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LAST STAGES IN THE BIOSYNTHESIS OF ANTIRRHINOSIDE*

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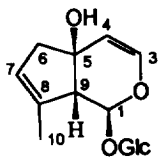
Abstract—Feeding experiments with ^{2}H-labelled precursors have now shown that the last steps in the biosynthesis of antirrhinoside in *Antirrhinum majus* involve an initial hydroxylation of the 6-position of 6,10-dideoxyaucubin to give linaride (10-deoxyaucubin), followed by epoxidation to give 10-deoxycatalpol (5-deoxyantirrhinoside) and finally hydroxylation of the 5-position to give antirrhinoside. 10-Deoxycatalpol was prepared by epoxidation of 10-deoxyaucubin with H_2O_2/WO_3. Additionally, the iridoid content of two other species of *Antirrhinum*, namely *A. speciosum* and *A. sicculum* was investigated. In the first of these 10-deoxcatalpol was isolated for the first time from a plant together with antirrhinoside, linaride (10-deoxyaucubin) and macfadienoside. *Antirrhinum sicculum* contained 5-glucosyl antirrhinoside as the main iridoid together with antirrhinoside and macfadienoside.

INTRODUCTION

Antirrhinoside (**1**) is an iridoid glucoside consistently found in plants belonging to the Scrophularioideae–Antirrhineae [1] and it is the main iridoid in *Antirrhinum majus* [2]. We have earlier investigated the biosynthesis of this compound in some detail [2–5] and have shown it to proceed via 8-epi-deoxyloganic acid (**2**), deoxygeniposidic acid (**3**) and 6,10-dideoxyaucubin (**4**). The present paper deals with the last steps in the biosynthetic sequence. The conversion of **4**, the last known precursor, to **1** requires oxidation at (i) C-5, (ii) C-6 and (iii) the 7,8 double bond, although not necessarily in this sequence. However, the most likely sequence of these oxidations is (ii), (iii) and (i) (i.e. giving the sequence of intermediates **4** → **5** → **6** → **1** for the following reasons: the first intermediate, linaride (10-deoxyaucubin; **5**), is known from the genus *Linaria* [6], which is closely related to *Antirrhinum*, whereas the two alternatives, **7** and **8** have not have been described from plants. Assuming **5** to be the first intermediate, the next step in the biosynthesis of antirrhinoside (**1**) might be either an epoxidation or a hydroxylation of C-5. We have earlier shown [7] that the hydroxylation of C-5 is the last step in the biosynthesis of ipolamiide (**9**). Thus 10-deoxycatalpol (**6**) is the most likely intermediate. At the outset of this work, **6** was not known. Recently, however, an ester of **6** was reported from *Linaria japonica* [8] and when investigating two other species of *Antirrhinum* we found 10-deoxycatalpol (**6**) as a minor constituent of *A. speciosum*. The iridoid content of the two plants is also reported in this paper.



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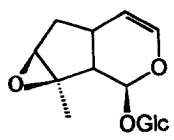

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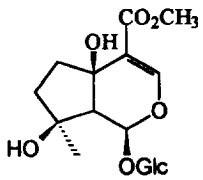

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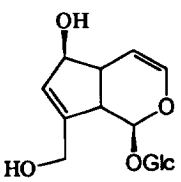

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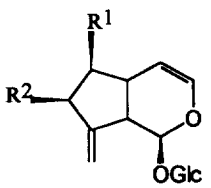
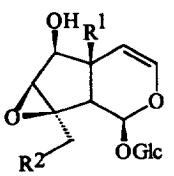
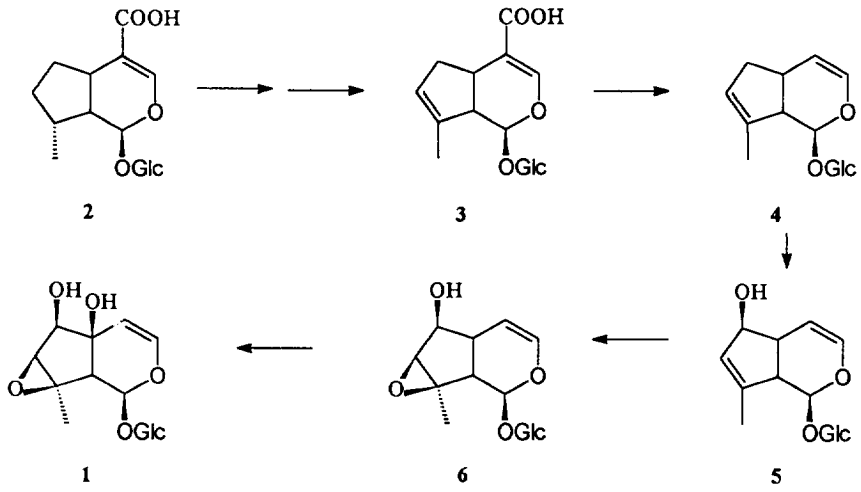


