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SYNTHESIS OF GLUCOSYL CONJUGATES OF [17-$^2H_2$]-LABELLED AND UNLABELLED GIBBERELLIN A_{34}

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Key Word Index—[$^2H_2$]-Labelled; gibberellins; GA_{34} glucosyl conjugates; synthesis.

Abstract—The synthesis of GA_{34}-β-D-glucopyranosyl ester and of GA_{34}-2-*O*-β-D-glucopyranoside starting from GA_{34}-16-norketone methyl ester are described. The structures of the synthesized compounds were confirmed by NMR and by electrospray ionization-mass spectrometry.

INTRODUCTION

After feeding gibberellin A_4 (GA_4) to various plant tissues, among other metabolites, the formation of presumptive GA_{34}-*O*-glucoside has been reported [1–3]. These identifications, however, were based on the characterization of the parent GA_{34} after hydrolysis of polar fractions only [1, 2]. In order to provide appropriate standards for the identification of metabolically formed GA_{34} glucosyl conjugates, we synthesized both GA_{34}-2-*O*-β-D-glucoside **7a** and GA_{34}-β-D-glucosyl ester **5a**. Moreover, the corresponding [17-$^2H_2$]-labelled conjugates **7b** and **5b** were synthesized to serve as internal standards for intended quantitative analysis.

RESULTS AND DISCUSSION

The aglucones **2a**, **2b** and **3a**, **3b** were obtained from GA_{34}-16-norketone methyl ester **1**, prepared according to the procedure of Beeley and MacMillan [4]. Methylenation of **1** was accomplished using Lombardo's reagent Zn–$TiCl_4$–CH_2Br_2($C^2H_2Br_2$) [5]. This method, previously applied to other gibberellins, provided **2a** and **2b** in yields higher than 90% and specific incorporation of the deuterium at the 17-position. Demethylation of **2a** and **2b** with lithium *S*-propyl thiolate [6] led to the free acids **3a** and **3b**. The spectral data of **2a** and **3a** were in agreement with those given in the literature [4, 7].

Reaction of **3a** and **3b** with equimolar amounts of α-acetobromoglucose in dichloroethane in the presence of Ag_2CO_3 followed by deacetylation gave the GA_{34}-β-D-glucosyl esters **5a** and **5b** with 20% and 19% total yield, respectively. In the ^1H NMR spectra of **5a** and **5b**, the anomeric proton H-1′ appeared at δ 5.53 as a doublet with a coupling constant of 8.2 Hz, indicating the 1′,2′-*trans*-glucosidic linkage. In the positive-ion ESI-mass spectra, the $[M + Na]^+$ ions at *m/z* 533 and 535, respectively, appeared with the highest abundance. In the negative-ion spectra, the favoured fragmentation into aglucone and glucosyl moiety was indicated by the base peaks at *m/z* 347 and 349, respectively [8].

By glucosylation of **2a** and **2b** under similar conditions, but with an excess of the glucosyl donor, and by subsequent deacetylation and demethylation, the compounds **7a** and **7b** were obtained with a 6–7% overall yield. The presence of one glucose unit was indicated by the $[M + Na]^+$ (100) ions at *m/z* 533 and 535, respectively, of the positive ion ESI-mass spectra and by the only signal for an anomeric proton at δ 4.38 (*d*, 1H, $J = 7.9$ and 7.6 Hz, respectively) in the ^1H NMR spectra. ^1H NMR investigations of **8a** obtained by acetylation of **7a** confirmed the structure as the 2-*O*-glucoside because of the downfield shift of the 3-H signal to δ 5.19. The 2-H signal was unaffected.

EXPERIMENTAL

^1H and ^{13}C NMR spectra were recorded at 500 and 125 MHz, respectively. All chemical shifts δ (ppm) are referenced to TMS. EIMS were measured at 70 eV. Flash chromatography was performed on Kieselgel 60, 230–400 mesh (Merck) using N_2 positive pressure. HPLC separations were carried out on a LiChrospher 100 RP 18 (250 × 10 mm i.d., 10 μm particle size) column. Elutions were performed with the given solvents at a flow rate of 3 ml min^{-1} and UV detection at 210 nm. α-Acetobromoglucose was purchased from Fluka. $C^2H_2Br_2$ (99% ^2H-enriched) was obtained from Aldrich.

ent-2α,3α,10β-Trihydroxy-20-norgibberell-16-ene-7,19-dioic acid 7-methyl ester-19,10-lactone (*GA_{34} methyl ester*) (**2a**). To a stirred soln of **1** (100 mg) in CH_2Cl_2 (15 ml) the methylenation reagent (4 ml), prepared from Zn, $TiCl_4$ and CH_2Br_2 in THF, was

