Preparation of Some New C19-Gibberellin Derivatives for Biochemical Studies

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Introduction

The gibberellins are a widespread group of plant hormones [1]. They were first recognized as metabolites of the fungus, Gibberella fujikuroi. Later on they were also found 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 gibberellins plant hormones are known and numbered gibberellin A1–An [6]. They can be divided into two families: a C19 series exemplified by gibberellic acid (GA3) (1) and a C20 series exemplified by gibberellin A13 (GA13) (11). They were originally discovered as the phytotoxic metabolites of a rice pathogen G. Fujikuroi [7–8].

Among the C19 series of gibberellins, GA1 (1) and GA4 (2) and GA7 (3) can easily be obtained from the fungus Gibberella fujikuroi. The gibberellin A4 (2) cannot easily be separated from gibberellin A7 (3), we obtained it (2) by treating the mixture of GA4/GA7 with acetic anhydride in pyridine. In this way GA4 was separated from GA7 as its acetate derivative 4 and characterized by its proton NMR spectroscopy.

Results and Discussion

Preparation of GA4(2) derivatives

The carboxyl group of GA4 acetate 4 was reduced to primary alcohol by treating it with ethyl chloroformate and triethylamine and then with sodium borohydride. In the first step the precipitates of triethylamine hydrochloride were removed by filtration to leave a solution of mixed anhydride which was used directly for sodium borohydride reduction to afford C-l primary alcohol 5. The micro-analysis showed five oxygen atoms in the molecule which confirmed the conversion of carboxyl function into primary alcohol. The 13C NMR spectrum showed the absence of signal for the COOH group and appearance of an additional CH2 at 62.6 ppm assigned to C-1(CH2OH). The structure was further confirmed by its 'H NMR, which displayed multiplet at δ 3.69 due to the CH2OH (C-7).

Ozonization of 5 yielded 6 via ozonoide (7). The 6 was assigned as ent-3α-acetoxy-7-hydroxy-16-oxo-20-norgibberell-19-oic acid 19–10/5 lactone on the basis of its 'H and 13C NMR spectroscopy. The 13C NMR spectrum exhibited the loss of exomethylene carbon signal, which appeared in the precursor compound 5 at 106.2 ppm and the spectrum showed a new carbonyl carbon resonance at 222.6 ppm related to C-16. There were
no exomethylene resonances observed in the $^1$H NMR spectrum. The structure was further confirmed by micro-analysis, which showed loss of one carbon, two hydrogens and one additional oxygen atom in the molecule (see Scheme 1).

Crude mixture of GA$_3$/GA$_7$ supported by the appearance of a pair of doublets at $\delta$ 2.70 (5.2 Hz) and 2.98 (5.2 Hz) due to the epoxy ring (H-17) (see Scheme 2).

**Preparation of GA$_3$(1) derivative**

Acetylation of gibberellic acid (GA$_3$) 1 with acetic anhydride in pyridine yielded the diacetate 8, which was confirmed by the resonances at $\delta$ 2.05 (OAc) and 2.15 (OAc) in the $^1$H NMR spectrum. Reduction of C-7 carboxyl to C-7 primary alcohol was performed by treating the diacetate 8 with ethyl chloroformate, triethylamine and then with sodium borohydride. The conversion of acid into alcohol was confirmed by the appearance of a multiplet at $\delta$ 3.78 in the $^1$H NMR spectrum of 9. Treatment of alcohol 9 with $m$-chloroperbenzoic acid, afforded $ent$-3$\alpha,13$-diacetoxy-7-hydroxy-16,17-epoxy-20-norgibberell-1-en-19-oic acid 19$\rightarrow$10 lactone 10. The micro-analysis showed an extra oxygen atom in the molecule. The $^{13}$C NMR did not show the resonances associated with exomethylene function (C-17) or $sp^2$ hybridized quaternary carbon atom (C-16). The structure was further supported by the appearance of a pair of doublets at $\delta$ 2.70 (5.2 Hz) and 2.98 (5.2 Hz) due to the epoxy ring (H-17) (see Scheme 2).

The prepared new derivatives (5, 6, 10) will be used as precursors for biochemical preparations of plant growth regulating inhibitors.