11 R^1=OH; R^2=H
12 R^1=H; R^2=OH



13 R^1=OGlc; R^2=H
14 R^1=OH; R^2=OH
15 R^1=OGlc; R^2=OH
16 R^1=H; R^2=OH

*In honour of Professor Horst Rimpler's sixtieth birthday.



Scheme 1. Last steps in the biosynthesis of antirrhinoside (**1**).

RESULTS AND DISCUSSION

Deuterium-labelled 10-deoxyaucubin pentaacetate (*d*-**5a**) was prepared in 23% yield from aucubin hexaacetate (**10a**) by catalytic transfer deuteration with DCOOD and Pd/C [9]. Additionally, 6,10-dideoxyaucubin tetraacetate (**4a**, 28%),linarioloside pentaacetate (**11a**, 5%) and unconverted starting material (**10a**, 20%) was isolated. Deacetylation of *d*-**5a** with methoxide gave *d*-**5**. Reduction of **10** with Li in liquid NH_3 containing ethanol-*d* gives a higher chemical yield of **5** [10], but the incorporation of deuterium (< 5%) was, owing to exchange with the NH_3, too low for use in biosynthetic experiments.

10-Deoxycatalpol (**6**) was prepared from 10-deoxyaucubin (**5**) by epoxidation with hydrogen peroxide and wolfram trioxide [11–13] after epoxidation of 10-deoxyaucubin pentaacetate (**5a**) with metachloroperbenzoic acid had failed. The best yield obtained was 25% (7% of the starting material was recovered). The low yield may be explained by non-specific oxidation of the iridoid by the peroxide and may also be due to degradation catalysed by the strong acids tungstic and pertungstic acid [12]. Sufficient material for the biosynthetic experiments could, however, easily be prepared. Both the ^2H-labelled precursors *d*-**5** and *d*-**6** were efficiently incorporated into **1** in *Antirrhinum majus*: 10-deoxyaucubin (**5**) gave a 24% incorporation and 10-deoxycatalpol (**6**) gave a 26% incorporation strongly indicating that they were intermediates in the biosynthesis of antirrhinoside (**1**). The final proof was obtained by feeding a large amount of ^2H-labelled 6,10-dideoxyaucubin (*d*-**4**) to *A. majus* and isolating the metabolites after a short period of time. Following this protocol, ^2H-labelled **5** and **6** could be isolated from the feeding experiment showing that these compounds had been formed from labelled **4**. As 6,10-dideoxyaucubin (**4**) has been shown to be an intermediate in the biosynthesis of **1** [5], we have thus proved that the subsequent steps are hydroxylation of the 6-position to give **5** followed by epoxidation to **6** and that the 5-hydroxy-group is the last to be introduced in the antirrhinoside (**1**) molecule.

Concurrently with the biosynthesis experiments, the iridoid content of *A. speciosum* and *A. sicculum* were investigated. Only three other *Antirrhinum* species have been investigated for iridoids up to now. *Antirrhinum majus* and *A. tortuosum* both contain **1** as the major iridoid [14] together with small amounts of antirrhide (**12**) [15] and 5-glucosylantirrhinoside (**13**) [16], whereas *A. orontium* contains large amounts of macfadienoside (**14**) together with small amounts of calycinoside (= 5-glucosylmacfadienoside, **15**) and aucubin (**10**) [17]. In the present work, **1** was the main iriodid in *A. speciosum* but **14**, linaride (**5**) and 10-deoxycatalpol (**6**) were also isolated, the latter for the first time from a natural source. The identity of **6** was confirmed by comparison (^1H and ^{13}C NMR) with the synthetic material above and with the published [8] data for **6** obtained by hydrolysis of the bisirioid compound iridolinarin A from *Linaria japonica*. The finding of **5** and **6** together with antirrhinoside (**1**) further underlines the biosynthetic pathway **5** → **6** → **1**. That macfadienoside (**14**) is also present in this plant indicates that this compound is formed by hydroxylation of **1** in *Antirrhinum*, particularly when catalpol (**16**) has not been detected in the genus. The last plant investigated, *A. sicculum*, contained 5-glucosylantirrhinoside (**13**) as the major iridoid together with **1** and **14**.

