Quinones and Related Compounds in Higher Plants. Part 11.[1] Role of 2-Carboxy-2,3-dihydro-1,4-naphthoquinone and 2-Carboxy-2-(3-methylbut-2-enyl)-2,3-dihydro-1,4-naphthoquinone † in the Biosynthesis of Naphthoquinone Congeners of *Catalpa ovata* Callus Tissues [2]

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Dilution analyses after administration of ^{14}C-labelled 4-(2-carboxyphenyl)-4-oxobutanoic acid (3) to the callus tissues of *Catalpa ovata* demonstrated the following results: (i) 2-Carboxy-4-oxo-1-tetralone (COT), 2-carboxy-4-hydroxy-1-tetralone (CHT), prenyl-COT (4), and prenyl-CHT (5) are on the biosynthetic pathway of naphthoquinones; (ii) the route passing through COT ⟶ prenyl-COT (4) ⟶ catalponone (6) is the main pathway of the biosynthesis of naphthoquinones, whereas there exists a subsidiary route passing through CHT ⟶ prenyl-CHT (5) ⟶ catalponol (2); (iii) regarding the key intermediates prenyl-COT (4) and catalponone (6), (2*S*)-prenyl-COT [(2*S*)-(4)] and (2*R*)-catalponone [(2*R*)-(6)] participate in the biosynthesis and both prenylation and decarboxylation proceed stereoselectively; and (iv) the acid (3) was incorporated into menaquinone-1 (7), 1-hydroxy-2-methylanthraquinone (8), 3-hydroxydehydro-iso-α-lapachone (9), *etc.*, in the callus tissues. Furthermore, administration of 4-([*carboxy*-^{14}C]-2-carboxyphenyl)-4-hydroxy[4-^{3}H]butanoic acid (32) to the original *Catalpa* plant and the callus tissues revealed that (3) is cyclised to COT and then reduced to CHT.

In the course of studies on the biosynthesis of prenylnaphthoquinone congeners including catalpalactone (1) and catalponol (2), the constituents of *Catalpa ovata* G. Don (Bignoniaceae), we have elucidated that both compounds (1) and (2) are biosynthesised *via* 4-(2-carboxyphenyl)-4-oxobutanoic acid (3) and that the prenylation takes place at the position equivalent to C-2 of (3) without aromatisation of the ring formed by cyclisation of 3.[3-5] We have also established that, of the probable intermediates inferred from these findings, 2-carboxy-4-hydroxy-1-tetralone (CHT) was incorporated into the constituents described above.[6,7]

This paper deals with (i) the demonstration of the intermediacy of 2-carboxy-4-oxo-1-tetralone (COT), a possible important precursor ranked with CHT, and their probable successors 2-carboxy-4-oxo-2-prenyl-1-tetralone (prenyl-COT) (4) and 2-carboxy-4-hydroxy-2-prenyl-1-tetralone (prenyl-CHT) (5) for the biosynthesis of prenylnaphthoquinone congeners, especially the corroboration of the key intermediary role of COT and (4) in view of the stereochemistry of the prenylation and the successive decarboxylation to catalponone (6); (ii) concurrent verification of the incorporation of (3) into several quinonoids; and (iii) new evidence for the biosynthetic pathway from (3) to COT.

Judging from the structures, the main subjects of this work, COT, (4), and (5), seem to be very unstable, as is CHT.[8] For this reason, usual feeding experiments of (4) and (5) labelled with isotopes are likely to be of no use, since spontaneous decarboxylation of the substances fed would inevitably preclude elucidation of the results. We therefore attempted to prove the intermediacy of COT, CHT, (4), and (5) by trapping them in the form of the methyl ester and the decarboxylation product after the administration of labelled precursor (3) to the plant. It seemed impracticable, however, to use the wood which has been used as the plant material in the previous experiments, since the labile intermediates would not survive the process of extraction. Fortunately, we succeeded in obtaining *Catalpa ovata* callus tissues, in which menaquinone-1 (7), 1-hydroxy-2-methylanthraquinone (8), and four dehydro-iso-α-lapachone derivatives including 3-hydroxydehydro-iso-α-lapachone (9) were detected along with catalpalactone (1), and four α-lapachones including α-lapachone (10), the known constituents of the wood of the plant.[1] These callus tissues served for the trapping experiments described below.

The seven probable biosynthetic intermediates used for the experiments were CHT methyl ester (11), 4-oxo-1-tetralone (12), 4-hydroxy-1-tetralone (13), prenyl-CHT methyl ester (14), 2-*epi*-prenyl-CHT methyl ester (15), 2-*epi*-prenyl-CHT lactone (16), and prenyl-COT methyl ester (17). These substances, excluding the previously reported (11), (12), and (13)[8] were synthesised by the following methods. The starting material for all these substances was CHT methyl ester (11). Reaction of (11) with 1-bromo-3-methylbut-2-ene in the presence of potassium t-butoxide in t-butyl alcohol yielded two major products (14) and (15) along with a minor product (16). The product (14), $C_{17}H_{20}O_4$, was an oily substance whose spectral data indicated the presence of prenyl, methoxycarbonyl, hydroxy, and aryl ketone groups. Accordingly, the substance was assumed to be prenyl-CHT methyl ester. Substance (15) was also an oil having the same composition and similar spectral properties suggesting it to be a stereoisomer of (14). In fact, the C-4 proton signal of (14) and (15) appeared at δ 5.15 (dd, *J* 11.0 and 5.0 Hz) and 4.94 (t, *J* 5.0 Hz), respectively, the same as those of catalponol (2) [δ 5.00 (dd, *J* 10.0 and 5.0 Hz)] and epicatalponol (18) [δ 4.95 (t, *J* 4.0 Hz)].[9] As it is known that both (2) and (18) have conformations

† Referred to throughout this paper by the trivial names 2-carboxy-4-oxo-1-tetralone (COT) and 2-carboxy-2-prenyl-4-oxo-1-tetralone (prenyl-COT) respectively. Other trivial names employed are 2-carboxy-4-hydroxy-1-tetralone (CHT) (for 4-hydroxy-1-oxo-1,2,3,4-tetrahydronaphthalene-2-carboxylic acid) and 2-carboxy-4-hydroxy-2-prenyl-1-tetralone (prenyl-CHT) [for 4-hydroxy-2-(3-methylbut-2-enyl)-1-oxo-1,2,3,4-tetrahydronaphthalene-2-carboxylic acid].

allowing the prenyl groups to occupy equatorial positions,[10] these n.m.r. data suggest the corresponding conformations for compounds (14) and (15), respectively. Furthermore, the downfield shift of the C-4 proton signal of (14) by 0.21 p.p.m. from that of (15) should be ascribed to the anisotropic effect of the axial methoxycarbonyl group on C-2, which, in turn, is compatible with the foregoing assignment. It can be concluded, therefore, that the prenyl group on C-2 of (14) and that of (15), both being in equatorial conformations, assume *cis* and *trans* configuration to the hydroxy-group on C-4, respectively. Another product (16) showed an n.m.r. spectrum similar to that of (15) except for the absence of a methoxy-signal. It further showed an i.r. absorption at 1 780 cm^{-1} assignable to a five-membered lactone. Accordingly, it was assumed that (16) contains a lactone ring formed from (15) by transesterification between the methoxycarbonyl group on C-2 and the hydroxy-group on C-4. As was expected, alkali treatment of (15) yielded (16) quantitatively. This result verifies the postulated relative configuration of substituents on C-2 and C-4 in prenyl-CHT methyl ester (14) and the 2-epimer (15). Finally, Jones oxidation of (14) and (15) gave the same desired (±)-prenyl-COT methyl ester (17).

Optical resolution of this compound was carried out as follows: At first, an additional chiral centre was introduced into (17) through reduction with $LiAl(OBu^t)_3H$. The reduction, when conducted at −30 °C for 1.5 h, yielded two products (19) and (20) in the ratio 1 : 2, while at 40 °C for 1 h the same products were obtained in the ratio 19 : 1. The n.m.r. spectrum of (19) showed a methine proton signal at δ 5.15 (d, J 4.0 Hz) which collapsed to a singlet on addition of D_2O, indicating coupling with a hydroxy-proton. On the other hand, the spectrum of (20) showed the corresponding proton signal at δ 4.88 (s). These findings imply that both substances are stereoisomers regarding the C-1 position of 2-methoxycarbonyl-4-oxo-1-tetralol (19) formed by the reduction of (17). It is known that the anisotropic effect of the C-1 hydroxy-group on the C-8 proton of 1-tetralol depends upon the conformation of the hydroxy-group. In the spectra of (19) and (20), however, the definite assignment of the C-8 proton signal was impossible because of the overlapping of this signal with those of the C-6 and C-7 protons. As the signal pattern of the aromatic proton region in the spectrum of (20) was, however, superimposable on that of isocatalponol (21) [10,11] comprising the 1-tetralol structure with a quasi-equatorial hydroxy-group, the C-1 hydroxy-group appeared likely to assume a quasi-equatorial position in compound (20), and hence a quasi-axial conformation in compound (19). For the corroboration of this inference and the elucidation of the relative configuration of the substituents on C-1 and C-2, both compounds (19) and (20) were converted into the corresponding cyclic compounds, respectively. Thus, (19) was treated with *m*-chloroperbenzoic acid in the presence of toluene-*p*-sulphonic acid resulting in epoxidation of the double bond at C-2′ followed by cyclisation to give (22). On the other hand, (20) was treated successively with the same peracid and BF_3 giving rise to (23). In both transformations, the desired compounds were obtained in relatively good yields, along with some minor products, which were, however, not examined. In spite of the close resemblance of the n.m.r. spectra of both cyclic compounds (22) and (23), long-range coupling between one of the C-2 methylene protons and the C-1 proton (J 1.2 Hz; confirmed by the spin-decoupling experiment) was observed only in the spectrum of (22). This is caused presumably by the difference of the steric position of the C-1 protons of both compounds: in compound (22), the long-range coupling across the *W*-path seems to be possible between the quasi-equatorial C-1 proton and the equatorial C-3 proton, but not in (23) because of the quasi-axial position of the C-1 proton. Regarding compound (19), there still remains the problem of the relative configuration of the prenyl group to the hydroxy-group, to which a quasi-axial position was assigned. If it is *cis*, (19) can cyclise with retention of conformation, whereas if it is *trans*, the compound cannot cyclise without changing conformation. In the latter case, the long-range coupling between the C-1 and C-3 protons of the cyclisation product (22) should not be allowed, because the C-1 proton must adopt the quasi-axial position. As that was not the case, the *cis* configuration of the C-1 hydroxy- and C-2 prenyl groups of (19) and hence the *trans* orientation of both groups of (20) were concluded.