**Experimental**

Melting points were determined on a Kofler Hotstage apparatus and are uncorrected. Infra red spectra were recorded using Nujol mulls or chloroform on a Perkin-Elmer 597,1710 FTIR spectrometer. $^1$H and $^{13}$C NMR spectra were obtained on Bruker WM 360 (360 MHz) spectrometer. All NMR spectra were recorded in deuteriated chloroform with TMS as an internal standard.
Preparation of ent-3α-acetoxy-7-hydroxy-20-norgibberell-16-en-19-oic acid 19→10 lactone (5)

The crude mixture of GA$_3$/A$_7$ (6.0 gm) was treated with acetic anhydride (20 ml) in pyridine (40 ml) overnight. The reaction mixture was diluted with water and extracted with ethyl acetate. The organic layer was washed with saturated copper sulphate solution and then with water. This was dried over sodium sulphate and the solvent was removed by evaporation under reduced pressure.

The obtained gum (5.7 g), was dissolved in dry THF (105 ml) and then treated with triethylamine (3 ml) and ethyl chloroformate (2 ml) under nitrogen with continuous stirring at room temperature overnight. The precipitates of triethylamine hydrochloride were removed by filtration from the reaction mixture which were formed during the reaction.

The resulted filtrate was directly treated with sodium borohydride (2 g) for four hours. Water was added and the product was extracted with ethyl acetate. The organic phase was washed with water and dried over anhydrous sodium sulphate. The solvent was removed by evaporation and the gummy product was chromatographed on silica gel. Elution with 40% ethyl acetate in light petroleum afforded, ent-3α-acetoxy-7-hydroxy-16-oxo-20-norgibberell-19-oic acid 19→10 lactone (6) (2.4 g), as a foam. IR (Nujol) $v_{\text{max}}$: 3442, 1739 (br.) cm$^{-1}$; (Found: C, 63.41; H, 7.15. C$_{20}$H$_{26}$O$_{6}$ requires C, 63.14; H, 7.41%); $^1$H NMR (CDCl$_3$, 360 MHz): $\delta$ 1.14 (3H, s, H-18), 2.03 (3H, s, OAc), 3.53 (1H, dd, $J$ = 10.9, 3.3 Hz, H-7') and 4.84 (1H, dd, $J$ = 3.4, 1.5 Hz, H-3); $^{13}$C NMR (CDCl$_3$, 90.5 MHz): 27.3 (C-1), 25.3 (C-2), 72.1 (C-3), 53.5 (C-4), 48.5 (C-5), 50.2 (C-6), 62.4 (C-7), 49.6 (C-8), 53.4 (C-9), 39.7 (C-10), 16.4 (C-11), 25.1 (C-12), 44.4 (C-13), 35.9 (C-14), 49.1 (C-15), 222.6 (C-16), 14.9 (C-18), 170.0 (C-19), 21.0 (CH$_3$-CO) and 177.1 (CH$_3$-COO) ppm.

Acetylation of gibberellic acid (1)

Gibberellic acid (2.4 g) was dissolved in pyridine (15 ml) and treated with acetic anhydride (10 ml) at room temperature overnight. The reaction mixture was then diluted with water and extracted with ethyl acetate. The organic layer was washed with a saturated solution of copper sulphate and then with water. The solvent was removed by evaporation under reduced pressure. The crude material was chromatographed using ethyl acetate and light petroleum to give diacetate derivative of gibberellic acid (8) (2.0 g).

M.P.: 176–177 °C; IR (CHCl$_3$) $v_{\text{max}}$: 3210, 1770, 1735, 1705 and 860 cm$^{-1}$; $^1$H NMR (CDCl$_3$, 360 MHz): $\delta$ 1.18 (3H, s, H-18), 2.05 (3H, s, OAc), 3.69 (2H, m, H-7), 4.92 (1H, br.s, H-17), 4.93 (1H, br.s, H-17') and 4.94 (1H, br.s, H-3); $^{13}$C NMR (CDCl$_3$, 62.8 MHz): 27.5 (C-1), 25.4 (C-2), 72.3 (C-3), 53.2 (C-4), 47.3 (C-5), 50.2 (C-6), 62.6 (C-7), 50.9 (C-8), 53.0 (C-9), 94.0 (C-10), 15.8 (C-11), 31.3 (C-12), 38.7 (C-13), 38.7 (C-14), 42.7 (C-15), 157.2 (C-16), 106.2 (C-17), 15.0 (C-18), 170.1 (C-19), 21.1 (CH$_3$-CO) and 177.5 (CH$_3$-COO) ppm.

