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BIOSYNTHESIS OF PRENYLNAPHTHOQUINONE CONGENERS IN CALLUS CULTURES OF CATALPA OVATA

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Regarding the biosynthesis of catalpalactone derivatives in *Catalpa ovata* G. Don[1], we have examined the naphthoquinone congeners of the callus tissues which were induced from the seedlings of the plant and subcultured on the Linsmaier-Skoog medium. Mainly using the GC-MS technique, we have succeeded in identifying seven known constituents previously obtained from the wood of the intact plant[2], catalpalactone (1), catalponol (2), catalponone (3) and four α-lapachones including α-lapachone (4) itself as well as six congeners, menaquinone-1 (5), 1-hydroxy-2-methylanthraquinone and four dehydro-iso-α-lapachones including 2R,3R-3-hydroxy-dehydro-iso-α-lapachone (6)[3] among the constituents of the callus tissues.

This paper describes the dilution analysis demonstrating the incorporation of [2'-^{14}C-carboxy]-4-(2'-carboxyphenyl)-4-oxo-butanoic acid (7) into the above-mentioned compounds 1-6 as well as a detailed study establishing the intermediacy of 2-carboxy-4-oxo-α-tetralone (COT), 2-carboxy-4-hydroxy-α-tetralone (CHT), 2-prenyl-COT (8) and 2-prenyl-CHT (9) for the biosynthesis of these compounds. Since 8 and 9, like COT and CHT, would probably be highly unstable, we attempted to detect 8, 9 as well as CHT as their methyl esters through the extraction of a portion of the callus cells with MeOH followed by immediate treatment of the extract with diazomethane and to detect COT and CHT as their decarboxylation products by extraction of the rest of the callus cells as they are with benzene. 2-Prenyl-CHT methyl ester (10) was obtained along with 2-epiprenyl-CHT methyl ester (11) and 2-epiprenyl-CHT lactone (12) by the condensation of CHT methyl ester (13)[1] with dimethylallyl bromide in the presence of KOBu^t, while 2-prenyl-COT methyl ester (14) was prepared by the Jones oxidation of 11.

In the first administration experiment (experiment A), an aqueous solution of [2'-^{14}C-carboxy]-7 was poured on the *Catalpa ovata* callus tissues (two weeks after subculturing, 36 g

wet weight when harvested) and the cultures were kept at 25° in the dark for 5 days. Half of the callus tissues were homogenized in cold MeOH in a glass homogenizer and centrifuged. The resulting supernatant was immediately treated with excess diazomethane. The other half of the callus tissues were extracted immediately with benzene. 2-Prenyl-COT methyl ester (10), 2-epiprenyl-CHT methyl ester (11), CHT methyl ester (13) and 2-prenyl-COT methyl ester (14) as well as 2-epiprenyl-CHT lactone (12) were added to the diazomethane-treated mixture, while catalponol (2R,4S) (2)[4], catalponone (2R) (3), menaquinone-1 (5), 4-hydroxy-α-tetralone (15), 4-oxo-α-tetralone (16) and 2-epicatalponol (2S,4S) (17) were added to the benzene extract.

