, or 2 deuterium atoms were computed from the Linc 8 computer listings and used in the formula: Number of deuterium atoms per molecule = 0.01 [percentage of molecules containing $^2H_1$ + 2 (percentage of molecules containing $^2H_2$)]. This method was used only for the data in parentheses in Table 1.

Exploratory Reductions.—(a) *With sodium borohydride–lithium bromide.* To the solvent (0.5 ml) was added anhydrous lithium bromide (7 mg, 0.08 mmol) and sodium borohydride (3 mg, 0.08 mmol). After stirring at 0 °C for 10 min the enone (4) or (5) (0.08 mmol) was added and stirring was continued at 0 °C for 1 h. After addition of water and acidification to pH 3.0 with 2M-hydrochloric acid, the products were recovered in ethyl acetate and identified by g.l.c.–mass spectrometry of the TMSi-ethers using a 2% SE-33 column. The relative amounts of each product were estimated by g.l.c. of the TMSi-ethers on a 2% SE-33 column, isothermally at 225 °C.

(b) *With lithium tri-t-butoxyaluminium hydride.* To a suspension of lithium aluminium hydride (48 mg, 1.26 mmol) in the chosen solvent (1 ml) at 0 °C was added dry t-butyl alcohol (320 μl). After 10 min the enone (4) (50 mg, 0.106 mmol) was added and stirring was continued at 0 °C for 1.5 h. The products were isolated, identified, and the relative yields estimated as in (a).

ent-13-*Acetoxy*-10β-*hydroxy*-3-*oxo*-20-*norgibberella*-1,16-*diene*-7,19-*dioic Acid* 7-*Methyl Ester* 19,10-*Lactone* (*Methyl* 3-*Didehydrogibberellin* A_3 13-*Acetate*) (4).—Gibberellin A_3 (1) (10.0 g) was methylated with diazomethane and the product in dioxan (450 ml) was stirred for 22 h with manganese dioxide [18] (102 g). The usual work-up gave a gum (11.5 g) which was crystallised from acetone–light petroleum to give the enone (3), m.p. 184—186 °C (lit.,[19] 186—187 °C).

This enone and toluene-*p*-sulphonic acid (35 mg) in acetic anhydride (50 ml) were left at room temperature for 24 h. The solution was poured into water at 0 °C; extraction with ethyl acetate (sodium hydrogencarbonate wash) and recovery gave the required *acetate* (4) (10.4 g), m.p. 164—166 °C (from acetone–light petroleum) (Found: C, 65.75; H, 6.0%; M^+, 400.153. $C_{22}H_{24}O_7$ requires C, 66.0; H, 6.0%; M^+, 400.152); $\nu_{max.}$ 1 785, 1 730, 1 695, and 1 660(sh) cm^{-1}; δ 1.28 (s, 18-H_3), 2.05 (s, OCOMe), 2.90 (d, *J* 10.5 Hz, 6-H), 3.54 (d, *J* 10.5 Hz, 5-H), 3.76 (s, CO_2Me), 5.03 and 5.20 (each t, *J* 2 Hz, 17-H_2), 6.05 (d, *J* 9.5 Hz, 2-H), and 7.25 (d, *J* 9.5 Hz, 1-H); *m/e* 400 (M^+ 21%), 358 (24), and 43 (100).

ent-10β-*Hydroxy*-3-*oxo*-20-*norgibberella*-1,16-*diene*-7,19-*dioic Acid* 7-*Methyl Ester* 19,10-*Lactone* (*Methyl* 3-*Didehydrogibberellin* A_7) (5).—A mixture (1.0 g) of GA_4 (32) and GA_7 (2) (85%) was methylated with diazomethane and the product in dioxan (50 ml) was stirred overnight at room temperature with manganese dioxide [18] (12 g). The usual work-up gave a gum which was separated by p.l.c. with ethyl acetate–light petroleum (1 : 1) into GA_4 methyl ester (14) (50 mg, R_F 0.50) and the required enone (5) (815 mg, R_F 0.75), m.p. 133—135 °C (lit.,[20] 139—140 °C); δ 1.19 (s, 18-H_3), 2.82 (d, *J* 11 Hz, 6-H), 3.40 (d, *J* 11 Hz, 5-H), 3.72 (s, CO_2Me), 4.84 and 4.96 (each br s, 17-H_2), 5.98 (d, *J* 9 Hz, 2-H), and 7.20 (d, *J* 9 Hz, 1-H).

