The Esterification of Gibberellins with Dimethylformamide Dimethyl Acetal (DMFDMA)

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Gibberellins, Esterification, Dimethylformamide Dimethyl Acetal

The key step is to prepare rare gibberellins by converting the more readily available gibberellins (GA_3, GA_4, GA_7 and GA_{13}) into their methyl esters. The DMFDMA is employed as an esterifying reagent to avoid the diazomethane due to its toxic, explosive and hazardous nature. It is observed that DMFDMA apart from esterification can also be used as acetylating and epimerizing reagent.

Introduction

The gibberellins are a α widespread group of plant hormones [1]. They were first recognized as metabolites of the fungus, *Gibberella fujikuroi.* Later on they were also found to present in higher plants [1–4]. The main interest in the gibberellins is due to their role as natural plant growth regulating hormones e.g. gibberellic acid (**1**) [5]. Nearly 80 gibberellin plant hormones are known and numbered gibberellin $A_1 \cdots An$ [6]. They are characterized by a common carbon skeleton but differ from one another in the position and the number of their functional groups. They can be divided into two families: A C_{19} series examplified by gibberellic acid (**1**) and a C_{20} series examplified by gibberellin A_{13} (**2**). They were originally discovered as the phytotoxic metabolites of a rice pathogen, *G. fujikuroi* [7–8].

Esterification of gibberellins

The conversion of the more readily available gibberellins, gibberellic acid (**1**), gibberellin A_4 and A_7 (**3** and **4**) and gibberellin A_{13} (**2**) to the rare gibberellins is often accomplished *via* their methyl esters. Indeed much of the Chemistry of the gibberellin has been studied with their methyl esters. The esters are easier to crystallize and chromatograph. Furthermore, many of the reactions involved in the conversion to other gibberellins require the conversion of the hydroxyl groups at C-3 and C-13 to chloro and other derivatives by reagents which would also react with the carboxyl OH. The methyl esters are commonly and easily prepared with diazomethane. This reagent suffers from the disadvantages of toxicity and it is explosive presenting a hazard when the reaction is carried out on a large scale. Other methods of esterification for example the Fischer-Speier method using acid involve conditions that would lead to the rearrangement of the gibberellins. Thus ring A of gibberellic acid (**1**) undergoes aromatization to form allogibberic acid (**5**) in the presence of acid at room temperature whilst under slightly more vigorous conditions the C/D ring system undergoes a Wagner-Meerwein rearrangement to form the 8:13-isogibberellins, e.g. gibberic acid (**6**).

Utility of DMFDMA

Hence, a mild, non-toxic reagent was required. Dimethyl formamide dimethyl acetal (**7**) has been recommended as a mild esterifying reagent. In 1965 Eschenmoser *et al.* [9] described the reaction of formamide acetals with carboxylic acids. It was found to be a facile method for the preparation of ester. The key step in the proposed mechanism involves the cyclic displacement of dimethylformamide as shown in Scheme 1. It is the object of this work to establish suitable conditions for the esterification of the gibberellins and to see the behaviour of this reagent (DMFDMA) on gibberellins at low (room temperature) and at high temperatures (reflux).

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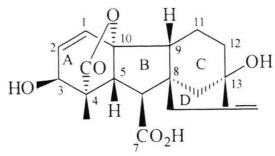

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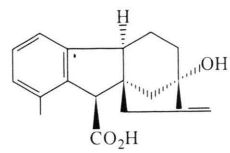

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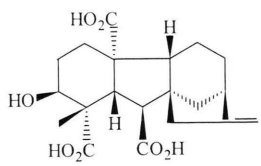

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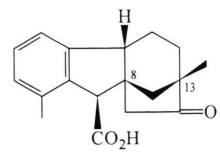

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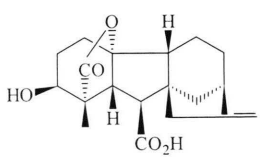

