# Stereochemistry of Ring A in Gibberellic Acid as Indicated by Use of Nuclear Overhauser Effects

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The α-orientation of 1 → 4a lactone in ring A of gibberellic acid derivatives was determined by exploiting the dipole-dipole interactions of C-1 methyl and C-2 hydrogens that are spacially proximated.

The experiments on nuclear Overhauser effects led to establishing the ring A stereochemistry in solutions and the results are in complete agreement with the structure deduced by X-ray crystallographic data.

The structure and absolute configuration of gibberellic acid, a highly active plant growth-regulating hormone, have now been firmly established by the chemical and combined physical methods (Grove, 1961; Grigor'eva and Kucherov, 1966) such as ultraviolet, infrared, nuclear magnetic resonance (NMR), optical rotatory dispersion, and circular dichroism studies. However, the absolute stereochemistry of ring A, and particularly the orientation of the 1 → 4a lactone ring, in gibberellic acid (IA or IB) was the
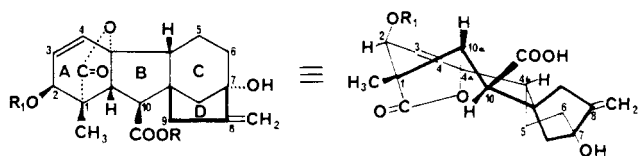
IA

IB
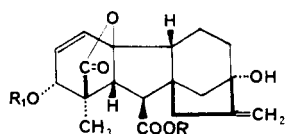
R=R₁=H GIBBERELLIC ACID

R=CH₃; R₁=H METHYL GIBBERELLATE

R=CH₃; R₁=COCH₃ METHYL ACETYLGIBBERELLATE

source of some controversy for quite a long time. This has finally been resolved by X-ray analysis (McCapra et al., 1966; Hartsuck and Lipscomb, 1963) that confirmed the structure IA wherein the orientation of lactone is α to ring A with C-1 methyl in quasi-equatorial and C-2 hydroxyl in quasi-axial disposition.

A very useful method for NMR spectral assignments applying the nuclear Overhauser effect (NOE) was pioneered by Anet and Bourn (1965); and recently this effect was successfully utilized to solve configurational, conformational, and stereochemical problems (Hart and Davis, 1969). This paper describes NOE experiments which, although remarkably simple, provided a direct approach to understanding the ring

A stereochemistry of gibberellic acid in solution. Results obtained are in accordance with the structure IA deduced in the solid state by X-ray diffraction method.

EXPERIMENTAL

Methyl gibberellate was prepared (Cross, 1954) by using 316 mg. of gibberellic acid. The methyl ester (220 mg.) was obtained as needles after two recrystallizations from benzene-methanol, m.p. 210° to 211° C; reported m.p. 209° to 210° C.

Methyl acetylgibberellate (130 mg.) was obtained as prisms by treatment of methyl gibberellate (160 mg.) in 2 ml. of pyridine with 1 ml. of acetic anhydride for 40 hours and was recrystallized from benzene-light-petroleum ether (b.p. 60° to 80° C), m.p. 178° to 180° C; reported m.p. 180° and 181° C.

NMR spectra were recorded on a Varian HA-100 NMR spectrometer, equipped with a Varian Model V-3521A integrator/decoupler, operating in frequency sweep mode. The NOE data were obtained on the same spectrometer in a freshly prepared solution (10 to 15%) with either tetramethyl silane or benzene as an internal field-frequency lock, and the samples were thoroughly degassed before use. Double resonance experiments were performed by the use of the same spectrometer with a Model 200J audio-oscillator and Model 5212 electronic counter, both from Hewlett-Packard.

PROCEDURE

The experimental procedure, for example, in methyl gibberellate, consisted of first recording the proton resonance spectrum and then of measuring the individual areas under the signals for methyl, C-2, and C-10a protons. Then, a second (H₂) saturating field was applied to the transitions of spacially proximate nuclei. The effect of H₂ irradiation is expressed either as the percentage of peak-height difference between the peaks, or as the percentage area increase under the peaks, before and during irradiation. The results in Table II are derived from the percentage increase in the integrated areas (average of 5 scans) and a positive enhancement greater than 4% was taken as evidence for a NOE. It should be emphasized that this error is a measure of the poorest reproducibility and not an average deviation. Care has been taken to distinguish between an increase in signal height due to removal of unresolved coupling (in which case the band width is reduced and the signal area remains unchanged) and the increase in signal height resulting from NOE (in which case the signal area increases proportionately, but the band width remains essentially unchanged).

Table I. NMR Spectra (100 MHz) of Gibberellic Acid Derivatives (p.p.m.)

