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2-CARBOXY-4-HYDROXY-α-TETRALONE, A PRECURSOR FOR CATALPONOL BIOSYNTHESIS*

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Key Word Index—*Catalpa ovata*; Bignoniaceae; biosynthesis; 2-carboxy-4-hydroxy-α-tetralone; catalponol; catalpalactone; 4,9-dihydroxy-α-lapachone.

Abstract—2-Carboxy-4-hydroxy-α-tetralone (**5**) and its methyl ester (**10**) were incorporated into catalponol (**1**) in *Catalpa ovata* with retention of C-4 and C-8 tritium atoms. Incorporation of the former two substances into catalpalactone (**2**) and 4,9-dihydroxy-α-lapachone (**12**) was also demonstrated.

INTRODUCTION

In previous work [1], we demonstrated the following intermediate steps in the biosynthesis of catalponol (**1**), catalpalactone (**2**) and α-lapachones in *Catalpa ovata*: (i) catalponol (**1**) and catalpalactone (**2**) are biosynthesized via 4-(2′-carboxyphenyl)-4-oxobutanoic acid (**3**). (ii) Prenylation of **1** and **2** occurs at the position corresponding to C-2 of 4-(2′-carboxyphenyl)-4-oxobutanoic acid (**3**). (iii) In the formation of catalponol (**1**), prenylation occurs on the B-ring without aromatization of the hydronaphthalene skeleton. This type of prenylation may also occur in the formation of catalpalactone (**2**) or α-lapachones.

These findings strongly suggest that catalponol (**1**) and/or catalponone (**4**) occur as intermediates between 4-(2′-carboxyphenyl)-4-oxobutanoic acid (**3**) and catalpalactone (**2**) or α-lapachones. This consideration led us to suppose that 2-carboxy-4-hydroxy-α-tetralone (**5**) and/or 2-carboxy-4-oxo-α-tetralone (**6**) could be reasonable intermediates between 4-(2′-carboxyphenyl)-4-oxobutanoic acid (**3**) and catalponol (**1**) and/or catalponone (**4**).

This paper deals with the results of the administration of 2-carboxy-4-hydroxy-α-tetralone (**5**) and its derivatives to *C. ovata* to examine the intermediacy of **5** for the biosynthesis of catalponol (**1**) and other naphthoquinone congeners in this plant.

RESULTS AND DISCUSSION

Details of the chemical properties and the labelling methods of 2-carboxy-4-hydroxy-α-tetralone (**5**) and related substances have been reported in the preceding paper [2] of this series. Tritium labelled precursors were synthesized according to the method employed in the previous report (Scheme 1).

Me 4-(2′-carbomethoxy-3′-(1″-phenyl-5″-tetrazolyloxy)-phenyl)-4-oxobutyrate (**7**) prepared by several steps from 3-methoxyphthalic anhydride was subjected to catalytic reduction [3] over Pd-C in an atmosphere of tritium to give [3,7-$^3H_2$]-3-(2′-carbomethoxyethyl)-phthalide (**8**) and Me [3′,4,4-$^3H_3$]-4-(2′-carbomethoxyphenyl)-butryate (**9**). Dieckmann condensation of the phthalide (**8**) in the presence of potassium *t*-butoxide in toluene yielded [4,8-$^3H_2$]-2-carbomethoxy-4-hydroxy-α-tetralone (**10**) as the main product. This substance (**10**) was hydrolyzed with dilute sodium hydroxide at room temperature to give [4,8-$^3H_2$]-2-carboxy-4-hydroxy-α-tetralone (**5**) which decarboxylated spontaneously to [4,8-$^3H_2$]-4-hydroxy-α-tetralone (**11**) on standing overnight.