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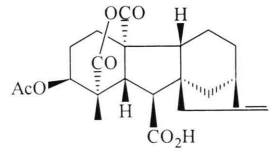

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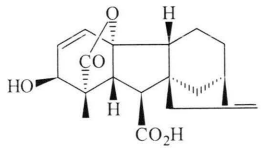

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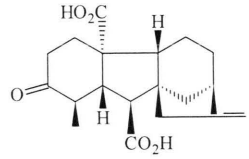

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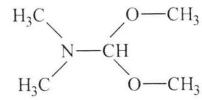

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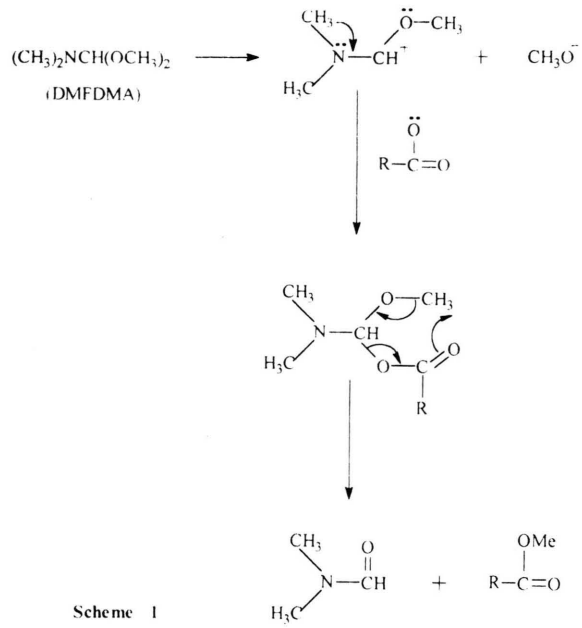
Results and Discussion

In order to establish the utility of DMFDMA for compounds in the gibberellin series, acetate-anhydride **8**, keto-acid **9**, GA_{13} (**2**) and GA_3 (**1**) were treated under reflux and at room temperatures with this reagent. The products, thus obtained, were identified spectroscopically.

Preparation of acetate-anhydride **8** *and treatment with DMFDMA*

The acetate-anhydride **8** was prepared by treating the GA_{13} (**2**) with acetic anhydride under reflux for 1 h. The product **8** obtained, was confirmed by its proton NMR and infra-red spectroscopy. The ^1H NMR spectrum showed an additional methyl singlet at δ 1.96 due to the presence of acetate group at C-3 whereas the normal Me-18 resonated at δ 1.05 as a singlet. The spectrum also exhibited a pair of broad singlets at δ 4.82 and 4.99 related to the olefinic protons. The infra-red spectrum displayed four carbonyl absorptions at 1801, 1763, 1750 and 1725 cm^{-1}. The former two absorptions belong to the anhydride function and the latter two absorptions correspond to the OAc and carboxyl

functions. Thus the above mentioned spectral data confirmed the structure **8**.



Scheme 1

When **8** was treated with DMFDMA under reflux, two compounds **10** and **11** were formed which were separated by column chromatography. The compound **10** which was eluted first found to be the 3-acetoxy derivative of GA_{13} trimethyl ester on the basis of its proton NMR spectroscopy. The spectrum showed five methyl signals at δ 1.11, 2.09, 3.60, 3.67 and 3.72 as singlets related to the Me-18, OAc and three OMe, respectively. The two doublets at δ 2.52 and 3.88 with coupling constant 12.6 Hz were assigned to H-5 and H-6, respectively.

The second product **11** eluted from the same column afforded trimethyl ester of GA_{13} which showed three methoxyl signals at δ 3.59, 3.66 and 3.71 in the proton NMR spectrum. The signal at δ 3.96 (t, J=6.0, 3.0 Hz) was assigned to H-3. The same H-3 signal in **10** was observed at δ 5.21. The downfield shift of H-3 signal in **10** is due to the presence of acetyl group. The structures of the compounds **10** and **11** were further confirmed by treating GA_{13} (**2**) with etherreal diazomethane to afford a compound identical to **11** which on acetylation yielded **10**.

