Conversion of Trachylobanic Acid into Novel Pentacyclic Analogues of Gibberellins by *Gibberella fujikuroi*, Mutant B1-41a

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Summary 12,16-Cyclogibberellins A_9 and A_{12} have been isolated from the microbiological transformation of trachylobanic acid by *Gibberella fujikuroi*, mutant B1-41a, thus providing a notable example of the non-specificity of enzymes, catalysing the biosynthesis of fungal gibberellins; the 12,16-cyclogibberellin A_9 retains gibberellin-like biological activity.

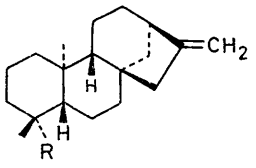
GIBBERELLA fujikuroi 'mutant B1-41a' which is blocked[1] for gibberellin biosynthesis between *ent*-kaurenal (**1**) and *ent*-kaurenoic acid (**2**) has previously been shown[2,3] to metabolise a range of analogues of *ent*-kaurenoic acid (**2**) into the corresponding analogues of the normal fungal gibberellins. This remarkable degree of non-specificity for substrates containing the *ent*-kauranoid skeleton is now

shown to extend to the *ent*-12,16-cyclokaurane (trachylobane) ring system.

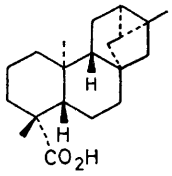
Trachylobanic acid (**3**), isolated[4] from flowers of *Helianthus annuus*, was incubated with pigmented mycelium of *G. fujikuroi* mutant B1-41a, re-suspended in nitrogen-free medium. G.l.c.–m.s. analysis of the acidic metabolites as the Me esters (2% QF-1) and trimethylsilyl derivatives (2% SE-33) indicated the presence, *inter alia*, of the 12,16-cyclo-analogues of GA_4, GA_9 GA_{12}, GA_{13}, GA_{14}, GA_{15}, GA_{24}, GA_{25}, GA_{37}, and GA_{47}.[5] The proportions of these metabolites varied with length of incubation, pH value of the resuspension medium, and the concentration of the substrate. On a preparative scale 12,16-cyclogibberellin A_9 (**4**) (26 mg), m.p. 114—116 °C, 12,16-cyclogibberellin A_{12} (**5**) (35 mg), m.p. 237—240 °C, and a mixture (19 mg) of the 12,16-analogues of gibberellins A_4 and A_{14} were isolated by p.l.c. of the metabolites from a 5-day shake culture of trachylobanic acid (220 mg), distributed between 110 × 100 ml conical flasks, each containing pigmented mycelium of the mutant B1-41a and nitrogen-free medium (25 ml), buffered at pH 7·0.

12,16-Cyclogibberellins A_9 (**4**) and A_{12} (**5**) were characterised by ^1H and ^{13}C n.m.r. spectroscopy. The ^{13}C-spectra showed no olefinic carbon signals; signals at δ 25·6 p.p.m. for compound (**4**) and at δ 25·4 p.p.m. for compound (**5**) were assigned to C-16, the corresponding C-16 signal in trachylobanic acid (**3**) occurring at δ 22·4 p.p.m. (*cf.* ref. 6). The ^1H-n.m.r. spectra of (**4**) and (**5**) contained no vinylic protons, two cyclopropyl proton signal in the range δ 0·5—0·9, two methyl singlets for (**4**), and three methyl singlets for (**5**). In the ^1H spectrum of (**5**) in C_5D_5N, the 6-H doublet occurred at unexpectedly low field (δ 4·05, *J* 13 Hz). This signal also occurred at low field for C_5D_5N solutions of the *ent*-gibberellane-7,19-dioic acids, gibberellin A_{12} (**6**) (δ 4·08), gibberellin A_{13} (**7**) (δ 5·04), and gibberellin A_{14} (**8**) (δ 4·22). The shift to lower field of the 6-protons in C_5D_5N solution, compared to that for the methyl esters in $CDCl_3$ solution, appears to be a useful diagnosis of the presence of a 19-oic acid in *ent*-gibberellanes and *ent*-12,16-cyclogibberellanes.

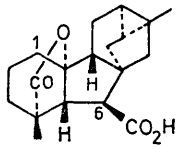
The novel pentacyclic system, as represented by 12,16-cyclogibberellin A_9 (**4**) retains gibberellin-type biological activity in the lettuce hypocotyl, cucumber hypocotyl, and dwarf rice bio-assays. Whether 12,16-cyclogibberellins occur naturally remains to be determined but preliminary studies indicate that they are not present in *H. annuus*.



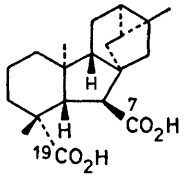
(1) R = CHO
(2) R = CO_2H



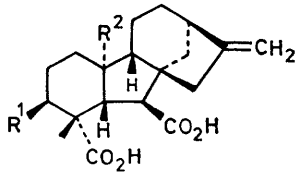
(3)



(4)



(5)



(6) R^1 = H, R^2 = Me
(7) R^1 = OH, R^2 = CO_2H
(8) R^1 = OH, R^2 = Me

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