Reduction of ent-10β-*Hydroxy*-3-*oxo*-20-*norgibberella*-1,16-*diene*-7,19-*dioic Acid* 7-*Methyl Ester* 19,10-*Lactone* (5) *in Tetrahydrofuran.*—(a) *With sodium borohydride and protium work-up.* To a suspension of sodium borohydride (40 mg) and anhydrous lithium bromide (87 mg) in dry tetrahydrofuran (10 ml), cooled to 0 °C, was added the enone (5) (400 mg). After 1 h at 0 °C, the mixture was poured into water which was acidified with hydrochloric acid and extracted with ethyl acetate. P.l.c. of the recovered product with ethyl acetate–light petroleum (7 : 3) gave, from the R_F band at 0.45, 3-*epi*-GA_4 methyl ester (19) (183 mg), m.p. 170—171 °C (from acetone–light petroleum) (lit.,[21] m.p. 166—167 °C); δ 1.18 (s, 18-H_3), 2.54 (d, *J* 11 Hz, 6-H), 2.79 (d, *J* 11 Hz, 5-H), 3.69 (m, 3-H), 3.74 (s, CO_2Me), and 4.87 and 4.95 (each br s, 17-H_2); *m/e* (MeTMSi) 418 (M^+, 17%), 289 (87), 261 (25), 233 (31), 225 (31), 129 (67), 75 (72), and 73 (100).

Extraction of the band at R_F 0.5 gave a mixture of GA_4 methyl ester (14) and 3-*epi*-GA_7 methyl ester (8) in the ratio 1 : 3 (g.l.c.–mass spectrometry). Another p.l.c. of this mixture as before gave 3-*epi*-GA_7 methyl ester (8) (34 mg)

as a gum which, by evaporation of an acetone solution, gave a solid foam, m.p. 55—65 °C (Found: C, 69.3; H, 7.2. $C_{20}H_{24}O_5$ requires C, 69.8; H, 7.0%); $\nu_{max.}$ 3 540 (br), 1 772, 1 658, and 870 cm^{-1}; *m/e* (MeTMSi-ether) 416 (M^+, 5%), 311 (41), 223 (56), 222 (94), 193 (16), 157 (30), 75 (43), and 73 (100).

(b) *With sodium borodeuteride and protium work-up.* To a suspension of sodium borodeuteride (25 mg) and anhydrous lithium bromide (50 mg) in tetrahydrofuran (5 ml) at 0 °C was added the enone (5) (200 mg). After 1 h at 0 °C the product was recovered and fractionated as in (a) to give the 3α-[1β,3β-$^2H_2$]alcohol (20) (118 mg, 1.83 atoms deuterium per molecule) from the band at R_F 0.45. Extraction of the band at R_F 0.50 gave a gum (32 mg), shown by g.l.c.–mass spectrometry to be a mixture (1 : 1) of the methyl ester of [1β,3α-$^2H_2$]GA_4 (35) and the [3β-^2H]-3α-allylic alcohol (10) with deuterium-contents of 1.76 and 0.91 atoms per molecule, respectively.