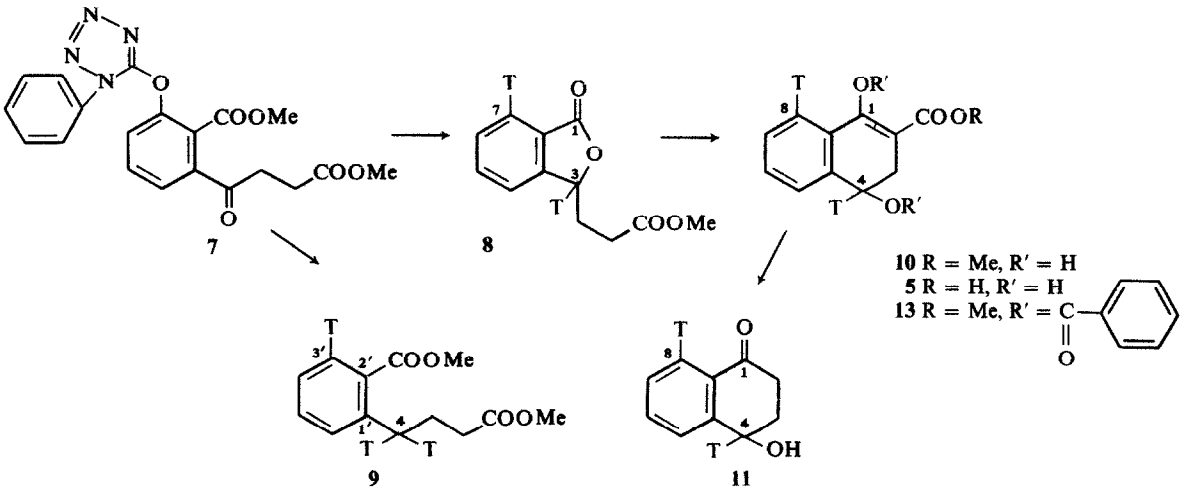
These labelled substances, [4,8-$^3H_2$]-2-carbomethoxy-4-hydroxy-α-tetralone (**10**), [4,8-$^3H_2$]-2-carboxy-4-hydroxy-α-tetralone (**5**) and [4,8-$^3H_2$]-4-hydroxy-α-tetralone (**11**) were administered hydroponically to separate branches of *C. ovata*. After a week, catalponol (**1**), catalpalactone (**2**) as well as 4,9-dihydroxy-α-lapachone (**12**) were isolated from the plant. At this time, **10** was administered to the plant in the expectation that it would be metabolized after *in vivo* hydrolysis.

The 4-^{3}H/8-^{3}H ratio of [4,8-$^3H_2$]-**10** was estimated from the radioactivity of the purified dibenzoate (**13**) [4] of **10** and that of phthalic anhydride which was obtained by alkali treatment followed by permanganate oxidation of the dibenzoate (**13**). The same 4-^{3}H/8-^{3}H ratio was assumed for [4,8-$^3H_2$]-**5**. Catalponol (**1**) isolated in this experiment was converted to 4-(4′-nitrophenylazo)-benzoate (**14**) or oxidized with Jones reagent to catalponone (**4**) and purified. The 4-^{3}H/8-^{3}H ratio of **1** was estimated from the radioactivity of the 4-(4′-nitrophenylazo)-benzoate (**14**) and that of catalponone (**4**). Catalpalactone (**2**) was purified by recrystallization and 4,9-dihydroxy-α-lapachone (**12**) was purified after conversion to the diacetate (**15**). The incorporation ratios into the three substances **1**, **2** and **12** were estimated on the basis of the amount of 8-^{3}H [5].

The incorporation ratios and specific incorporation ratios of 2-carboxy-4-hydroxy-α-tetralone (**5**) and 2-carbomethoxy-4-hydroxy-α-tetralone (**10**) into catalponol (**1**), catalpalactone (**2**) and 4,9-dihydroxy-α-lapachone (**12**) as well as the 4-^{3}H/8-^{3}H ratios of **5**, **10** and **1** are shown in Table 1, while the corresponding data on 4-hydroxy-α-tetralone (**11**) are shown in Table 2.

As expected, both **5** and **10** were incorporated into the

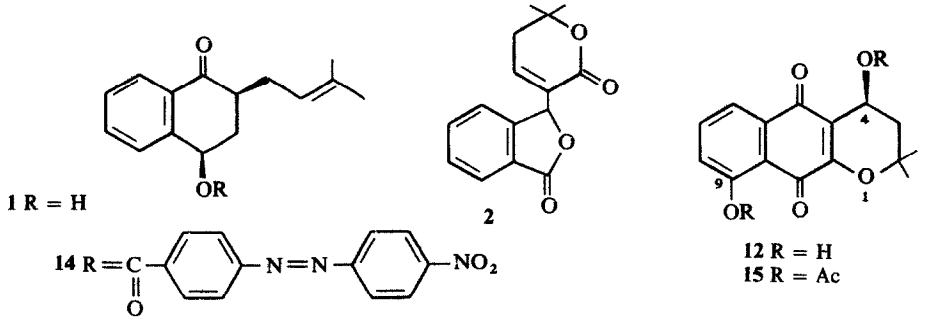
* Quinones and related compounds in higher plants 6. For part 5 see ref. [1].



