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(2*R*)-CATALPONONE, A BIOSYNTHETIC INTERMEDIATE FOR PRENYLNAPHTHOQUINONE CONGENERS OF THE WOOD OF *CATALPA OVATA**

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Key Word Index—*Catalpa ovata*; Bignoniaceae; catalponone; prenylnaphthoquinones; biosynthesis.

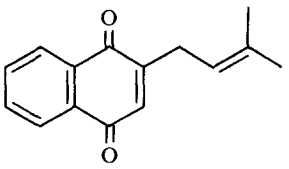
Abstract—In accord with the results of experiments using callus tissues of *Catalpa ovata*, the simultaneous administration of (2*R*)-[1-^{14}C]catalponone and (2*S*)-[8-^{3}H]catalponone to the wood of the same plant demonstrated that the main pathway for the biosynthesis of naphthoquinone congeners, including catalpalactone, catalponol and 4,9-dihydroxy-α-lapachone, proceeds through the 2*R*-enantiomer of catalponone.

INTRODUCTION

In previous publications, we have reported the isolation of prenylnaphthoquinone congeners including catalpalactone (**1**) [1], catalponol (**2**) [2], (2*R*)-catalponone [(2*R*)-**3**], deoxylapachol (**4**) [3] and five derivatives of α-lapachone (**5**) [4] from the wood of *Catalpa ovata* G. Don. More recently, we have detected dehydro-*iso*-α-lapachone (**6**) and four of its derivatives (some of these are found in the fruits of *C. ovata*) and menaquinone-1 (**7**) along with four known α-lapachones, and **1**, **2**, **3** and **5** in the benzene extract of the callus tissues induced from the seedlings of *C. ovata*. In addition, we have also isolated some of the dehydro-*iso*-α-lapachones from callus tissues [5].

Our studies on the biosynthesis of the naphthoquinones in *C. ovata* have shown that [6–9]: (1) the main biosynthetic pathway to **1**, **2** and **5** passes through 4-(2′-carboxyphenyl)-4-oxobutanoic acid (**8**), 2-carboxy-4-oxo-1-tetralone (COT), 2-prenyl-2-carboxy-4-oxo-1-tetralone (**9**) and catalponone (**3**); (2) there also exists a subsidiary route from COT to catalponol (**2**) via 2-carboxy-4-hydroxy-1-tetralone (CHT) and 2-prenyl-2-carboxy-4-hydroxy-1-tetralone (**10**); (3) interconversions between the above-mentioned two routes may be possible. Furthermore, based on the stereochemical examination of the biosynthesis in callus tissues, we demonstrated that both (2*S*)-prenyl-2-carboxy-4-oxo-1-tetralone (**9**) and (2*R*)-catalponone [(2*R*)-**3**] are biosynthetic intermediates for quinones, but their enantiomers are not.

In the present work, we have demonstrated through the simultaneous administration of two differently labelled enantiomers of catalponone (**3**) to the wood of *C. ovata* that (2*R*)-catalponone [(2*R*)-**3**] also plays a key intermediary role in the biosynthesis of the naphthoquinone congeners in the original *Catalpa* plant.



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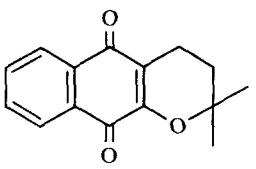

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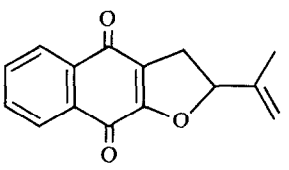

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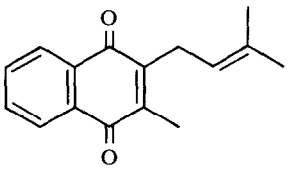

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* Part 15 in the series "Quinones and Related Compounds in Higher Plants". For Part 14 see Inouye, H., Matsumura, H., Kawasaki, M., Inoue, K., Tsukada, M. and Tabata, M. (1981) *Phytochemistry* **20**, 1701.

RESULTS AND DISCUSSION

The ^{14}C- and ^{3}H-labelled enantiomers of catalponone (**3**) were obtained in the following way. [2′-Carboxy-^{14}C]-**8** [10] and [3′-^{3}H]-**8** [11] prepared by the reported

