Determination of Stereochemistry at C-16 of Dihydrogibberellin A₁ Methyl Ester (Methyl Tetrahydrogibberellate) and Its 16-Epimer†

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Stereochemistry at C-16 of dihydrogibberellin A₁ methyl ester (methyl tetrahydrogibberellate, III) and its 16-epimer was elucidated. NMR analysis employing a shift reagent, Eu(thd)₃ or Eu(fod)₃, was found to be very effective for settling this stereochemical problem.

In the course of structure determination of gibberellin A₃ (gibberellic acid, I) an extensive investigation on the stereostructures of hydrogenation products was carried out^{1~7)} especially in connection with stereochemistry of ring A.^{3~6)} Catalytic hydrogenation of the methylene group exocyclic to ring D generates a new asymmetric center at C-16 (gibberellane numbering), and gibberellin A₃ methyl ester (II) affords, upon reduction, dihydrogibberellin A₁ methyl ester (methyl tetrahydrogibberellate, III) and its 16-epimer (IV) in almost equal amount as neutral products.²⁾ No configurational assignment was made for the 17-methyl group of this pair of epimers partly because it was of little significance for the determination of stereostructure of the original gibberellin molecule (I) and partly because of the lack of an appropriate methodology to solve this problem. In this paper we describe the determination of stereochemistry at C-16 of these two reduction products as shown in III and IV by means of NMR analysis employing lanthanide paramagnetic shift reagents.

Dihydrogibberellin A₁ methyl ester (methyl tetrahydrogibberellate, III) and its 16-epimer

(IV) were prepared as described by Cross.²⁾ They were converted to dihydrogibberellin A₂₀ methyl ester (V)⁸⁾ and its 16-epimer (VI), respectively, by the known method.⁹⁾ Removal of the hydroxyl group at C-3 of III or IV was important for the subsequent NMR analysis which depended on the presence of the bridgehead hydroxyl group at C-13. Presence of another hydroxyl at C-3 might complicate the analysis. Two steviol derivatives with known stereochemistry at C-16 were prepared as reference compounds by the method of Mosettig *et al.*¹⁰⁾ Thus hydrogenation of steviol (VII) followed by methylation afforded dihydrosteviol A methyl ester (IX), while that of stevioside (VIII) followed by acid hydrolysis and methylation gave dihydrosteviol B methyl ester (X). The α -configuration of the 17-methyl group of dihydrosteviol B had been proved by its conversion to (–)-“ β ”-dihydrokaurene (XI)¹⁰⁾ which in turn had been correlated with a veatchine derivative with an α -methyl at C-16.¹¹⁾

An inspection of molecular models of V and VI indicates that the distance (*R*) from the center of the circle of rotation of the 17-methyl protons to the hydroxyl oxygen atom in these two molecules is not equal: $R=3.3\text{\AA}$

† Gibberellins: Part XXV. For Part XXIV see: K. Mori, *Tetrahedron*, **27**, 4907 (1971).

in V while $R=2.7\text{ \AA}$ in VI. This is also true in the case of the steviol derivatives: $R=3.3\text{ \AA}$ in IX while $R=2.7\text{ \AA}$ in X. Recent remarkable development in the use of paramagnetic shift reagents¹²⁾ such as $\text{Eu}(\text{thd})_3$, $\text{Eu}(\text{DPM})_3$ ¹³⁾ or $\text{Eu}(\text{fod})_3$ ¹⁴⁾ provided the most powerful tool for discerning these two stereoisomers. On the basis of the fact that the magnitude of the paramagnetic shift to lower field induced by a shift reagent is in direct proportion to the spatial proximity of the protons (in this case protons of 17-methyl) to the site of co-ordination (hydroxyl at C-13),^{12,13,15,16)} it can be expected that the 17-methyl group in VI suffers a more significant shift than that in V.

