Pergamon

Tetrahedron Letters 39 (1998) 3877–3880

TETRAHEDRON LETTERS

Biosynthetic origin of the antheridiogen, gibberellin A_{73} methyl ester, in ferns of the *Lygodium* genus

Graham M. Wynne,[a] Lewis N. Mander,[a]* Nobuyuki Goto,[b] Hisakazu Yamane[b]* and Toshio Omori[b]

[a]Research School of Chemistry, Institute of Advanced Studies, Australian National University, Canberra, ACT, 0200, Australia
[b]The Biotechnology Research Centre, The University of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo, 113, Japan

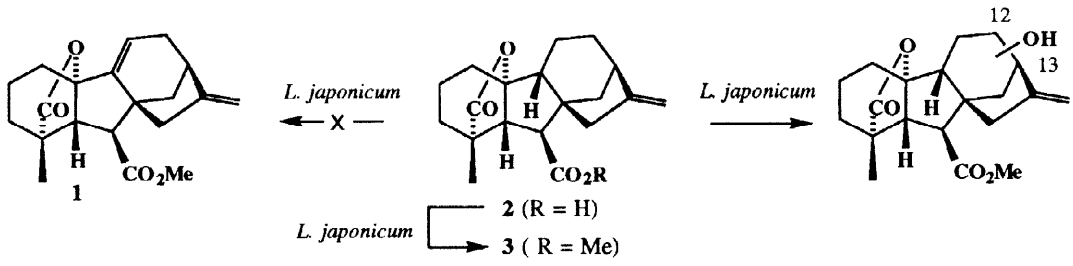
Received 27 February 1998; accepted 17 March 1998

Abstract

9,11-Didehydrogibberellin A_{24} has been prepared from GA_3 and shown to be the biosynthetic precursor of the potent antheridiogen, gibberellin A_{73} methyl ester, in prothallia of the ferns *Lygodium flexuosum* and *Lygodium circinnatum*. © 1998 Elsevier Science Ltd. All rights reserved.

Keywords: Biogenesis; Biologically active compounds; Diazo compounds; Terpenoids.

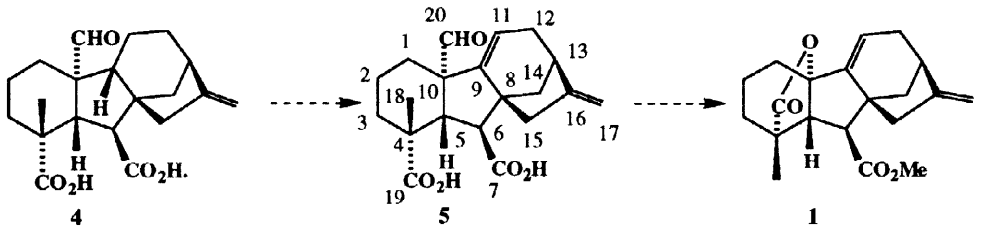
The methyl ester of gibberellin A_{73} ("GA_{73}") (**1**), first isolated in trace quantities (3-4 nanograms per litre of culture filtrate) from prothallia of the fern *Lygodium japonicum* [1] has been shown to be a potent antheridiogen, inducing antheridia in prothallia of *L. japonicum* at femtomolar concentrations [2]. Diene **1** has subsequently been isolated in greater quantities from *L. flexuosum* and *L. circinnatum*, with several hydroxy derivatives also being obtained from the latter species [3]. GA_9 (**2**) would appear to be the most obvious biosynthetic precursor of **1**, but it has not been possible to demonstrate incorporation of isotopically labelled material by prothallia of *L. japonicum* [4,5]. Although methylation of the carboxy group to form **3** occurred, this was then followed by hydroxylation at C(12) or C(13) (Scheme 1). More recently, however, metabolic experiments with labelled precursors have shown that GA_{24} (**4**) is a biosynthetic precursor to **1** in this fern genus [6].



Scheme 1

* Corresponding authors. E-mail: mander@rsc.anu.edu.au; ayamane@hongo.ecc.u-tokyo.ac.jp.