(c) *With borodeuteride and deuterium work-up.* The enone (5) (650 mg) in tetrahydrofuran (15 ml) was reduced at 0 °C for 1 h with sodium borodeuteride (70 mg) and lithium bromide (155 mg). Deuterium oxide (3 ml) was added and the pH was adjusted to 3.0 with deuterium chloride. Work-up as in (a) gave the [1β,2ξ,3β-$^2H_3$]-3α-alcohol (21) (345 mg, R_F 0.45, 2.43 atoms deuterium per molecule) and a mixture (180 mg, R_F 0.50) containing (g.l.c.–mass spectrometry) mainly the [3β-^2H]-3α-alcohol (10) with 0.89 atoms deuterium per molecule.

(d) *With borohydride and deuterium work-up.* Reduction as in (a), except that borohydride (20 mg) and lithium bromide (43 mg) were used and that deuterium oxide (1.5 ml) and deuterium chloride were added to the reaction mixture, gave the [2ξ-^2H]-3α-alcohol (22) (78 mg, 0.77 atoms deuterium per molecule) and a mixture (44 mg) containing (g.l.c.–mass spectrometry) mainly the unlabelled 3α-allylic alcohol (8).

Hydride Reduction of ent-13-*acetoxy*-10β-*hydroxy*-3-*oxo*-20-*norgibberella*-1,16-*diene*-7,19-*dioic Acid* 7-*Methyl Ester* 19,10-*Lactone* (4) *in Pyridine.*—(a) *With borodeuteride and protium work-up.* To dry pyridine (1 ml) at 0 °C was added sodium borodeuteride (3 mg) and lithium bromide (7 mg). After 10 min the enone (4) (30 mg) was added. After 1 h at 0 °C the reaction mixture was poured into water, which was acidified with concentrated hydrochloric acid and extracted with ethyl acetate. The recovered products were analysed by g.l.c.–mass spectrometry of the TMSi-derivatives and shown to contain the 3α-alcohol (16) and the 3α-allylic alcohol (9), containing 1.72 and 0.91 atoms deuterium per molecule, respectively.

(b) *With borodeuteride and deuterium work-up.* As in (a) except that deuterium oxide (1 ml) was added to the reaction solution after 1 h at 0 °C and that deuterium chloride was used to adjust the pH to 3.0. G.l.c.–mass spectrometry of the TMSi-derivatives of the recovered products identified the 3α-alcohol (17) containing 2.72 atoms deuterium per molecule.

(c) *With borohydride and deuterium work-up.* As in (b) replacing the sodium borodeuteride by sodium borohydride, gave the 3α-alcohol (18) and 3α-allylic alcohol (7) with 0.95 and 0.00 atoms deuterium per molecule, respectively.

Reduction of ent-13-*Acetoxy*-10β-*hydroxy*-3-*oxo*-20-*norgibberella*-1,16-*diene*-7,19-*dioic Acid* 7-*Methyl Ester* 19,10-*Lactone* (4) *in Di*-(2-*methoxyethyl*) *Ether.*—(a) *With sodium borohydride and protium work-up* (*with P. S. Kirkwood*). Anhydrous lithium bromide (87 mg) and sodium borohydride (40 mg) in the solvent (5 ml) were stirred at 0 °C for 10 min. The enone (4) (400 mg) was added and stirring at 0 °C was continued for 1 h. The mixture was poured into water, which was acidified to pH 3.0 by adding concentrated hydrochloric acid, and the aqueous suspension was extracted with ethyl acetate. Recovery from the ethyl acetate extract yielded an oil which was fractionated by p.l.c. with ethyl acetate–light petroleum (7 : 3). The band at R_F 0.45 gave 3-epi-GA_1 *methyl ester* 13-*acetate* (15) (240 mg), m.p. 143—145 °C (from ethyl acetate–light petroleum (Found: C, 65.8; H, 7.0. $C_{22}H_{28}O_7$ requires C, 63.35; H, 6.9%); δ 1.16 (s, 18-H_3), 2.02 (s, OCOMe), 2.56 (d, *J* 10 Hz, 6-H), 2.77 (d, *J* 10 Hz, 5-H), 3.63 (m, 3-H), 3.75 (s, CO_2Me), 4.99 (br s, 17-H), and 5.15 (br s, 17-H); *m/e* (TMSi by g.l.c.–mass spectrometry) 476 (M^+, 12%), 434 (20), 347 (39), 129 (66), 75 (48), 73 (100), 44 (18), and 43 (42).