The NMR measurement of two stereoisomers of dihydrogibberellin A_{20} methyl ester (V and VI) was carried out with or without addition of $\text{Eu}(\text{thd})_3$. As can be seen from Table I, the 17-methyl group of the so-called

16-epi ester (8-epi ester in earlier literatures^{2-6,9)}) showed a shift to much lower field than the other, *ca.* 0.5 ppm lower than the down field shift observed with its isomer. Thus the stereochemistry represented by VI was assigned to the 16-epi ester, while V was assigned to dihydrogibberellin A_{20} methyl ester. Therefore the parent compounds, dihydrogibberellin A_1 methyl ester (methyl tetrahydrogibberellate) and its 16-epimer, possess the stereostructures III and IV, respectively. This conclusion was supported by the result obtained with the two steviol derivatives. The 17-methyl group of dihydrosteriol B methyl ester (X) suffered a more significant paramagnetic shift than that of the epimer (IX) when their NMR spectra were measured in the presence of $\text{Eu}(\text{fod})_3$. This is in accord with their stereostructures established by chemical correlation.

In the above arguments the presence of other

TABLE I. NMR DATA FOR THE GIBBERELLIN AND STEVIOL DERIVATIVES^{a)}

Compound	17-Me	18-Me	20-Me	7-CO ₂ Me	19-CO ₂ Me
Dihydrogibberellin A ₁ Me ester (III) in (CD ₃) ₂ CO ^{b)}	0.95, d ^{e)}	1.04, s		3.68, s	
16-Epidihydrogibberellin A ₁ Me ester (IV) in (CD ₃) ₂ CO ^{b)}	0.92, d	1.05, s		3.67, s	
Dihydrogibberellin A ₂₀ Me ester (V) in CDCl ₃ (16.6 mg of V) ^{c)}	1.00, d	1.09, s		3.76, s	
with Eu(thd) ₃ (10.5 mg)	2.50, d	1.39, s		4.22, s	
$J_{Eu} = \delta_{CDCl_3} - \delta_{Eu(thd)_3}$	-1.50	-0.30		-0.46	
16-Epidihydrogibberellin A ₂₀ Me ester (VI) in CDCl ₃ (16.5 mg of VI) ^{c)}	0.97, d	1.10, s		3.76, s	
with Eu(thd) ₃ (10.5 mg)	3.03, d	1.44, s		4.31, s	
$J_{Eu} = \delta_{CDCl_3} - \delta_{Eu(thd)_3}$	-2.06	-0.34		-0.55	
Dihydrosteviol A Me ester (IX) in CDCl ₃ (2.5 mg of IX) ^{c)}	0.98, d	1.18, s	0.82, s		3.66, s
with Eu(fod) ₃ (ca. 3.8 mg)	2.67, d	1.95, s ^{d)}	1.85, s ^{d)}		4.40, s
$J_{Eu} = \delta_{CDCl_3} - \delta_{Eu(fod)_3}$	-1.69	-0.77	-1.03		-0.74
Dihydrosteviol B Me ester (X) in CDCl ₃ (2.5 mg of X) ^{c)}	0.94, d	1.18, s	0.84, s		3.66, s
with Eu(fod) ₃ (3.8 mg)	2.94, d	1.93, s ^{d)}	1.90, s ^{d)}		4.48, s
$J_{Eu} = \delta_{CDCl_3} - \delta_{Eu(fod)_3}$	-2.00	-0.85	-1.06		-0.82

a) Values are given in δ units (ppm) with TMS as an internal standard.

b) Recorded on a Jeol NM-4H 100 spectrometer at 100 MHz. The compds were insoluble in CDCl₃.

c) Recorded on a Varian XL-100 spectrometer at 100.1 MHz. Eu(thd)₃=Tris (2,2,6,6-tetramethyl-3,5-heptanedionato)-europium (III)=Eu(DPM)₃=Tris (dipivalomethanato)-europium (III). Eu(fod)₃=Tris (1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,6-octanedionato)-europium (III).

d) A 3H singlet at a higher field was assumed to be due to 20-Me.