methods were administered separately to branches of *C. ovata* to give 1-^{14}C- and 8-^{3}H-labelled catalponol (**2**), respectively. Jones oxidation of [1-^{14}C]-**2** afforded (2*R*)-[1-^{14}C]catalponone [(2*R*)-**3**], whereas conversion of [8-^{3}H]-**2** to its C-2 epimer through alkaline treatment followed by Jones oxidation gave the enantiomeric (2*S*)-[8-^{3}H]catalponone [(2*S*)-**3**]. A mixture (^{3}H/^{14}C = 5.06) of (2*R*)-[1-^{14}C]catalponone and (2*S*)-[8-^{3}H]-catalponone was administered to a branch of *C. ovata*, and after 10 days catalpalactone (**1**), catalponol (**2**) and 4,9-dihydroxy-α-lapachone (**11**) were isolated. The incorporation data (based on ^{14}C) and the ^{3}H/^{14}C ratios of the purified compounds (Table 1) clearly indicated that (2*R*)-catalponone [(2*R*)-3] was the preferred enantiomer for the biosynthesis of **1**, **2** and **11**, though the incorporation of (2*S*)-**3** was not negligible. This could be explained by isomerization of (2*S*)-**3** to (2*R*)-**3**. In view of the co-occurrence of catalponol (**2**) and epicatalponol (**12**) in a congeneric plant, *Catalpa bignonioides* Walt. (H. Inouye and T. Hayashi, unpublished work), it is conceivable that administered (2*S*)-**3** was metabolized, not only by isomerization to (2*R*)-**3**, but also through reduction to epicatalponol (**12**) followed by isomerization to catalponol (**2**) and further oxidation to (2*R*)-**3**, which finally leads to **1**, **11**, etc. (Scheme 1). In any case, the results unequivocally established that (2*R*)-catalponone [(2*R*)-**3**] is on the main biosynthetic pathway to the quinones.

The recent isolation of (2*R*)-catalponone [(2*R*)-**3**] in very low yield from the wood of *C. ovata* [3], the findings described at the beginning of this paper, and the results of the experiments just described all strongly suggest that the biosynthesis of naphthoquinone congeners follows the same stereochemical course in the original plant as in the callus tissues.

EXPERIMENTAL

Column chromatography was carried out on Si gel AR-100 (Mallinckrodt) or acetylated polyamide prepared by the acetylation of polyamide (Wako C-200, 100 g) with Ac_2O and pyridine (400 ml each). Si gel GF_{254} (Merck) was used for TLC of non-radioactive materials and Si gel 60 F_{254} plates (Merck) for that of radioactive ones. Si gel 60 PF_{254} (Merck) was employed for prep. TLC of non-radioactive materials and Si gel F_{254} plates (Merck) (2.0 mm in thickness) for that of radioactive ones. Spots and bands were detected under a UV light or by exposure to I_2 vapour. Radioactive spots were detected by using a chromatogram scanner. Radioactivity was measured by liquid scintillation spectrometry. Sp. activities given are those before dilution. *Catalpa ovata* branches were taken from a plant grown in the campus of Kyoto University.

Preparation of (2R)-[1-^{14}C]*catalponone* [(2R)-**3**]. A soln of [2′-carboxy-^{14}C]-**8** [6] (30 mg, 2.0 mCi) in aq. EtOH (0.5 ml EtOH and 5.0 ml H_2O) was administered hydroponically to a 4-year-old branch (with 9 leaves) of *C. ovata*. After a week, the wood (fr. wt 130 g) was cut into pieces, soaked in C_6H_6 (250 ml) and set aside overnight. It was further extracted with C_6H_6 (2 × 250 ml) under reflux and the combined extracts were concd *in vacuo*. The residue (208 mg) was chromatographed on Si gel (20 g) eluted successively with C_6H_6 (200 ml), C_6H_6–EtOAc (49:1, 200 ml) and C_6H_6–EtOAc (24:1). Fractions showing a spot at R_f 0.49 on TLC (C_6H_6–EtOAc, 4:1) were combined and concd *in vacuo*. The residue was digested with a small vol. of MeOH and insoluble materials were filtered off. The filtrate gave on concn *in vacuo* a radioactive substance (8.05 mg) which on TLC co-chromatographed with authentic catalponol (**2**). The [1-^{14}C]-**2** was dissolved in Me_2CO and Jones reagent was added dropwise with stirring until the yellow colour of the soln persisted. After stirring for a further 5 min, the mixture was diluted with H_2O and extracted with $CHCl_3$ (4 × 5 ml). The combined $CHCl_3$ extracts were washed with H_2O, dried and concd *in vacuo* to give a crystalline residue (7.30 mg), which was chromatographed on Si gel (5 g) with C_6H_6 as eluant collecting 7 ml fractions. Evapn of the combined fractions 8–10 *in vacuo* afforded a radioactive compound (6.27 mg), which was identical with catalponone (**3**) on TLC (C_6H_6–EtOAc, 4:1, R_f 0.75). The sp. activity was 6.29 × 10^{7} dpm/nmol. This substance, after dilution with the carrier (1.48 mg), was used in the feeding experiment.

