COMMUNICATION

C-18 Hydroxylation of gibberellins

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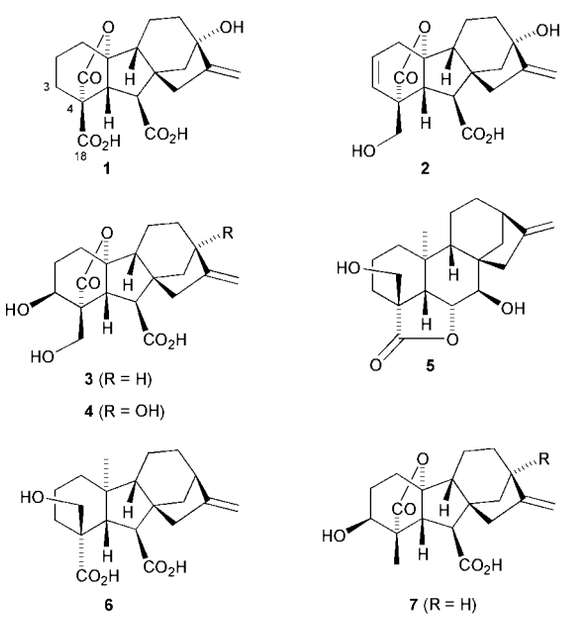
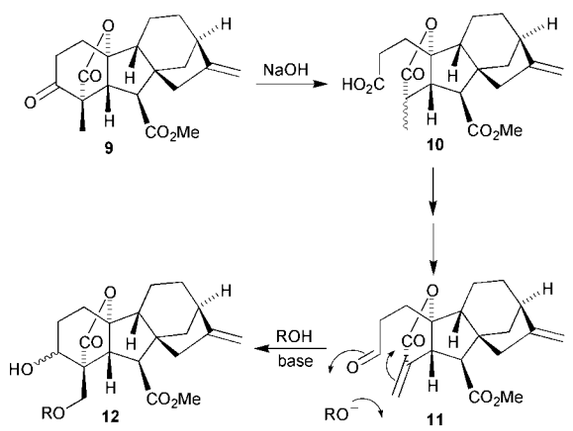
A protocol for the hydroxylation of the 18-methyl group in gibberellins has been developed, as demonstrated by the successful synthesis of 18-hydroxy GA_4 methyl ester by means of a tandem process involving the conjugate addition of alkoxide to the α-methylene lactone moiety of a ring A-seco-gibberellin followed by an intramolecular aldol reaction.

Gibberellins ("GAs") in which the 18-methyl group has undergone oxidation have been isolated from immature seeds of sword bean (*Canavalia gladiata*), *e.g.* GA_{21} (**1**) and GA_{22} (**2**),[1] and from germinating barley grain (*Hordeum vulgare*), *e.g.* the 18-hydroxy derivatives of GA_4 (**3**) and GA_1 (**4**).[2] In the case of 18-hydroxy GA_4 (**3**), the structure was determined by converting 7β,18-dihydroxykaurenolide (**5**) into 18-hydroxy GA_{12} (**6**) and then carrying out the metabolic transformation of this material to a series of 18-hydroxy C_{19} GAs with the fungus *Gibberella fujikuroi* (B1-41a mutant).[3] In order to confirm these assignments and, more importantly, provide sufficient quantities of this type of GA for more extensive biological studies, we initiated a study aimed at establishing a general procedure for the synthesis of these compounds from the fungal GAs, GA_4 (**7**) and GA_1 (**8**). The successful outcome of our efforts in the GA_4 series is reported in this Communication.



Our synthetic plan is outlined in Scheme 1, the proposed tandem transformation **11**→**12** being based on the well established aldol process that has been shown to be quite general for forming the C3–C4 bond of both C_{19} and C_{20} gibberellins.[4] This plan was then rendered to practice as indicated in Scheme 2.

