# The Stereochemistry of Reduction of A Gibberellin $\Delta^1$-Unsaturated Ketone

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Whilst sodium borodeuteride reduction of gibberellin $A_3$ 3-ketone which affords gibberellin $A_1$ and its 3-epimer, proceeds stereospecifically from the β-face at C-1, the reaction has only limited stereoselectivity at C-2 affording in the case of 3-*epi*-gibberellin $A_1$ methyl ester, a 2α:2β-$^2$H ratio of 0.35—0.5:1 thus revising a previous conclusion.

In the course of the preparation of gibberellin $A_9$ and $A_{20}$ from gibberellic acid we described [1] the conjugate reduction of the unsaturated ketone (1) with sodium borodeuteride–copper(I) chloride utilizing the initial observations of Gurvich *et al.*[2] Based on an examination of changes in the $^1$H n.m.r. spectrum of the resultant 3-*epi*-gibberellin $A_1$ methyl ester, we concluded that the proton which was deuteriated at C-1 was the 1β (axial) proton whilst at C-2 it was the 2β (equatorial) proton which bore the label and hence reduction had taken place from the β-face of the molecule. MacMillan examined [3] the stereochemistry of borodeuteride reduction of the gibberellin $A_7$ methyl ester 3-ketone by analysing the deuterium content of the metabolites formed from the labelled gibberellins in cultures of *Gibberella fujikuroi*. Subsequently he has extended this work in the 13-desoxy series and has informed us that he did not agree with our stereochemical conclusions.† In view of the importance of gibberellins labelled on ring A in metabolic studies, we have re-examined the problem using $^2$H n.m.r. spectroscopy.

Our analysis of the ring A $^1$H n.m.r. resonances rested on the assignment of the 2-H signals in [2,2,6-$^2H_3$]-3-*epi*-gibberellin $A_4$ methyl ester (2). This assignment was incorrect in the case of the 2α-proton. Retro-aldol cleavage and re-formation of ring A in 3-hydroxygibberellins affords a means of labelling C-2 *via* the intermediate 3,4-seco-3-aldehyde.[4] Treatment of 3-*epi*-gibberellin $A_4$ methyl ester (2) with methan[$^2$H]olic sodium methoxide under reflux afforded [2,2,6-$^2H_3$]gibberellin $A_4$ methyl ester (3) ($M^+$, 349) and [2,2,6-$^2H_3$]-3-*epi*-gibberellin $A_4$ methyl ester (2) ($M^+$, 349). Whilst the $^2$H n.m.r. spectrum of [2,2,6-$^2H_3$]gibberellin $A_4$ methyl ester had signals at δ 1.78 and 2.66 (ratio 2 : 1) that of [2,2,6-$^2H_3$]-3-*epi*-gibberellin $A_4$ methyl ester had signals at δ 1.41, 2.14, and 2.68 (ratio 1 : 1 : 1). A re-determination of the 360 MHz $^1$H n.m.r. spectrum of [2,2,6-$^2H_3$]-3-*epi*-gibberellin $A_4$ methyl ester showed that it lacked signals at δ 2.2 and 1.45 (assigned to the 2-H resonances) and at δ 2.66 (d, *J* 10.3 Hz, 6-H) whilst the 5-H signal (δ 2.53) appeared as a singlet. Signals at δ 1.57 and 2.13 collapsed to doublets (*J* 14 Hz) and were consequently assigned to the 1-H resonances. Although these could be assigned to the 1β-(axial) and 1α-(equatorial) proton resonances respectively on the basis of the generalization [5] that axial protons resonate at higher field than their equatorial counterparts, we nevertheless sought confirmation of this by spin-decoupling experiments. These were based on irradiation at δ 3.65, 2.23, and 1.45 in 3-*epi*-gibberellin $A_1$ methyl ester (4) and led to the coupling pattern shown in the Figure.

