Pergamon

Tetrahedron Letters, Vol. 37, No. 5, pp. 723-726, 1996

0040-4039/96 $15.00 + 0.00

0040-4039(95)02252-X

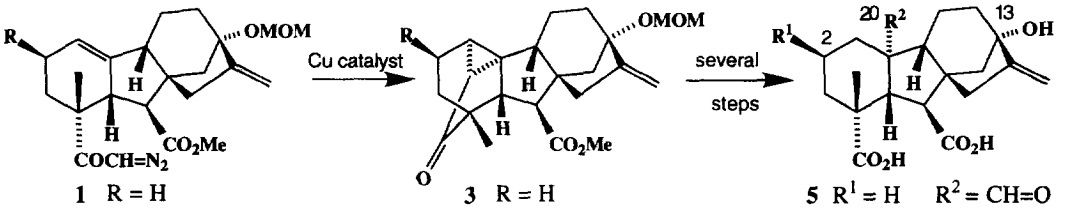
Structure Determination and Synthesis of a New Gibberellin, GA_{99}, from Spinach Plants: 2β-Hydroxy-GA_{19}

Lewis N. Mander* and David J. Owen

Research School of Chemistry, Institute of Advanced Studies, Australian National University, and the Co-operative Research Centre for Plant Sciences, Canberra, ACT, 0020, Australia.

Abstract: The structure of a new gibberellin, GA_{99}, isolated from Spinach plants has been determined to be 2β-hydroxy-GA_{19} (**6**) by synthesis from gibberellic acid. The key steps in the preparation involved the reduction under Luche conditions of a $\Delta^{1(10)}$-2-one derivative to furnish the critical 2β-stereochemistry, intramolecular cyclopropanation of the $\Delta^{1(10)}$ bond by a 4α-diazoacetyl group followed by Li-NH_3 reduction, and then oxidative cleavage of the resulting cyclopentanone by oxygenation of the derived potassium enolate.