Cleavage of the A-ring was readily achieved by means of a retro-Claisen reaction on ketone **9**[5] induced by brief treatment (8 minutes) with NaOH in aqueous THF.[6] Under these conditions a 9:1 mixture of C4 epimers **10** was obtained with the *endo*-methyl isomer predominating. Extended reaction times led to a major increase in the proportion of the *exo*-isomer and hydrolysis of the methyl ester function. Next, the reduction of the 3-carboxy in the mixture of **10** epimers was effected by $NaBH_4$ treatment of the mixed anhydride formed from ethyl chloroformate,[7] only the *endo*-epimer (**13**) being isolated. In view of the modest yield of **13** (*ca.* 70% based on 55% conversion), alternative activating groups were explored, *e.g.* benzotriazolyl[8] and pentafluorophenyl,[9] but no improvement was obtained. Recovered starting material (**10**) could, however, be easily recycled and sufficient quantities of the alcohol **13** duly obtained. It was envisaged that formation of the α-methylene lactone group in **11** could be achieved by the replacement of H-4 with a suitable leaving group, followed by elimination, thereby allowing the direct functionalisation of C-18 *via* the proposed tandem transformation **11**→**12**. Protection of the free hydroxy group in **13** was initially thought to be necessary and accordingly the corresponding *tert*-butyldimethylsilyl ether **14** was formed using standard conditions (quantitative yield).[10] Subsequent treatment of **14** with LDA followed by tetrabromomethane[11] afforded bromo lactone **15** in excellent yield (99%). Reaction on the *exo*-face of the enolate to introduce the bromo substituent *syn* to H-5β was expected to ensure that the subsequent elimination of HBr would involve the 4-methyl group and afford the desired methylene lactone.[12] In the event, treatment of **15** with TBAF[10] induced deprotection with concomitant elimination in the desired sense, thereby rendering alcohol **16** directly in 62% yield. Alternatively, bromination of unprotected **13** was carried out to give bromo lactone **17** in good yield (73%) which, after treatment with TBAF, now gave **16** in two steps from **13** (55% overall). Formation of aldehyde **11** was smoothly achieved by Dess–Martin periodinane oxidation[13] of **16** (89%) as a prelude to carrying out the desired tandem transformation.



Scheme 1

It was hoped that hydroxide itself might undergo conjugate addition to **16** and thence give the target compound (**20**) directly, but the reaction was unsuccessful. Treatment of aldehyde **11** with potassium carbonate (5 equivalents) in methanol, however, gave an approximately 1:1 ratio of 3α- and

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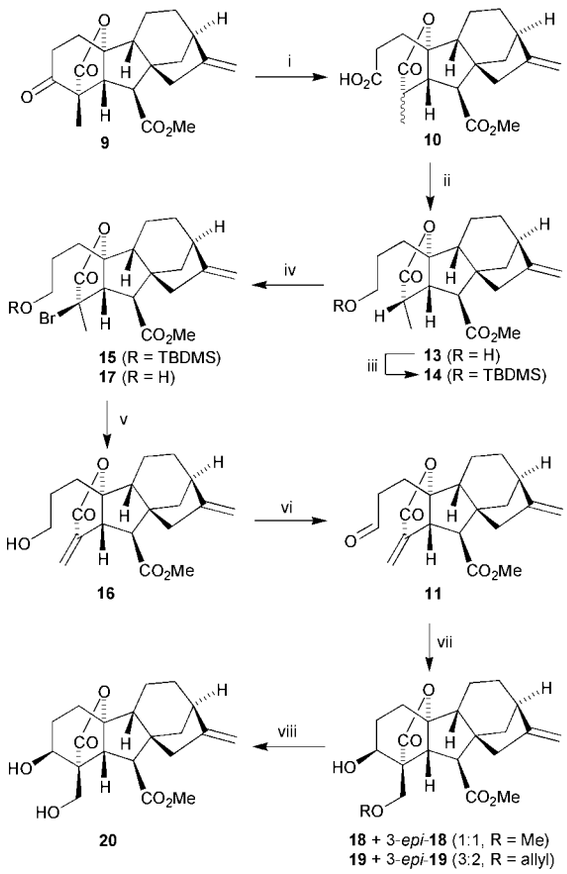

Scheme 2 *Reagents and conditions*: i, 1.0 M NaOH, THF, 0 °C, 8 min, 87%; ii, EtOCOCl, Et_3N, THF, 0 °C, 30 min to room temp., 4 h, then $NaBH_4$–EtOH, 0 °C, 70% (based on 55% conversion); iii, TBSCl, Et_3N, imidazole, DMF, room temp., 2.5 h, 100%; iv, LDA, THF, −78 °C, 25 min, then CBr_4, −78 °C, 40 min, 99% (R = TBDMS) or 73% (R = H); v, TBAF, THF, 0 °C to room temp., 4 h, 62% (R = TBDMS) or 75% (R = H); vi, Dess–Martin periodinane, CH_2Cl_2, room temp., 20 min, 89%; vii, R = Me: K_2CO_3, MeOH, room temp., 10 min, 50% (1 : 1); R = allyl: K_2CO_3, allyl alcohol, room temp., 80 min, 56% (3 : 2); viii, $RhCl(PPh_3)_3$, DABCO, 10% aq. EtOH, 75 °C, 24 h, then 1.0 M HCl, room temp., 30 min, 37% (based on 77% conversion).