Reduction of the αβ-unsaturated ketone (1) with sodium borohydride–copper(I) chloride in methan[$^2$H]ol gave [2-$^2$H]-3-*epi*-gibberellin $A_1$ methyl ester ($M^+$, 363) whilst reduction with sodium borodeuteride–copper(I) chloride in methanol gave [1,3-$^2H_2$]-3-*epi*-gibberellin $A_1$ methyl ester ($M^+$, 364).

† See preceding papers.

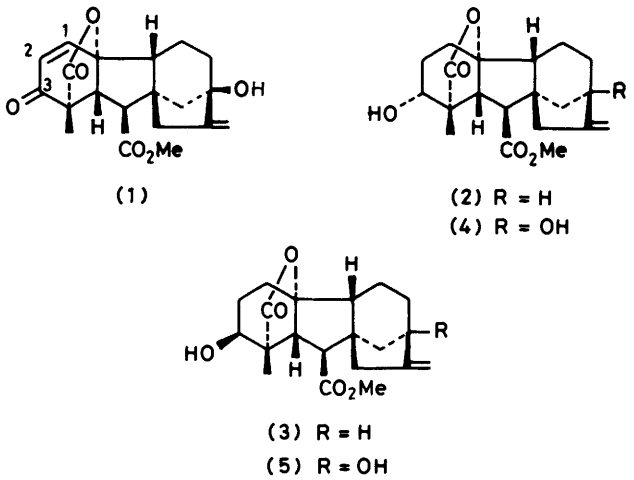
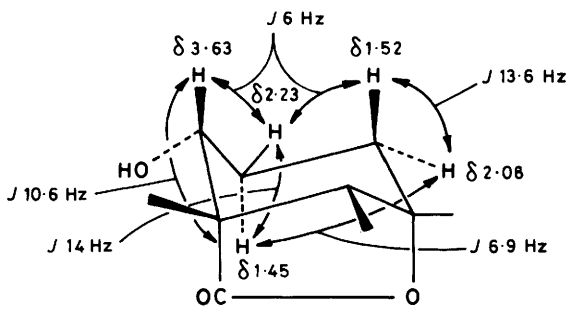



**Figure.** 1-H, 2-H, and 3-H Coupling constants and chemical shifts in 3-*epi*-gibberellin $A_1$ methyl ester




Reduction with sodium borodeuteride–copper(I) chloride in methan[$^2$H]ol gave [1,2,3-$^2H_3$]-3-*epi*-gibberellin $A_1$ methyl ester ($M^+$, 365). In each case the 3-*epi*-gibberellin $A_1$ methyl ester was accompanied by a small amount of gibberellin $A_1$ methyl ester (5). The sites of deuteriation had been located in our previous work [1] from the changes in the $^{13}$C n.m.r. spectra. The stereochemistry of the labels followed from an examination of the $^2$H n.m.r. spectra (see Table). The stereochemistry of deuteriation at C-1 by reduction with sodium borodeuteride was clear-cut. The $^2$H n.m.r. spectrum showed signals at δ 1.52 (1β-H) and at δ 3.63 (3β-H) with no evidence of a signal at δ 2.1 (1α-H). Hence the label has been introduced stereospecifically at 1β-H in agreement with previous conclusions. However the labelling at 2-H is less clear-cut. Examination of the $^2$H n.m.r. spectra, which were not available in our previous work, revealed some label at both 2α-H and 2β-H.

Table. $^2H$ N.m.r. Spectra of deuteriated gibberellins determined at 55.28 MHz in $CHCl_3$ (95%), $CDCl_3$ (5%)

| Reagent | Resonance (relative integral) 1-H | 2-H | 3-H |
|---|---|---|---|
| Gibberellin $A_1$ methyl ester | | | |
| $MaB^2H_4$ : MeOH | 1.74 (1) | | 3.80 (1.05) |
| $NaBH_4$ : $MeO^2H$ | | 1.80 (6.9) | 3.79 (0.5) |
| 3-*epi*-Gibberellin $A_1$ methyl ester | | | |
| $NaB^2H_4$ : MeOH | 1.52 (1.1) | | 3.62 (1) |
| $NaBH_4$ : $MeO^2H$ | 1.46 (3.38) | 2.21 (6.76) | 3.63 (1) |
| $NaB^2H_4$ : $MeO^2H$ | 1.51 (2.8) | 2.21 (1) | 3.63 (2.3) |