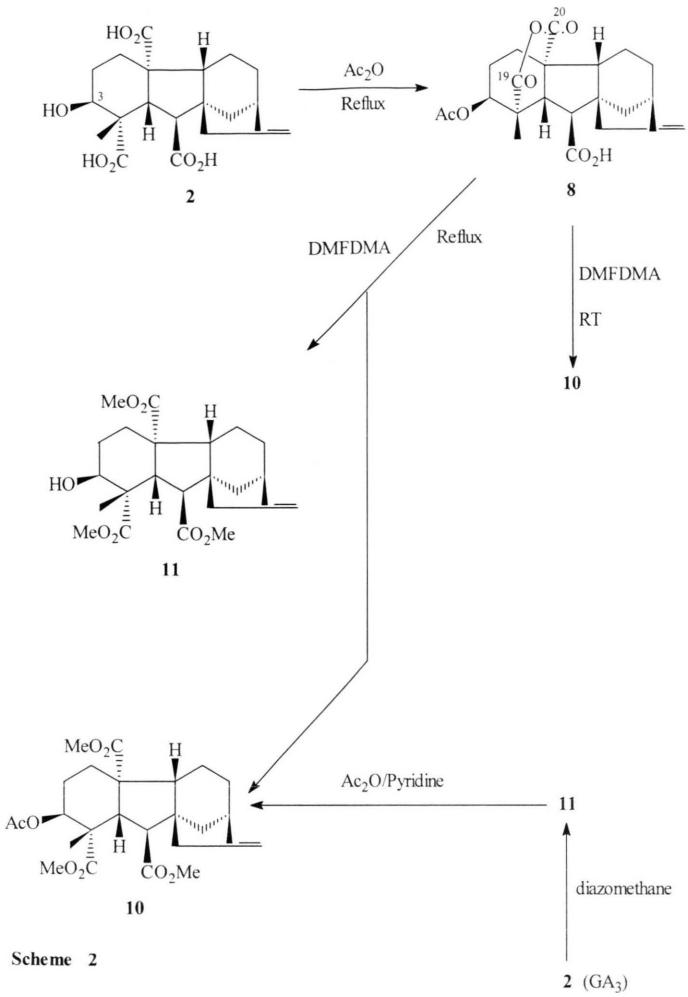
When acetate-anhydride was treated with DMFDMA at room temperature only one product was isolated, the ^{1}H NMR spectrum of which exactly matched with that of **10** previously obtained from the same reaction when it was performed under reflux. The teatment of DMFDMA with acetate-anhydride and the products formed during this reaction have been illustrated in Scheme 2.

Preparation of 19-nor, 3-oxo derivative of GA_{13} **9** *and treatment with DMFDMA*

Oxidation of GA_{13} (**2**) with 8 N chromium trioxide give an unstable β-keto-acid **1** which was confirmed by the disappearance of the carbinylic proton at δ 3.96 in the proton NMR spectrum. The β-keto-acid was easily decarboxylated on heating to afford *ent*-3-oxo-19-nor-gibberell-16-en-7,20-dioic acid **9** which was easily recognized by the appearance of methyl doublet in ^{1}H NMR spectrum. When 19-nor-keto-acid **9** was treated with DMFDMA under reflux and at room temperature, the same product was isolated from both the reactions which was assigned to the *ent*-3-oxo-19-nor-gibberell-16-en-7,20-dioic acid-7,20-dimethyl ester **13**. The infra-red spectrum did not show the hydroxyl absorption while on the other hand the proton NMR spectrum exhibited two methoxyl signals at δ 3.68 and 3.77 and confirmed the structure **13** (see Scheme 3).