Alkali hydrolysis of (19) gave an acid (24), which was resolved in the form of quinine salts. The resulting salts of both enantiomers, on work-up with dilute hydrochloric acid, yielded the corresponding enantiomers of the free acid (24). On treatment with diazomethane, both acids gave the methyl esters (+)- and (−)-(19), respectively. The optical purity of both esters was

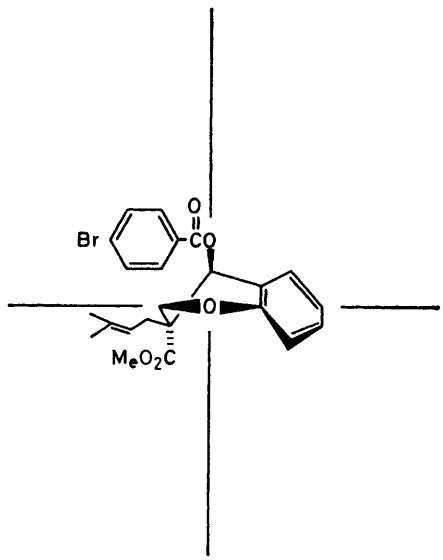
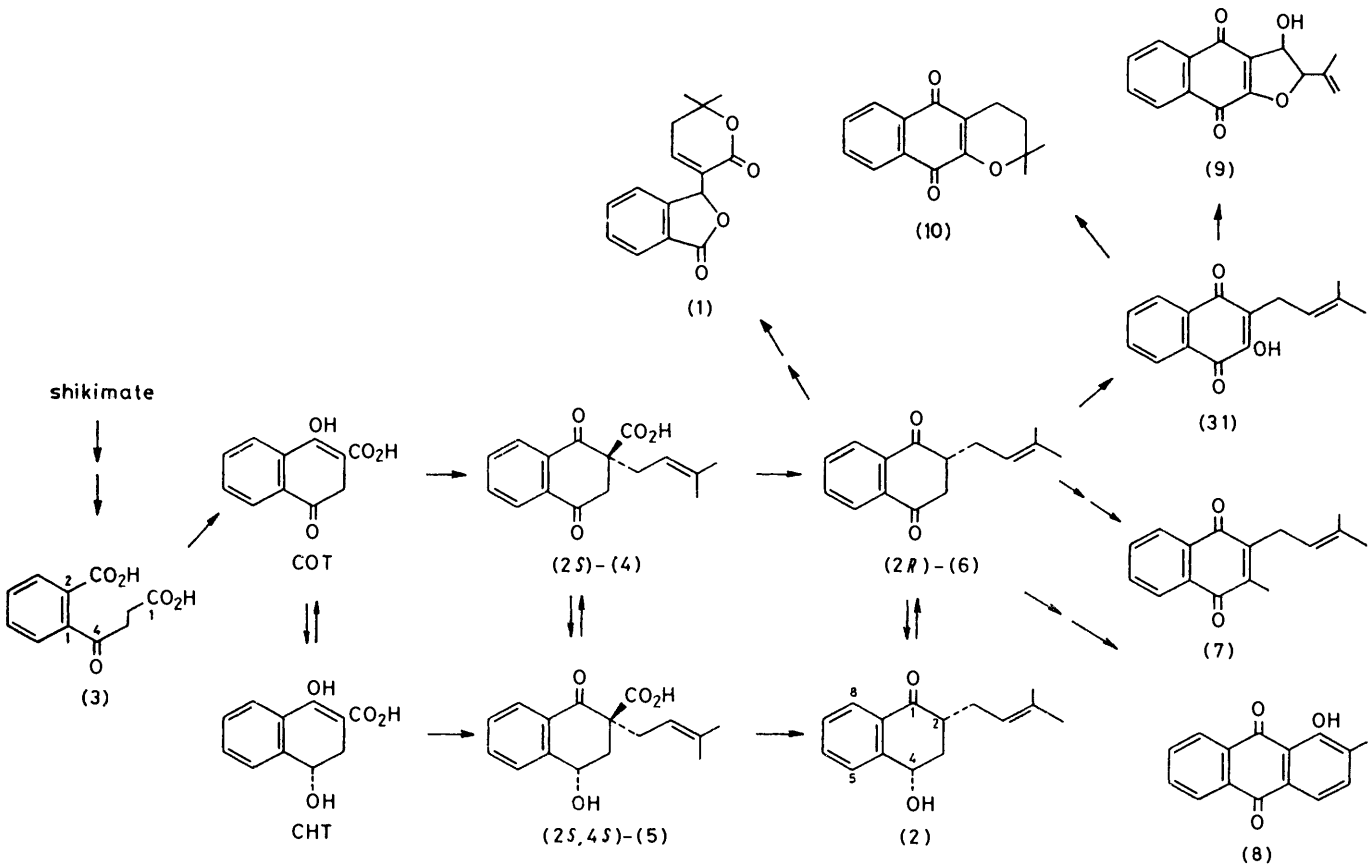


FIGURE. Projection showing the conformation of an aryl ketone enantiomer (+)-(25)

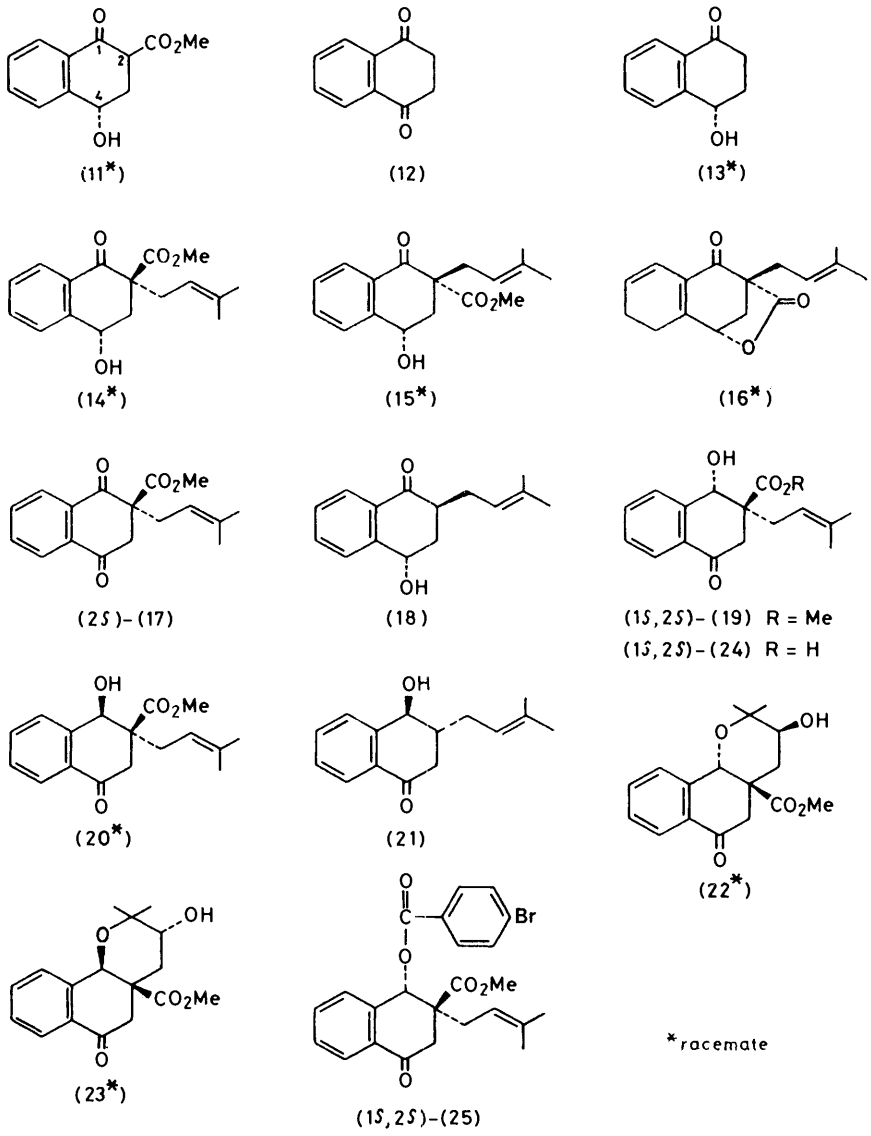
verified by the n.m.r. spectra measured in the presence of 0.63 mol equiv. of the chiral shift reagent tris[trifluoroacetyl-(+)-camphorato]europium(III) [Eu(tfac)$_3$] [12] showing single signals for the C-1 proton at δ 5.56 [for (−)-(19)] and 5.66 [for (+)-(19)].

The absolute structures of both enantiomers of (19) were obtained by applying the exciton chirality method [13] to the c.d. spectra of both *p*-bromobenzoates (25) which are mirror images of each other; *e.g.* *S*-configuration was assigned to the chiral centre C-1 of (+)-(25) which showed the positive first Cotton effect at 254.5 nm, while *R*-configuration was assigned to C-1 of (−)-(25). These findings, together with the foregoing *cis* orientation of the C-1 hydroxy- and C-2 prenyl groups of (19), imply that the absolute configuration of (+)- and (−)-(19) should be 1*S*,2*S* and 1*R*,2*R*, respectively. Along with that, (+)-(25) indicated a positive Cotton effect at 325 nm owing to the carbonyl $n\rightarrow\pi^*$ transition, while (−)-(25) showed a negative effect. Accordingly, by application of the aryl ketone chirality rule,[14] the half-chair conformations, one of which is drawn in the Figure, were assigned to both enantiomers, respectively; this is consistent with the above conclusion. Finally, Jones oxidation of (1*S*,2*S*)-(+)- and (1*R*,2*R*)-(−)-(19) gave (2*S*)-(−)- and (2*R*)-(+)-prenyl-COT methyl ester [(2*S*)- and (2*R*)-(17)], respectively.

On carrying out the dilution analyses, we first centred our examination on the incorporation of 4-([*carboxy*-^{14}C]-2-carboxyphenyl)-4-oxobutanoic acid (3) {hereafter referred to as [*carboxy*-^{14}C]-(3)} into prenyl-COT (4) and prenyl-CHT (5) (both in the form of racemates). We also examined the incorporation of the administered substance into quinonoids which had been detected in the callus tissues. The feeding experiments were carried out twice. In the first experiment (Experiment A), [*carboxy*-^{14}C]-(3) was administered to callus tissues of *Catalpa ovata* (wet weight 36 g) grown for two weeks after being transferred, and incubated in the dark at 24 °C for five days. One half of the callus tissues, after addition of methanol, was homogenised with cooling (freezing mixture) and centrifuged. The supernatant was worked up immediately with an excess of an ethereal solution of diazomethane under continuous cooling. The other half of the callus tissues was extracted as it was with benzene. If the cells contain CHT, prenyl-COT (4), and prenyl-CHT (5), they must be converted in the diazomethane-treated solution into the corresponding methyl esters, (11), (17), and (14), while in the benzene extract into the decarboxylation products, 4-hydroxy-1-tetralone (13), catalponone (6), and catalponol (2), respectively. If the 2-*epi*-prenyl-CHT (26) exists as an intermediate, it must be converted in the diazomethane-treated solution into the 2-*epi*-prenyl-CHT methyl ester (15) and the 2-*epi*-prenyl-CHT lactone (16), while in the benzene extract



SCHEME Biosynthetic pathway for naphthoquinone congeners in callus tissues of *Catalpa ovata*



into 2-*epi*-catalponol (18). Furthermore, COT would be decarboxylated to give 4-oxo-1-tetralone (12) in the benzene extract. Thus, (11), (14), (15), (16), and (17) were added as carriers to the foregoing diazomethane-treated solution, while (2), (6), (12), (13), and (18) were added to the benzene extract. Meanwhile, menaquinone-1 (7) as a representative of the quinonoids was added to the latter extract to demonstrate the incorporation of (3) into quinonoids in the cultured cells. The residues obtained after concentration of both solutions *in vacuo* were chromatographed on silica gel to re-isolate the components.