Scheme 1. Synthesis of tritium labelled substances administered to *C. ovata*.

Table 1. Administration of [4,8-$^3H_2$]-2-carboxy- (**5**) and [4,8-$^3H_2$]-2-carbomethoxy-4-hydroxy-α-tetralone (**10**) to *C. ovata*

	Sp. act. dpm/mmol (amount mg)	Incorp. % (Sp. incorp. %)	4-^{3}H/8-^{3}H
2-Carboxy-4-hydroxy-α-tetralone (**5**)	1.85×10^{10} (0.805)		0.420
Catalponol (**1**)	2.41×10^5 (80.5)	0.26 (0.0015)	0.255
Catalpalactone (**2**)	3.27×10^4 (50.8)	0.013 (0.00025)	
4,9-Dihydroxy-α-lapachone (**12**)	7.44×10^4 (9.34)	0.005 (0.00057)	
2-Carbomethoxy-4-hydroxy-α-tetralone (**10**)	1.85×10^{10} (0.860)		0.420
Catalponol (**1**)	9.43×10^5 (50.0)	0.66 (0.0059)	0.225
Catalpalactone (**2**)	1.38×10^5 (22.3)	0.023 (0.0011)	
4,9-Dihydroxy-α-lapachone (**12**)	2.34×10^5 (1.06)	0.0018 (0.0018)	

