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THE CHEMICAL AND MICROBIOLOGICAL SYNTHESIS OF BEYERGIBBERELLIN A_9

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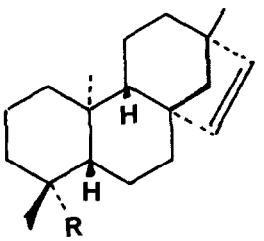
Abstract: Beyergibberellin A_9 has been prepared by the microbiological transformation of the parent hydrocarbon,ent-beyer-15-ene,with Gibberella fujikuroi revealing the lack of substrate:specificity in the latter and in confirmation of its structure, the beyergibberellin has also been obtained by chemical synthesis from methyl gibberellate via an 8,13-isogibberellin.

Amongst tetracyclic diterpenoids, those with the ent-beyer-15-ene carbon skeleton are a large class second in number only to those with an ent-kaurenoid skeleton. Despite the fact that ent-beyer-15-ene diterpenoids are known to occur with a hydroxylation pattern reminiscent of gibberellin biosynthetic intermediates, no beyergibberellins - i.e. gibberellin plant hormones with the beyerene arrangement of rings C and D - have hitherto been isolated. The biosynthesis of the gibberellins has been extensively studied but there is no evidence to preclude the formation of these compounds. Consequently we have prepared and incubated the parent hydrocarbon, ent-beyer-15-ene (1)[1] with Gibberella fujikuroi to see if it can be metabolized along the gibberellin pathway. Previous studies have shown that a mutant of Gibberella fujikuroi can accept the 19-carboxylic acid, isosteviol,[2] whilst we have shown that the wild-type fungus will metabolize substrates with the trachylobane[3] and atiserene[4] carbon skeleton to afford the corresponding trachyloba- and atisa-gibberellins.

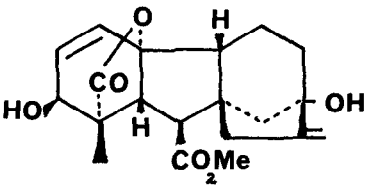
The detection of the metabolites of artificial substrates in Gibberella fujikuroi is facilitated by carrying out the incubations in the presence of AMO-1618 which blocks the formation of endogenous ent-kaur-16-ene[5] and hence the biosynthesis of the natural tetracyclic diterpenoid metabolites. Both ent-beyer-15-ene (1) and ent-beyer-15-en-19-ol (2)[1,6] were examined as artificial substrates in shake cultures of Gibberella fujikuroi grown in the presence of the inhibitor. The fermentations were harvested after 5 - 6 days and the metabolites were isolated. The acidic fractions were methylated with diazomethane and the methyl esters separated by chromatography on silica gel. The major product in both cases was identified as the methyl ester of beyergibberellin A_9 (10) (Found: 330.1831, $C_{20}H_{26}O_4$ requires M^+ 330.1831), δ_{CDCl_3} 1.08 (3H,s,19-H), 1.17 (3H,s,17-H), 2.46 (1H,d, J 7 Hz, 5-H), 2.78 (1H,d, J 7 Hz, 6-H), 3.69 (3H,s, OMe), 5.5 (2H,s, 15- and 16-H). A number of other beyergibberellin metabolites were obtained in smaller amounts.

The structure of the major metabolite was confirmed by the partial synthesis of its methyl ester from methyl gibberellate (3). Methyl gibberellate was converted[7] to gibberellin A_1 methyl ester and thence by rearrangement with trifluoroacetic acid to the 8,13-isogibberellin (4).[8] The use of trifluoroacetic acid for this step avoids epimerization at C-9. The 3-desoxy compound (11) was prepared in a similar manner via gibberellin A_{20} methyl ester. The ring A hydroxyl group in (4) was protected as the trimethylsilylethoxymethyl ether whilst the 16-carbonyl group was reduced with sodium borohydride to the 16α-alcohol (5). The latter was then converted to the 16α-toluene-p-sulphonate (6). Elimination with collidine gave a mixture. The protecting group was removed with fluoride ion and the ring A hydroxyl group was then acetylated to afford a mixture of acetates. These were separated by careful chromatography on $AgNO_3$:SiO_2 into the 3-acetate of beyergibberellin A_4 methyl ester (7) and the acetate of gibberellin A_4 methyl ester together with its endocyclic isomer. The 3-acetate of beyergibberellin A_4 methyl ester (7) was hydrolysed to afford the methyl ester of beyergibberellin A_4 (8). The hydrolysis was accompanied by some epimerization at C-3 to afford (9). Beyergibberellin A_9 methyl ester (10), identical to the material isolated from the fermentations, was obtained from the



1 R = Me

2 R = CH_2OH



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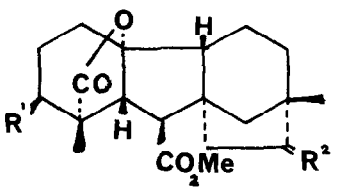
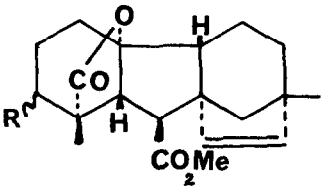


4 R^1 = OH; R^2 = =O.

5 R^1 = OSEM; R^2 = α-OH,β-H.

6 R^1 = OSEM; R^2 = α-OTs,β-H

11 R^1 = H; R^2 = =O.



7 R = β-OAc

8 R = β-OH

9 R = α-OH

10 R = H

SEM = $(CH_3)_3SiCH_2CH_2OCH_2$-

3-epimer (9) by reduction of its thiocarbonylimidazole derivative with tributyltin hydride. Alternatively the 3-desoxy compound (11)[8] was reduced to the 16α-alcohol. Elimination of the corresponding methanesulphonate or of the alcohol itself on alumina again afforded a mixture from which beyergibberellin A_9 methyl ester (10) could be separated by chromatography on $AgNO_3$:SiO_2. Evidence for the stereochemistry of the 16-alcohol and the elimination reaction will be presented in our full paper.

This work illustrates the ability of Gibberella fujikuroi to metabolize an unnatural hydrocarbon along the complete gibberellin pathway and thus to make available novel gibberellins by 'analogue biosynthesis'. Since beyer-15-enes are quite widespread it suggests that beyergibberellins may eventually be found to occur naturally.

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