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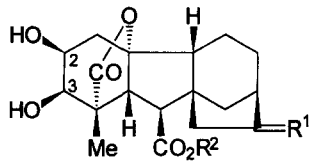
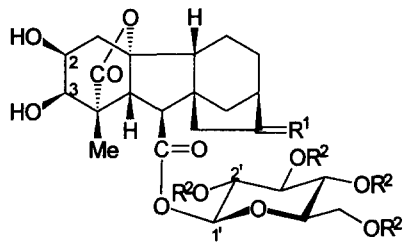
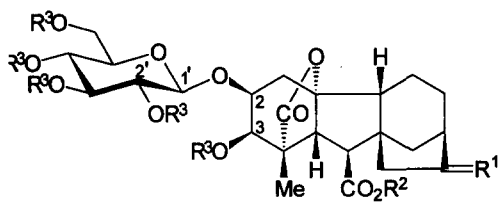


	R^1	R^2
1	O	Me
2a	CH_2	Me
2b	C^2H_2	Me
3a	CH_2	H
3b	C^2H_2	H



	R^1	R^2
4a	CH_2	Ac
5a	CH_2	H
5b	C^2H_2	H



	R^1	R^2	R^3
6a	CH_2	Me	H
7a	CH_2	H	H
7b	C^2H_2	H	H
8a	CH_2	H	Ac

added at room temp. under an Ar atmosphere [5]. After stirring for 2 hr at room temp., the mixt. was dropped into a slurry of 3 g $NaHCO_3$ in 1.5 ml H_2O under vigorous stirring. The clear organic soln was sepd and the aq. residue extracted ×6 with EtOAc. The comb. organic solns were dried and evapd to yield 96 mg of crude product. Flash CC with $CHCl_3$–EtOAc (2:3) yielded **2a** as amorphous solid (93 mg, 94%). $\nu_{max}^{CHCl_3}$ cm^{-1}: 3409–3544, 1766, 1732. $[\alpha]_D^{25}$: −28.1° (MeOH, *c* 0.5). ^1H NMR ($CDCl_3$): δ 1.17 (*s*, 18-H_3), 2.66 (*d*, *J* = 10.7 Hz, 6-H), 3.24 (*d*, *J* = 10.7 Hz, 5-H), 3.65 (*d*, *J* = 4 Hz, 3-H), 3.71 (*s*, CO_2Me), 3.79 (*m*, 2-H), 4.86 and 4.98 (each *br*, 17-H_2). ^{13}C NMR ($CDCl_3$): δ 14.7 (C-18), 16.0 (C-11), 31.3 (C-12), 35.9 (C-1), 36.8 (C-14), 38.5 (C-13), 44.5 (C-15), 50.7 (C-5), 50.8 (C-6), 52.0 (OMe), 52.2 and 53.2 (C-8 and C-4), 52.9 (C-9), 67.1 (C-2), 72.0 (C-3), 94.0 (C-10), 107.5 (C-17), 156.5 (C-16), 173.0 (C-7), 177.4 (C-19). EIMS, *m/z* (rel. int.): 362 $[M]^+$ (10), 344 (4), 330 (100), 312 (10), 302 (21), 284 (67), 240 (59), 228 (57).

ent - [17 - $^2H_2$] - 2α,3α,10β - *Trihydroxy* - 20 - *nor* - *gibberell* - 16 - *ene* - 7,19 - *dioic acid* - 7 - *methyl ester* - 19,10-*lactone* ([17 - $^2H_2$] GA_{34} *methyl ester*) (**2b**). The norketone **1** (90 mg) was treated as described above with the methylenation reagent prepd from $C^2H_2Br_2$ to yield **2b** (84 mg, 93%). **2b** contains 96 atoms % $[^2H_2]$, 2 atoms % $[^2H_1]$ and 2 atoms % $[^2H_0]$. $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400–3543, 1767, 1732. $[\alpha]_D^{27}$: −18.2° (MeOH, *c* 0.5). ^1H NMR ($CDCl_3$): δ 1.19 (*s*, 18-H_3), 2.67 (*d*, *J* = 10.7 Hz, 6-H), 3.25 (*d*, *J* = 10.7 Hz, 5-H), 3.71 (*s*, CO_2Me), 3.75 (*d*, *J* = 4 Hz, 3-H), 3.91 (*m*, 2-H). EIMS, *m/z* (rel. int.): 364 $[M]^+$ (10), 346 (4), 332 (100), 314 (6), 304 (19), 286 (43), 242 (32), 230 (30).