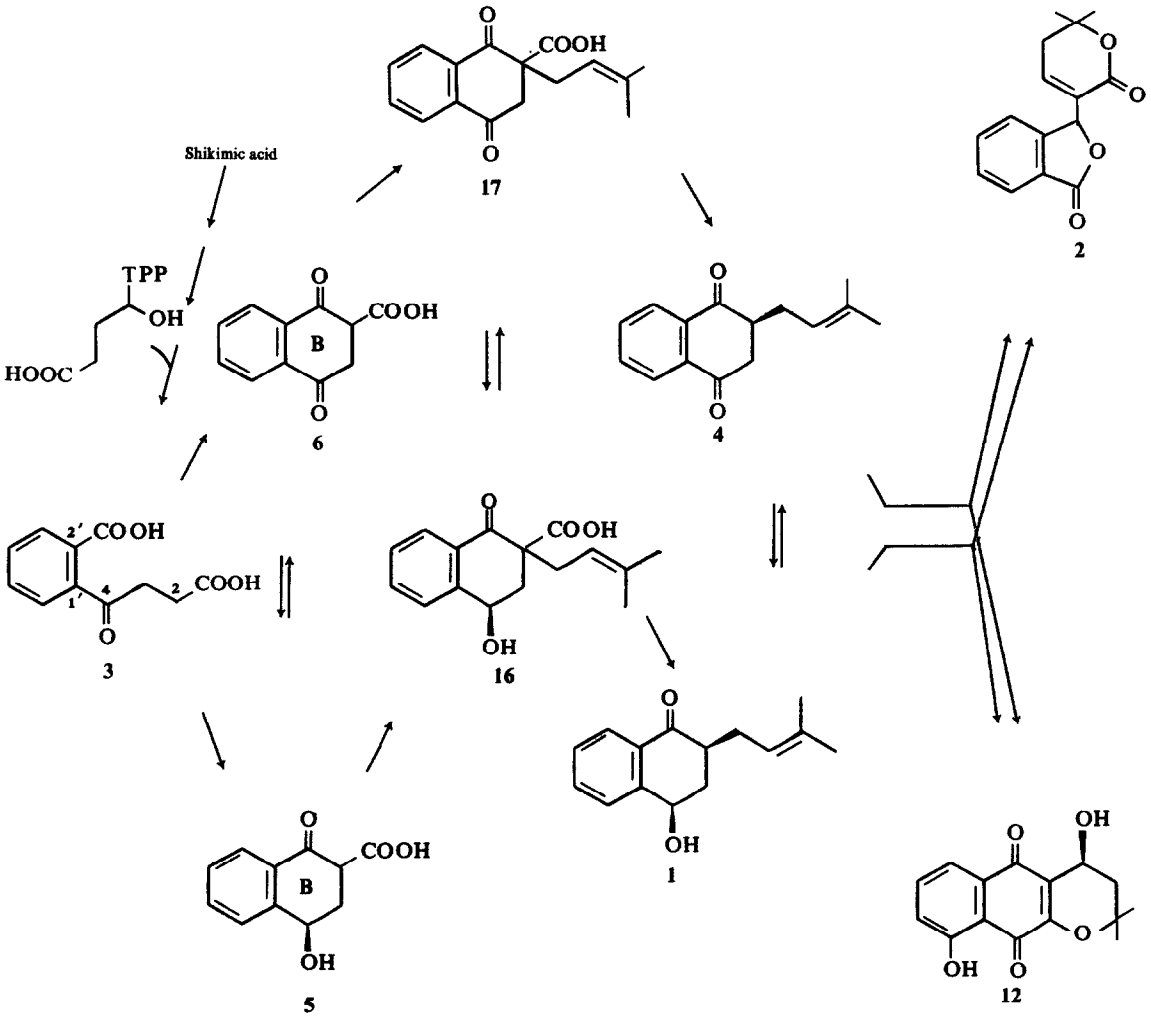


Scheme 2.

Table 2. Administration of [4,8-$^3H_2$]-4-hydroxy-α-tetralone (11) to *C. ovata*

	Sp. act. dpm/mmol (amount mg)	Incorp. × 10^3% (Sp. incorp. × 10^3%)
4-Hydroxy-α-tetralone (11)	1.30 × 10^{10} (0.83)	
Catalponol (1)	6.56 × 10^3 (72.0)	6.17 (0.050)
Catalpalactone (2)	2.46 × 10^3 (53.3)	0.76 (0.019)
4,9-Dihydroxy-α-lapachone (12)	5.26 × 10^3 (4.10)	0.12 (0.040)

3 above-mentioned substances including catalponol (1). However, the incorporation ratios and specific incorporation ratios of 5, a supposed normal biosynthetic intermediate, into 1 and 2 were much less than those of its Me ester (10). These results would be ascribable to the decomposition of most of the labile substance (5) before its arrival at the biosynthetic site. The extremely low and negligible incorporations of 11 into these 3 substances as compared with those of 5 and 10 support this concept and suggest that prenylation would precede the decarboxylation. It was also found that the incorporation ratios and specific incorporation ratios of 2-carboxy-4-hydroxy-α-tetralone (5) and 2-carbomethoxy-4-hydroxy-α-tetralone (10) into catalponol (1) were much higher than those of both precursors into catalpalactone (2) and 4,9-dihydroxy-α-lapachone (12). These results suggest that 1 would be located closer to the precursor (5) on the biosynthetic pathway than the other two metabolites and that, as described earlier in this paper, it is possible that the administered materials are



Scheme 3. Biosynthesis of catalponol (1), catalpalactone (2) and 4,9-dihydroxy-α-lapachone (12).

