An efficient synthesis of (2, 3 and 22, 23)-diepoyxbrassinosteroids and their plant
growth promoting activity†


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A highly efficient synthesis of (2,3 and 22,23)-diepoxybrassinosteroids using molecular oxygen, benzaldehyde and transition metal oxides as catalysts has been achieved and they showed moderate plant growth promoting activity.

Brassinolide 1 and its analogues, collectively known as brassinosteroids2, are a new class of steroidal phytohormones3 with high growth promoting and antistress activity. Brassinosteroids find extensive use in boosting yields2c•2e of several agricultural crops and for this, most synthetic efforts are focused on generating new analogues and study their efficacy in the fields. It has been reported recently that brassinosteroids with an epoxy function at 2, 23 [both (22R, 23R) and (22S, 23S)-epoxides] and 2α, 3α-dihydroxy functionality, protected as acetates in compounds 2 and 3 have growth promoting effect5 on the first internode, as well as the second internode of young soybean plant. 

Brassinolide 1, effectively promoted the elongation of the first internode but failed5 to elongate the second internode. Again, compound 4, with (22R, 23R)-epoxide and the 2, 3-dihydroxy group protected as 2, 3-isopropylidenedioxy, is completely inactive under standard brassinosteroids bioassay systems but, under field conditions6, a greater increase in yield was obtained as compared with brassinolide 1. This suggests that the epoxy function in the side chain may be the biosynthetic precursor of the corresponding diol and 2, 3-diacetoxy or 2, 3-isopropylidenedioxy functionality got change into an active diol, slowly in the plant body under field trial conditions. This also explain longer-lasting biological activity of compound 4 as compared with 1, which gets deactivated within a few days.

Isolation and characterization of secasterone 5 [(22R, 23R, 24S)-2β, 3]-epoxy-22, 23-dihydroxy-24-methyl-5α-cholestan-6-one7 (Figure 1) represents the first naturally occurring brassinosteroid with a 2, 3-epoxy function. Synthesis and biological activity5 of the epimeric brassinosteroids 6, 7 and 8 with a 2, 3-epoxy function related to castasterone and 24-epi-castasterone have also been documented. The bioactivity of these 2, 3-epoxy brassinosteroids may be due to an in vivo biotransformation in the plant material leading to active ring A hydroxylated brassinosteroids. Synthesis of 2α, 3α-epoxy-5α-spirostan-6, 23-dione9 and spirostan-12, 23-dione10 which combine some of the structural features present in certain naturally occurring brassinosteroids have also been very recently documented.

With these points in view we would like to report here a highly efficient synthesis of brassinosteroids 10 and 11 with epoxy functions at 2, 3 and 22, 23 positions and their plant growth promoting activity under actual field cultivation conditions.

The known11 (22E)-stigmasta-2, 22-dien-6-one 9 was synthesised from stigmasterol12 in four steps with an increased overall yield of 63% (Scheme I). Bubbling oxygen to a solution of freshly distilled benzaldehyde in 1, 2-dichloroethane containing catalytic amount of Fe2O3, CoO3 or MnO2 at 30°C for 5 hr to 18 hr (Table 1), the formation of peroxoenoic acid is complete13. To this preformed peroxoenoic acid, dienone 9 in 1, 2-dichloroethane was added and the reaction mixture was stirred at 30°C for a period of 4 days to afford the diepoxyalactone 12 (26%) along with a mixture of diepoxyketones 10 and 11 (42%). Carrying out the reaction at 30°C for longer time or at a higher temperature or using excess of reagent the yield of the diepoxyalactone 12 could not be increased. On the
Reagents and conditions: (a) Benzaldehyde, Dichloroethane, Catalyst (Table 1), Molecular Oxygen, 30°C, Stirring (Table 1), Compound 9, Stirring (Table 1) or 4 days for compound 12

Scheme 1

contrary the yields of these products decreased and the formation of more polar products arising from the opening of the A ring 2α, 3α-epoxide were observed. Epoxidation of alkenes and Baeyer-Villiger oxidation of ketones with molecular oxygen, aldehydes and transition metal oxides as catalyst have been well documented in the literature. One-step epoxidation of (22E)-olefin and Baeyer-Villiger
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Table I—Synthesis of diepoxyketones 10 and 11

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>Time required for the formation of peroxybenzoic acid</th>
<th>Reaction time</th>
<th>Yield of 10 + 11</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Fe2O3</td>
<td>1 : 6 : 0.3, Catalyst 20h 9</td>
<td>24h</td>
<td>68%</td>
</tr>
<tr>
<td>2</td>
<td>Fe2O3</td>
<td>1 : 10 : 0.3, Catalyst 20h 10</td>
<td>24h</td>
<td>52%</td>
</tr>
<tr>
<td>3</td>
<td>CoO3</td>
<td>1 : 9 : 0.3, Catalyst 5h 11</td>
<td>18h</td>
<td>90%</td>
</tr>
<tr>
<td>4</td>
<td>MnO2</td>
<td>1 : 6 : 0.3, Catalyst 5h 11</td>
<td>18h</td>
<td>99%</td>
</tr>
</tbody>
</table>

Table II—Effect of synthetic brassinosteroids 10 and 11 on the yield of tomato (marketable fruits) under field conditions, season 1999/2000

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>Number of fruits per plant</th>
<th>Average fruit weight (g)</th>
<th>Fruit weight per plant (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (water spray)</td>
<td>15.83</td>
<td>60.5</td>
<td>958</td>
</tr>
<tr>
<td>0.5 ppm (22S,23S)-28-homobrassinolide</td>
<td>16.17</td>
<td>80.4</td>
<td>1300</td>
</tr>
<tr>
<td>0.5 ppm compound 10</td>
<td>16.21</td>
<td>70.3</td>
<td>1140</td>
</tr>
<tr>
<td>0.5 ppm compound 11</td>
<td>17.17</td>
<td>60.06</td>
<td>1041</td>
</tr>
</tbody>
</table>

*Each compound was sprayed twice i.e. 44 days after transplanting (flowering stage) and 54 days after transplanting (fruit setting stage).