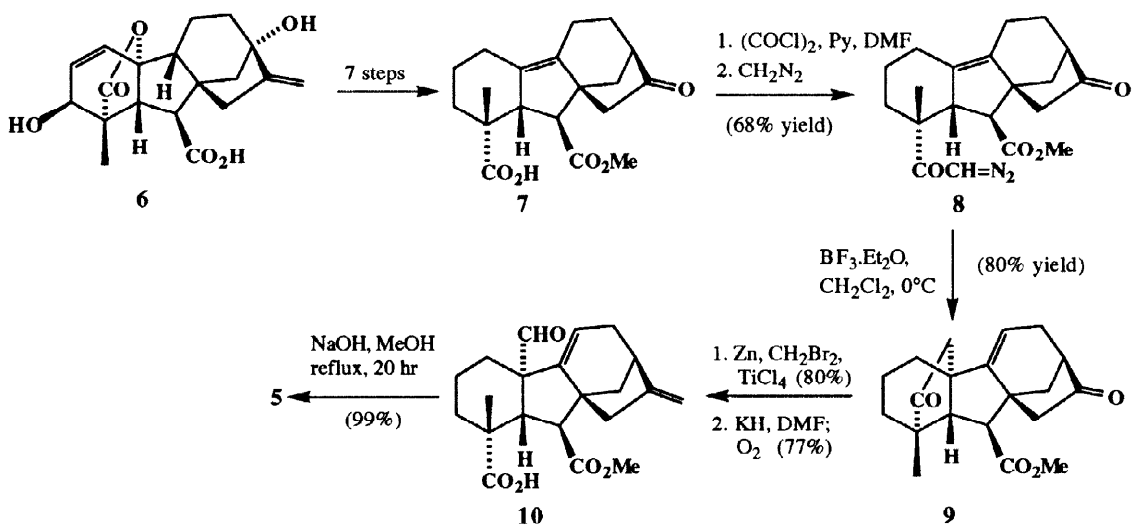
0040-4039/98/$19.00 © 1998 Elsevier Science Ltd. All rights reserved.
PII: S0040-4039(98)00606-6



Scheme 2. Possible biosynthetic pathway to gibberellin A_{73} methyl ester

These results naturally raised the possibility that an intermediate in the formation of **1** might be 9,11-didehydro-GA_{24} (**5**) (Scheme 2). Having recently established a new approach to the synthesis of C_{20}-gibberellins that also introduces a 9(11)-alkene bond, we have tested this hypothesis by preparing a deuterium labelled sample of **5** and feeding it to prothallia of *L. flexuosum* and *L. circinnatum*. The details of the synthesis and incorporation studies are disclosed in this Letter.

Our first approach [7] to the synthesis of C-ring functionalised C_{20}-gibberellins was an amalgam of earlier procedures that had addressed separately the problems of introducing a functional group into the C-ring [8,9] and of incorporating a formyl group at C(10) [10]. The resulting syntheses required *ca* 18 steps, however. Arising out of a search for a more direct approach to this class of gibberellins, we can now report that the Lewis acid-catalysed cyclisation of diazoketone **8** (obtained in 9 steps from GA_3 (**6**) [11]) affords **9** in good yield (Scheme 3), thereby achieving in the one process, the introduction of both the C(20) carbon atom and functionality into the C-ring. After reconstitution of the 17-methylene group by a regioselective Lombardo methylenation [12], oxidative cleavage, as applied to similar compounds [13], gave access to 9,11-didehydro-GA_{24} (**5**).* The overall sequence required only 13 steps and was readily modified to allow preparation [12] of [17,17-$^2H_2$]-**5**.

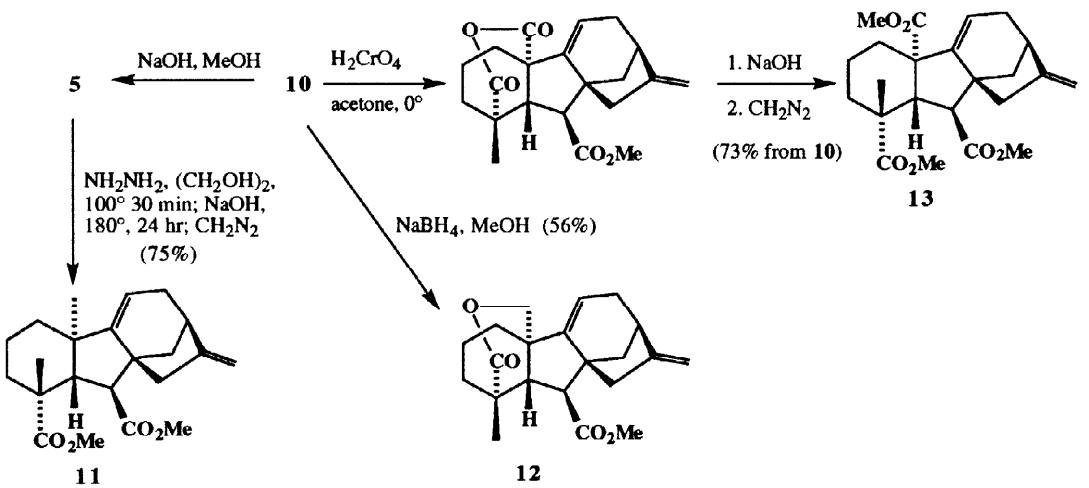


Scheme 3. Synthesis of 9,11-didehydrogibberellin A_{24} (5).