In the $^2H$ n.m.r. spectra determined at 55.28 MHz, it was not possible to resolve 1β-H (δ 1.52) from 2α-H (δ 1.45). In the sample of [2-$^2$H]-3-*epi*-gibberellin $A_1$ methyl ester, there was clearly some label at 3β-H which was presumably introduced by reduction with sodium borodeuteride that had been formed by exchange between the sodium borohydride and the methan[$^2$H]ol.[6] This would introduce an equivalent amount of deuterium at 1β-H (δ 1.52). Subtraction of this amount in the nominally [2-$^2$H]-3-*epi*-gibberellin $A_1$ methyl ester gives a ratio of 0.35 : 1 between 2α- and 2β- whilst application of the same calculation to the sample of [1,2,3-$^2H_3$]-3-*epi*-gibberellin $A_1$ methyl ester gives a ratio of 0.5 : 1. An interesting feature of the results with [1,2,3-$^2H_3$]-3-*epi*-gibberellin $A_1$ methyl ester is that less deuterium is incorporated in total at C-2, compared to 1-H and 3-H which could be taken to suggest that an isotope effect exists in the proteolysis of an intermediate enol-borate. Deuterium is not removed from C-2 under the reaction conditions and work-up since treatment of [2,2,6-$^2H_3$]-3-*epi*-gibberellin $A_4$ methyl ester with sodium borohydride–copper chloride in methanol gave back the starting material with the deuterium labels at C-2 and C-6 in the same ratio.

The $^2H$ n.m.r. spectrum of [2,2,6-$^2H_3$]-gibberellin $A_4$ methyl ester (3) contained resonances at δ 1.78 (2-$^2H_2$) and 2.66 (6-H) with integrals in the ratio of 2 : 1. Comparison of the $^1H$ n.m.r. spectrum of this sample with that of undeuteriated gibberellin $A_4$ methyl ester revealed the disappearance of signals from the multiplets at δ 1.8 whilst the AB-system (*J* 13.7 Hz) (δ 1.98 and 1.74) which was assigned to the 1α-H and 1β-H protons became clear. The sample of [1,3-$^2H_2$]-gibberellin $A_1$ methyl ester (5) showed signals at δ 1.75 and 3.80 only corresponding to stereospecific 1β-deuteriation. The sample of [2-$^2$H]-gibberellin $A_1$ methyl ester showed a $^2H$ n.m.r. signal at δ 1.80 together with a small signal at δ 3.80 arising by deuteriation with sodium borodeuteride formed during the reduction. However since the 2α-H and β-resonances are almost co-incident, it was not possible to assign the stereochemistry at this position.

In conclusion reduction takes place stereospecifically at C-1β from the β-face of the molecule but it is only stereoselective to a relatively limited extent at C-2. The lack of correlation between the stereochemistry of the new C–H bonds at C-1, C-2, and C-3 in the gibberellin $A_1$ and 3-*epi*-gibberellin $A_1$ methyl esters suggests that these bonds are formed at different stages in the reduction and in particular it militates against the *cis*-hydroboronation of a $\Delta^{2,3}$-enol borate favoured in our previous work.[1]

The $^{13}C$ n.m.r. spectrum of 3-*epi*-gibberellin $A_4$ methyl ester was compared to that of [2,2,6-$^2H_3$]-3-*epi*-gibberellin $A_4$ methyl ester. The changes on deuteriation (see Experimental section) permit a more confident assignment [7] to be made to the $^{13}C$ resonances of C-2 and C-6.

## Experimental

$^1H$ and $^2H$ N.m.r. spectra were determined on a Bruker WH 360 spectrometer at the University of Edinburgh through the courtesy of Dr. I. H. Sadler and his collaborators.