In the second experiment (Experiment B), [*carboxy*-^{14}C]-(3) was administered to the callus tissues grown for three weeks after transfer and work-up in an analogous way to that described above. Compounds (14), (15), (16), and (17) were added to the diazomethane-treated methanol extract. Besides (2) and (6) (for trapping the biosynthetic intermediates), compounds (1), (9), and (10) were added to the benzene extract for detecting the metabolites. They were then re-isolated. Of the compounds re-isolated in both experiments, six crystalline compounds, catalpalactone (1),[15] (2*R*)-(−)-catalponone [(2*R*)-(6)],[9] 3-hydroxydehydro-iso-α-lapachone (9),[16] α-lapachone (10),[17] 4-oxo-1-tetralone (12),[7] and 2-*epi*-prenyl-CHT lactone (16), were purified as they were, while non-crystalline and hardly crystallisable substances were purified after conversion into crystalline derivatives in the following ways: (±)-CHT methyl ester (11) was converted into the dibenzoate (27), 4-hydroxy-1-tetralone (13) into 4-oxo-1-tetralone (12),[8] and (±)-prenyl-CHT methyl ester (14) and (±)-prenyl-COT methyl ester (17) * were converted into the same

* As one of the carbonyl groups of (17) was reduced on catalytic hydrogenation, subsequent oxidation was necessary to obtain (28).

(±)-isopentyl-COT methyl ester (28) through catalytic reduction followed by oxidation. Moreover, catalponol (2) was converted into (2*R*)-catalponone (2*R*)-(6), menaquinone-1 (7) into the leucoactate (29), (±)-2-*epi*-prenyl-CHT methyl ester (15) into (±)-2-*epi*-prenyl-CHT lactone (16), and epicatalponol (18) into (2*S*)-(+)-catalponone [(2*S*)-(6)].[9] In both experiments, unmetabolised OSB was recovered using chromatography.

Incorporation ratios of (3) into the foregoing substances in these experiments clearly demonstrate that (3) was incorporated not only into catalpalactone (1), α-lapachone (10), and (2*R*)-(−)-catalponone [(2*R*)-(6)], but also into the constituents characteristic of the cultured cells such as menaquinone-1 (7) and 3-hydroxydehydro-iso-α-lapachone (9) (Table 1). Furthermore, the incorporation

TABLE 1

Dilution analyses of constituents and intermediates after administration of 4-([*carboxy*-^{14}C]-2-carboxyphenyl)-4-oxobutanoic acid (3) (8.44 mg, 0.5 mCi each) to *Catalpa ovata* callus cultures

Compound	Incorporation (%) [a] [total activity (d.p.m.)] Experiment A	Experiment B
(±)-(11)	0.0025 [7.38×10^3]	
(±)-(14)	0.0013 [3.71×10^3]	0.0015 [5.04×10^3]
(±)-(15)	0.000 05 [b] [1.52×10^2]	0.000 04 [b] [1.45×10^2]
(±)-(17)	0.0042 [1.22×10^4]	0.0033 [1.12×10^4]
(2)	0.0048 [1.40×10^4]	0.022 [7.49×10^4]
(−)-(2*R*)-(6)	0.046 [1.35×10^5]	0.31 [1.06×10^6]
(12)	0.021 [6.20×10^4]	
(±)-(13)	0.0056 [1.63×10^4]	
(18)	0.000 31 [9.09×10^2]	
(1)		0.29 [9.74×10^5]
(7)	0.0083 [2.43×10^4]	
(9)		0.29 [9.94×10^5]
(10)		1.09 [3.67×10^6]

[a] Percentage incorporation was calculated on the basis of the balance between the radioactivity of (3) administered and that recovered. The balances for Experiments A and B were 2.92×10^8 and 3.38×10^8 d.p.m. mmol^{-1}, respectively. [b] Figure includes the incorporation into (±)-2-*epi*-prenyl-CHT lactone (16).

ratios of (3) into (12) and (13), above all, show that (3) was incorporated into COT in a 3.75 times higher ratio than into CHT, provided that the degradation ratios of COT to (12) and that of CHT to (13) were the same. The fact that the incorporation ratio of (3) into (11) is less than half of that into (13), although both (11) and (13) were derived from CHT, may be explained by degradation of most of the CHT giving rise to (13) during the course of the extraction. It was also found that (3) was further incorporated into prenyl-COT methyl ester (17) as well as into prenyl-CHT methyl ester (14) in a ratio similar to that for the incorporation of (3) into COT to that into CHT. These facts evidently demonstrate that four compounds, COT, CHT, prenyl-COT (4), and prenyl-CHT (5), are involved in the biosynthetic pathway for naphthoquinones and that COT and prenyl-COT (4) act as biosynthetic intermediates in a more important role than CHT and prenyl-CHT. Considering these findings together with the extremely low content of catalponol (2) in the callus cultures used in the present experiment, it may safely be said that, in the callus cultures, the route, COT ⟶ prenyl-COT (4) ⟶ catalponone (6), is the main pathway, while the route, CHT ⟶ prenyl-CHT (5) ⟶ catalponol (2), is a subsidiary one. Between both pathways, interconversions such as prenyl-COT (4) ⇌ prenyl-CHT (5) and catalponone (6) ⇌ catalponol (2) would also be possible. On the other hand, virtually no radioactivity was detected in the 2-*epi*-prenyl-CHT methyl ester (15) and 2-*epi*-prenyl-CHT lactone (16) which would be formed spontaneously from 2-*epi*-prenyl-CHT (26) through the extraction procedure. Accordingly, 2-*epi*-prenyl-CHT (26) seems to be ruled out as an intermediate for the biosynthesis of catalponol (2). Considering the steric configuration at C-4 of (2), it could therefore be concluded that, of all the four stereoisomers of prenyl- and 2-*epi*-prenyl-CHT, (2*S*,4*S*)-prenyl-CHT [(2*S*,4*S*)-(5)] can alone be the intermediate for the biosynthesis of (2), and that decarboxylation of this compound would proceed with retention of configuration giving rise to catalponol (2). It was also presumed, therefore, that the C-2 position of prenyl-COT (4), an intermediate in the main biosynthetic route, would also assume *S*-configuration and that of catalponone (6), the decarboxylation product, would adopt the *R*-configuration.

To verify this presumption, dilution analyses with optically active intermediates were then performed. The experiments were also carried out twice (Experiments C and D) in the same way as described earlier by adding carriers of optically active prenyl-COT methyl esters [(2*R*)- and (2*S*)-(17)] and catalponones [(2*R*)- and (2*S*)-(6)] to extracts of the callus cultures, to which (3) was administered. At the same time, trapping experiments of some constituents of the callus cultures were also undertaken.

In Experiment C, [*carboxy*-^{14}C]-(3) was administered to the callus tissues (wet weight, 56 g) of *C. ovata* cultured for a month after transferring. After incubation of the callus at 24 °C in the dark for 40 h, one half of the callus tissues was extracted with methanol and the extract was immediately treated with diazomethane, while the other half of the tissues was extracted with benzene in the same way as in the preceding experiments. The diazomethane-treated solution was divided into two halves. Then, (2R)-(+)-prenyl-COT methyl ester (2*R*)-(17) was added to one half, while (2*S*)-(−)-prenyl-COT methyl ester (2*S*)-(17) and methyl 4-(2-methoxycarbonylphenyl)-4-oxobutanoate (30) was added to the other half. The benzene extract was also divided into two halves and (2*S*)-(+)-catalponone [(2*S*)-(6)] and 3-

hydroxydehydro-iso-α-lapachone (9) were added to one half, while (2*R*)-(−)-catalponone [(2*R*)-(6)] and catalpalactone (1) were added to the other half.

In Experiment D, [*carboxy*-^{14}C]-(3) was fed to the callus cultures (wet weight, 60 g) grown for 1½ months after transferring; the mixture was then worked up in the same way as in Experiment C. The dilution analysis was carried out by adding carriers of (2*S*)-(−)-prenyl-COT methyl ester [(2*S*)-(17)], (2*R*)-(+)-prenyl-COT methyl ester [(2*R*)-(17)], and methyl 4-(2-methoxycarbonylphenyl)-4-oxobutanoate (30) to the diazomethane-treated methanol extract and (2*R*)-(−)- and (2*S*)-(+)-catalponone [(2*R*)- and (2*S*)-(6)], catalpalactone (1), menaquinone-1 (7), 1-hydroxy-2-methylanthraquinone (8), and lapachol (31) to the benzene extract.

Each extract of both experiments mixed with the carriers, after concentration, was subjected to chromatography on silica gel to re-isolate the components. Of the isolated compounds, crystallisable (2*R*)-(−)- and (2*S*)-(+)-catalponone [(2*R*)- and (2*S*)-(6)], 3-hydroxy-dehydro-iso-α-lapachone (9), catalpalactone (1), 1-hydroxy-2-methylanthraquinone (8), and lapachol (31) were purified by recrystallisation, while non-crystallisable compounds, both enantiomers of prenyl-COT methyl ester [(2*S*)- and (2*R*)-(17)], menaquinone-1 (7), and methyl 4-(2-methoxycarbonylphenyl)-4-oxobutanoate (30), were purified after conversion into crystalline substances, (1*S*,2*S*)-(+)-(19), (1*R*,2*R*)-(−)-(19), leucoacetate (29), and (3),[5] respectively. The incorporation ratios of (3) into the substances obtained in these experiments are shown in Table 2. These values demonstrate that (3) was incorporated in relatively high ratios into the constituents of comparatively high content including 3-hydroxy-dehydro-iso-α-lapachone (9) and lapachol (31), and that it was incorporated even into the constituents of comparatively low content including catalpalactone (1), menaquinone-1 (7), and 1-hydroxy-2-methylanthraquinone (8). Regarding prenyl-COT (4), the intermediate for the biosynthesis of these constituents, the incorporation of (3) into the 2*S*-form was 24—30 times higher than that into the 2*R*-form. Furthermore, the incorporation into (2*R*)-catalponone [(2*R*)-(6)] was 12—20 times higher than that into the (2S)-form. These facts clearly demonstrate that, as was expected, COT was stereoselectively prenylated to give (2*S*)-prenyl-COT [(2*S*)-(4)], which, in turn, was decarboxylated with retention of the configuration to yield (2*R*)-(−)-catalponone [(2*R*)-(6)], the key intermediate for the biosynthesis of prenylnaphthoquinones.*

TABLE 2

Dilution analyses of nine compounds after administration of 4-([*carboxy*-^{14}C]-2-carboxyphenyl)-4-oxobutanoic acid (3) (4.58 mg; 0.27 mCi each) to *Catalpa ovata* callus cultures

	Incorporation (%) [a] [total activity (d.p.m.)]	
	Experiment C	Experiment D
(−)-(2*S*)-(17)	0.0084 [3.17 × 10^4]	0.0058 [2.50 × 10^4]
(+)-(2*R*)-(17)	0.000 35 [1.32 × 10^3]	0.000 19 [8.25 × 10^2]
(−)-(2*R*)-(6)	0.045 [1.68 × 10^5]	0.18 [7.87 × 10^5]
(+)-(2*S*)-(6)	0.0022 [8.25 × 10^3]	0.015 [6.43 × 10^4]
(1)	0.063 [2.38 × 10^5]	0.23 [9.61 × 10^5]
(9)	3.26 [1.23 × 10^7]	
(7)		0.0090 [3.85 × 10^4]
(8)		0.0057 [2.46 × 10^4]
(31)		0.73 [3.12 × 10^6]

[a] Percentage incorporation was calculated on the basis of the balance between the radioactivity of (3) administered and that recovered. The balances for Experiments C and D were 3.78 × 10^8 and 4.28 × 10^8 d.p.m. mmol^{-1}, respectively.