ent - 2α,3α,10β - *Trihydroxy* - 20 - *norgibberell* - 16-*ene* - 7,19 - *dioic acid* - 19,10 - *lactone* (GA_{34}) (**3a**). **2a** (55 mg) in HMPT (0.5 ml) was treated with 5–6 equivalents of Li *S*-propyl thiolate in HMPT [6] at room temp. under an Ar atmosphere for 4 hr. The reaction was stopped by addition of HOAc and the evapd reaction mixt. was subjected to DEAE-Sephadex A-25 (15 ml). The column was eluted with 50 ml aliquots of MeOH and MeOH–HOAc (2:1). Evapn of the acid frs yielded 48 mg product, which was further purified by flash CC with EtOAc–hexane–HOAc (30:20:1) to give pure **3a** (40 mg, 75%). ν_{max}^{KBr} cm^{-1}: 3423 *br*, 1757, 1717. $[\alpha]_D^{28}$: −21.8 (MeOH, *c* 0.5). ^1H NMR (Me_2CO-d_6): δ 1.16 (*s*, 18-H_3), 2.55 (*d*, *J* = 11 Hz, 6-H), 3.26 (*d*, *J* = 11 Hz, 5-H), 3.63 (*d*, *J* = 4 Hz, 3-H), 3.74 (*m*, 2-H), 4.84 and 4.96 (each *br*, 17-H_2). ^{13}C NMR (Me_2CO-d_6): δ 15.4 (C-18), 16.7 (C-11), 32.2 (C-12), 36.8 (C-1), 37.6 (C-14), 39.8 (C-13), 45.1 (C-15), 51.7 (C-5), 52.1 (C-6), 52.5 (C-8), 53.6 (C-9), 54.0 (C-4), 67.8 (C-2), 73.0 (C-3), 94.3 (C-10), 107.3 (C-17), 158.3 (C-16), 174.4 (C-7), 177.8 (C-19). EIMS, *m/z* (rel. int.): 348 $[M]^+$ (32), 330 (80), 312 (52), 303 (50), 284 (100), 268 (89), 256 (32), 240 (51), 223 (70).

ent - [17 - $^2H_2$] - 2α,3α,10β - *Trihydroxy* - 20 - *nor* - *gibberell* - 16 - *ene* - 7,19 - *dioic acid* 19,10 - *lactone* ([17 - $^2H_2$] GA_{34}) (**3b**). **3b** was prepd from **2b** (50 mg) by the method described in the previous expt; 35 mg (73%) of an amorphous solid containing 96 atoms % $[^2H_2]$, 2 atoms % $[^2H_1]$ and 2 atoms % $[^2H_0]$ were obtained. ν_{max}^{KBr} cm^{-1}: 3447 *br*, 1751, 1718. $[\alpha]_D^{29}$: −9.7 (MeOH, *c* 0.5). ^1H NMR (Me_2CO-d_6): δ 1.16 (*s*, 18-H_3), 2.58 (*d*, *J* = 11 Hz, 6-H), 3.27 (*d*, *J* = 10.7 Hz, 5-H), 3.63 (*d*, *J* = 4 Hz, 3-H), 3.75 (*m*, 2-H). EIMS, *m/z* (rel. int.): 350 $[M]^+$ (11), 332 (100), 314 (50), 304 (32), 286 (89), 270 (84), 258 (22), 242 (43).

ent - 2α,3α,10β - *Trihydroxy* - 20 - *norgibberell* - 16 - *ene* - 7,19 - *dioic acid* - 19,10 - *lactone* - 7 - *O* - β - D - *glucopyranosyl ester* (GA_{34} - β - D - *glucopyranosyl*