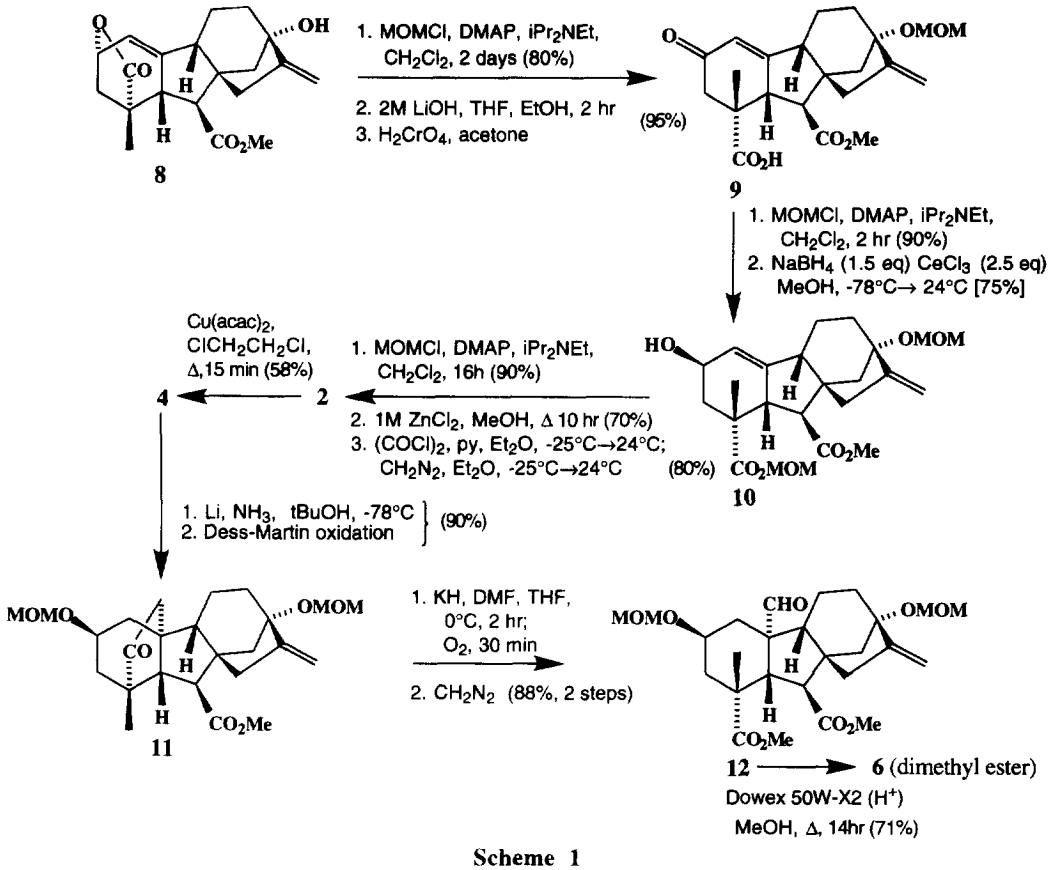
Several 2β-hydroxy C-20 gibberellins, *e.g.* 2β-hydroxy-GA_{53} (**7**), have been tentatively identified from a number of plant sources, including tomato,[1] barley[2] and *Silene armeria*.[3] In order to gain access to workable amounts of these gibberellins, to confirm their structural identity, and to explore there biosynthetic origins, we have undertaken the synthesis of 2β-hydroxy GA_{19} (**6**) in the expectation that it would be possible to convert this compound into a range of C-20 gibberellins with varying oxidation levels at C(20), with and without the 13-hydroxyl. In the event, the synthesis of the dimethyl ester derivative of the target aldehyde has made it possible to establish that **6** occurs naturally in spinach (*Spinacia oleracea* L.).[4] The planned route to **6** was based on an earlier study in which GA_{19} (**5**) had been prepared *via* the intramolecular cyclopropanation reaction of diazoketone **1** to afford **3**.[5] Thus, adaptation of the previous procedures to a 2β-substituted analogue could be expected to afford **4**, and thence 2β-hydroxy-GA_{19} (**6**). The realisation of this plan is outlined in Scheme 1, beginning with lactone **8**, the preparation of which has been described previously.[6,7]