Finally, the problem regarding the position of CHT in the biosynthetic pathway of quinonoids should be considered. As described above, CHT seems to intervene between COT and prenyl-CHT (5) in the subsidiary route COT ⟶ prenyl-CHT (5) ⟶ catalponol (2). However, there still remains the possibility that COT is derived *via* CHT, which, in turn, is formed through cyclisation of (32), the reduction product of (3), as in the chemical conversion † of (3) into CHT methyl ester. To examine this possibility, administration of 4-([*carboxy*-^{14}C]-2-carboxyphenyl)-4-hydroxy[4-^{3}H]butanoic acid (32), obtained by reduction of [*carboxy*-^{14}C]-(3) with NaB^3H_4, to the callus cultures was carried out. A feeding experiment of the same labelled compound to the wood of this plant was also performed to examine the biosynthetic route in the wood.

The results obtained (Table 3) indicate that (32) was incorporated into catalponone (6), catalponol (2), catalpalactone (1), α-lapachone (10), and 4,9-dihydroxy-α-lapachone (34) in the callus tissues as well as in the wood, while the tritium label had completely disappeared in compound (2). Catalponol (2) isolated during the experiment was purified after catalytic reduction to the crystalline dihydrocatalponol (35), and 4,9-dihydroxy-α-lapachone (34) after conversion into the diacetate (36).[7] In the experiment using callus cultures, [*carboxy*-^{14}C]-(3) formed through oxidation of (32) accompanied with loss of a ^{3}H-atom was recovered in 15% yield. These facts definitely show that (32) was metabolised into (6) and (2) after the oxidation to (3). It is thus demonstrated that COT is biosynthesised both in callus tissues and wood through direct cyclisation of (3), but not *via* CHT.

On the basis of all the foregoing results, it is concluded that the principal biosynthetic pathway for prenylnaphthoquinones and their congeners in the callus cultures for *C. ovata* passes through COT, (2*S*)-prenyl-COT [(2*S*)-(4)], and (2*R*)-catalponone [(2*R*)-(6)] (Scheme).

The formation of menaquinone-1 (7) through this route is highly suggestive of the biosynthetic pathway of

* The comparison of incorporation of (2*R*)-catalponone [(2*R*)-(6)] and (2*S*)-catalponone [(2*S*)-(6)] into quinonoids in the wood of *C. ovata*, which support this conclusion, will be reported elsewhere.

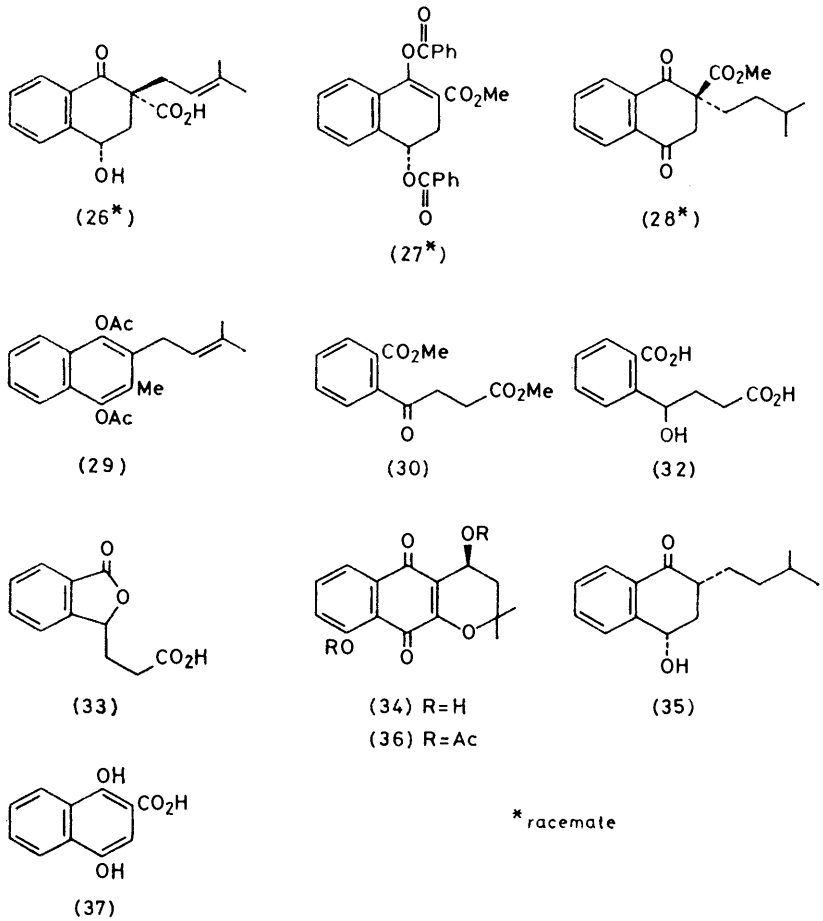
† Actually, in the chemical conversion of (3) into CHT methyl ester, the methyl ester of phthalide (33), an equivalent of the methyl ester (30) of (32), was used.

TABLE 3

Percent incorporation of 4-([*carboxy*-^{14}C]-2-carboxyphenyl)-4-hydroxy[4-^{3}H]butanoic acid (32) [a] into five constituents of *Catalpa ovata*

	Callus tissues [b]		Wood		
Constituent	Incorporation (%) [c] [total activity (d.p.m.)]	^{3}H/^{14}C	Incorporation (%) [c] [spec. incorp. (%)]	Spec. activity [c] (d.p.m. mmol^{-1}) [amount (mg)]	^{3}H/^{14}C
(1)	0.348 [1.42 × 10^{5}]	0	0.20 [0.0050]	1.45 × 10^{6} [35.5]	0
(2)	0.016 [6.39 × 10^{3}]	0	0.40 [0.0073]	2.12 × 10^{6} [42.0]	0
(6)	0.059 [2.41 × 10^{4}]	0			
(10)	0.781 [3.19 × 10^{5}]	0			
(34)			0.0059 [0.0055]	1.62 × 10^{6} [1.0]	0

[a] The acid (32) with the specific activity of ^{14}C 2.92 × 10^{10} d.p.m. and ^{3}H/^{14}C ratio of 0.814 was fed to the callus tissues, while the acid with the same specific activity of ^{14}C and ^{3}H/^{14}C ratio of 3.58 was administered to the wood. [b] Percentage incorporation was calculated on the basis of the balance between the radioactivity of the precursor and that of (33) recovered. [c] Incorporation and specific incorporation as well as total and specific activities of isolated substances were calculated on the ^{14}C basis.



vitamins K (phylloquinone and menaquinones). Young [18] and Bentley *et al.*[19,20] postulated that 1,4-dihydroxy-2-naphthoic acid (37) is an intermediate for menaquinone biosynthesis in *Escherichia coli*. Young *et al.*[21] further demonstrated the presence of 1,4-dihydroxy-2-naphthoate octaprenyltransferase in cell extracts of *E. coli*. It could easily be presumed, however, that vitamins K (especially in view of plant origin, phylloquinone) are also formed through the same prenylation mechanism as described above.

EXPERIMENTAL

M.p.s were taken on a Yanagimoto micromelting-point apparatus. I.r. spectra were determined with a Hitachi 215

grating spectrophotometer, ^{1}H n.m.r. spectra, unless otherwise stated, with a Varian A-60 or HA-100 spectrometer for solutions in deuteriochloroform with tetramethylsilane as internal standard, low resolution mass spectra with a Hitachi RMU 6D or JEOL JMS-01SG-2 spectrometer, and high resolution spectra with the latter. Column chromatography was performed on silica gel (Mallinckrodt AR-100) or on acetylated polyamide obtained by acetylation of polyamide (Wako C-200) (100 g) with acetic anhydride (400 ml) and pyridine (400 ml) in the usual way. Silica gel 60 GF_{254} (Merck) was used for t.l.c. and spots were visualised by irradiation with u.v. light or by exposure to iodine vapour. Silica gel PF_{254} (Merck) was used for preparative t.l.c. and bands were visualised by irradiation with u.v. light. Solvent mixtures are given on a volume/volume basis. Solutions in organic solvents were dried over anhydrous magnesium sulphate. Radioactivity was measured by a Beckman LS-230 liquid scintillation counter with samples dissolved in a scintillation fluid consisting of toluene (10 ml), PPO (40 mg), and POPOP (0.5 mg). The specific activities are calculated on the basis of the activities obtained after recrystallisation to constant value and expressed as values before dilution. T.l.c. plates were scanned with an Aloka JTC-201 radiochromatogram scanner.

Prenylation of (±)-4-*Hydroxy*-2-*methoxycarbonyl*-1-*tetralone* (11).—A solution of (±)-CHT methyl ester (11) (220 mg) in dry t-butyl alcohol (5 ml) was added dropwise to a stirred solution of potassium t-butoxide (165 mg) in dry t-butyl alcohol (15 ml) under nitrogen and 1-bromo-3-methylbut-2-ene (0.2 ml) was then added in one portion to the mixture. After being stirred for 30 min, the mixture was poured into ice–water, adjusted to pH 3 with 1N-hydrochloric acid, and extracted with ether. The extract was washed with brine, dried, and concentrated *in vacuo*. The residue (225 mg) was chromatographed on silica gel (10 g), eluting with benzene, to give crystals (12.0 mg) which were recrystallised from methanol to yield (±)-2-*epi*-prenyl-CHT lactone (16) as needles, m.p. 123—124 °C (Found: C, 74.75; H, 6.3. $C_{16}H_{16}O_3$ requires C, 74.98; H, 6.29%); $\nu_{max.}$ (Nujol) 1 780, 1 688, and 1 603 cm^{-1}; δ 1.65br (3 H, s, $=CCH_3$), 1.69 (3 H, d, *J* 0.5 Hz, $=CCH_3$), 2.64 (1 H, d, *J* 12.0 Hz, 3-H_{ax}), 2.78 (2 H, d, *J* 8.0 Hz, $-CH_2CH=$), 2.93 (1 H, dd, *J* 12.0 and 6.0 Hz, 3-H_{eq}), 5.07 (1 H, deformed t, *J* 8.0 Hz, $-CH_2CH=$), 5.50 (1 H, d, *J* 6.0 Hz, 4-H), 7.30—7.73 (3 H, m, 5-, 6-, and 7-H), and 7.98—8.10 (1 H, m, 8-H). Elution with benzene–ethyl acetate (98 : 2) gave (±)-prenyl-CHT methyl ester (14) (81.6 mg) as an oil, (Found: *m/e* 288.1347. $C_{17}H_{20}O_4$ requires *M*, 288.1361); $\nu_{max.}$ ($CHCl_3$) 3 400—3 600, 1 728, 1 682, and 1 600 cm^{-1}; δ 1.67br [6 H, s, $=C(CH_3)_2$], 2.03 (1 H, dd, *J* 13.0 and 11.0 Hz, 3-H_{ax}), 2.30br (1 H, s, OH, disappeared on treatment with D_2O), 2.72 (2 H, d, *J* 7.0 Hz, $-CH_2CH=C<$), 2.78 (1 H, dd, *J* 13.0 and 5.0 Hz, 3-H_{eq}), 3.63 (3 H, s, CO_2CH_3), 5.04 (1 H, m, $-CH_2CH=C<$), 5.15 (1 H, dd, *J* 11.0 and 5.0 Hz, 4-H), 7.31—7.72 (3 H, m, 5-, 6-, and 7-H), and 7.95—8.16 (1 H, m, 8-H). Further elution with the same solvent gave the methyl ester (15) (84.0 mg) of the (±)-2-epimer of prenyl-CHT (5) as an oil (Found: *m/e* 288.1330. $C_{17}H_{20}O_4$ requires *M*, 288.1361); $\nu_{max.}$ ($CHCl_3$) 3 400—3 600, 1 722, 1 682, and 1 598 cm^{-1}; δ 1.67 and 1.69 [each 3 H, d, *J* 1.0 Hz, $=C(CH_3)_2$], 2.40 and 2.74 (each H, dd, *J* 15.0 and 5.0 Hz, 3-H_2), 2.70br (2 H, d, *J* 8.0 Hz, $-CH_2CH=$), 2.80br (1 H, s, OH, disappeared on treatment with D_2O), 3.68 (3 H, s, CO_2CH_3), 4.94 (1 H, t, *J* 5.0 Hz, 4-H), 5.09 (1 H, deformed t, *J* 8.0 Hz, $-CH_2CH=$), 7.30—7.70 (3 H, m, 5-, 6-, and 7-H), and 7.94—8.15 (1 H, m, 8-H).