Action of DMFDMA on gibberellin A_{13} (**2**)

The fungal metabolite gibberellin A_{13} (**2**) was treated with DMFDMA at room temperature and the product, thus obtained, was assigned as *ent*-3β-hydroxy-gibberell-16-en-7,19,20-trioic acid 7,19-dimethyl ester, 20⟶3 lactone **14** which had previously been obtained by the reduction of the 3-keto-ester **12** with sodium borohydride [10]. The infra-red spectrum showed absence of hydroxyl absorption while on the other hand NMR spectrum showed the presence of two methoxyl groups in the molecule which appeared at δ 3.63 and 3.69. The absence of hydroxyl group in the molecule clearly explains the formation of lactone ring across 20⟶3. A plausible explanation may be that the rate of methylation of the carboxylic acid groups at positions C-7 and C-19 was faster than of the group at C-20 and also faster than the rate of formylation of the 3-hydroxyl group; hence the 7,19-dimethyl ester was formed first. Once the 3-hydroxyl group has been formylated, the carboxyl-



Scheme 2

ate anion at C-20 would then act as an internal nucleophile to displace the formimido group at C-3 and hence, form the *ent*- 20→3β lactone. The epimerization at C-3 can be explained by Scheme 4. The same compound **14** was formed when the GA_{13} was treated with DMFDMA under reflux.

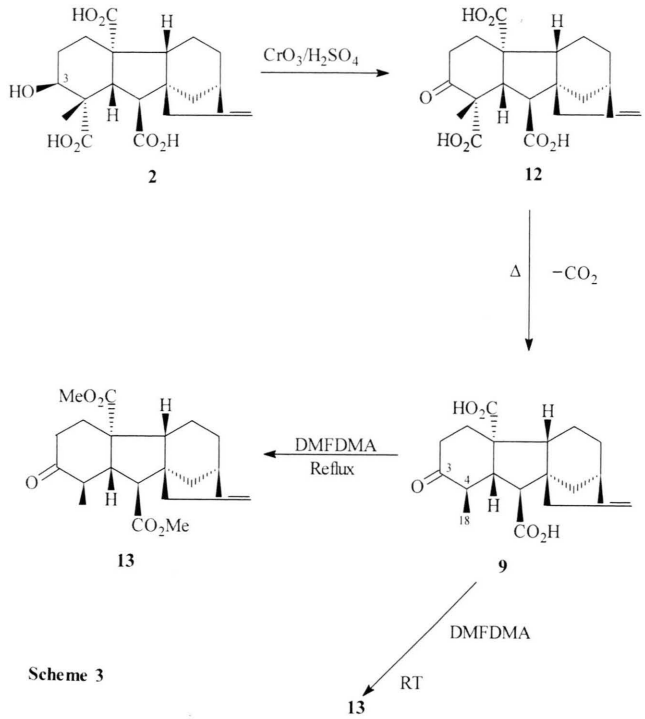
Action of DMFDMA on gibberellic acid (**1**)

3-Formyl, 7-methyl ester **15** was obtained when gibberellic acid **1** was treated with DMFDMA at room temperature. The same product was also observed when this reaction was performed under reflux. The ^{1}H NMR spectrum of **15** exhibited a downfield singlet at δ 7.89 which was assigned to formyl proton. The presence of an extra carbon atom due to the formyl group was confirmed by microanalysis which showed 21 carbons in the molecule. The proton NMR spectrum also displayed a signal at δ 3.72 as a singlet assigned for methoxyl group. The exomethylene protons resonated at δ 4.94 (s) and 5.21 (br, s) while the other protons associated with endocyclic double bond showed their resonances at δ 5.87 (dd, J=9.3, 3.6 Hz, H-2) and 6.36 (dd, JH=9.3, 0.9 Hz, H-1).

Experimental

Preparation of acetate-anhydride **8** *and treatment with DMFDMA*

The gibberellin A_{13} (**2**) (1.57 g) isolated from the fungus *Gibberella fujikuroi* was refluxed with acetic anhydride (10 ml) for 1 h. Then diluted with



Scheme 3

water, and the product was recovered in ethyl acetate. The ethyl acetate layer was washed with water and dried over sodium sulphate. The solvent was removed by evaporation under reduced pressure which afforded a gum. This was chromatographed on silica using ethyl acetate and light petroleum as a mobile phase. Elution with 55% ethyl acetate in light petroleum gave acetate-anhydride of GA_{13} (**8**) (1.11 g) as a white powder.