e) The coupling constant for these doublets is $J=7$ Hz.

functional groups, that is, an ester and a lactone, was ignored to simplify the situation. An ester carbonyl group also complexes with a shift reagent. However, its characteristic shift magnitude is smaller than that of a hydroxyl group as shown in the order $-NH_2 > -OH > C=O > -O- > -CO_2R > -CN$.^{13,17)} Indeed in the present case the most remarkable shift was always observed with the 17-methyl near the hydroxyl at C-13, although shifts were also seen with other methyl groups near the carbonyl groups. This justified our simplification to neglect the effect of carbonyl groups on 17-methyl as the first approximation.

In the absence of a shift reagent the α -methyl group at C-16 of IV, VI and X always

absorbed at a lower field (0.03~0.04 ppm) than that of the β -methyl group at C-16 of III, V and IX.

The result of GLC analysis of V, VI, IX and X are summarized in Table II. The β -methyl epimers (V and IX) exhibited longer retention times than the α -methyl epimers (VI and X). This indicates that a compound with a hydroxyl group in less crowded environment is retained more tightly on the column. This is in agreement with Cross's observation that dihydrogibberellin A₁ methyl ester (methyl tetrahydrogibberellate, III) moves more slowly than its 16-epimer (IV) when chromatographed over alumina.²⁾

In conclusion hitherto unknown configuration of the 17-methyl group of hydrogenation

TABLE II. GLC OF THE GIBBERELLIN AND STEVIOL DERIVATIVES

Compound	Retention times (min) ^{a)}
Dihydrogibberellin A ₂₀ Me ester (V) ^{b)}	19.9
16-Epidihydrogibberellin A ₂₀ Me ester (VI) ^{b)}	18.8
Dihydrosteviol A Me ester (IX) ^{c)}	40.8
Dihydrosteviol B Me ester (X) ^{c)}	39.8

^{a)} All retention times were determined on a Yanaco G 80 gas chromatograph equipped with an SE-30 column: 5% SE-30 on Celite 545, 80~100 mesh, 0.75 m $\times$ 3 mm i.d.

^{b)} Column temp 238°C; Carrier gas N₂, 0.7 kg/cm²

^{c)} Column temp 215°C; Carrier gas N₂, 0.6 kg/cm²

products of gibberellin A₃ (I) was determined. In view of the chemical correlation⁵⁾ of various hydrogenolysis products with dihydrogibberellin A₁ methyl ester (methyl tetrahydrogibberellate, III) or its 16-epimer (IV, named as 8-epimer in the earlier literatures^{2~6,9)}), the present work has removed one of the few uncertainties remaining unsolved after the completion of structure determination of gibberellin A₃ (I).

EXPERIMENTAL

All mps were uncorrected. Substances for study were synthesized according to the literatures cited in the text. Eu(thd)₃ was prepared by the method of Eisentraut and Sievers.¹⁸⁾ Eu(fod)₃ was purchased from Merck, Sharp and Dohme of Canada Limited, Montreal, Canada.

Methyl tetrahydrogibberellate (dihydrogibberellin A₁ methyl ester, III). Mp 270~271°C (lit.²⁾ 260~264°C).

Methyl 16-epitetrahydrogibberellate (16-epidihydrogibberellin A₁ methyl ester, IV). Mp 247~249°C (lit.²⁾ 233~236°C).

Dihydrogibberellin A₂₀ methyl ester (V). Mp 235~237°C (lit.⁹⁾ 233~235°C).

16-Epidihydrogibberellin A₂₀ methyl ester (VI). Mp 205~206°C lit.⁹⁾ 205~206°C).

Dihydrosteviol A methyl ester (IX). Mp 110~114°C (lit.¹⁰⁾ 116~119°C). This could not be purified completely because of the limited availability of steviol, the starting material.

Dihydrosteviol B methyl ester (X). Mp 105~112°C (lit.¹⁰⁾ 132~134°C). This contained IX as impurity

and could not be purified completely because of the scarcity of the material.

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