	C-1 CH₃	H-2	H-3	H-4	H-8	H-10	H-10a	Others
Methyl gibberellate (acetone-d_6)	1.15 (s)[a]	4.04 (d) (J = 4 c/s)	5.88 (2d) (J = 4 and 9 c/s)	6.36 (bs) (J = 9 c/s)	4.87 (bs) 5.2 (bs)	3.26 (J = 11 c/s)	2.68 (J = 11 c/s)	3.7 (s) (OCH₃)
Methyl acetyl gibberellate (CDCl₃)	1.14 (s)	5.33 (d) (J = 4 c/s)	5.82 (2d) (J = 4 and 9 c/s)	6.3 (d) (J = 9 c/s)	4.93 (t) 5.24 (t) (J = 1.5 c/s)	3.32 (J = 11 c/s)	2.75 (J = 11 c/s)	3.68 (s) (OCH₃) 2.06 (s) (acetate)

[a] (s) singlet; (bs) broad singlet; (d) doublet; (t) triplet; H-10 and H-10a appear as an AB quartet.

RESULTS AND DISCUSSION

The NMR spectral data of gibberellins at 40 and 60 MHz were reported earlier (Sheppard, 1960; Hanson, 1965) and the characteristic signals at 100 MHz for methyl gibberellate and methyl acetylgibberellate are now given in Table I. Hanson (1965) made a detailed study of the effect of solvents such as chloroform, pyridine, and acetone on the shielding of protons in gibberellins. He concluded that pyridine shows a deshielding effect whereas acetone shields the protons when each is compared with chloroform. However, we did not find any significant difference in the chemical shifts of protons between the solutions in chloroform-d and acetone-d_6.

With most organic compounds, the main relaxation mechanism contributing to the spin-lattice relaxation time, T_2, is the direct dipole-dipole interaction. If intermolecular contributions can be made very small by dissolving the compound in a magnetically inert solvent, the main contribution to the relaxation of a relevant proton will arise from the closely situated protons in the same molecule. When the latter protons are saturated, an increase in the integrated intensity of the signal due to the relevant proton is observed. Application of this method, therefore, should indicate the lactone orientation in ring A of gibberellic acid.

Complete saturation of C-1 methyl singlet (in the two samples) by double irradiation should change the integrated intensity of either C-2 hydrogen only or both H-2 and H-10a without affecting spin-decoupling and line-widths. The increase in the integrated intensity of H-2 signal was about 22% in methyl gibberellate and methyl acetylgibberellate, whereas no significant change in intensity of H-10a was observed (Table II). Similarly, saturation of H-2 increased the intensity of methyl resonance by about 20% with simultaneous spin-decoupling and enhanced intensity of H-3. These results indicate clearly that the methyl group is in close proximity only with the H-2 and not with H-10a. Examination of molecular models further reveals the following information. In conformation IA, for example, the methyl protons and H-2 are approximately 2.45 Å apart, measured as internuclear distance on Drieding models, assuming the C—C and C—H bond lengths correspond closely to the reported values from X-ray data, whereas the methyl hydrogens and H-10a are about 2.9 Å apart (while the distance between the methyl hydrogens and H-10 is about 1.96 Å). In conformer IB, the internuclear distance between methyl hydrogens and H-10a is about 2.26 Å which is much shorter than that of the methyl and H-2. Furthermore, H-4 comes very close to H-5 (distance 1.40 Å) in this conformation and thereby a strong relaxation between these is expected. The slight differences in the internuclear distances seem to affect the dipole-

Table II. Results of Double-Resonance Experiments

Compound	Proton Irradiated[a]	Proton Observed	Enhancement, %
Methyl gibberellate	C-1 methyl	H-2	23
	H-2	C-1 methyl	23
		H-3	15
	H-3	H-2	22
		H-4	15
Methyl acetyl-gibberellate	C-1 methyl	H-2	22
	H-2	C-1 methyl	20
		H-3	15
	H-3	H-2	21
		H-4	20

[a] During irradiation of H-2 and H-3 protons, both decoupling and NOE effects were noticed.

dipole interactions considerably and thus cause considerable changes in the relaxation mechanism.

In summary, the C-1 methyl group appears to be in close proximity with the H-2 and farther apart from the H-10a when the lactone ring is in α-orientation to ring A (IA). If the lactone has β-orientation, then the methyl group which would be axially situated would be closer to H-10a. Absence of NOE between C-1 methyl and H-10a is clear evidence that ring A of gibberellic acid in solution should assume structure IA with both methyl and H-2 in the quasi-equatorial disposition. For this reason, the lactone ring assumes the α-orientation. This method provides direct evidence for the ring A stereochemistry of gibberellic acid as unequivocally determined by the X-ray diffraction method.

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