incorporated into **2** and **12** via catalponol (**1**). It should be emphasized that tritium at C-4 of **5** or **10** was definitely incorporated into the corresponding position of catalponol (**1**). Regardless of whether or not 2-carboxy-4-hydroxy-α-tetralone (**5**) is an obligatory intermediate in the biosynthetic pathway to **1**, **2**, **12** etc., this fact clearly indicates that of the two enantiomers of **5**, the 4*R* isomer is the precursor of catalponol (**1**). The 4-^{3}H/8-^{3}H ratio of **5** or **10** administered to the plant was reduced by half in catalponol (**1**) isolated during the administration experiment. This reduction may be due to the release of tritium at the C-4 position (benzylic position) or to the coexistence of a route to catalponol (**1**) through a substance bearing a C-4 carbonyl group such as 2-carboxy-4-oxo-α-tetralone (**6**) together with another route to catalponol (**1**) where the C-4 proton is retained. Although Dieckmann condensation of the diMe ester of 4-(2′-carboxyphenyl)-4-oxobutanoic acid (**3**) afforded indanedioneacetic acid Me ester as described in the preceding paper, it is highly probable that in the enzymatic reaction **3** is directly converted into the 6-membered ring substance **6** which is further reduced to **5**. Assuming as described above, that 4*R*-**5** is a specific precursor for catalponol (**1**) etc., the role of 2-carboxy-4-oxo-α-tetralone (**6**) as a normal biosynthetic intermediate cannot be ruled out. As an intermediate between **1** and **5**, 2-carboxy-2-dimethylallyl-4-hydroxy-α-tetralone (**16**) may be reasonably considered. Another route from **6** to catalponol (**1**) via 2-carboxy-2-dimethylallyl-4-oxo-α-tetralone (**17**) and catalponone (**4**) not passing through **5** could also be considered. As described before, there might be a route **5** → **6** → **17** → **4** → **1** or a converse one such as **5** → **16** → **1** → **4**. Supposed biosynthetic pathway of catalponol (**1**), catalpalactone (**2**), 4,9-dihydroxy-α-lapachone (**12**) etc. based upon these considerations are shown in Scheme 3, where the statement concerning 2-carboxy-4-oxo-α-tetralone (**6**) and its Me ester is only speculation as the synthesis of these substances has not yet been successful, presumably due to their instability. The compound, 1,4-dihydroxy-2-naphthoic acid (**18**) corresponding to **6** has so far been assumed to be an intermediate subsequent to 4-(2′-carboxyphenyl)-4-oxobutanoic acid (**3**) on the biosynthetic route to menaquinone [6, 7, 8]. Recently, Young [9] and Bentley *et al.* [10] independently reported that this substance (**18**) was detected as an intermediate in menaquinone (**19**) biosynthesis in *E. coli*. This finding may well be in accord with our results if **18** is considered to have been formed by the enolization of **6** in the course of extraction. In recent studies [11, 12], they also showed that **18** was incorporated into menaquinone (**19**) in *E. coli*. In this series of substances, however, many examples of such non-physiological reactions have been observed. For example, in the same reports, they demonstrated the incorporation of 1,4-naphthoquinone into menaquinone (**19**) and we [13] also observed good incorporation of deoxylapachol (**20**) into catalponol (**1**) in *C. ovata*. But it is obvious that these incorporations do not occur in the normal biosynthetic pathway. Accordingly, the prenylation mechanism in the biosynthesis of the vitamin K group is most likely identical with that in the cases of **1**, **2**, **12** etc. in *C. ovata*. Establishment of the main pathway among those shown in Scheme 3 as well as the elucidation of the detailed prenylation mechanism in the biosynthesis of the vitamin K group are fascinating problems yet to be examined.

EXPERIMENTAL

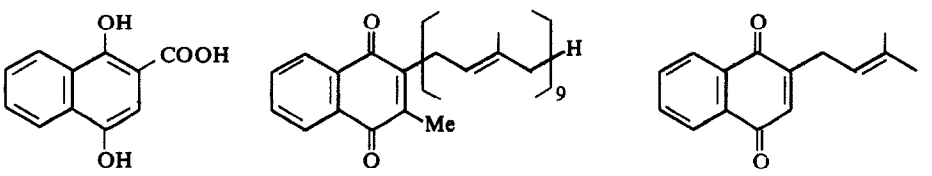
General procedures. Column chromatography was carried out on Si gel or acetylpolyamide prepared by the conventional acetylation of polyamide. TLC and PLC were run on Si gel GF_{254} and spots or bands were visualized by exposure to I_2 vapour or under UV light (254 nm). Unless otherwise noted, the solvent system employed was C_6H_6–Et_2O (7:3). The activated charcoal employed was Darco G-60. Radioactivity was measured by liquid scintillation counting in toluene (10 ml) + PPO (40 mg) and POPOP (0.5 mg). The sp. act. are expressed as values before dilution. Radioactive spots on TLC were monitored by a chromatogram scanner.

Plant material. Branches (8-yr old) from *Catalpa ovata* G. Don were taken from a plant grown in the campus at Kyoto University.

Preparation of [3,7-$^3H_2$]-3-(2′-carbomethoxyethyl)-phthalide (**8**). To a soln of Me 4-(2′-carbomethoxy-3′-(1″-phenyl-5″-tetrazolyloxy)phenyl)-4-oxobutyrate (**7**) (96 mg) in MeOH (15 ml) was added Pd-C (10%) prepared from 10% $PdCl_2$–aq. HCl (0.55 ml) and activated charcoal (45 mg) and the mixture was stirred under $^3H_2$ (2 Ci) for 5 hr at room temp. Subsequently, a large excess of H_2 was introduced into the reaction mixture and stirring was continued for another 9 hr. The reaction mixture was centrifuged and the ppt. washed with MeOH (3 × 5 ml). All the supernatants were combined and concentrated *in vacuo* to give a residue, which was chromatographed on Si gel (15 g, 1.5 × 15 cm) and eluted with C_6H_6 to give oily **9** as a minor product, yield 14 mg (70 mCi), TLC: R_f 0.75. Parts of subsequent fractions eluted with C_6H_6–Et_2O (49:1 and 97:3) were combined and concd *in vacuo* to give **8** as pillars, yield 39.8 mg (160 mCi), TLC: R_f 0.52.