The band at R_F 0.5 yielded a mixture (44 mg) of 3-*epi*-GA_3 methyl ester 13-acetate (7) and GA_1 methyl ester 13-acetate (12) in the ratio 1 : 10 (by g.l.c.–mass spectrometry). A repeat p.l.c. of the mixture with the same solvent system gave GA_1 *methyl ester* 13-*acetate* (12) (35 mg) as a gum (Found: M^+, 404.185. $C_{22}H_{28}O_7$ requires *M*, 404.184); δ 1.14 (s, 18-H_3), 2.03 (s, OCOMe), 2.70 (d, *J* 10 Hz, 6-H), 3.23 (d, *J* 10 Hz, 5-H), 3.75 (s, CO_2Me), 3.86 (br s, 3-H), 5.01 and 5.17 (both br s, 17-H_2); $\nu_{max.}$ ($CHCl_3$) 3 450 (br), 1 769, and 1 732 cm^{-1}; *m/e* (TMSi-ether, g.l.c.–mass spectrometry) 476 (M^+, 7%), 434 (18), 347 (23), 282 (49), 219 (52), 75 (100), 73 (82), 44 (52), and 43 (56).

(b) *With sodium borodeuteride and deuterium work-up.* The conditions for reduction and work-up were the same as the corresponding reduction in pyridine. The quantities were: sodium borodeuteride (40 mg), lithium bromide (87 mg), the enone (4) (400 mg), and di-(2-methoxyethyl) ether (5 ml). The products were the [1β,3β-$^2H_2$]-3α-alcohol (16) (225 mg, 1.78 atoms deuterium per molecule) and a 12 : 1 mixture (84 mg), by g.l.c.–mass spectrometry, of the [1β,3α-$^2H_2$]-derivative of the 3β-alcohol (12) and the [3β-^2H]-3α-allylic alcohol (9), with 1.80 and 1.76 atoms deuterium per molecule, respectively.

(c) *With borodeuteride and deuterium work-up.* Conditions and work-up were identical to the corresponding reduction of the enone (4) in pyridine except that the solvent was di-(2-methoxyethyl) ether (5 ml). The 3α-alcohol (16) had 1.83 atoms deuterium per molecule.

(d) *With borohydride and deuterium work-up.* As in (b) except that sodium borohydride was used in place of sodium borodeuteride. Analysis by g.l.c.–mass spectrometry showed that the products contained no deuterium. This experiment was repeated with the same result.

Treatment of ent-2ξ-*Deuterio*-3β,10β-*dihydroxy*-20-*norgibberella*-16-*ene*-7,19-*dioic Acid* 7-*Methyl Ester* 19,10-*Lactone* (18) *with Aqueous Alkali.*—A solution of the labelled alcohol (18) (10 mg, 0.77 deuterium atoms per molecule) in methanol (10 ml) and 2M-sodium hydroxide (10 ml) was refluxed for 8 h, then poured into water. Acidification with concentrated hydrochloric acid and extraction with ethyl acetate gave a gum, which was heated to 80 °C for 20 min, then methylated and trimethylsilylated. The product was shown to be a mixture of GA_4 (32) (0.08 deuterium atoms per molecule) and 3-*epi*-GA_4 (25) (0.11 deuterium atoms per molecule) in the ratio 1 : 9 by g.l.c.–mass spectrometry of the methyl ester TMSi-ethers.