IR *($CHCl_3$)* ν_{max}: 3246 (br.), 1801, 1763, 1750, 1725 and 1678 cm^{-1}; ***^{1}H NMR*** ($CDCl_3$, 60 MHz): δ 1.05 (3H, s, H-18), 1.96 (3H, s, OAc), 4.82 (1H, br. s, H-17), 4.99 (1H, br. s, H-17′) and 5.11 (1H, t, J=4.7, 2.7 Hz, H-3).

The compound **8** (500 mg) was refluxed with DMFDMA (10 ml) for 2 h. The DMFDMA was removed by evaporation under reduced pressure and the gum thus obtained, was chromatographed on silica. Elution with 25–30% ethyl acetate in light petroleum gave 3-acetoxy derivative of GA_{13} trimethyl ester **10** (330 mg) as a gum.

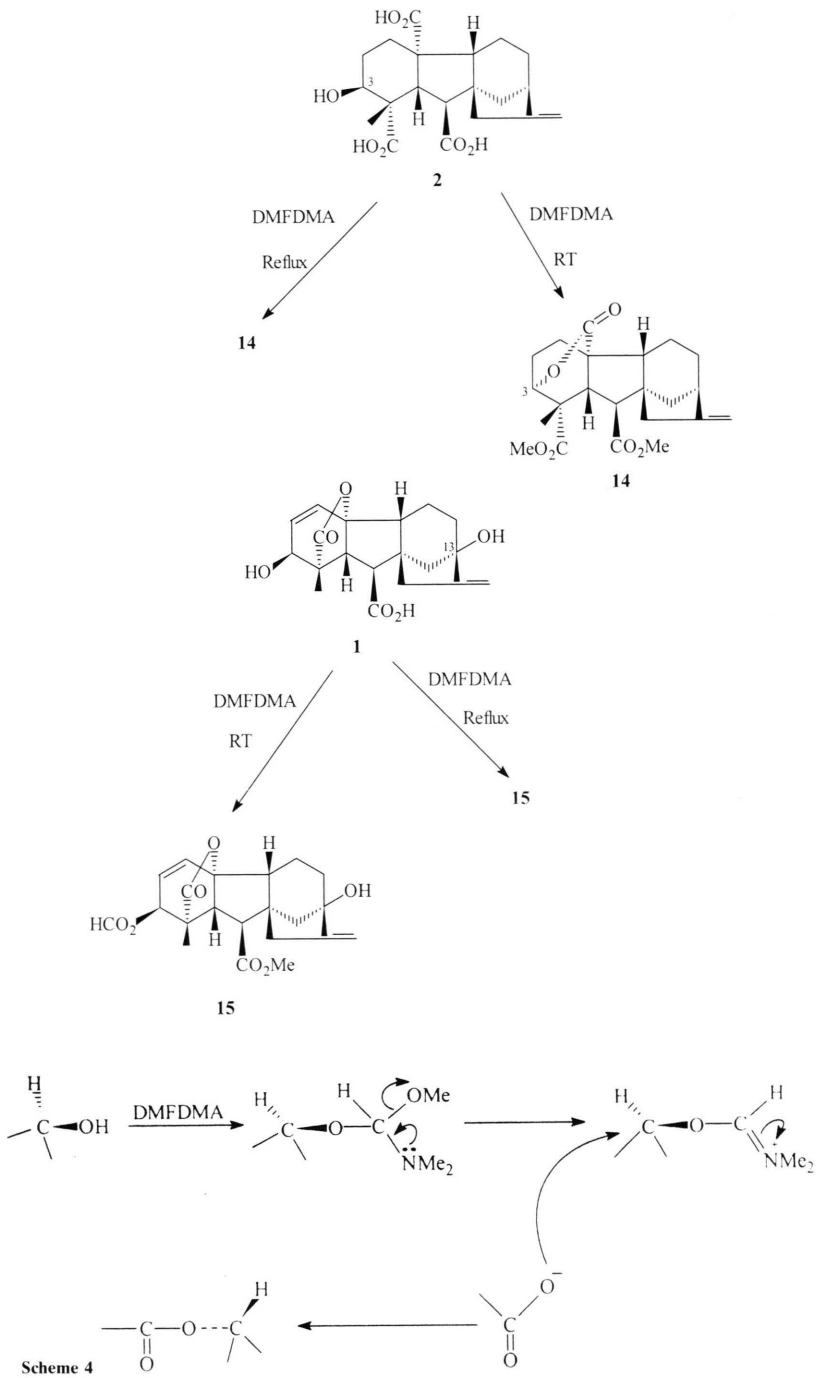
^{1}H NMR *($CDCl_3$, 360 MHz)*: δ 1.11 (3H, s, H-18), 2.09 (3H, s, OAc), 2.52 (1H, d, J=12.6 Hz, H-5), 3.60 (3H, s, OMe), 3.67 (3H, s, OMe), 3.72 (3H, s, OMe), 3.88 (1H, d, J=12.6 Hz, H-6), 4.81 (1H, s, H-17), 4.89 (1H, s, H-17′) and 5.21 (1H, t, J=4.9, 2.5 Hz, H-3).