EXPERIMENTAL

Preparative chromatography: Merck Lobar reverse phase columns (C_{18}) eluted with H_2O–MeOH mixts as specified. Peaks were detected by UV simultaneously at 206 and 254 nm. ^2H NMR: 76.8 MHz at 300 K in H_2O with 0.016%. ^2H of natural abundance as int. standard. *Antirrhinum majus*, *A. speciosum* (IOK 18-94) and *A. sicculum* (IOK 17-94) were obtained from the experimental station of the Botanical Garden of Copenhagen in Tåstrup near Copenhagen. Voucher specimens are deposited at the Botanical Museum, The University of Copenhagen.

Deuterated 10-*deoxyaucubin* (d-**5**). Aucubin hexaacetate (**10a**, 2.65 g) was dissolved in dioxane (65 ml). After

flushing with N_2, the soln was heated to 98°. Pd–C (5%; 650 mg) was added together with DCO_2D (637 mg, 3 eq). After 45 min the reaction mixt. was cooled to room temp, filtered and the solvent evapd. The product mixt. was treated with NaOMe in MeOH (0.3 M, 70 ml). After 2 hr the soln was neutralized with HOAc and the solvent evapd. The crude iridoid mixt. was applied to a reverse phase C-column and chromatographed with H_2O–MeOH (25:1 → 1:1) giving aucubin (**10**, 307 mg, 20%), *d*-**5** (336 mg, 23%), *d*-**11** (73 mg, 5%) and *d*-**4** (389 mg, 28%). Both *d*-**5** and *d*-**4** contained 0.23 ^2H in the 10-position as calculated from the 500 MHz ^1H NMR spectra.

Deuterated 10-*deoxycatalpol* (*d*-**6**). Deuterated 10-deoxyaucubin from above (*d*-**5**, 120 mg) was dissolved in H_2O (10 ml) and WO_3 40 mg and aq. H_2O_2 (30%; 0.7 g) was added. The suspension was stirred for 22 hr after which time activated C (0.7 g) was added to absorb the iridoids. The carbon was washed with H_2O (50 ml) which was discarded and then with MeOH (50 ml). The MeOH-fraction was evapd and applied to a B-column. Chromatography with H_2O–MeOH (25:1) gave *d*-**6** (31.5 mg, 25%) with 0.23 ^2H in the 10-position.

General procedure for the administration of labelled precursors and for the isolation of iridoids. The experiments were carried out as described earlier [4]. The crude glucoside fraction was applied to a B-column and antirrhinoside (**1**) was eluted with H_2O–MeOH (15:1). It was contaminated with a small amount of antirrhide (**12**). As this compound does not interfere in the ^2H NMR spectra, further purification was omitted. The incorporations were calculated as described previously [4].

Feeding experiment with a small amount of d-**4**. The precursor (16.5 mg) was fed to 5 g of plant. After 90 hr, work-up gave **1** (130 mg) with an incorporation of 26% This served as a control experiment.

Feeding experiment with d-**5**. The precursor (16.5 mg) was fed to 7.5 g plant. After 140 hr, work-up gave **1** (140 mg) with an incorporation of 24%.

Feeding experiment with d-**6**. The precursor (16.5 mg) was fed to 5.1 g plant. After 144 hr, work-up gave **1** (134 mg) with an incorporation of 26%.