3β-hydroxy methyl ethers **18** in modest yield (50%). Evidence for the formation of the products was provided by ^{1}H NMR spectroscopy,† with the appearance of a singlet at 3.29 ppm (3H), a pair of AB doublets at 3.59 and 3.75 ppm (J_{gem} = 10.0 Hz) associated with the 18-methylene group and the return of the AB spin system arising from H-5 and H-6 (3.42 and 2.76 ppm, J = 10.6 Hz, for the 3β-epimer) that is characteristic of intact gibberellins.[14] As expected, deprotection of the methyl ethers[10] could not be achieved, but the successful addition of methoxide to the system had shown that the strategy was feasible and accordingly we began searching for an alternative alkoxide. 2,2,2-Trichloroethanol was successfully added to **11** but attempts to liberate the free hydroxy group with Zn–AcOH[10] were unsuccessful. It was thought that there would be a good chance of removing the corresponding 4-methoxybenzyl ether,[10] but 4-methoxybenzyl oxide failed to add. Success was finally achieved by means of the addition of allyl oxide to **11**, which afforded a 2 : 3 ratio of 3α- and 3β-OH allyl ethers **19** in moderate yield (56%). Following separation, treatment of the desired 3β-OH allyl ether **19** with $RhCl(PPh_3)_3$ and DABCO, followed by acidic workup[15] resulted in liberation of the free hydroxy group at C-18, giving our target compound (**20**) ‡ in an unoptimised 37% yield (based on 77% conversion). The structure of the endogenous GA was then confirmed as 18-hydroxy GA_4 by GC-MS comparison[16] § of the derived methyl ester with the synthetic product (**20**).

The successful synthesis of 18-OH GA_4 methyl ester (**20**), in seven steps from 3-oxo-GA_4 methyl ester (**9**), is the first example in which the unactivated 18-methyl group of a gibberellin has been functionalised. Current efforts are being directed towards refining this methodology, applying it to the synthesis of the more complex 18-hydroxy GAs, *e.g.* **4**, and obtaining sufficient quantities of these 18-hydroxy GAs in order to carry out extensive biological studies.[2]

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Notes and references

† All new compounds were fully characterised by ^{1}H and ^{13}C NMR spectroscopy, mass spectrometry, elemental analysis and/or high resolution mass spectrometry.

‡ Selected data for **20**: mp 166–167 °C (from EtOAc–petroleum spirits bp 60–80 °C); Found: C, 66.0; H, 7.4. Calcd. for $C_{20}H_{26}O_6$: C, 66.3; H, 7.2%; ν_{max}/cm^{-1} 3441, 3065, 2944, 2880, 1766, 1735, 1658, 1438, 1382, 1267, 1199, 1019, 888; δ_H(300 MHz; $CDCl_3$) 1.30–2.15 (m, 13H), 2.63 (t, J = 7.1 Hz, 1H, H-13), 2.86 (d, $J_{6,5}$ = 11.4 Hz, 1H, H-6), 3.35 (d, $J_{5,6}$ = 11.4 Hz, 1H, H-5), 3.66 (d, J_{gem} = 12.6 Hz, 1H, H-18), 3.76 (s, 3H, -CO_2CH_3), 3.99 (d, 12.7 Hz, 1H, H′-18), 4.28 (d, J = 2.4 Hz, 1H, H-3), 4.89 (s, 1H, H-17), 5.02 (s, 1H, H′-17). δ_C(75 MHz; $CDCl_3$) 16.1 (C-11), 27.2 and 27.3 (C-1 and C-2), 31.4 (C-12), 36.1 (C-14), 38.2 (C-13), 43.7 (C-15), 48.1 (C-9), 50.6 (C-6), 50.9 (C-8), 52.4 (-CO_2CH_3), 53.5 (C-5), 58.0 (C-4), 64.0 (C-18), 70.0 (C-3), 94.5 (C-10), 107.9 (C-17), 156.7 (C-16), 174.8 (C-19), 175.3 (C-7). *m/z* (E/I) 362 (M^+, 12%), 344 (18), 330 (100), 312 (92), 284 (60), 266 (60), 240 (60), 195 (20), 155 (30), 129 (43), 115 (34), 91 (78), 79 (47).

§ Selected mass spectral data from GC-MS analysis of TMS derivatised **20** and natural sample (ref. 16); 18-OH GA_4-Me-TMS (synthetic): 506 (M^+, 6%), 591 (36), 474 (70), 431 (16), 369 (61), 341 (78), 317 (100), 266 (68), 223 (85), 181 (19), 129 (26), 73 (53). 18-OH GA_4-Me-TMS (natural): 506 (M^+, 16%), 591 (60), 474 (64), 431 (13), 369 (38), 341 (54), 317 (67), 266 (35), 223 (44), 181 (21), 129 (28), 73 (100).

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