Experimental Section

Oxidation of B-ring ketone of (22E)-2α, 3α-diacetoxy-5α-stigmasta-22-en-6-one with trifluoroperoxy acetic acid in good yield has been reported. Adding a solution of 2, 22-dien-6-one 9 in 1, 2-dichloroethane to a preformed peroxybenzoic acid using MnO2 as a catalyst (Table I, entry 4), stirring the reaction mixture for 18 hr at 30°C, an excellent yield (99%) of the diepoxyketones 10 and 11 have been realised. The diepoxyketones 10 and 11 have very close Rf values and we failed to separate them by flash column chromatography. However, they could be separated readily by preparative HPLC. The (22R, 23R)-epoxide 10 and (22S, 23S)-epoxide 11 are formed in the ratio 73 : 27.

The plant growth promoting activity of compounds 10 and 11 was evaluated in comparison with (22S, 23S)-28-homobrassinolide, by the foliage treatment of tomato plant under field conditions. Compound 10 was found to be more active than compound 11. The results are summarised in Table II.

In conclusion a highly efficient synthesis of (2,3 and 22,23)-diepoxybrassinosteroids has been achieved which has the potential to be used for the enhancement of yields of a variety of agricultural crops.

General Procedure

In a 100 mL two necked round bottom flask fitted with a bubbler and condenser, a solution of benzaldehyde (0.616 g, 6 mmole) in 1,2-dichloroethane (40 mL) was stirred at 40°C. To it, MnO2 (0.026 g, 0.3 mmol) was added and oxygen was
bubbled through the solution till peroxycbenzoic acid formation was complete (5 hr). A solution of compound 9 (0.410 g, 1 mmol) in 1,2-dichloroethane (12 mL) was added to the reaction mixture and stirring was continued at 40° C with passage of oxygen for 18 hr. To the reaction mixture, 20% sodium metabisulphite solution (10 mL) was added, stirred for 10 min, it was then filtered through celite and the filtrate was extracted with dichloromethane (3 x 50 mL). The organic extract was washed with water (2 x 25 mL), aqueous sodium bicarbonate (2 x 25 mL), water (3 x 25 mL) and brine (2 x 25 mL). Dichloromethane extract was dried over anhyd. sodium sulphate and the crude product was isolated by removal of solvent. This was purified by flash column chromatography using silica gel to furnish pure product (0.438 g, 99%) which was found to be a mixture of compounds 10 and 11. These two compounds were separated by preparative HPLC using gas pressure 61 Kg and flow rate 1.5 mL/min. Retention time for compound 10 was 7.49 min. while that of compound 11 was 9.06 min.

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(22R,23R)-2a,3a,22,23-Diepoxy-5\alpha\text{-stigmast-6-one} 10: \text{m.p. 143-45° C (diethyl ether-hexane); IR : 1703} \ (\text{C}=0), 1257, 1093, 1022, 904, 804, 727, 607, 472 \ cm^{-1} ; \ ^1\text{HNMR (200 MHz, CDCl}_3\text{: } \delta = 0.64 \ (s, 3H, 18-CH}_3), 0.69 \ (s, 3H, 19-CH}_3), 0.89-1.00 \ (3d, Jt, 12H, 26, 27, 29 and 21-CH}_3), 2.33 \ (m, 2H, 5-CH and 7-CH), 2.47 \ (m, 1H, 23-CH), 2.72 \ (m, 1H, 22-CH), 3.10 \ (m, 1H, 2-CH), 3.26 \ (m, 1H, 3-CH) ; \ ^{13}\text{CNMR (75 MHz, CDCl}_3\text{:吻 = 0.7, CHCl}_3) ; \ MS m/z : 442 (M^+, 6%), 427 (2%), 411 (2%), 400 (1%), 358 (100%), 340 (5%), 329 (8%), 313 (35%), 295 (27%), 285 (25%), 271 (14%), 259 (11%), 245 (8%), 227 (16%), 213 (9%), 201 (9%), 187 (15%), 173 (33%), 159 (23%), 147 (28%), 135 (32%), 121 (32%), 109 (49%), 95 (26%). \text{Anal. Calcd. for } C_{29}H_{46}O_3 : \ C, 78.68; \ H, 10.47. \text{Found: } C, 78.51; \ H, 10.75%.
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Acknowledgement

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References


4 Godrej Agrovet Ltd. Mumbai, India, has commercialized (22S,23S)-homobrassinolide for the enhancement of yields of paddy, wheat, tomato, cabbage, cauliflower, potato, groundnut (peanut), cotton, grapes and tea.


7 Scimidi J, Spengler B, Yokota T, Nakayama M, Takatsuto S,
13 Quantitative formation of peroxybenzoic acid was found on iodometric titration of an aliquot of the solution.