1 R = H
2 R = OMOM

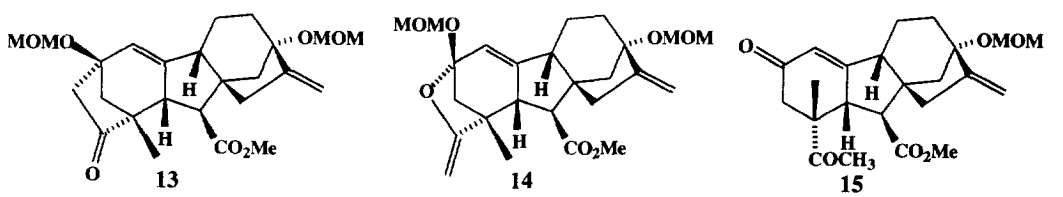
3 R = H
4 R = OMOM

5 R^1 = H R^2 = CH=O
6 R^1 = OH R^2 = CH=O
7 R^1 = OH R^2 = Me

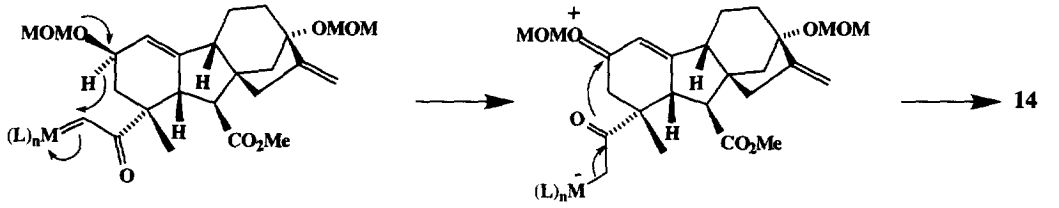


Scheme 1

After protection of the 13-hydroxyl as the methoxymethyl ether, the A-ring lactone function was hydrolysed and the resulting hydroxy acid oxidised with Jones' reagent.[8] The 2-oxo group in the resulting enone **9** was surprisingly resistant to reduction, possibly due to *in situ* ketol formation, but the derived methoxymethyl ester was reduced satisfactorily to the desired 2β-epimer **10** with a combination of cerium chloride and sodium borohydride, conditions that were chosen to promote approach of the hydride reagent to the more hindered lower face of the A-ring.[9] Following protection of the 2β-hydroxyl, the methoxymethyl ester was selectively cleaved with zinc chloride, the diazoketone **2** prepared, and the cyclopropanation reaction undertaken with copper-bronze as the catalyst. In the conversion of **1** to **3** this catalyst had afforded an 87% yield,[5] but in the present case, only 46% of **4** was obtained, the balance of material being accounted for by the formation of the CH insertion product **13** (38% yield) and the unstable acetal **14** (15% yield).



The identity of the latter compound was apparent from ^{1}H and ^{13}C NMR spectra, the former showing resonances at δ3.82 and 4.27 (br s.) for the methylene group (*cf.* δ3.80 for 2-methoxypropene);[10] the latter spectrum displayed signals at δ82.4 and 163.3 for the enol ether function, and at δ106.4 for the C2 acetal carbon. Acetal **14** was rapidly hydrolysed on silica gel to methyl ketone **15**. A 2-CH insertion product had been evident in the preparation of **3**, and so the greater relative amount of **13** formed from **2** was not surprising, given the expected activation from the 2β-substituent.[11] The transfer of hydride implicit in the formation of **14** (Scheme 2) has precedent in the work of Doyle[12] and Lee[13] and has been observed by us with a number of other substrates in which stabilisation of an incipient cation by neighbouring functionality could occur.[14] Some improvement in the yield of **4**[15,16] (to 58%) could be obtained with $Cu(acac)_2$ as the catalyst, while **13** was the major product (75%) with $Rh_2(OAc)_4$.



Scheme 2

In a continuation of the synthesis, **4** was reduced by lithium-NH_3(l)-*t*-BuOH to **11**[15,17] with some over-reduction to the 19-carbinol, a problem that was readily corrected by oxidation of the crude product with the Dess-Martin reagent.[18] *A priori*, it had appeared possible that some hydrogenolysis of the 2β-substituent could have occurred, but this reaction was not in evidence. Oxidative cleavage of the cyclopentanone ring in **11** by oxygenation of the potassium enolate[19] proceeded smoothly and after methylation of the product to aid purification, dimethyl ester **12** was obtained in excellent yield. The dimethyl ester of the target aldehyde **6**[15,20] was then obtained by hydrolysis of the ether protecting groups.[21] Comparison of the GCMS of the trimethylsilylated derivative of **6** dimethyl ester with a similarly derivatised natural gibberellin isolated from spinach[4] has established that the synthetic and endogenous gibberellins are identical, so **6** has been designated as GA_{99}.[22] Further studies stemming from the availability of **6** have shown that this gibberellin is representative of several 2β-hydroxy C-20 gibberellins isolated from a variety of plant sources, details of which will be submitted for publication shortly.[23]

Acknowledgements. We thank Dr Jan Zeevaart for undertaking the GC-MS comparison of the endogenous GA_{99} from spinach with the synthetic sample, and Abbott Laboratories for a generous gift of gibberellic acid.

REFERENCES AND NOTES

1. Koshioka, M.; Nishijima, T.; Yamazaki, H.; Liu, Y.; Nonaka M.; Mander L. N. *J. Hort. Sci.* **1994**, 69, 171-179.
2. Croker, S. J.; Hedden, P.; Lenton, J. R.; Stoddart, J. L. *Plant Physiol.* **1990**, 94, 194-200.
3. Zeevaart, J. A. D.; Talon, M.; Wilson, T. M. in *Gibberellins*; Takahashi, N.; Phinney, B. O.; MacMillan, J., Eds.; Springer-Verlag: New York, 1991; pp 273-279.