ester) (**5a**). (a) GA_{34}-β-D-(2′,3′,4′,6′-tetra-*O*-acetyl)-glucopyranosyl ester (**4a**). To a stirred soln of **3a** (20 mg) in dichloroethane (2.5 ml) Ag_2CO_3–Celite [9] (35 mg) was added under an Ar atmosphere. The mixt. was treated with α-acetobromoglucose (25 mg) in dichloroethane (200 μl) at boiling temp. After 10 min (the H_2O formed was removed azeotropically), the mixt. was diluted with EtOAc and filtered. The filtrate was evapd to dryness. The residue was dissolved in MeOH (1 ml) and subjected to DEAE-Sephadex A-25 (7 ml). The column was eluted with 30-ml aliquots of MeOH and MeOH–HOAc (2:1). Evapn of the neutral fr. gave crude **4a**, which was purified by flash CC with hexane–EtOAc (9:11) to yield pure **4a** (18 mg, 46%). From the acid fr. *ca* 50% of unchanged starting material **3a** could be recovered. ^{1}H NMR ($CDCl_3$): δ 1.17 (*s*, 18-H_3), 2.01, 2.04, 2.06 (4*s*, 4 Ac), 2.67 (*d*, *J* = 11 Hz, 6-H), 3.24 (*d*, *J* = 11 Hz, 5-H), 3.76 (*d*, *J* = 3.7 Hz, 3-H), 3.90 (*m*, 2-H), 4.82, 4.96 (each *br*, 17-H_2), 5.79 (*d*, *J* = 8.2 Hz, 1′-H). ESIMS (pos.) *m/z* (rel. int.): 701 $[M + Na]^+$ (100). ESIMS (neg.) *m/z* (rel. int.): 713 $[M + Cl]^-$ (5), 677 $(M - H]^-$ (6), 389 (61), 347 $[M - C_{14}H_{19}O_9]^-$ (100). (b) **4a** (18 mg) was dissolved in MeOH (5 ml) and treated with 0.05 N NaOMe soln (1 ml). After 15 min at room temp., HOAc was added and the reaction mixt. evapd to dryness. By flash CC with EtOAc–MeOH (17:3) and further purification by HPLC with MeOH–H_2O (2:3), pure **5a** (6 mg, 44%) was obtained as an amorphous solid. ^{1}H NMR (Me_2CO-d_6): δ 1.13 (*s*, 18-H_3), 2.66 (*d*, *J* = 11 Hz, 6-H), 3.30 (*d*, *J* = 11 Hz, 5-H), 3.62 (*d*, *J* = 4.3 Hz, 3-H), 3.73 (*m*, 2-H), 5.53 (*d*, *J* = 8.2 Hz, 1′H), 4.831 and 4.95 (each *br*, 17-H_2). ESIMS (pos.) *m/z* (rel. int.): 533 $[M + Na]^+$ (100), 549 $[M + K]^+$ (15). ESIMS (neg.) *m/z* (rel. int.): 545 $[M + Cl]^-$ (28), 509 $[M - H]^-$ (4), 389 (20), 347 $[M - C_6H_{11}O_5]^-$ (100).

ent - [17 - $^2H_2$] - 2α,3α,10β - *Trihydroxy* - 20 *nor* - *gibberell* - 16 - *ene* - 7,19 - *dioic acid* - 19,10 - *lactone* - 7 - *O* - β - D - *glucopyranosyl ester* ([17 - $^2H_2$] GA_{34} - β-D-*glucopyranosyl ester*) (**5b**). **5b** (7 mg) was obtained as above from **3b** (25 mg), α-acetobromoglucose (31 mg) and Ag_2CO_3–Celite (43.5 mg) as an amorphous solid (19%). ^{1}H NMR (Me_2CO-d_6): δ 1.13 (*s*, 18-H_3), 2.66 (*d*, *J* = 11 Hz, 6-H), 3.30 (*d*, *J* = 11 Hz, 5-H), 3.62 (*d*, *J* = 4.3 Hz, 3-H), 3.73 (*M*, 2-H), 5.53 (*d*, *J* = 8.2 Hz, 1′H). ESIMS (pos.) *m/z* (rel. int.): 535 $[M + Na]^+$ (100), 551 $[M + K]^+$ (12). ESIMS (neg.) *m/z* (rel. int.): 547 $[M + Cl]^-$ (19), 511 $[M - H]^-$ (20), 391 (60), 349 $[M - C_6H_{11}O_5]^-$ (100).