Dilution experiment. Three small shoots of *A. majus* (5.2 g) were immersed in an aq. soln (1.0 ml) of *d*-**4** (137 mg). After 4 hr two thirds of the soln had been taken up by the plants. Unlabelled **6** (10.0 mg) in H_2O (0.5 ml) was added, the plants were left for 2.5 hr more, by which time all of the soln had been absorbed and subsequently worked-up. Additional unlabelled **6** (10 mg) was added as a carrier. The crude glucoside fraction was applied to a size C-column and eluted with H_2O–MeOH (25:1–2:1). Compound **6** (16 mg; incorporation 1.2%) was eluted with 25:1, followed by **1** (70 mg; incorporation 1.6%) with 15:1, **5** (*ca* 1 mg; incorporation 1.4%) with 3:1, and recovered **4** (89 mg, 66% of applied material) with 2:1. Compound **6** was contaminated with a small amount of a deuterated impurity. This was removed by addition of unlabelled **6** (40 mg) and rechromatography on a size C-column.

Antirrhinum speciosum. Fresh aerial parts of the plant (275 g) were homogenized with EtOH and the concd extract partitioned in Et_2O–H_2O. Evapn of the aq. phase followed by treatment with a small amount of activated C gave a crude extract (10 g). A part of this (4 g) was applied to a size C-column. Elution with H_2O–MeOH (25:1 → 2:1) gave three fractions. The first fraction (A,180 mg) contained a mixture of three compounds, the second antirrhinoside (**1**, 2.06 g 1.9%) and the third 10-deoxyaucubin (linaride, **5**, 80 mg, 0.07%). Fraction A was rechromatographed on the size C column with H_2O–MeOH (25:1 → 10:1) to give macfadienoside (**14**, 20 mg, 0.02%), 10-deoxycatalpol (**6**, 40 mg, 0.04%) and antirrhinoside (**1**, 70 mg).

Antirrhinum sicculum. Frozen aerial parts of the plant (400 g) were worked-up as above to give a crude extract (14.2 g). A part of this (4.77 g) was applied to a size C-column and eluted with H_2O–MeOH (15:1) to give macfadienoside (**14**, 90 mg, 0.007%), a mixture of antirrhinoside and 5-glucosylantirrhinoside (**1** and **13**, 1:1, 460 mg, 0.34%) and pure 5-glucosylantirrhinoside (**13**, 250 mg, 0.2%).

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REFERENCES

1. Kooiman, P. (1970) *Acta Bot. Neerl.* **19**, 329.
2. Damtoft, S. (1983) *Phytochemistry* **22**, 1929.
3. Damtoft, S., Jensen, S. R. and Nielsen, B. J. (1983) *Biochem. Soc. Trans.* **11**, 593.
4. Breinholt, J., Damtoft, S., Demuth, H., Jensen, S. R. and Nielsen, B. J. (1992) *Phytochemistry* **31**, 795.
5. Damtoft, S., Jensen, S. R. and Jessen, C. U. (1993) *Phytochemistry* **33**, 1087.
6. Bianco, A., Guiso, M., Iavarone, C., Passacantilli, P. and Trogolo, C. (1977) *Gazz. Chim. Ital.* **107**, 83.
7. Damtoft, S., Jensen, S. R., Nielsen, B. J. and Thorsen, J. (1992) *Phytochemistry* **31**, 3839.
8. Otsuka, H. (1994) *J. Nat. Prod.* **57**, 357.
9. Jensen, S. R., Kirk, O. and Nielsen, B. J. (1989) *Phytochemistry* **28**, 97.
10. Birch, A. J., Grimshaw, J. and Juneja, H. R. (1961) *J. Chem. Soc* 5194.
11. Payne, G. B., Smith, C. W. (1957) *J. Org. Chem.* **22**, 1682.
12. Prat, D. and Lett, R. (1986) *Tetrahedron Letters* **27**, 707.
13. Prat, D., Delpech, B. and Lett, R. (1986) *Tetrahedron Letters* **27**, 711.
14. Scarpati, M. L., Guiso, M. and Esposito, P. (1968) *Gazz. Chim. Ital.* **98**, 177.
15. Scarpati, M. L. and Guiso, M. (1969) *Gazz. Chim. Ital.* **99**, 807.
16. Guiso, M. and Scarpati, M. L. (1969) *Gazz. Chim. Ital.* **99**, 800.
17. Bianco, A., Guiso, M., Iavarone, C., Poccia, L. and Trogolo, C. (1979) *Gazz. Chim. Ital.* **109**, 561.