Lactonisation of the Methyl Ester (15).—A solution of the methyl ester (15) (20 mg) in dry toluene (1 ml) was added to a suspension of potassium t-butoxide (9 mg) in dry toluene (2 ml) and the mixture was stirred at room temperature for 5 min. After addition of water under ice-cooling, the mixture was neutralised with 1N-hydrochloric acid and extracted with ether. The extract was washed with brine, dried, and concentrated *in vacuo*. Recrystallisation of the residue (19 mg) from methanol afforded needles, m.p. 123—124 °C, which were identified with an authentic sample of the lactone (16) (i.r. and n.m.r. spectra and mixed m.p.).

Jones Oxidation of (±)-4-*Hydroxy*-2-*methoxycarbonyl*-2-*prenyl*-1-*tetralone* (14) *and its* 2-*Epimer* (15).—Jones reagent was added dropwise to an ice-cooled solution of the methyl ester (14) (25 mg) in acetone (0.8 ml) with stirring until the yellow colour of the solution persisted. After being stirred for a further 5 min, the mixture was diluted with water (5 ml) and extracted with ether. The extract was washed with brine, dried, and concentrated *in vacuo*. The residue (22 mg) was recrystallised from methanol to give (±)-*prenyl*-COT *methyl ester* (17) as prisms, m.p. 75—76 °C (Found: C, 71.05; H, 6.4. $C_{17}H_{18}O_4$ requires C, 71.31; H, 6.34%); $\nu_{max.}$ ($CHCl_3$) 1 738, 1 700, and 1 598 cm^{-1}; δ 1.62br and 1.69br [each 3 H, s, $=C(CH_3)_2$], 2.78 (2 H, d, *J* 7.0 Hz, $-CH_2CH=$), 2.88 and 3.45 (each H, d, *J* 16.0 Hz, 3-H_2), 3.63 (3 H, s, CO_2CH_3), 5.06 (1 H, deformed t, *J* 7.0 Hz, $-CH_2CH=C<$), and 7.55—8.20 (4 H, A_2B_2, 4 ArH). The 2-epimer (15) was also oxidised by the same procedure as described above to yield the same product (17).

Reduction of (±)-*Prenyl-COT Methyl Ester* (17) *with Lithium Tri-t-butoxyaluminium Hydride*.—A solution of (±)-prenyl-COT methyl ester (17) (125 mg) in dry tetrahydrofuran (1 ml) was added dropwise under nitrogen at −30 °C to a stirred solution of lithium tri-t-butoxyaluminium hydride prepared from lithium aluminium hydride (20 mg) and t-butyl alcohol (0.14 ml) in dry tetrahydrofuran (1 ml). After stirring for a further 1.5 h, the reaction mixture was diluted with water, adjusted to pH 3 with 1N-hydrochloric acid and extracted with ether. The extract was washed with brine, dried, and concentrated *in vacuo* to furnish an oily residue which was subjected to preparative t.l.c. [light petroleum–ether (7 : 3), six developments]. The residue from the eluate of the less polar band (R_F 0.65) was crystallised from ether–light petroleum to yield (±)-2-*methoxycarbonyl*-4-*oxo*-2-*prenyl*-1α-*tetralol* (19) (37.5 mg) as needles, m.p. 81—82 °C (Found: C, 71.0; H, 7.2. $C_{17}H_{20}O_4$ requires C, 70.81; H, 6.99%); $\nu_{max.}$ (Nujol) 3 300, 1 715, 1 655, and 1 585 cm^{-1}; δ 1.55 and 1.68 [each 3 H, d, *J* 1.0 Hz, $=C(CH_3)_2$], 2.53 (2 H, d, *J* 8.0 Hz, $-CH_2CH=$), 2.92 (2 H, s, 3-H_2), 3.25 (1 H, d, *J* 4.0 Hz, OH, disappeared on treatment with D_2O), 3.55 (3 H, s, CO_2CH_3), 5.10 (1 H, deformed t, *J* 8.0 Hz, $-CH_2CH=C<$), 5.15 (1 H, d, *J* 4.0 Hz, 1-H, collapsed to a singlet on treatment with D_2O), 7.15—7.65 (3 H, m, 6-, 7-, and 8-H), and 7.82—8.05 (1 H, m, 5-H). The more polar band (R_F 0.55) yielded (±)-2-*methoxycarbonyl*-4-*oxo*-2-*prenyl*-1β-*tetralol* (20) (72.0 mg) as an oil (Found: *m/e*, 288.1350. $C_{17}H_{20}O_4$ requires *M*, 288.1361); $\nu_{max.}$ ($CHCl_3$) 3 450, 1 720, 1 700, 1 680, and 1 598 cm^{-1}; δ 1.57 and 1.73 [each 3 H, d, *J* 1.0 Hz, $=C(CH_3)_2$], 2.50 (2 H, d, *J* 8.0 Hz, $-CH_2CH=$), 2.60 and 3.21 (each H, d, *J* 17.0 Hz, 3-H_2), 3.60 (3 H, s, CO_2CH_3), 3.75br (1 H, s, OH, disappeared on treatment with D_2O), 4.88 (1 H, s, 1-H), 5.13 (1 H, deformed t,

J 8.0 Hz, $-CH_2CH=C<$), 7.22—7.80 (3 H, m, 6-, 7-, and 8-H), and 7.87—8.10 (1 H, m, 5-H).

(±)-2-*Carboxy*-4-*oxo*-2-*prenyl*-1α-*tetralol* [(±)-(24)].—A solution of (±)-prenyl-COT methyl ester [(±)-(17)] (2.50 g) in dry tetrahydrofuran (10 ml) was added dropwise over a period of 30 min to a stirred solution of lithium tri-t-butoxyaluminium hydride [from lithium aluminium hydride (400 mg) and t-butyl alcohol (2.80 ml)] in dry tetrahydrofuran (20 ml) under nitrogen at 40 °C. After stirring for a further 30 min, the mixture was worked up as described above to give a residue (2.46 g) which contained (±)-(19) and (±)-(20) in a ratio of 95 : 5 (g.l.c.). The residue, without further purification, was dissolved in methanol (10 ml) and stirred at 40 °C with 2N-sodium hydroxide (10 ml) for 2 h. The usual work-up gave a substance (2.32 g) which was recrystallised from methanol–chloroform to yield the (±)-*acid* [(±)-(24)] (2.09 g) as needles, m.p. 182—183 °C (Found: C, 70.2; H, 6.65. $C_{16}H_{18}O_4$ requires C, 70.06; H, 6.61%); $\nu_{max.}$ (Nujol) 3 300, 3 100—2 200, 1 685, 1 650, and 1 582 cm^{-1}; δ (CD_3OD) 1.67 and 1.73 [each 3 H, d, *J* 1.0 Hz, $=C(CH_3)_2$], 2.62 (2 H, d, *J* 8.0 Hz, $-CH_2CH=$), 2.78 and 2.89 (each H, d, *J* 17.0 Hz, 3-H_2), 5.00 (1 H, s, 1-H), 5.18 (1 H, deformed t, *J* 8.0 Hz, $-CH_2CH=C<$), 7.12—7.80 (3 H, m, 6-, 7-, and 8-H), and 7.80—8.06 (1 H, m, 5-H).

Conversion of the Methyl Ester (±)-(19) *into the Tricyclic Compound* (±)-(22).—A solution of *m*-chloroperbenzoic acid (66 mg) in chloroform (3.3 ml) was added to a solution of (±)-(19) (48 mg) in chloroform (3.3 ml) containing toluene-*p*-sulphonic acid (3.3 mg). After stirring at room temperature for 3 h, the mixture was diluted with chloroform, washed successively with 10% aqueous sodium hydrogensulphite, saturated aqueous sodium hydrogencarbonate, and brine, dried, and concentrated *in vacuo*. The residue (51 mg) was purified by preparative t.l.c. [benzene–ethyl acetate (6 : 4)] and the main product obtained from the band at R_F 0.30 was recrystallised from chloroform to give the tricyclic compound (±)-(22) (25 mg) as *needles*, m.p. 131—133 °C (Found: C, 67.0; H, 6.6. $C_{17}H_{20}O_5$ requires C, 67.09; H, 6.62%); $\nu_{max.}$ (Nujol) 3 360, 1 718, 1 680, and 1 595 cm^{-1}; δ 1.32 and 1.47 [each 3 H, s, $>C(CH_3)_2$], 1.91 (1 H, dd, *J* 13.0 and 6.0 Hz, 1′-H_{eq}), 2.27 (1 H, dd, *J* 13.0 and 11.0 Hz, 1′-H_{ax}), 1.90—2.35 (1 H, m, OH, disappeared on treatment with D_2O), 2.83 (1 H, dd, *J* 17.0 and 1.2 Hz, 3-H_{eq}), 3.27 (1 H, d, *J* 17.0 Hz, 3-H_{ax}), 3.52 (3 H, s, CO_2CH_3), 5.03 (1 H, d, *J* 1.2 Hz, 1-H), 7.25—7.65 (3 H, m, 6-, 7-, and 8-H), and 7.92—8.13 (1 H, m, 5-H). A minor product that appeared at R_F 0.42 on t.l.c. was not examined.

Conversion of the Methyl Ester (±)-(20) *into the Tricyclic Compound* (±)-(23).—*m*-Chloroperbenzoic acid (68 mg) was added to a solution of (±)-(20) (60 mg) in chloroform (3 ml) and the mixture was stirred at room temperature for 1.5 h. After dilution with chloroform, the solution was washed successively with 10% aqueous sodium sulphite, saturated aqueous sodium hydrogencarbonate, and brine, dried, and concentrated *in vacuo*. The residue (63 mg) was subjected to preparative t.l.c. [benzene–ethyl acetate (7 : 3)] to give two bands at R_F 0.26 and 0.42. The latter, which was the main band, was eluted with chloroform to afford the 2′,3′-epoxy-derivative (45 mg) of (±)-(20) as a powder, δ 1.20 and 1.30 [each 3 H, s, $>C(CH_3)_2$], 2.02 (2 H, d, *J* 6.0 Hz, 1′-H_2), 2.72 and 3.43 (each H, d, *J* 17.0 Hz, 3-H_2), 2.87 (1 H, t, *J* 6.0 Hz, 2′-H), 3.62 (3 H, s, CO_2CH_3), 3.52—3.98br (1 H, OH, disappeared on treatment with D_2O), 5.03br (1 H, d, *J* 8.0 Hz, 1-H, collapsed to a singlet on treatment with D_2O), and 7.25—8.08 (4 H, m, 4 ArH). The epoxide in dry benzene (2 ml) was treated with boron trifluoride–ether (0.2 ml) at room temperature for 5 h. After addition of water, the benzene phase was separated and the remaining aqueous phase was extracted twice with benzene. The combined organic extracts were washed successively with saturated aqueous sodium hydrogencarbonate and water, dried, and concentrated *in vacuo*. The resulting residue (40 mg) was purified by preparative t.l.c. [benzene–ethyl acetate (7 : 3)] and the main band at R_F 0.28 was eluted with chloroform to afford the tricyclic compound (±)-(23) as a powder (23 mg) (Found: C, 66.9; H, 6.5. $C_{17}H_{20}O_5$ requires C, 67.09; H, 6.62%); $\nu_{max.}$ ($CHCl_3$) 3 350, 1 710, 1 680, and 1 590 cm^{-1}; δ 1.37 and 1.40 [each 3 H, s, $>C(CH_3)_2$], 1.73 (1 H, dd, *J* 13.0 and 12.0 Hz, 1′-H_{ax}), 1.83—2.07br (1 H, OH, disappeared on treatment with D_2O), 2.38 (1 H, dd, *J* 13.0 and 5.0 Hz, 1′-H_{eq}), 2.52 and 3.13 (each H, d, *J* 17.5 Hz, 3-H_2), 3.44 (3 H, s, CO_2CH_3), 3.99 (1 H, dd, *J* 12.0 and 5.0 Hz, 2′-H), 4.96br (1 H, s, 1-H), 7.22—7.73 (3 H, m, 6-, 7-, and 8-H), and 7.87—8.08 (1 H, m, 5-H).