Ozonolysis of alcohol (5)

The alcohol 5 (2.8 g) was dissolved in ethyl acetate (50 ml) and ozone gas was passed into it at $-70$ °C until the dark blue colour persisted. The ozonide (7) thus obtained, was reduced by treating it with zinc-dust (1.0 g) and acetic acid (2 ml) under continuous stirring at room temperature overnight. Zinc-dust was removed by filtration and the solvent was removed by evaporation under reduced pressure. The resulting gum was chromatographed on a silica gel column. Elution with 50% ethyl acetate in light petroleum afforded, ent-3α-acetoxy-7-hydroxy-16-oxo-20-norgibberell-19-oic acid 19→10 lactone (6) (2.4 g), as a foam.

Preparation of ent-3α,13-di-acetoxy-7-hydroxy-20-norgibberell-1,16-dien-19-oic acid 19→10 lactone (9)

The diacetate 8 (2.0 g) was dissolved in dry THF (18 ml) and treated with triethylamine (0.8 ml) and ethyl chloroformate (0.6 ml) under nitrogen overnight. The precipitates formed were removed by filtration from the reaction mixture. The filtrate was directly treated with sodium borohydride (800 mg) or four hours. Water was added and the organic material was extracted by ethyl acetate. This was dried over anhydrous sodium sulphate, solvent was removed by evaporation to afford a gum, which was chromatographed on silica gel. Elution with 50% ethyl acetate in light petrol yielded ent-3α,13-di-acetoxy-7-hydroxy-20-norgib-
berell-1,16-dien-19-oic acid 19→10 lactone (9) (800 mg).

**M.P.** 150–152 °C; **IR** (CHCl₃) $v_{\text{max}}$: 3475, 1760, 1732, 895 cm⁻¹; $^1$H **NMR** (CDCl₃, 360 MHz): $\delta$ 1.27 (3H, s, H-18), 1.97 (3H, s, OAc), 2.10 (3H, s, OAc), 3.78 (2H, m, H-7), 4.97 (1H, br.s., H-17), 5.10 (1H, br.s, H-17'), 5.30 (1H, d, $J$ = 4.0 Hz, H-3), 5.78 (1H, dd, $J$ = 9.0, 4.0 Hz, H-2) and 6.35 (1H, $J$ = 9.0 Hz, H-1).

**Epoxidation of alcohol (9)**

The alcohol 9 (800 mg) was dissolved in dry chloroform (30 ml) and treated with $m$-chloroperbenzoic acid (800 mg) at 0 °C overnight. Saturated solution of sodium sulphite was then added in order to destroy the extra amount of $m$-chloroperbenzoic acid. The product was recovered in chloroform, washed with water and dried over sodium sulphate. On concentration yielded a gum, which was chromatographed on silica gel. Elution with 60% ethyl acetate in light petroleum afforded ent-3α, 13-diacetoxy-7-hydroxy-16,17-epoxy-20-norgibberell-1-19-oic acid 19→10 lactone 10 (600 mg), as a foam.

**IR** (CHCl₃) $v_{\text{max}}$: 3500, 1772, 1737, and 1639 cm⁻¹. (Found: C, 62.34; H, 6.56. C₂₃H₂₈O₈ requires C, 62.57; H, 6.62%); $^1$H **NMR** (CDCl₃, 360 MHz): $\delta$ 1.16 (3H, s, H-18), 1.88 (3H, s, OAc), 2.00 (3H, s, OAc), 2.70 (1H, d, $J$ = 5.2 Hz, H-17), 2.98 (1H, d, $J$ = 5.2 Hz, H-17'), 3.57 (1H, dd, $J$ = 11.0, 7.4 Hz, H-7), 3.72 (1H, dd, $J$ = 11.0, 3.3 Hz, H-7'), 5.20 (1H, d, $J$ = 3.7 Hz, H-3), 5.73 (1H, dd, $J$ = 9.2, 3.7 Hz, H-2) and 6.32 (1H, d, $J$ = 9.2 Hz, H-1); $^{13}$C **NMR** (CDCl₃, 62.8 MHz): 134.4 (C-1), 128.6 (C-2), 70.6 (C-3), 52.1 (C-4), 46.8 (C-5), 51.3 (C-6), 61.4 (C-7), 48.7 (C-8), 51.4 (C-9), 90.3 (C-10), 16.9 (C-11), 40.6 (C-12), 80.1 (C-13), 41.1 (C-14), 32.6 (C-15), 66.6 (C-16), 50.1 (C-17), 14.5 (C-18), 177.5 (C-19), 20.5 (CH₃CO), 21.3 (CH₃CO), 169.9 (CH₃CO), and 170.0 (CH₃CO) ppm.

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