Further elution with 40% ethyl acetate in light petroleum gave GA_{13} trimethyl ester **11** (142 mg), which was crystallized from chloroform as plates.

M.p.: 153–154 °C; ***IR*** ($CHCl_3$) ν_{max}: 3400, 1730 (br.), 1660, 880 cm^{-1}; ***^{1}H NMR*** ($CDCl_3$, 360 MHz): δ 1.23 (3H, s, H-18), 2.58 (1H, d, J=12.7 Hz, H-5), 3.59 (3H, s, OMe), 3.66 (3H, s, OMe), 3.71 (3H, s, OMe), 3.87 (1H, d, J=12.7 Hz, H-6), 3.96 (1H, t, J=6.0, 3.0 Hz, H-3), 4.80 (1H, s, H-17) and 4.88 (1H, br. s, H-17′).

Compound **8** was treated with DMFDMA at room temperature overnight. DMFDMA was removed under reduced pressure without heating. The product was purified by column chromatography. Elution with 25–30% ethyl acetate in light petroleum gave the same product **10** which was formed when the same reaction was performed under reflux.

The ^{1}H NMR spectra of GA_{13} trimethyl ester and its derivative 3-acetoxy exactly matched with the products **11** and **10** which were formed during the reaction between acetate anhydride **8** and DMFDMA.



2 **14** **14** **1** **15** **15**

Scheme 4

*Preparation of 19-nor,3-oxo derivative of GA_{13} (**9**) and treatment with DMFDMA*

GA_{13} (**2**) (1.0 g) was dissolved in acetone (20 ml) and treated with 8 N chromium trioxide (Jone's reagent) dropwise until the yellow colour persisted for ½ h. Then methanol was added in order to destroy the extra amount of Jone's reagent. The solvent was removed under reduced pressure.

The crude material was diluted with water and the product was recovered in ethyl acetate. On evaporation it gave a gum which was redissolved in minimum amount of acetone and then, water (50 ml) was added. The mixture was refluxed for 1 h and the product was again recovered in ethyl acetate. After concentration, it gave a solid which was chromatographed on silica gel column. Elution with 30% methanol in ethyl acetate afforded ent-3-oxo-19-nor-gibberell-16-en-7,20-dioic acid **9** (800 mg) as a white powder. M.p.: 248 °C; IR (Nujol) ν_{max}: 3410, 1710, 1660 cm^{-1}.