[4,8-$^3H_2$]-2-Carbomethoxy-4-hydroxy-α-tetralone (**10**). To a suspension of K *t*-butoxide (140 mg) in dry toluene (1 ml) heated at 110–115° under N_2 was added dropwise a soln of [3,7-$^3H_2$]-3-(2′-carbomethoxyethyl)-phthalide (**8**) (50 mg, 2 mCi) in dry toluene (1 ml) with stirring over a period of 45 min. After gentle heating of the mixture under reflux for another 45 min, H_2O (5 ml) was added to the reaction soln cooled with ice. The soln was adjusted to pH 2 with N HCl and extracted with Et_2O (5 ml × 5). The Et_2O layers were washed with satd aq. NaCl, dried and concd to give a residue (42 mg) which was subjected to PLC. A band around R_f 0.28 was scraped off and extracted with MeOH. The MeOH extract was concd *in vacuo* to give 2-carbomethoxy-4-hydroxy-α-tetralone (**10**) (8.6 mg) which gave a single radioactive spot on TLC. The sp. act. was 1.85 × 10^{10} dpm/mmol.

[4,8-$^3H_2$]-2-Carboxy-4-hydroxy-α-tetralone (**5**). To [4,8-$^3H_2$]-**10** (0.86 mg, sp. act. 1.85 × 10^{10} dpm/mmol) was added N NaOH (0.6 ml) and the mixture stirred for 1 hr at room temp.



18 **19** **20**

Scheme 4.

After the mixture was adjusted to pH 6 with N HCl at 0°, it was diluted with 0.2 M Pi buffer (pH 6.7, 6 ml) and used immediately for the administration expt. The mixture was assumed to contain 0.805 mg of [4,8-$^3H_2$]-**5** (sp. act. 1.85×10^{10} dpm/mmol) as a preliminary cold run of the hydrolysis of **10** gave **5** in a quantitative yield, TLC: H_3PO_4–Si gel (0.5:99.5), R_f 0.35.

[4,8-$^3H_2$]-4-Hydroxy-α-tetralone (**11**). To a soln of [4,8-$^3H_2$]-**10** (sp. act. 1.85×10^{10} dpm/mmol, 1.3 mg) was added N NaOH (0.5 ml) and the mixture stirred at room temp. for 1 hr. The reaction soln was cooled to 0°, adjusted to pH 2 with N HCl and extracted with Et_2O (5 ml × 4). The Et_2O layers were washed with satd aq. NaCl, dried and concd *in vacuo* to give a crystalline residue of **5**. After spontaneous decarboxylation of this substance (**5**) by standing 18 hr at room temp., the product was subjected to PLC in C_6H_6–EtOAc (7:3). A band *ca* R_f 0.25 was scraped off and extracted with MeOH. The MeOH extract was concd *in vacuo* to give an oily residue (0.83 mg), TLC of which indicated a single radioactive spot corresponding to that of an authentic sample of 4-hydroxy-α-tetralone (**11**). The sp. act. was 1.85×10^{10} dpm/mmol.

Benzoylation of [4,8-$^3H_2$]-2-carbomethoxy-4-hydroxy-α-tetralone (**10**). **10** (0.43 mg, sp. act. 1.85×10^{10} dpm/mmol) diluted with the carrier (40 mg) was subjected to conventional benzoylation with Py (1 ml) and benzoyl chloride (0.06 ml). The crystalline reaction product (38.2 mg) was chromatographed on a Si gel column (5 g, 1.6 × 6 cm) with C_6H_6 and radioactive dibenzoate (**13**) was recrystallized from Et_2O to constant activity. The sp. act. was 2.13×10^8 dpm/mmol.