*^{1}H NMR (dimethyl ester) (300 MHz, $CDCl_3$): δ1.21 (3H, s, 4-Me), 2.20 (1H, dt, J = 15.9, 2.7 Hz, H-15), 2.39 (1H, d, J = 12.5 Hz, H-5), 2.54 (1H, br d, J = 15.0 Hz, H-15), 3.66 (3H, s, OMe), 3.75 (1H, d, J = 12.5 Hz, H-6), 3.75 (3H, s, OMe), 4.87 (1H, br s, H-17), 4.96 (1H, br s, H'-17), 5.31 (1H, dd, J = 3.0, 3.2 Hz, H-11). ^{13}C NMR (75.5 MHz, $CDCl_3$) 21.5 (C-2), 29.0 (C-18), 32.0 (C-1), 38.3, 38.5, 40.1 (C-3, C-14, C-15), 41.8 (C-13), 45.4, 46.2 (C-4, C-8), 49.5 (C-6), 52.3 (2xCO_2**Me**), 55.1 (C-5), 60.9 (C-10), 107.6 (C-17), 123.6 (C-11), 146.8 (C-9), 155.3 (C-16), 174.4 (CO_2Me), 177.2 (CO_2Me), 195.8 (C-20).

To investigate the biosynthetic pathway leading to the methyl ester of GA_{73} (**1**), the candidate precursor, [17,17-$^2H_2$]-9,11-didehydro-GA_{24}, was added to 5-week-old prothallia of *L. circinnatum* and *L. flexuosum*, respectively, and the cultures maintained for a further 10 days. In these experiments,† as in the case of our previous study [6], the prothallia were cultured in the presence of 1μg/mL of uniconazole-P, an inhibitor of GA biosynthesis [14] used to reduce the levels of native gibberellin-derived antheridiogens and to promote the metabolism of the fed substrate [15]. [$^2H_2$]-GA_{73} methyl ester was identified as a major metabolite in both *L. flexuosum* and *L. circinnatum* by full-scan GC-MS, no other metabolites being detected in either species. Thus, these results, together with the earlier experiments [6], provide strong circumstantial evidence that GA_{73}-Me is formed from GA_{24} *via* 9,11-didehydro-GA_{24} (**5**) in these *Lygodium* ferns. In addition, the absence of [17,17-$^2H_2$]-9,11-didehydro-GA_{24} methyl ester from the culture medium, provides support for our earlier conclusion [6], that the C-7 carboxy group was methylated after conversion of GA_{24} (**4**) into GA_{73} (**1**).

To date, three biosynthetic pathways have been established from C_{20}-GAs to C_{19}-GAs in higher plants, *i.e.* "non-early 3β,13-hydroxylation","early 3β-hydroxylation" and "early 13-hydroxylation" [16]. The results from the present study indicate a sub-branch in the non-3β,13-early hydroxylation pathway that operates in these fern gametophytes. It may possibly occur in higher plants as well, given the isolation of GA_{73} and its 3β-hydroxy derivative (GA_{88}) from developing apple seeds [17,18]. A search for further 9,11-didehydro-C_{20}-GAs appears to be warranted, and so we have prepared several analogues of **5**, namely **11**, **12** and



Scheme 4. Synthesis of 9,11-didehydrogibberellin A_{12}, A_{15} and A_{25} methyl esters

†Spores of *L. flexuosum* (20 mg) and *L. circinnatum* (8 mg) were aseptically inoculated and cultured for 5 weeks on 20 and 8 Petri dishes (3 cm diameter), respectively, each containing 6 mL of 1/10 strength Murashige and Skoog's mineral salts [19] medium containing uniconazole-P (1μg/mL) solidified with 0.5 agar, at 25°C under continuous white light (5W.m^{-2}). Ten μg of [17,17-$^2H_2$]-9,11-didehydro-GA_{24} in MeOH (5μL) was aseptically added to a 50 mL conical flask containing 10 mL of the same medium as above, but without agar. To each conical flask, the 5-week-old prothallia collected from 4 Petri dishes were transferred and incubated at 25°C under continuous white light (5Wm^{-2}). After 10 days the medium was separated from the prothallia by filtration. The filtrate was adjusted to pH 3 with 1M HCl and extracted with EtOAc (3x). The combined EtOAc phase was dried over Na_2SO_4 and evaporated to dryness. The residue was dissolved in EtOAc (1 mL) and passed through a short column containing 0.5 g of silica gel. The column was eluted with EtOAc (3x1ml), then the combined eluate evaporated to dryness. The residue was trimethylsilylated with MSTFA at 70°C for 20 min and analyzed by GC-MS as described previously [6]. Mass spectral data:
$^2H_2$-GA_{73}-Me (*L. flexuosum*): 330 (M^+; 1%), 299 (5), 286 (84), 271 (12), 241 (23), 227 (100), 183 (58); KRI: 2324;
$^2H_2$-GA_{73}-Me (*L. circinnatum*): 330 (M^+; 1%), 299 (5), 286 (85), 271 (12), 241 (24), 227 (100), 183 (65); KRI: 2326.
GA_{73}-Me (*reference spectrum*) [3]: 328 (M^+; 1%), 297 (14), 284 (100), 269 (17), 241 (21), 225 (95), 183 (29); KRI: 2320.