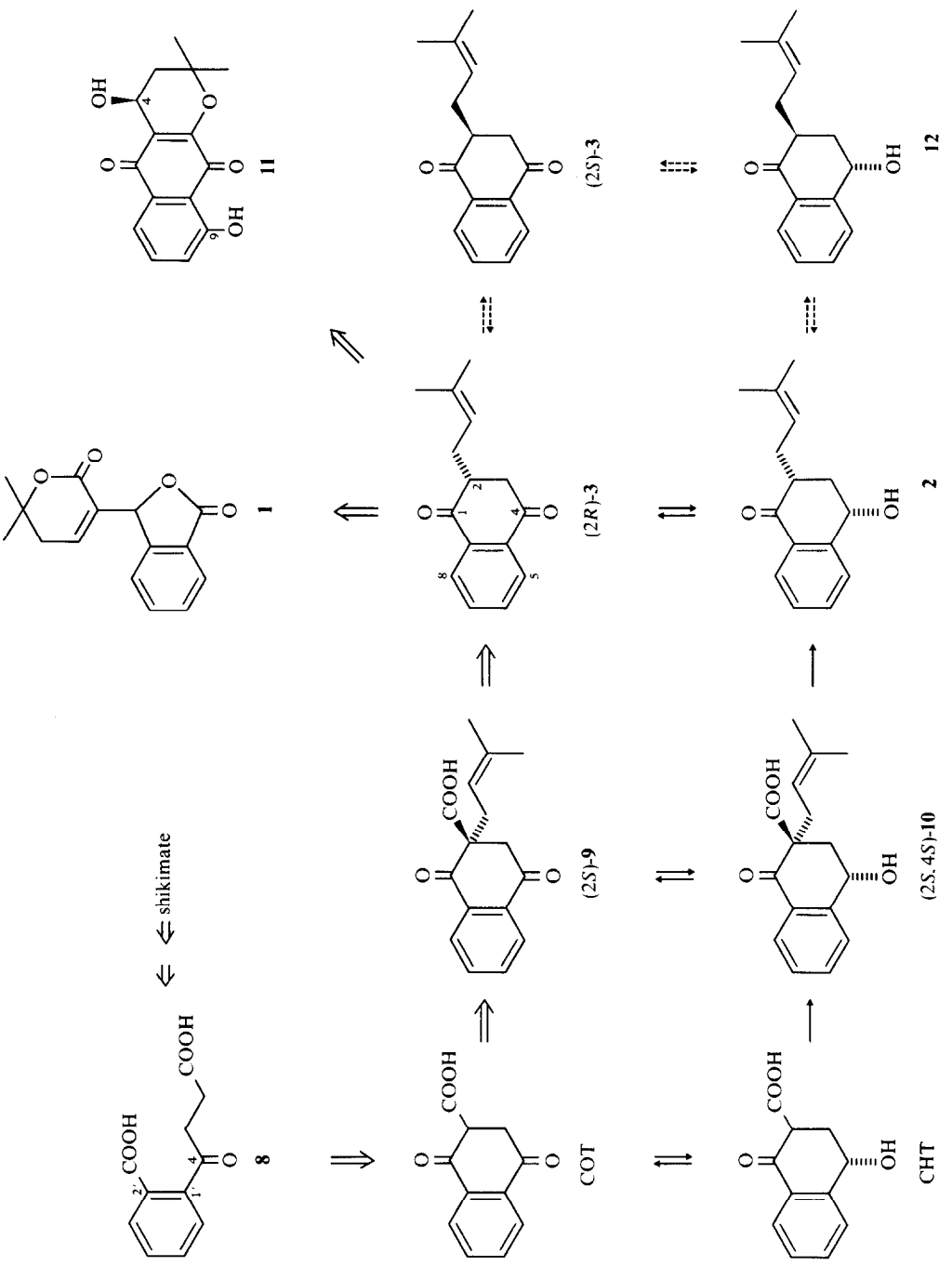
Preparation of (2S)-[8-^{3}H]*catalponone* [(2S)-**3**]. A soln of [3′-^{3}H]-**8** [11] (9.0 mg, 5.0 mCi) in aq. EtOH (0.5 ml EtOH and 5.0 ml H_2O) was administered hydroponically to a 4-year-old branch (with 7 leaves) of *C. ovata*. After a week, the wood (110 g) was worked up the same as in the above experiment to furnish a radioactive substance (15.0 mg) with the TLC properties of catalponol (**2**). [8-^{3}H]-**2** thus obtained was dissolved in MeOH (0.5 ml) and 0.1 M NaOH (0.5 ml) was added. After stirring at room temp. for 5 min, the reaction was adjusted to pH 3 with M HCl and extracted with $CHCl_3$ (4 × 5 ml). The combined $CHCl_3$ extracts were washed with H_2O, dried and concd *in vacuo*. Prep. TLC of the residue with C_6H_6–EtOAc (8:2) gave two main bands (R_f 0.37 and 0.49). The polar band afforded radioactive epicatalponol (**12**), whereas the less polar one furnished the starting material (**2**), which was reacted again to give more **12**. Radioactive **12** was oxidized with Jones reagent as for [1-^{14}C]-**2**, to give a crude oxidation product (9.20 mg), which was chromatographed on Si gel (5 g) with C_6H_6 as eluant, collecting