Alkali treatment of [4,8-$^3H_2$]-2-carbomethoxy-4-hydroxy-α-tetralone dibenzoate (**13**) *and subsequent $KMnO_4$ oxidation.* To a soln of [4,8-$^3H_2$]-2-carbomethoxy-4-hydroxy-α-tetralone dibenzoate (**13**) (35 mg) in MeOH (1 ml) was added N NaOH (2 ml) and stirred at 80° for 2 hr. The reaction mixture was diluted with H_2O and extracted with EtOAc (7 ml × 3). The EtOAc extracts after washing with H_2O were concd *in vacuo* to give a residue which consisted primarily of **11**. This residue was mixed with 0.1 N NaOH (1 ml) and $KMnO_4$ (80 mg) was gradually added to the mixture with stirring. After stirring for 2 hr, the resulting ppt. of MnO_2 was dissolved by addition of $NaHSO_3$. The soln was acidified with N HCl and extracted with EtOAc (5 ml × 4). The EtOAc layers were washed with satd aq. NaCl, dried and evapd *in vacuo* to give colourless plates, yield 12 mg. Sublimation of this substance at 180°/0.8 mm Hg afforded phthalic anhydride (8.03 mg), which was diluted with the carrier (66.9 mg) and repeatedly sublimed to constant activity. The sp. act. was 1.50×10^8 dpm/mmol.

Administration of [4,8-$^3H_2$]-2-carbomethoxy-4-hydroxy-α-tetralone (**10**) *to* C. ovata *and isolation of radioactive catalponol* (**1**), *catalpalactone* (**2**) *and 4,9-dihydroxy-α-lapachone* (**12**) *and preparation of their derivatives.* A soln of [4,8-$^3H_2$]-2-carbomethoxy-4-hydroxy-α-tetralone (**10**) (0.86 mg, sp. act. 1.85×10^{10} dpm/mmol) in H_2O (6 ml) containing Tween 80 (3 drops) placed in a glass tube was administered hydroponically to a branch with 8 leaves remaining (85 cm in length) at the flowering stage in May. During administration, H_2O was added to the glass tube in order to allow the remaining labelled substance to be absorbed into the plant. After a week, the plant (fr. wt 79 g) was cut into pieces and extracted with C_6H_6 (200 ml × 3) under reflux. The extracts were combined and concd *in vacuo* to give a residue (530 mg), which was chromatographed on a Si gel column (20 g, 2 × 16 cm). After elution with C_6H_6 (350 ml), the column was eluted with a mixture of C_6H_6–Et_2O (49:1) and 25 ml fractions were collected. Fractions 16–20 of the C_6H_6–Et_2O eluate gave catalponol (**1**) (50 mg), TLC: C_6H_6–EtOAc (4:1), R_f 0.49. Fractions 24–27 gave a yellow residue (31 mg), which was dissolved in a small vol. of aq. MeOH, transferred to an acetylpolyamide column (20 g, 1.6 × 25 cm) and eluted with H_2O. Fractions of 20 ml each were collected. Fractions 6–8 were combined and extracted with $CHCl_3$ (10 ml × 3). The $CHCl_3$ extracts were dried and concd to give colourless pillars (22.3 mg), which were identical with catalpalactone (**2**) according to TLC: C_6H_6–EtOAc (4:1), R_f 0.36. This substance was repeatedly recrystallized from MeOH to constant activity (sp. act. 1.38×10^5 dpm/mmol). Fractions 12–15 of the same eluate were extracted with $CHCl_3$ (10 ml × 3), the extracts dried and the solvent removed *in vacuo* to give a mixture of α-lapachones as a yellow crystalline residue (1.27 mg).

Conversion of [4,8-$^3H_2$]-catalponol (**1**) *to 4-(4′-nitrophenylazo)-benzoate* (**14**). To an ice cooled soln of an aliquot of (4,8-$^3H_2$]-catalponol (**1**) (10.6 mg) in Py (1 ml) was added 4-(4′-nitrophenylazo)-benzoyl chloride (33 mg) and the mixture left standing 18 hr at room temp. After extraction of the mixture with C_6H_6 (5 ml × 4), the C_6H_6 layers were washed with H_2O, dried and concd *in vacuo* to give a red crystalline residue (20 mg). The residue was chromatographed on Si gel (12 g, 1.6 × 14 cm) with C_6H_6 to give the benzoate as red needles (17.5 mg) which were recrystallized from petrol to constant activity (sp. act. 9.43×10^5 dpm/mmol).

Conversion of [4,8-$^3H_2$]-catalponol (**1**) *into [8-^3H]-catalponone* (**4**). To an ice cooled soln of an aliquot of [4,8-$^3H_2$]-**1** (10 mg) excess Jones reagent was added dropwise and stirring was continued for a further 5 min. After dilution with H_2O, the reaction mixture was extracted with Et_2O (10 ml × 3). The Et_2O layers were washed with satd aq. NaCl, dried and concd *in vacuo* to give a residue (8 mg). The residue was chromatographed on Si gel (10 g, 1.6 × 12 cm) with C_6H_6 to give colourless needles (7.2 mg), which were identical with an authentic sample of catalponone (**4**) on TLC: C_6H_6–EtOAc (4:1), R_f 0.75. This substance was recrystallized from petrol to constant activity (sp. act. 7.7×10^5 dpm/mmol).