13 (Scheme 4), in order to assist these future investigations. Thus, the 10α-formyl group in 9,11-didehydro-GA_{24} was deoxygenated by means of a Wolff-Kishner reduction, using a procedure developed for similar GAs [20]. The GA_{12} analogue **11** was obtained subsequently after methylation with diazomethane. Reduction of the half ester **10** with sodium borohydride furnished the GA_{15} derivative **12**, while Jones' oxidation [21] of **10** gave rise to the 19,20-anhydride, from which the methyl ester of 9,11-didehydro-GA_{25} **13** was formed after hydrolysis and methylation.

Acknowledgements.

The authors are indebted to Bruce Twitchin for technical assistance, and to Heinar Streimann (Australian National Botanic Gardens) and Professor Helmut Schraudolf (University of Ulm) for the collection of *Lygodium* spore.

References

[1] Yamane H, Satoh Y, Nohara K, Nakayama M, Murofushi N, Takahashi N, Takeno K, Furuya M, Furber M, Mander LN. Tetrahedron Lett. 1988; 29: 3959.

[2] Takeno K, Yamane H, Yamauchi T, Takahashi N, Furber M, Mander LN. Plant Cell Physiol. 1989; 30: 201-205.

[3] Yamauchi T, Oyama N, Yamane H, Murofushi N, Schraudolf H, Pour M, Furber M, Mander LN. Plant Physiol. 1996; 111: 741-745.

[4] Yamane H, Takahashi N, Takeno K, Furuya M. Planta, 1979; 147: 251-256.

[5] Sato Y, Yamane H, Kobayashi M, Yamaguchi I, Takahashi N. Agric. Biol. Chem. 1985; 49: 255-258.

[6] Yamauchi T, Oyama N, Yamane H, Murofushi N, Schraudolf H, Pour M, Mander LN, Seto H. Plant Physiol. 1997; 113: 773-778.

[7] Mander LN, Owen, DJ. Tetrahedron, 1997; 53: 2137-2162.

[8] Chu A, Mander LN. Tetrahedron Lett. 1988; 29: 2727-2730.

[9] Bhaskar KV, Mander LN. Tetrahedron Lett. 1996; 37: 719-722.

[10] Dawe RD, Mander LN, Turner JV. Tetrahedron Lett. 1985; 26: 363-366.

[11] Furber M, Kraft-Klaunzer P, Mander LN, Pour M, Yamauchi T, Murofushi N, Yamane H, Schraudolf H. Aust. J. Chem. 1995; 48: 427-444.

[12] Lombardo L. Tetrahedron Lett. 1982; 23: 4293.

[13] Dawe RD, Mander LN, Turner JV, Xinfu P. Tetrahedron Lett. 1985; 26: 5725-5728.

[14] Izumi K, Kamiya, Y, Sakurai A, Oshio H, Takahashi N. Plant Cell Physiol. 1985; 26: 821-827.

[15] Nakayama M, Yamane H, Murofushi N, Takahashi N, Mander LN, Seto H. J. Plant Growth Regul. 1991; 10: 115-119.

[16] MacMillan J. Natl. Prod. Rep. 1997; 14: 205-308.

[17] Oyama N, Yamauchi T, Yamane H, Murofushi N, Agatsuma M, Pour M, Mander LN, Biosci. Biotech. Biochem. 1996, 60, 305-308.

[18] Hedden P, Hoad GV, Gaskin P, Lewis MJ, Green JR, Furber M, Mander LN. Phytochemistry, 1993; 32: 231-237.

[19] Murashige T, Skoog F. Physiol. Plant. 1962; 15: 473.

[20] Mander LN, Owen DJ, Twitchin B. Aust. J. Chem. 1996; 49: 249-253.

[21] Bowden K, Heilbron IM, Jones ERH, Weedon BCL. J. Chem. Soc. 1946; 39-45.