4. Talon, M.; Zeevaart, J. A. D.; Gage, D. A., unpublished results. See ref. 23.
5. Dawe, R. D.; Mander, L. N.; Turner, J. V. *Tetrahedron Lett.* **1985**, 26, 363-366.
6. Beale, M. H.; MacMillan, J.; Makinson, I. K.; Willis, C. L. *J. Chem. Soc. Perkin Trans. 1* **1991**, 1191-1195.
7. Nakayama, M.; Yokota, T.; Sohma, R.; Mander, L. N.; Twitchin, B.; Komatsu, H.; Matsui, H.; Bukovac, M. J. *Phytochemistry*, submitted for publication.
8. Bowers, A.; Halsall, T. G.; Jones, E. R. H; Lemin, A.J. *J. Chem. Soc.* **1953**, 2548.
9. Gemal, A. L.; Luche, J. L. *J. Am. Chem. Soc.* **1981**, 103, 5454-5459.
10. Pouchert, C. J., Ed. *The Aldrich Library of NMR Spectra Ed 2* Vol 1, Aldrich Chemical Company: Milwaukee, 1983, p 181B.
11. Taber, D. F.; Ruckle Jr., R. E. *J. Am. Chem. Soc.* **1986**, 108, 7686-7693. Spero, D. M.; Adams, J. *Tetrahedron Lett.* **1992**, 33, 1143-1146. Weng, P.; Adams, J. *J. Am. Chem. Soc.* **1994**, 116, 3296-3305.
12. Doyle, M. P.; Dyatkin, A. B.; Autry, C. L. *J. Chem. Soc. Perkin. Trans 1* **1995**, 619-621.
13. Lee, E.; Choi, I.; Song, S. Y. *J. Chem. Soc. Chem. Commun.* **1995**, 321-322.
14. Morris, J. C. *Stable Norcaradienes: A New Approach to the Synthesis of Kaurenoid Diterpenes,* Australian National University, 1994. Rogers, D. H.; Morris, J. C.; Roden, F. S.; Frey; King, G. R.; Russkamp, F.-W.; Bell, R. A.; Mander, L.N. *Pure Appl. Chem.* in press.
15. All new compounds were characterised by ^{1}H and ^{13}C-NMR spectra, mass spectra, high resolution mass measurements and/or satisfactory microanalyses.
16. *Methyl* ent-*2α,13-di(methoxymethoxy)-19-oxo-1β,20-cyclo-19,20-cyclogibberell-16-en-7-oate* (**4**): mp 104.5-106°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.83 (3H, s, H-18), 2.37 (1H, d, J = 9.9 Hz, H-5), 3.00 (1H, d, J = 9.9 Hz, H-6), 3.35, 3.37 (2x3H, s, OMe), 3.70 (3H, s, CO_2Me), 4.29 (1H, ddd, J_1 = 8.8 Hz, J_2 = 3.9 Hz, J_3 = 1.9 Hz, H-2α), 4.53, 4.76 (2x1H, ABd, J = 7.1 Hz, OCH_2OMe), 4.61 (2H, s, OCH_2OMe), 5.05 (1H, br s, H-17), 5.13 (1H, br s, H'-17). MS (CI) *m/z* 447(M^++H 12%), 416 (52), 402 (53), 38)(100), 295 (30), 277 (13), 265 (10), 245 (17), 193 (10). HRMS (EI) *m/z* calcd for M^+, $C_{25}H_{34}O_7$: 446.2305; found: 446.2305. $C_{25}H_{34}O_7$ req C 67.24, H 7.67; found: C 67.40, H 7.90.