ent-1α-*Deuterio*-3α,10β-*dihydroxy*-20-*norgibberell*-16-*ene*-7,19-*dioic Acid* 19,10-*Lactone* ([1β-$^2H_1$]*Gibberellin* A_4) (33).—A solution of [1β,3β-$^2H_2$]-3-*epi*-GA_4 methyl ester (20) (118

mg, 1.83 atoms deuterium per molecule) in methanol (120 ml) and 2M-sodium hydroxide (120 ml) was refluxed for 16 h. After removal of the methanol *in vacuo* the aqueous residue was acidified with concentrated hydrochloric acid and extracted with ethyl acetate. The gum, recovered from the ethyl acetate, was heated at 80 °C for 30 min to ensure complete re-lactonisation, and dissolved in acetone (10 ml). To this solution, cooled to 0 °C, was added an excess of Jones reagent and, after 30 min at 0 °C, normal work-up and p.l.c. gave, from the band at R_F 0.6, [1β-^{2}H]-3-oxo-GA_4 (36) (26 mg, 0.74 deuterium atoms per molecule).

Aluminium foil (715 mg) and mercuric chloride (10 mg) were suspended in propan-2-ol (10 ml). After heating to reflux, carbon tetrachloride (60 μl) was added and the solution was refluxed for 3 h. The above [1β-^{2}H]ketone (26 mg) was added and refluxing was continued. After 3 h, addition to dilute hydrochloric acid and extraction with ethyl acetate gave an oil, which was fractionated by p.l.c. Recovery from the band at R_F 0.3 gave the required [1β-^{2}H]GA_4 (33) (4 mg, 0.81 deuterium atoms per molecule) and recovery from the band at R_F 0.20 yielded [1β-^{2}H]-3-*epi*-GA_4 (23) (9.3 mg, 0.81 deuterium atoms per molecule).

ent-1α,2ξ-*Dideuterio*-3α,10β-*dihydroxy*-20-*norgibberell*-16-*ene*-7,19-*dioic Acid* 19,10-*Lactone* ([1β,2ξ-$^2H_2$]*Gibberellin* A_4) (34).—A solution of [1β,2ξ,3β-$^2H_3$]-3-*epi*-GA_4 methyl ester (21) (80 mg, 2.43 deuterium atoms per molecule), in dichloromethane (15 ml), was stirred at room temperature with dihydropyran (80 μl) and toluene-*p*-sulphonic acid (1 crystal). After 3 h, water was added and the gum, recovered from the organic layer, was fractionated by p.l.c. with ethyl acetate–light petroleum (2 : 3). Recovery from the band at R_F 0.4 gave [1β,2ξ,3β-$^2H_3$]-3-*epi*-gibberellin A_4 methyl ester 2-tetrahydropyranyl ether (88 mg) as an equal mixture of diastereoisomers; δ 1.11 and 1.21 (18-H_3), 2.52 and 2.56 (5-H, *J* 11 Hz), 2.68 (6-H, *J* 11 Hz), 3.71 (CO_2Me), 4.63 (br), 4.75 (br), 4.84 (br, 17-H), and 4.97 (br, 17-H).

A solution of the tetrahydropyranyl ether (80 mg) in methanol (70 ml) and 2M-sodium hydroxide (70 ml) was refluxed overnight. After removal of the methanol *in vacuo*, the aqueous residue was acidified with concentrated hydrochloric acid and extracted with ethyl acetate. The recovered product in acetone–methanol (5 : 1) was stirred with toluene-*p*-sulphonic acid (2 mg) for 1 h (t.l.c. monitoring). The solution was then concentrated, diluted with water, and extracted with ethyl acetate to give [1β,2ξ,3β-$^2H_3$]-3-*epi*-GA_4 (26) (50 mg). This (50 mg), in acetone (10 ml), was oxidised at 0 °C for 1 h with Jones reagent in the usual manner. The crude product (40 mg) was separated and purified by p.l.c. The [$^3H_2$]keto-acid (37) (22 mg) was recovered from the band at R_F 0.60 and added in propan-2-ol (1 ml) to a refluxing solution prepared from propan-2-ol (10 ml), mercuric chloride (10 mg), aluminium foil (800 mg), and carbon tetrachloride (60 μl) as described in the previous experiment. After 3 h, dilute hydrochloric acid was added and the product, recovered in ethyl acetate, was purified by p.l.c. The required [1β,2ξ-$^2H_2$]GA_4 (34) (4 mg, 1.42 deuterium atoms per molecule) was obtained from the band at R_F 0.35 and [1β,2ξ-$^2H_2$]-3-*epi*-GA_4 (27) (11 mg, 1.40 deuterium atoms per molecule) was recovered from the band at R_F 0.25.