Acetylation of mixture of radioactive α-lapachones and isolation of [6-^3H]-4,9-dihydroxy-α-lapachone diacetate (**15**). Conventional acetylation of the mixture of α-lapachones (1.27 mg) with 0.2 ml each of Py and Ac_2O gave 1.56 mg of the reaction product. The product was chromatographed on Si gel (5 g, 1.6 × 5 cm) with C_6H_6 to give yellow needles, which were identical with an authentic sample of 4,9-diacetoxy-α-lapachone (**15**) according to TLC: C_6H_6–EtOAc (4:1), R_f 0.45. The yield was 1.38 mg. After dilution with the carrier diacetate (7 mg), this substance was recrystallized from MeOH to constant activity (sp. act. 2.34×10^5 dpm/mmol). The acetate of another α-lapachone occurring together with 4,9-dihydroxy-α-lapachone (**12**) was not examined further due to the scarcity of the sample.

Administration of [4,8-$^3H_2$]-2-carboxy-4-hydroxy-α-tetralone (**5**) *to* C. ovata, *isolation of radioactive catalponol* (**1**), *catalpalactone* (**2**) *and 4,9-dihydroxy-α-lapachone* (**12**) *and preparation of their derivatives.* A soln of [4,8-$^3H_2$]-**5** in 0.2 M phosphate buffer (pH 6.7, 6 ml) was administered to a branch with 11 leaves remaining (135 cm in length) as described for the administration of [4,8-$^3H_2$]-**10**. After isolation, the radioactive substances, catalponol (**1**) (80.5 mg), catalpalactone (**2**) (50.8 mg) and a mixture of α-lapachones (17.5 mg) were obtained. Catalpalactone (**2**) was purified as such (sp. act. 3.27×10^4 dpm/mmol). Catalponol (**1**) was purified after conversion into the 4-(4′-nitrophenylazo)-benzoate (**14**) (sp. act. 2.41×10^5 dpm/mmol) or catalponone (**4**) (sp. act. 1.92×10^5 dpm/mmol. The mixture of α-lapachones was acetylated, subjected to column chromatography on Si gel and 4,9-diacetoxy-α-lapachone (**15**) (12.2 mg) was isolated (sp. act. 7.44×10^4 dpm/mmol).

Administration of [4,8-$^3H_2$]-4-hydroxy-α-tetralone (**11**) *to* C. ovata, *isolation of radioactive catalponol* (**1**), *catalpalactone* (**2**) *and 4,9-dihydroxy-α-lapachone* (**12**) *and preparation of their derivatives.* A soln of [4,8-$^3H_2$]-**11** (0.83 mg, sp. act. 1.30×10^{10} dpm/mmol) in aq. EtOH (EtOH 0.5 ml, H_2O 6 ml) was administered hydroponically to a branch (110 cm in length) with 6 leaves remaining. After the same work-up as described for the administration of **10** or **5**, catalponol (**1**) (72 mg), catalpalactone (**2**) (53.3 mg) and a mixture of α-lapachones (12.2 mg) were obtained. Catalpalactone (**2**) was purified as such (sp. act. 2.46×10^3 dpm/mmol), and catalponol (**1**) was purified after oxidation to catalponone (**4**) (sp. act. 6.56×10^3 dpm/mmol). The mixture of α-lapachones was purified after conversion into the acetate and yielded 4,9-diacetoxy-α-lapachone (**15**) (5.37 mg) (sp. act. 5.26×10^3 dpm/mmol).

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4. As reported in the preceding paper of this series, **10** exists mainly in the enol form in solution.
5. In our previous report the observed incorporation ratios of racemic materials into substances **1**, **2** and **12** were doubled because it was assumed that only the 4*R* isomer could be incorporated as about half of the 4-^{3}H of [4,8-$^3H_2$]-**5** or [4,8-$^3H_2$]-**10** was retained in catalponol (**1**) (*vide infra*). However, as it is considered that the 4*S* isomer is also incorporated via **6**, the observed values are now reported without modifications; cf. Inoue, K., Ueda, S., Shiobara, Y. and Inouye, H. (1976) *Tetrahedron Letters* 1795.
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