ent - 2α,3α,10β - *Trihydroxy* - 20 - *norgibberell* - 16 - *ene* - 7,19 - *dioic acid* - 19,10 - *lactone* - 2 - *O* - β - D - *glucopyranoside* (*GA_{34}* - 2 - *O* - β - D - *glucopyranoside*) (**7a**). (a) GA_{34}-2-*O*-β-D-glucopyranoside methyl ester **6a**. **2a** (70 mg) in dichloroethane (3.5 ml) was treated with α-acetobromoglucose (400 mg) in dichloroethane (1 ml) in the presence of Ag_2CO_3–Celite (560 mg) under reflux as described above. After filtration and evapn of the filtrate to dryness, the residue was dissolved in MeOH (1 ml) and deacetylated by adding 0.5 N NaOMe soln (4 ml). After 1 hr, the reaction was stopped by addition of HOAc. Flash CC with $CHCl_3$–MeOH (9:1), yielded crude **6a**. For analytical purposes a small sample was purified by HPLC with MeOH–H_2O (3:2). ^{1}H NMR (MeOH-d_4): δ 1.13 (*s*, 18-H_3), 2.60 (*d*, *J* = 10.6 Hz, 6-H), 3.28 (5-H), 3.70 (*s*, CO_2Me), 3.83 (3-H), 3.86 (*m*, 2-H), 4.38 (*d*, *J* = 7.8 Hz, 1′-H), 4.86 and 4.98 (each *br*, 17-H_2). ESIMS (pos.) *m/z* (rel. int.): 547 $[M + Na]^+$ (100), 413 (59). ESIMS (neg.) *m/z* (rel. int.): 559 $[M + Cl]^-$ (85), 523 $[M - H]^-$ (100), 339 (47), 325 (53). (b) Crude **6a** was dissolved in HMPT (500 μl) and treated with a 1.5 N soln of Li *S*-propyl thiolate in HMPT (400 μl) under Ar for 4 hr. Work-up as described for **3a** gave crude **7a**, which was first subjected to a flash CC with $CHCl_3$–MeOH–HOAc (90:15:1) and then purified by HPLC with MeOH–0.2% aq. HOAc (11:9) to yield pure **7a** (7 mg, 7% referred to **2a**). ^{1}H NMR (MeOH-d_4): δ 1.23 (*s*, 18-H_3), 2.45 (6-H), 3.27 (5-H), 3.83 (3-H), 3.87 (*m*, 2-H), 4.38 (*d*, *J* = 7.9 Hz, 1′-H), 4.79 and 4.90 (each *br*, 17-H_2). ^{13}C NMR (MeOH-d_4, derived from HMQC): δ 15.3 (C-18), 17.0 (C-11), 32.6 (C-12), 35.6 (C-1), 38.5 (C-14), 40.6 (C-13), 46.0 (C-15), 53.0 (C-5), 54.0 (C-9), 56.0 (C-6), 62.5 (C-6′), 70.9 (C-3), 71.4 (C-4′), 75.1 (C-2′), 76.4 (C-2), 77.7 (C-3′), 78.0 (C-5′), 103.0 (C-1′), 106.7 (C-17). ESIMS (pos.) *m/z* (rel. int.): 533 $[M + Na]^+$ (100), 549 $[M + K]^+$ (26), 413 (31). ESIMS (neg.) *m/z* (rel. int.): 545 $[M + Cl]^-$ (5), 509 $[M - H]^-$ (100).

ent - 3α - *Acetoxy* - 2α,10β - *trihydroxy* - 20 - *nor* - *gibberell* - 16 - *ene* - 7,19 - *dioic acid* - 19,10 - *lactone* - 2′,3′,4′,6′ - *tetra* - *O* - *acetyl* - 2 - *O* - β - D - *glucopyrano* - *side* (3 - *O* - *acetyl* - *GA_{34}* - 2 - *O* - β - D - (2′,3′,4′,6′ - *tetra* - *O*-*acetyl*-*glucopyranoside*) (**8a**). **7a** (1 mg) was acylated with Ac_2O in pyridine to yield **8a**. ^{1}H NMR (MeOH-d_4): δ 1.08 (*s*, 18-H_3), 1.94, 2.0, 2.02, 2.06, 2.09 (5 *s*, 5 Ac), 2.45 (*br*, 6-H), 3.17 (5-H), 3.90 (*m*, 2-H), 4.72 (*d*, *J* = 7.9 Hz, 1′-H), 4.81 and 4.93 (each *br*, 17-H_2), 5.19 (3-H).

ent - [17 - $^2H_2$] - 2α,3α,10β - *Trihydroxy* - 20 - *nor* - *gibberell* - 16 - *ene* - 7,19 - *dioic acid* - 19,10 - *lactone* - 2 - *O* - β - D - *glucopyranoside* ([17 - $^2H_2$] - *GA_{34}* - 2 - *O* - β - D - *glucopyranoside*) (**7b**). **7b** was prepd from **2b** (75 mg), α-acetobromoglucose (425 mg) and Ag_2CO_3–Celite (600 mg) according to the procedure described for **7a**; amorphous solid, yield 6.5 mg (6.2%). ^{1}H NMR (MeOH-d_4): δ 1.20 (*s*, 18-H_3), 2.52 (*d*, *J* = 10.7 Hz, 6-H), 3.26 (5-H), 3.83 (3-H), 3.86 (*m*, 2-H), 4.38 (*d*, *J* = 7.6 Hz, 1′-H). ESIMS (pos.) *m/z* (rel. int.): 535 $[M + Na]^+$ (100), 551 $[M + K]^+$ (16), 413 (20). ESIMS (neg.) *m/z* (rel. int.): 547 $[M + Cl]^-$ (14), 511 $[M - H]^-$ (100).

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