17. *Methyl* ent-*2α,13-di(methoxymethoxy)-19-oxo-19,20-cyclogibberell-16-en-7-oate* (**11**): mp 106-107°C. IR 1735 cm^{-1}; 1H NMR (300 MHz, $CDCl_3$) δ 0.92 (3H, s, H18), 2.39 (1H, d, J = 12.0 Hz, H5), 2.51 (1H, d, J = 12.0 Hz, H6), 3.31, 3.36 (2x3H, s, OMe), 3.69 (3H, s, CO_2Me), 3.69 (1H, m, H2), 4.55, 4.73 (2x1H, ABd, J = 7.1 Hz, OCH_2OMe), 4.58 (2H, s, OCH_2OMe), 5.00 (1H, br s, H17), 5.14 (1H, br s, H'17). MS (EI) *m/z* 448 (M^+, 2%), 345 (3), 167 (15), 149 (56), 129 (11), 113 (18), 111 (11), 105 (10), 98 (10), 97 (24), 85 (35), 71 (75), 57 (76), 55 (100). HRMS (EI) *m/z* calcd for M^+, $C_{25}H_{36}O_7$: 448.2461; found 448.2460. $C_{25}H_{36}O_7$ req C 66.94, H 8.09; found C 66.72, H 8.39.
18. Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, 48, 4155-4156. Dess, D. B.; Martin, J. C. *J. Am. Chem. Soc.* **1991**, 113, 7277-7287. Ireland, R. E.; Liu, L. *J. Org. Chem.* **1993**, 58, 2899.
19. Dawe, R. D.; Mander, L. N.; Turner, J.V.; Pan Xin-Fu *Tetrahedron Lett.* **1985**, *26*, 5725-5728.
20. *Dimethyl* ent-*2α,13-dihydroxy-20-oxo-gibberell-16-en-7,19-dioate* (**6**, GA_{99} dimethyl ester): ^{1}H NMR (300 MHz, $CDCl_3$) δ1.17 (3H, s, H-18), 2.31 (1H, d, J = 12.5 Hz, H-5), 2.40 (1H, ddd, J_{gem} = 12.9 Hz, $J_{2\alpha,3\alpha}$ = 4.4 Hz, $J_{1\alpha,3\alpha}$ = 1.6 Hz, H-3α), 2.62 (1H, ddd, J_{gem} = 12.0 Hz, $J_{1\alpha,2\alpha}$ = 4.7 Hz, $J_{1\alpha,3\alpha}$ = 1.7 Hz, H-1α), 3.65, 3.75 (2x3H, s, CO_2Me), 3.83 (1H, d, J = 12.5 Hz, H-6), 4.00 (1H, m, H-2), 4.95 (1H, br s, H-17), 5.19 (1H, br s, H'-17), 9.66 (1H, s, H-20). MS (EI) *m/z* 374 (M^+-MeOH, 100%), 356 (20), 342 (40), 328 (90), 314 (14), 300 (70), 269 (40), 135 (70), 105 (40), 91 (45). HRMS (EI) *m/z* calcd for M^+-MeOH, $C_{21}H_{26}O_6$: 374.1729; found 374.1729.
21. Seto, H., and Mander L.N. *Syn. Commun.* **1992**, 22, 2823-2828.
22. MacMillan, J; Takahashi, N. **1968** *Nature* 217, 170. Among recently assigned gibberellins, GA_{95} is 1,2-didehydro-GA_{20} (see ref. 7) and GA_{96} is 12β-hydroxy-GA_{73} (Furber, M.; Kraft-Klaunzer, P.; Mander, L. N.; Pour, M.; Yamauchi, T.; Murofushi, N.; Yamane, H.; Schraudolf, H. *Aust. J. Chem.*, **1995**, 48, 427-444). GA_{97} has been assigned to 2β-hydroxy-GA_{53} (**7**) and GA_{98} to 2β-hydroxy-GA_{44} (see ref.23).
23. Mander, L. N.; Owen, D. J.; Croker, S. J.; Gage, D.A.; Gaskin, P. A.; Hedden, P.; Lewis, M. J.; Talon, M.; Zeevaart, J. A. D.; Brenner, M. L.; Sheng, C. to be submitted to *Phytochemistry*).

(*Received in UK* 8 *November* 1995; *accepted* 24 *November* 1995)