ent-1α,3β-*Dideuterio*-3α,10β-*dihydroxy*-20-*norgibberell*-16-*ene*-7,19-*dioic Acid* 19,10-*Lactone* ([1β, 3α-$^2H_2$]*Gibberellin* A_4) (35).—A solution of [1β,3β-$^2H_2$]-3-*epi*-gibberellin A_4 methyl ester (20) (165 mg, 1.72 atoms deuterium per molecule) in methanol (150 ml) and 2M-sodium hydroxide (150 ml) was refluxed overnight. After removal of the methanol *in vacuo*, the product was recovered in the usual manner, heated at 80 °C for 30 min, and then partitioned between ethyl acetate and saturated aqueous sodium hydrogencarbonate solution. From the ethyl acetate layer, starting material (32 mg) was recovered. Acidification of the aqueous layer and extraction with ethyl acetate gave a gum which was fractionated by p.l.c. into [1β,3β-$^2H_2$]-3-*epi*-GA_4 (24) (72 mg, R_F 0.4, 1.70 deuterium atoms per molecule) and a gum (11 mg, R_F 0.5) which, after p.l.c. under the same conditions, gave [1β,3α-$^2H_2$]GA_4 (35) (6 mg, 1.70 deuterium atoms per molecule).

Microbiological Transformation of [1β-$^2H_1$]-, [1β,2ξ-$^2H_2$]-, *and* [1β,3α-$^2H_2$]*Gibberellin* A_4 *by* Gibberella fujikuroi, *Mutant* B1-41a.—The procedure used was that described previously [22] in which shake-flask cultures of the mutant B1-41a were grown at 25 °C for 3 d in ICI medium (40% nitrogen) (40 ml) in conical flasks (250 ml). The pigmented mycelium from each flask was then re-suspended in nitrogen-free ICI medium (50 ml) to which the substrate (1 mg) in acetone (100 ml) had been added; the suspension cultures in conical flasks (250 ml) were then shaken at 25 °C for 5 d. The culture filtrates were acidified with dilute hydrochloric acid to pH 3.0 and extracted with ethyl acetate. The total product from each substrate was methylated and then trimethylsilylated. The metabolites were identified by g.l.c.–mass spectrometry using a 2% QF-1 column run isothermally at 170 °C for 5 min then temperature-programmed to 240 °C at 2° min^{-1}. The deuterium content in the identified metabilites was calculated as described earlier. The results are shown in Table 3.

Treatment of ent-1α,2ξ-*Dideuterio*-3α,10β-*dihydroxy*-20-*norgibberell*-16-*ene*-7,19-*dioic Acid* 19,10-*Lactone* (34) *with Phosphoryl Chloride.* [1β,2ξ-$^2H_2$]GA_4 (34) (500 μg, 1.42 atoms deuterium per molecule) was methylated with diazomethane and the product, in pyridine (0.5 ml) was refluxed with phosphoryl chloride (5 μl) for 2 h. The normal work-up gave a gum shown, by g.l.c.–mass spectrometry, to consist mainly of [1β-^{2}H]-2,3-didehydro-GA_9 methyl ester (41) containing 1.14 atoms deuterium per molecule.

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