The keto-acid **9** (400 mg) was treated with DMFDMA (10 ml) under reflux for 2 h. The DMFDMA was removed under reduced pressure and the resulted gum was subjected to column chromatography. Elution with 50% ethyl acetate in light petroleum yielded ent-3-oxo-19-nor-gibberell-16-en-7,20-dioic acid 7,20-dimethyl ester **13** (190 mg), which was crystallized from diethyl ether as needles.

M.p.: 102 °C; ***^{1}H NMR*** ($CDCl_3$, 90 MHz): δ 0.96 (3H, d, J=6.6 Hz, H-18), 3.68 (3H, s, OMe), 3.77 (3H, s, OMe) and 4.88 (2H, br. d, H-17). The keto-acid **9** (400 mg) was treated with DMFDMA (10 ml) at room temperature overnight. The DMFDMA was removed under reduced pressure without heating. After chromatography it gave the same diester **13**.

Action of DMFDMA on gibberellin A_{13} (**2**)

Gibberellin A_{13} (**2**) (1.0 g) was treated with DMFDMA (10 ml) at room temperature. The reaction was checked by taking TLC at 15, 30 min and then, each 1 h intervals. After 3 h a major spot appeared. The DMFDMA was removed under reduced pressure without heating. The gum obtained, was subjected to column chromatography. Elution with 50% ethyl acetate in light petroleum yielded *ent*-3β-hydroxygibberell-16-en-7,19,20-trioic acid 20→3 lactone 7,19-dimethyl ester **14** (65 mg), which was crystallized from ethyl acetate as needles.

M.p.: 150 °C; ***IR*** ($CHCl_3$) ν_{max}: 1730 (br.), 1660, 883 cm^{-1}; ***^{1}H NMR*** ($CDCl_3$, 360 MHz): δ 1.42 (3H, s, H-18), 2.32 (1H, d, J=12.6 Hz, H-5), 2.95 (1H, d, J=12.6 Hz, H-6), 3.63 (3H, s, OMe), 3.69 (3H, s, OMe), 4.75 (1H, s, H-17), 4.81 (1H, br. s, H-3) and 4.88 (1H, br. s, H-17′).

Gibberellin A_{13} (**2**) (1.0 g) treated with DMFDMA (10 ml) under reflux for 2 h. DMFDMA was removed under reduced pressure gave a gum. The gum was loaded on silica gel column, which yielded the same δ-lactone **14** (800 mg).

Action of DMFDMA on gibberellic acid (**1**)

Gibberellic acid (**1**) (500 mg) was dissolved in THF (20 ml) and then treated with DMFDMA (15 ml) under reflux for 2 h. The DMFDMA was removed under reduced pressure to give a gum, which was chromatographed on silica gel column. Elution with 5% methanol in ethyl acetate gave 3-formyl 7-methyl ester derivative of gibberellic acid (**15**) (300 mg), which was crystallized from acetone as needles.

M.p.: 168–170 °C; ***IR*** (Nujol) ν_{max}: 3309, 1769, 1733 and 1661 cm^{-1}.

$C_{21}H_{24}O_7$ (388.4136)

Calcd	C 64.93	H 6.23%,
Found	C 64.54	H 6.77%.

^{1}H NMR (CD_3OD, 360 MHz): δ 1.16 (3H, s, H-18), 2.73 (1H, d, J=10.6 Hz, H-6), 3.24 (1H, d, J=10.6 Hz, H-5), 3.72 (3H, s, OMe), 3.92 (1H, br. s, H-3), 4.94 (1H, s, H-17), 5.21 (1H, br. s, H-17′), 5.87 (1H, dd, J=9.3, 3.6 Hz, H-2), 6.36 (1H, dd, J=9.3, 0.9 Hz, H-1) and 7.89 (1H, s, formyl group).

When gibberellic acid (500 mg) was treated with DMFDMA (15 ml) in THF (20 ml) at room temperature overnight, the same product **15** (205 mg) was obtained.

Acknowledgements

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