Resolution of (±)-2-*Carboxy*-4-*oxo*-2-*prenyl*-1α-*tetralol* [(±)-(24)].—Quinine (2.25 g) was added in several portions to a hot solution of (±)-(24) (2.00 g) in acetone (30 ml) and the resulting homogeneous solution was allowed to stand in a refrigerator overnight. The crystals were collected by filtration and the filtrate was concentrated *in vacuo* to half volume. The concentrated solution was again set aside in a refrigerator after seeding the crystals obtained above, and the precipitated crystals were collected by filtration. The work-up was repeated three times. The combined crude crystals (2.05 g) were recryallised three times from acetone to give pillars (1.54 g), m.p. 210—211 °C. A suspension of this substance in ice–water was acidified to pH 3 with 1N-hydrochloric acid and extracted with ethyl acetate. The extract was washed with 1N-hydrochloric acid and brine, dried, and concentrated *in vacuo*. The crystalline residue (670 mg) was recrystallised twice from chloroform–methanol to yield the acid (±)-(24) (416 mg) as needles, m.p. 204—205 °C, $[\alpha]_D^{22}$ +22.0° (*c*, 3.60 in methanol) whose i.r. and n.m.r. spectra were identical with those of the starting (±)-(24). The final mother-liquor, after filtration of the crude quinine salt, was concentrated *in vacuo* to give a residue (2.09 g), which was suspended in ice–water. After being acidified to pH 3 with 1N-hydrochloric acid, the suspension was extracted with ethyl acetate and the extract was washed successively with 1N-hydrochloric acid and brine, dried, and concentrated *in vacuo*. The residue (1.04 g) was recrystallised eleven times from chloroform–methanol to yield (−)-(24) (180 mg) as *needles*, m.p. 204—205 °C, $[\alpha]_D^{22}$ −22.8° (*c*, 3.22 in methanol), whose i.r. and n.m.r. spectra were identical with those of (±)-(24).

Methylation of (1S,2S)-(+)- *and* (1R,2R)-(−)-2-*Carboxy*-4-*oxo*-2-*prenyl*-1α-*tetralol* [(+)- *and* (−)-(24)].—An excess of ethereal diazomethane was added to a solution of the (+)-carboxylic acid (+)-(24) (60 mg) in methanol (2 ml) and the crude methylation product was recrystallised from ether–light petroleum to yield (1*S*,2*S*)-(+)-2-*methoxycarbonyl*-4-*oxo*-2-*prenyl*-1α-*tetralol* [(1*S*,2*S*)-(+)-(19)], m.p. 74—75 °C $[\alpha]_D^{22}$ +36.1° (*c*, 2.74 in methanol), whose i.r. and n.m.r. spectra were identical with those of (±)-(19) (Found: C, 70.65; H, 6.9. $C_{17}H_{20}O_4$ requires C, 70.81; H, 6.99%). The (−)-carboxylic acid [(−)-(24)] was also methylated to yield (1*R*,2*R*)-(−)-(19) as *needles*, m.p. 74—75 °C, $[\alpha]_D^{22}$ −35.5° (*c*, 2.70 in methanol) (Found: C, 70.75; H, 6.9).

p-*Bromobenzoylation of* (1S,2S)-(+)- *and* (1R,2R)-(−)-2-

Methoxycarbonyl-4-oxo-2-prenyl-1α-tetralol [(1S,2S)-(+)- *and* (1R,2R)-(−)-(19)].—*p*-Bromobenzoyl chloride (57 mg) was added to a solution of the (1*S*,2*S*)-(+)-methyl ester [(1*S*,2*S*)-(+)-(19)] (50 mg) in dry pyridine. After being set aside at room temperature overnight, the mixture was diluted with water and extracted with ether. The extract was washed successively with 1N-hydrochloric acid, saturated aqueous sodium hydrogencarbonate, and water, dried, and concentrated *in vacuo*. The residue (72 mg) was chromatographed on silica gel (5 g) with benzene to give the *p*-bromobenzoate (25) of (1*S*,2*S*)-(+)-(19) (62 mg) as an *oil* (Found: C, 61.0; H, 5.0. $C_{24}H_{23}O_5Br$ requires C, 61.16; H, 4.92%); $\nu_{max.}$ ($CHCl_3$) 1 725, 1 715, 1 680, and 1 585 cm^{-1}; δ 1.53 and 1.70 [each 3 H, d, *J* 0.5 Hz, $=C(CH_3)_2$], 2.55br (2 H, s, *J* 8.0 Hz, 1′-H_2), 3.00 (1 H, d, *J* 18.0 Hz, 3α-H), 3.24 (1 H, dd, *J* 18.0 and 1.5 Hz, 3β-H), 3.52 (3 H, s, CO_2CH_3), 5.10 (1 H, deformed t, *J* 8.0 Hz, 2′-H), 6.66 (1 H, d, *J* 1.5 Hz, 1-H), and 7.40—8.20 (8 H, m, 8 ArH); c.d. (2.335 × 10^{-3}M, methanol) $\lambda_{max.}$ (θ) 192 (−28 100), 196 (−47 100), 201 (0), 207 (+78 100), 214 (0), 216.5 (−8 000), 220 (−6 700), 236 (−46 300), 244.5 (0), 254.5 (+68 100), 272 (+7 490), 283 (+10 000), 290 (+9 580), 310 (+2 590), 325 (+3 250), 335 (+3 180), and 380 (0) nm. The (1*R*,2*R*)-(−)-methyl ester [(1*R*,2*R*)-(−)-(19)] (50 mg) was worked up in the same way as above giving rise to the p-*bromobenzoate* (62 mg) as a colourless oil (Found: C, 60.9; H, 4.8. $C_{24}H_{23}O_5Br$ requires C, 61.16; H, 4.92%); c.d. (1.717 × 10^{-3}M, methanol) $\lambda_{max.}$ (θ) 192 (+23 600), 195 (+43 600), 201 (0), 206.5 (−75 600), 214 (0), 215.5 (+8 800), 219 (+6 800), 236 (+42 300), 244.5 (0), 254 (−64 700), 272 (−7 080), 283 (−9 360), 290 (−9 120), 310 (−2 440), 325 (−3 030), 335 (−2 950), and 380 (0).

Jones Oxidation of (1S,2S)-(+)- *and* (1R,2R)-(−)-2-*Methoxycarbonyl-4-oxo-2-prenyl-1α-tetralol* [(1S,2S)-(+)- *and* (1R,2R)-(−)-(19)].—An excess of Jones reagent was added dropwise to a solution of (1*S*,2*S*)-(19) (50 mg) in acetone (2 ml) and the mixture was stirred at room temperature for 30 min. Usual work-up furnished a residue (48 mg) which was chromatographed on silica gel (5 g) with benzene as eluant to give (2*S*)-(−)-2-*prenyl*-COT *methyl ester* [(2*S*)-(−)-(17)] (43 mg) as an oil, $[\alpha]_D^{22}$ −64.2° (*c*, 3.37 in methanol) (Found: *m/e*, 286.1204. $C_{17}H_{18}O_4$ requires *M*, 286.1205). The i.r. and n.m.r. spectra of this substance were identical with those of (±)-(17). (1*R*,2*R*)-(−)-Methyl ester [(1*R*,2*R*)-(−)-(19)] was also oxidised with Jones reagent in the same way as above to give (2*R*)-(+)-2-*prenyl-COT methyl ester* [(2*R*)-(+)-(17)] as an oil, $[\alpha]_D^{22}$ +65.5° (*c*, 3.05 in methanol) (Found: *m/e*, 286.1230. $C_{17}H_{18}O_4$ requires *M*, 286.1205).

Benzoylation of (±)-*CHT Methyl Ester* (11).—Benzoyl chloride (0.06 ml) was added dropwise to an ice-cooled solution of CHT methyl ester (11) (35 mg) in dry pyridine (0.5 ml). After being set aside at room temperature for 2 h, the mixture was poured into ice–water and extracted with benzene. The extract was washed successively with 1N-hydrochloric acid, saturated aqueous sodium hydrogencarbonate, and brine, dried, and evaporated *in vacuo*. The crystalline residue (31.5 mg) was subjected to preparative t.l.c. [benzene–ether (7 : 3)] and the residue (26.0 mg) obtained from a band around R_F 0.86 was recrystallised from ether to afford the dibenzoate (27) as needles, m.p. 152—153 °C (Found: C, 73.1; H, 4.55. $C_{26}H_{20}O_6$ requires C, 72.89; H, 4.71%); $\nu_{max.}$ (Nujol) 1 742, 1 705, 1 633, and 1 601 cm^{-1}; δ 3.22 and 3.30 (each H, dd, *J* 17.5 and 5.0 Hz, 3-H), 3.65 (3 H, s, CO_2CH_3), 6.38 (t, *J* 5.0 Hz, 4-H), 7.30—7.74 (10 H, m, 10 ArH), and 7.99—8.38 (4 H, m, 4 ArH).

Jones Oxidation of 4-*Hydroxy*-1-*tetralone* (13).—4-Hydroxy-1-tetralone (13) (32 mg) was oxidised in cold acetone with Jones reagent in the usual way and the resulting crude product (25 mg) was recrystallised from n-hexane to afford 4-*oxo*-1-*tetralone* (12) as needles, m.p. 96—97 °C (Found: C, 75.2; H, 4.85. $C_{10}H_8O_2$ requires C, 74.99; H, 5.03%); $\nu_{max.}$ ($CHCl_3$) 1 682, 1 592, and 715 cm^{-1}; δ 3.08 (4 H, s, 2- and 3-H_2), 7.65—7.86 (4 H, 6- and 7-H_2), and 7.94—8.15 (4 H, m, 5- and 8-H_2).

Catalytic Hydrogenation of (±)-*Prenyl-CHT Methyl Ester* [(±)-(14)] *and Jones Oxidation of the Reduction Product.*—(±)-2-Prenyl-CHT methyl ester [(±)-(14)] (30 mg) was hydrogenated in methanol (2.5 ml) over platinum oxide (10 mg) at room temperature and atmospheric pressure. The catalyst was filtered off and the filtrate was concentrated *in vacuo*. The residue was oxidised in cold acetone (2 ml) with Jones reagent. The crude product (21 mg) was recrystallised from n-hexane to yield the *dihydro-derivative* (±)-(28) as colourless needles, m.p. 76—77 °C (Found: C, 70.65; H, 6.95. $C_{17}H_{20}O_4$ requires C, 70.81; H, 6.99%); $\nu_{max.}$ ($CHCl_3$) 1 735, 1 699, and 1 598 cm^{-1}; δ 0.92 (6 H, d, *J* 6.0 Hz, 2 CH_3), 1.10—2.30 (5 H, m, $-CH_2CH_2CH<$), 2.88 and 3.52 (each H, d, *J* 16.0 Hz, 3-H_2), 3.64 (3 H, s, CO_2CH_3), and 7.65—8.22 (4 H, A_2B_2, 4 ArH).