Table 1. Results of the simultaneous administration of (2*R*)-[1-^{14}C]catalponone (8.20 mg, 2.86 × 10^{8} dpm/mmol) and (2*S*)-[8-^{3}H]catalponone (8.20 mg, 5.67 × 10^{7} dpm/mmol) to the wood of *C. ovata*

Compound isolated	Wt (mg)	Sp. activity (dpm/mmol)*	Incorp. (%)*	Sp. incorp. (%)*	^{3}H/^{14}C†
Catalpalactone (**1**)	37.4	1.78 × 10^{4}	0.127	0.031	2.53
Catalponol (**2**)	45.0	5.87 × 10^{5}	5.68	1.04	1.80
4,9-Dihydroxy-α-lapachone (**11**)	2.77	1.16 × 10^{5}	0.058	0.204	2.30

* Expressed on basis of ^{14}C.

† Ratio for administered (2*R*,2*S*)-catalponone was 5.06.



Scheme 1.

7 ml fractions. Concn of the combined fractions 7–9 *in vacuo* afforded (2*S*)-[8-^{3}H]-**3** as colourless needles (8.20 mg, sp. activity 2.86×10^8 dpm/mmol). TLC, C_6H_6–EtOAc (4:1) R_f 0.75.

Administration of (2R)-[1-^{14}C]-**3** *and* (2S)-[8-^{3}H]-**3** *to the wood of* C. ovata *and isolation of catalpalactone* (**1**), *catalponol* (**2**) *and* 4,9-*dihydroxy-α-lapachone* (**11**). A mixture of (2*R*)-[1-^{14}C]-**3** (8.20 mg, 5.67×10^7 dpm/mmol) and (2*S*)-[8-^{3}H]-**3** (8.20 mg, 2.86×10^8 dpm/mmol) was emulsified in H_2O (5 ml) with Tween 80 (1 drop) and administered hydroponically to an 8-year-old branch (with 4 leaves) of *C. ovata*. After 10 days, the wood (fr. wt 156 g) was cut into pieces, soaked in C_6H_6 (300 ml) and set aside overnight. It was further extracted with C_6H_6 (2×300 ml) under reflux and the combined extracts were concd *in vacuo*. The residue (800 mg) was subjected to chromatography on Si gel (30 g) (chr 1), eluted successively with C_6H_6–EtOAc (49:1, 200 ml) and C_6H_6–EtOAc (24:1, 700 ml), collecting 50 ml fractions. Fractions 11–12 (chr 1-1) and 15–17 (chr 1-2) were combined and concd *in vacuo*. The residue of chr 1-1 was digested with a small vol. of MeOH and insoluble materials were filtered off. The filtrate was concd *in vacuo* to give a radioactive residue (45.0 mg) which was identical with an authentic sample of **2** on TLC. Radioactive **2** thus obtained was submitted to Jones oxidation in the usual way and the product (43.4 mg) was purified by chromatography on Si gel (7 g) with C_6H_6 as eluant to give radioactive (2*R*)-**3** as colourless needles. This compound was recrystallized from petrol to constant activity (39.0 mg). The sp. activity based on ^{14}C was 5.87×10^5 dpm/mmol. Next, the residue (47.0 mg) of chr 1-2 was chromatographed on acetylated polyamide (25 g) with H_2O as eluant, collecting 30 ml fractions. Fractions 5–7 (chr 1-2-1) and 8–11 (chr 1-2-2) were combined and extracted with $CHCl_3$. The $CHCl_3$ extract of chr 1-2-1 on concn *in vacuo* gave a residue (37.4 mg), which showed on TLC a single spot (R_f 0.36, C_6H_6–EtOAc, 4:1) corresponding to that of **1**. This substance was recrystallized from MeOH to constant activity giving radioactive **1** as colourless needles. The sp. activity was 1.78×10^4 dpm/mmol. The $CHCl_3$ extract of chr 1-2-2 was concd *in vacuo* to give a residue (7.90 mg), which was acetylated with 0.5 ml each of Ac_2O and pyridine in the usual way. The resulting acetate (7.70 mg) was chromatographed on Si gel (5 g)–C_6H_6. Fractions showing a single spot of R_f 0.45 on TLC (C_6H_6–EtOAc, 4:1) were concd *in vacuo* to give 4,9-diacetoxy-α-lapachone as yellow needles (3.62 mg). This compound, after dilution with the carrier (5.00 mg), was recrystallized from MeOH to constant activity. The sp. activity was 1.16×10^5 dpm/mmol.

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