*Deuteriation Experiments.*—(i) *ent*-10β,13-Dihydroxy-3-oxo-20-norgibberella-1,16-diene-7,19-dioic acid 19,10β-lactone 7-methyl ester (1) (400 mg) [8] in methan[$^2$H]ol (15 ml) was treated with sodium borohydride (300 mg) and copper(I) chloride (300 mg) at 0 °C for 30 min. The methanol was evaporated and the residue taken up in dilute hydrochloric acid and then extracted with ethyl acetate. The extract was washed with water and dried. The solvent was evaporated and the residue chromatographed on silica. Elution with 45% ethyl acetate–light petroleum gave *ent*-[2-$^2$H]-3α,10β-13-trihydroxy-20-norgibberell-16-ene-7,19-dioic acid 19,10β-lactone 7-methyl ester (5) (62 mg), m.p. 232—235 °C (lit.,[9] 233—235 °C) identified by its $^1H$ n.m.r. spectrum. Further elution with 45% ethyl acetate–light petroleum gave *ent*-[2-$^2$H]-3β,10β,13-trihydroxy-20-norgibberell-16-ene-7,19-dioic acid 19,10β-lactone 7-methyl ester (4) (250 mg), m.p. 188—189 °C (lit.,[2] 182—184 °C) identified by its $^1H$ n.m.r. spectrum. Each sample had a mass spectral peak of 363 for $C_{20}H_{25}{}^2HO_6$.

(ii) Repetition with the unsaturated ketone (1) (400 mg) in methanol (15 ml) with copper(I) chloride (300 mg) and sodium [$^2H_4$]borohydride afforded the ester (5) (30 mg) and the ester (4) (150 mg) identified as above. Each sample had a mass spectral peak of 364 for $C_{20}H_{24}{}^2H_2O_6$.

(iii) Repetition with the unsaturated ketone (1) (300 mg) in methan[$^2$H]ol (20 ml), copper(I) chloride (300 mg), and sodium [$^2H_4$]borohydride (150 mg) gave the [1,2,3-$^2H_3$]-ester (5) (42 mg) and the [1,2,3-$^2H_3$]-ester (4) (151 mg) identified as above. Each sample had a mass spectral peak of 365 for $C_{20}H_{23}{}^2H_3O_6$.

(iv) [2,2,6-$^2H_3$]-Gibberellin $A_4$ methyl ester and [2,2,6-$^2H_3$]-3-*epi*-gibberellin $A_4$ methyl ester were obtained as described previously. The latter had $^{13}C$ n.m.r. signals at (determined in $CDCl_3$) δ 12.52 (C-17), 15.84 (C-11), 28.5 (C-2, signal collapses on deuteriation), 29.72 (C-1), 31.09 (C-12), 36.80 (C-14), 38.61 (C-13), 44.24 (C-15), 51.32 (C-6, collapses on deuteriation), 51.66 ($OCH_3$), 52.19 (C-8), 53.09 (C-9), 54.12 (C-4), 56.44 (C-5), 72.56 (C-4), 92.64 (C-10), 107.10 (C-17), 156.24 (C-16), 172.88 (C-7), and 177.21 (C-19).

(v) [2,2,6-$^2H_3$]-3-*epi*-Gibberellin $A_4$ methyl ester (50 mg) in methanol (20 ml) was treated with sodium borohydride (30 mg) and copper(I) chloride (50 mg) for 45 min. The mixture was worked up as above to afford the starting material ($M^+$ 349) which was re-examined by $^2H$ n.m.r. spectroscopy. There was no change in the ratio of the signals.

## Acknowledgements

We thank Professor J. MacMillan and Dr. C. L. Willis for interesting discussions and exchange of information prior to publication. We thank Dr. I. H. Sadler, University of Edinburgh NMR Service for the determination of the n.m.r. spectra.

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*Received* 22*nd July* 1983; *Paper* 3/1256