Catalytic Hydrogenation of (±)-*Prenyl-COT Methyl Ester* [(±)-(17)] *and Jones Oxidation of the Reduction Product.*—(±)-2-Prenyl-COT methyl ester [(±)-(17)] (30 mg) was hydrogenated in methanol (1.5 ml) over platinum oxide (10 mg) at room temperature and atmospheric pressure. An oily product obtained by the same work-up as described above was oxidised with Jones reagent. Recrystallisation of the reaction product from n-hexane gave needles of the dihydro-derivative (±)-(28) which was identical with a sample of (±)-(28) derived from (±)-(14) (mixed m.p. and comparisons of i.r. and n.m.r. spectra).

Reductive Acetylation of Menaquinone-1 (7).—A mixture of menaquinone-1 (7) (120 mg), acetic anhydride (2 ml), zinc powder (120 mg), and triethylamine (1 drop) was stirred at room temperature for 15 min. The reaction mixture was poured into ice–water and extracted with chloroform. The extract was washed successively with saturated aqueous sodium hydrogencarbonate and brine, dried, and concentrated *in vacuo*. The residue (115 mg) was chromatographed on silica gel (5 g) with benzene as eluant to afford crystals (102 mg) which were recrystallised from methanol to yield the *leucoacetate* (29) as needles, m.p. 107—108 °C (Found: C, 73.45; H, 6.8. $C_{20}H_{22}O_4$ requires C, 73.60; H, 6.79%); $\nu_{max.}$ ($CHCl_3$) 1 750 and 1 600 cm^{-1}; δ 1.67 (3 H, d, *J* 1.2 Hz, $=CCH_3$), 1.75br (3 H, s, $=CCH_3$), 2.23 (3 H, s, $ArCH_3$), 2.41 (6 H, s, 2 CO_2CH_3), 3.40br (2 H, d, *J* 7.0 Hz, 1′-H_2), 5.08 (1 H, deformed t, *J* 7.0 Hz, 2′-H), and 7.32—7.84 (4 H, m, 4 ArH).

Cell Cultures.—Conditions for induction and culture of callus tissues of *Catalpa ovata* G. Don used for the present experiments have been described.[1]

Administration of 4-([carboxy-^{14}C]-2-*Carboxyphenyl*)-4-*oxobutanoic Acid* (3) *to the Callus Tissues and Detection of Naphthoquinone Congeners and their Biosynthetic Intermediates by Dilution Analyses.**—*Experiment A.* Callus tissues of *C. ovata* (2 weeks after subculturing, 36 g wet weight) were transferred into a 200 ml Erlenmeyer flask

* All the feeding experiments were carried out under sterile conditions. The activities shown for the trapped compounds in the following four experiments are values corresponding to the whole cells.

containing water (16 ml). A solution of [*carboxy*-^{14}C]-(3) (8.44 mg, 0.5 mCi) in 10% ethanol (15 ml) was sprinkled on the cultures which were then kept at 24 °C in the dark for 5 days. The cultures were divided into two equal parts. One half of the cultures, after addition of methanol (4 ml), was homogenised using a glass homogeniser under cooling with a freezing mixture at −10 °C. The supernatant obtained after centrifugation of the homogenate was immediately treated with ethereal diazomethane under cooling and the reaction mixture was concentrated *in vacuo* to *ca.* 20 ml. After addition of the carriers, (±)-(11) (20.0 mg), (±)-(14) (20.0 mg), (±)-(15) (15.0 mg), (±)-(16) (5.0 mg), and (±)-(17) (20.0 mg), the mixture was evaporated *in vacuo* and the residue was subjected to column chromatography on silica gel (8 g). Elution with benzene–ethyl acetate with increasing content of ethyl acetate gave (±)-(16) (4.85 mg), (±)-(17) (20.0 mg), and the dimethyl ester (30) of the unmetabolised (3). The alkaline hydrolysate of this ester (30) was diluted with the carrier of (3) (20 mg) and recrystallised from ether–light petroleum. Based on the activity of the obtained acid (3), the total radioactivity of the recovered (3) from the whole callus tissues amounts to 2.43×10^8 d.p.m. Further elution gave (±)-(14) (18.2 mg), (±)-(15) (11.0 mg), and (±)-(11) (20.0 mg). (±)-(14), (±)-(15), and (±)-(17), thus isolated, were converted according to the cold run into the respective crystalline derivatives. Thus, (±)-(11) (20.0 mg) was benzoylated with benzoyl chloride (0.04 ml) and pyridine (0.5 ml) in the usual way, and the product was purified by chromatography on silica gel yielding the dibenzoate (27) (24.2 mg) which, after dilution with the carrier (27) (40.0 mg), was recrystallised from methanol. The radioactive (±)-(14) (18.2 mg) was hydrogenated over platinum oxide and then oxidised with Jones reagent. The product was chromatographed on silica gel to yield (±)-(28) (12.7 mg) which, after addition of the carrier (20.0 mg), was recrystallised from n-hexane. Work-up of the radioactive (±)-(15) (11.0 mg) with potassium t-butoxide (4.5 mg) in toluene (2 ml) gave (±)-(16) (9.7 mg), which was combined with the substance (±)-(16) described above and recrystallised from methanol. The radioactive compound (±)-(17) was hydrogenated over platinum oxide (7.0 mg) in methanol (2 ml) and then oxidised with Jones reagent. The product (±)-(28), after dilution with the carrier (20.0 mg), was recrystallised from n-hexane. The other half of the callus tissues was left to stand in benzene at room temperature overnight and then extracted three times with the same solvent by warming at 80 °C for 1 h. The combined extracts were divided into two equal portions, one half of which was diluted with 20.0 mg each of (6), (2), and (13), and the other half with 20.0 mg each of (7), (12), and (18). The former mixture was chromatographed on silica gel eluted with benzene–ethyl acetate with increasing ethyl acetate content affording (6) (20.0 mg), (2) (20.0 mg), and (13) (17.8 mg). On the other hand, the latter mixture was chromatographed on silica gel (8 g) eluted successively with benzene and benzene–ethyl acetate giving rise to (7) (20.0 mg), (12) (18.8 mg), and (18) (19.5 mg). Of the materials thus isolated, (6) and (12) were recrystallised from light petroleum and n-hexane, respectively. The compounds (2), (7), (13), and (18) were transformed into suitable crystalline derivatives and purified by silica gel column chromatography followed by recrystallisation, respectively. Namely, compounds (2) (20.0 mg), (18) (19.5 mg), and (13) (17.8 mg) were subjected to Jones oxidation yielding (2*R*)-(6) (17.9 mg), (2*S*)-(6) (18.5 mg), and (12) (13.5 mg), respectively. (2*R*)- and (2*S*)-(6) were recrystallised from light petroleum, while (12), after addition of the carrier (20.0 mg), was recrystallised from n-hexane. (7) was subjected to reductive acetylation with acetic anhydride (0.5 ml), zinc powder (20 mg), and trimethylamine (1 drop) yielding (29) (21.5 mg) which, after addition of the carrier (40.0 mg), was recrystallised from methanol. The flasks used for the feeding were rinsed with methanol and the rinsings were treated with ethereal diazomethane. After removal of the solvent *in vacuo*, the residue was chromatographed on silica gel (5 g) using chloroform as eluant yielding the dimethyl ester (30) of the not metabolised [*carboxy*-^{14}C]-(3) which was purified after hydrolysis to the acid (3); total activity, 5.75×10^8 d.p.m. The total activity of [*carboxy*-^{14}C]-(3) recovered in Experiment A, therefore, amounted to 8.18×10^8 d.p.m.

Experiment B. The experiment was carried out in the same manner as in Experiment A. After administration of [*carboxy*-^{14}C]-(3) to the callus tissues (three weeks after subculturing; 25 g wet weight) under the same conditions as above, they were divided into two equal parts, and one half was extracted with methanol, and the other half with benzene. The methanolic extract was immediately treated with ethereal diazomethane and after addition of carriers (±)-(14) (20.0 mg), (±)-(15) (15.0 mg), (±)-(16) (5.0 mg), and (±)-(17) (20.0 mg), the mixture was concentrated *in vacuo*. The residue was chromatographed on silica gel (8 g) to afford (±)-(16) (5.0 mg), (±)-(17) (20.0 mg), (30) (0.6 mg), (±)-(14) (19.5 mg), and (±)-(15) (10.6 mg) in sequence. (30) was purified as (3) in the same way as described above; total activity, 3.24×10^8 d.p.m. On the other hand, (±)-(14), (±)-(15), (±)-(16), and (±)-(17) were converted into the crystalline derivatives and recrystallised. To the benzene extract was added 20.0 mg each of (1), (2), (6), (9), and (10), and the mixture was concentrated *in vacuo*. The residue was chromatographed on silica gel using benzene–ethyl acetate as eluant and (6) (20.0 mg), (10) (20.5 mg), (2) (18.5 mg), (1) (20.0 mg), and (9) (19.0 mg) were eluted in sequence. Compounds (1), (9), and (10) were recrystallised from methanol and (6) from light petroleum. Compound (2) was converted into (6) which was recrystallised from light petroleum. Finally, radioactive (3) was recovered from the flasks used for the feeding in the same way as in the foregoing experiment; total activity, 4.48×10^8 d.p.m. The total activity of all the recovered precursor (3) in this experiment amounted to 7.72×10^8 d.p.m.

Experiment C. A solution of [*carboxy*-^{14}C]-(3) (4.58 mg, 0.27 mCi) in 10% ethanol was poured onto the callus tissues of *C. ovata* (one month after subculturing; 56 g wet weight), soaked in water (8 ml), and the tissues were set aside at 24 °C in the dark for 40 h. One half of the tissues was homogenised in cold methanol (30 ml) (−10 °C) with a glass homogeniser and centrifuged. An excess of ethereal diazomethane was immediately added to the supernatant under continued cooling and the mixture was concentrated *in vacuo* to one third of the volume. The solution was divided into two equal parts and one half was diluted with (+)-(17) (50.0 mg), while the other half was diluted with (−)-(17) (50.0 mg) and (30) (20.0 mg). The former mixture was evaporated *in vacuo* and the residue was taken up in a small volume of chloroform. The insoluble material was filtered off and the filtrate was evaporated *in vacuo* to give a residue (fraction C-1) (68.2 mg). The latter mixture was worked up in the same way to give a residue (fraction C-2) (90.5 mg). The other half of the callus tissues was extracted with

benzene (2 × 50 ml) and the solution was divided into two halves. (2*S*)-(+)-(6) (20.0 mg) and (9) (20.0 mg) were added to one half, while (2*R*)-(−)-(6) (20.0 mg) and (1) (20.0 mg) were added to the other half. Both mixtures were concentrated *in vacuo* to leave residues [fractions C-3 (43.0 mg) and C-4, (42.5 mg), respectively]. Purification of the four fractions was carried out as follows. Fraction C-1 was chromatographed on silica gel (7 g) using benzene as eluant giving rise to (2*R*)-(+)-(17) (46.0 mg). This compound was reduced with lithium tri-t-butoxyaluminium hydride [from lithium aluminium hydride (8.0 mg) and t-butyl alcohol (0.056 ml)] and the product was purified by preparative t.l.c. [ether–light petroleum (3 : 7)] to yield (1*R*,2*R*)-(−)-(19) (41.5 mg), which was recrystallised from ether–light petroleum. Fraction C-2 was chromatographed on silica gel (7 g), eluted successively with benzene and benzene–ethyl acetate affording (2*S*)-(−)-(17) (48.1 mg) and (30) (19.1 mg). The latter, after alkaline hydrolysis to (3), was diluted with the carrier (20.0 mg) and recrystallised. Based on the radioactivity of the substance (3) thus obtained, the total activity of the recovered precursor for the whole tissues should be 2.24×10^8 d.p.m. On reduction with lithium tri-t-butoxyaluminium hydride, the foregoing compound (2*S*)-(−)-(17) gave (1*S*,2*S*)-(+)-(19) (40.8 mg) which, after preparative t.l.c. as for the enantiomer (2*R*)-(+)-(17), was recrystallised from ether–light petroleum. Fraction C-3 was chromatographed on silica gel (7 g) eluted successively with benzene (150 ml) and benzene–ethyl acetate (95 : 5), yielding (2*S*)-(+)-(6) (20.5 mg) and (9) (21.0 mg). The former was recrystallised from methanol, and the latter from light petroleum. Fraction C-4 was also chromatographed on silica gel (7 g) in the same way as described for fraction C-3 yielding (2*R*)-(−)-(6) (20.9 mg) and (1) (21.0 mg), which were recrystallised from light petroleum and methanol, respectively.

Experiment D. After administration of [*carboxy*-^{14}C]-(3) (4.58 mg, 0.27 mCi) to the callus cultures (one and a half month after subculturing; 60 g wet weight) and subsequent work-up as in the foregoing experiment, one half of the diazomethane-treated methanolic solution was diluted with (2*R*)-(+)-(17) (50.0 mg) and concentrated *in vacuo* to give fraction D-1 (60.5 mg), while the other half was diluted with (2*S*)-(−)-(17) (50.0 mg) and (30) (20.0 mg) and concentrated *in vacuo* to give fraction D-2 (82.0 mg). One half of the benzene extract diluted with (2*R*)-(−)-(6) (20.0 mg), (1) (20.0 mg), and (7) (20.0 mg) afforded fraction D-3 (63.4 mg), while the other half diluted with (2*S*)-(+)-(6) (20.0 mg), (8) (20.0 mg), and (31) (20.0 mg) afforded fraction D-4 (63.0 mg). Chromatography of each fraction on silica gel (7 g each) with benzene or benzene–ethyl acetate as eluant was performed as in the foregoing experiment. Of the re-isolated compounds, (2*R*)-(+)-(17) (49.6 mg), (2*S*)-(−)-(17) (49.0 mg), (1) (22.2 mg), (2*R*)-(−)-(6) (21.0 mg), (7) (20.5 mg), (2*S*)-(+)-(6) (20.5 mg), (8) (18.5 mg), and (31) (18.8 mg), both enantiomers of (17) and (6) as well as (30) were purified in the same way as above; total activity of (30), 1.75×10^8 d.p.m. Menaquinone-1 (7) was converted, after dilution with the carrier (40.0 mg), into the leucoacetate (29) (51.4 mg) which was recrystallised from methanol. The quinones (8) and (31) were recrystallised from methanol.

4-(2-Carboxyphenyl)-4-hydroxybutanoic Acid (32).—A solution of sodium borohydride (70 mg) in water (1 ml) was added dropwise to a solution of (3) (50 mg) in ethanol (2 ml). After standing at room temperature for 15 min, the mixture was diluted with water, neutralised with 1N-hydrochloric acid, and extracted with ether. The extract was washed with brine, dried, and evaporated *in vacuo*. Although the residue (47 mg) showed a single spot of R_F 0.30 on t.l.c. using a microcrystalline cellulose plate [ethanol–NH_4OH–water (20 : 4 : 1)], it transformed gradually into 3-(2-carboxyethyl)phthalide (R_F 0.55 on the above t.l.c.) while standing at room temperature.

Preparation of 4-([carboxy-^{14}C]-2-*Carboxyphenyl*)-4-*hydroxy*[4-^{3}H]*butanoic Acid* (32).—A solution of sodium borotritide (5 mg, *ca.* 1 mCi) in water (1 ml) was added dropwise to a solution of [*carboxy*-^{14}C]-(3) (0.87 mg, 2.92×10^{10} d.p.m. mmol^{-1}) in ethanol (0.5 ml). After standing at room temperature for 15 min, the mixture was diluted with water (3.5 ml) and neutralised with Amberlite IR-120 (H^+ form). The resulting solution showed a single radioactive spot whose R_F value was identical with that of (32) on t.l.c. (microcrystalline cellulose, ethanol–NH_4OH–water (20 : 4 : 1). This substance was used without further purification for the feeding experiments described below; specific activity 2.92×10^{10} d.p.m. mmol^{-1}; $^3H/^{14}C$ 0.814.

Hydrogenation of Catalponol (2).—Catalponol (2) (237 mg) was hydrogenated at room temperature and atmospheric pressure over platinum oxide (60 mg) in methanol. After removal of the catalyst, the filtrate was evaporated *in vacuo* and the crystalline residue (236 mg) was recrystallised from ether–light petroleum to yield dihydrocatalponol (35) as *needles*, m.p. 62—63 °C (Found: C, 77.7; H, 8.95. $C_{15}H_{20}O_2$ requires C, 77.55; H, 8.68%); $\nu_{max.}$ (Nujol) 3 250, 1 680, and 1 600 cm^{-1}; δ 0.92 (6 H, d, *J* 6.0 Hz, 2 CH_3), 1.10—2.70 (5 H, m, side chain 1-H_2, 2-H_2, and 3-H), 2.32br (1H, s, OH, disappeared on treatment with D_2O), 5.00 (1 H, d, *J* 11.0 and 5.0 Hz, 4-H), 7.20—7.85 (3 H, m, 5-, 6-, and 7-H), and 7.90—8.12 (1 H, m, 8-H).

Administration of 4-([carboxy-^{14}C]-2-*Carboxyphenyl*)-4-*hydroxy*[4-^{3}H]*butanoic Acid* (32) *to the Callus Tissues of* C. ovata *and Dilution Analyses with Catalpalactone* (1), *Catalponol* (2), *Catalponone* (6), *and α-Lapachone* (7).—The callus tissues of *C. ovata* (one month after subculturing; 37 g wet weight) were transferred to a flask containing water (10 ml) and a solution of [4-^{3}H, *carboxy*-^{14}*C*]-(32) [0.88 mg, specific activity (^{14}C) 2.92×10^{10} d.p.m. mmol^{-1}, $^3H/^{14}C$ 0.814] in 20% ethanol was sprinkled on the tissues. After being set aside at 25 °C for 3 days, the cultured cells were extracted successively with benzene (2 × 100 ml) and methanol (2 × 100 ml). Compounds (1), (2), (2*R*)-(6), and (10) (20.0 mg each) were added to the combined benzene extracts and the mixture was concentrated *in vacuo*. The residue (110.5 mg) was chromatographed on silica gel (15 g) eluted successively with benzene, benzene–ethyl acetate (98 : 2 and 97 : 3), and methanol. Benzene eluates gave (2*R*)-(6) (21.0 mg) and benzene–ethyl acetate (97 : 3) eluates afforded (10) (20.0 mg), (2) (19.1 mg), and (1) (21.9 mg) in sequence. Compounds (1) and (7) were recrystallised from methanol, and (6) from light petroleum. These three compounds were devoid of tritium labelling. The oily compound (2) was hydrogenated in methanol (3 ml) over platinum oxide (10 mg). After removal of the catalyst, the filtrate was evaporated *in vacuo* to give a residue (19.1 mg) which was chromatographed on silica gel (5 g) with benzene as eluant to give radioactive dihydrocatalponol (35) (15.1 mg) as needles. After addition of the carrier (20.0 mg), these were recrystallised from ether–light petroleum. On the other hand, the methanol extracts of the cultured cells, the methanol eluate of the chromatography of the benzene extracts of the tissues, and the methanol rinsings (3 × 15

ml) of the flasks used for feeding were combined and concentrated *in vacuo*. The residue (140 mg) was dissolved in chloroform and divided into two equal parts. The carrier (3) (100 mg) was added to one half of the solution and the mixture was evaporated *in vacuo*. The residue was chromatographed on silica gel (5 g) eluted successively with chloroform and chloroform–methanol (98 : 2) to give (3) (102.5 mg) which was recrystallised from chloroform–benzene; total activity (^{14}C) 6.13×10^6 d.p.m. $mmol^{-1}$. The carrier (33) (100 mg) was added to the other half of the foregoing chloroform solution and the mixture was chromatographed on silica gel (5 g) with chloroform as eluant to furnish (33) (102.0 mg) which was recrystallised from ether–light petroleum; total activity (^{14}C) 7.42×10^7 d.p.m. $mmol^{-1}$; $^3H/^{14}C$ 0.375. [The activity obtained was equal to that of the recovered (32), since (32) was easily convertible into (33) during the work-up.]

Administration of 4-([carboxy-^{14}C]-2-*Carboxyphenyl*)-4-*hydroxy*[4-^{3}H]*butanoic Acid* (32) *to the Wood of* C. ovata *and Isolation of Catalpalactone* (1), *Catalponol* (2), *and* 4,9-*Dihydroxy-α-lapachone* (36).—An aqueous solution of [4-^{3}H, *carboxy*-^{14}C]-(32) (0.76 mg, 2.92×10^{10} d.p.m. $mmol^{-1}$; $^3H/^{14}C$ 3.58) was fed hydroponically to a branch of *C.* ovata with leaves (in the flowering stage; 5 years old; length 45 cm). After 5 days, the branch (134 g wet weight) was cut into pieces and soaked in benzene (300 ml) at room temperature overnight and then extracted under reflux with benzene (2 × 300 ml). The combined extracts were concentrated *in vacuo* to give a residue (920 mg) which was chromatographed on silica gel (35 g) with benzene–ethyl acetate (96 : 4) as eluant to give a residue containing catalponol (2), which was triturated with methanol. After removal of the methanol-insoluble material by filtration, the filtrate was concentrated *in vacuo* to give an oil (42.0 mg) which showed on t.l.c. [benzene–ethyl acetate (8 : 2)] the same R_F value (0.49) as that of authentic catalponol (2). One half of the radioactive catalponol (2) thus obtained was subjected to hydrogenation in methanol over platinum oxide (10 mg) and the product was chromatographed on silica gel (8 g) with chloroform as eluant to yield dihydrocatalponol (35) (14.7 mg) as colourless needles. After addition of the carrier (20.0 mg), compound (35) was recrystallised from ether–light petroleum. Further elution gave a substance (55 mg) which was chromatographed on acetylated polyamide (20 g) with water as eluant. The aqueous eluate was extracted with chloroform and the chloroform layer was dried and concentrated *in vacuo* to afford a crystalline residue (35.5 mg) which showed the same R_F value (0.36) as that of catalpalactone (1) on t.l.c. [benzene–ethyl acetate (8 : 2)]. After addition of the carrier (35.0 mg), the substance was recrystallised from methanol. Further elution of the acetylated polyamide column gave a yellow mixture (5.92 mg) of α-lapachones. Purification after conversion into the acetate as described previously [7] gave 4,9-diacetoxy-α-lapachone (36) (1.28 mg) as yellow needles which, after addition of the carrier (10.0 mg), were recrystallised from methanol.

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