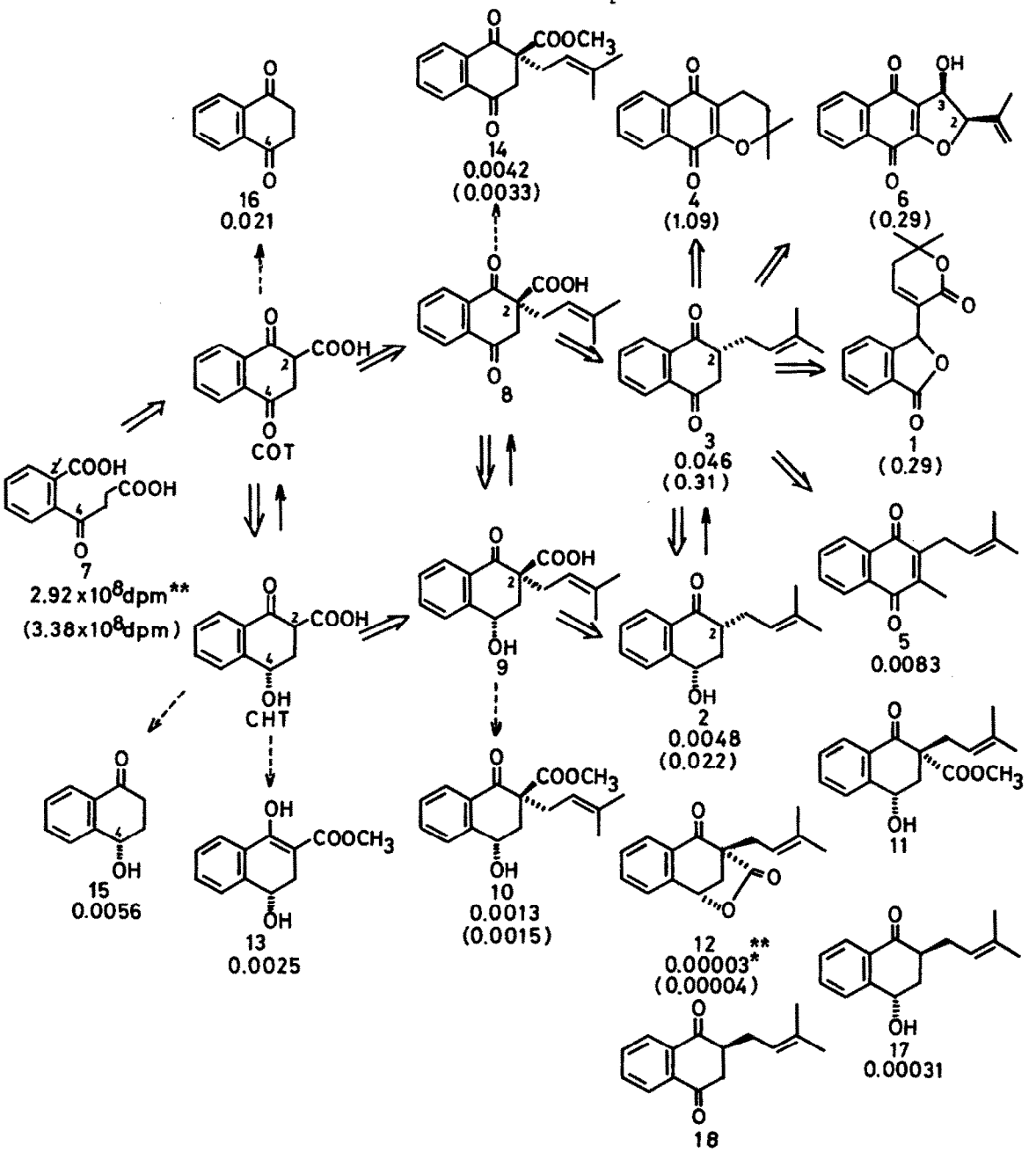
In the second experiment (experiment B), (three weeks after subculturing, 25 g wet weight when harvested), 10, 11, 12 and 14 were added to the diazomethane-treated mixture, while 1, 2, 3, 4 and 6 were added to the benzene extract.

In both experiments, the substances isolated by silica gel column chromatography were converted into suitable crystalline derivatives in the following ways: Compounds 2, 15 and 17 were oxidized with the Jones reagent to 3, 16 and catalponone (2S) (18), respectively; menaquinone-1 (5) was subjected to reductive acetylation with acetic anhydride and zinc powder to the leucoacetate; 13 was converted into the dibenzoate; 10 was hydrogenated over Pt and then oxidized with the Jones reagent to yield dihydroprenyl-COT methyl ester, while 14 was converted into the same compound by hydrogenation over Pt; 11 was successively worked up with $KOBu^t$ and HCl to give the lactone (12), which was combined with 12 reisolated from the callus extracts; Compounds 1, 3, 4, 6 and 16 were purified as they were by recrystallization.

The incorporations (%) of 7 into the substances examined in the above experiments are shown under the structural formulae in Scheme 1. The unbracketed figures are the values from experiment A, while the bracketed ones those from experiment B. From these values it clearly follows that 7 is incorporated into menaquinone-1 (5) and 3-hydroxy-dehydro-iso-α-lapachone (6), constituents characteristic of the callus cultures, as well as into catalpalactone (1), α-lapachone (4) and catalponone (3). Furthermore, the incorporation of the radioactivity of 7 into 16, 15, 13, 14 and 10 demonstrates that COT, CHT, prenyl-COT (8) and prenyl-CHT (10) are, as expected, on the biosynthetic route to naphthoquinone congeners.

On the other hand, the content of catalponol (2) in the callus cultures used for the present work was far lower than that in the intact plant; besides, the radioactivity of 13 and 15 derived from CHT, as well as that of 10 from 9 were considerably lower than those of 16 and 14 derived from COT and 8, respectively. Thus, it seems most probable that the main biosyn-

Scheme 1 Biosynthetic pathway of several naphthoquinone congeners in callus cultures of *Catalpa ovata**



* The compounds 10, 11, 12, 13, 14 and 15 used in these experiments were *dl* pairs.

** The radioactivity of 7 shown here was obtained after subtracting the activity of recovered 7 from the total activity administered.

*** As described in the text, incorporation ratio into the ester 11 was shown as the value into the lactone 12.

thetic pathway to 3 in the callus cultures is 7 → COT → 8 → 3, while the route CHT → 9 → 2 is a subsidiary one. Interconversions between both routes such as 3 ⇄ 2 would also be possible. Practically, no radiation was detected by the dilution analysis in 2-epiprenyl-CHT methyl ester (11) or in the corresponding lactone (12) which is readily formed from 11 during the isolation procedure. This fact, coupled with the absolute configuration of catalponol (2), implies that 9 possesses the 2S, 4S configuration and also implies that the decarboxylation of 9 to 2 proceeds with the retention of configuration. This inference is also supported by the fact that the incorporation of radioactivity into 2-epicatalponol (2S,4S) (17) is much lower than that into catalponol (2R,4S) (2). From the above results, it is further conceivable that catalponone (2R) (3) is formed by decarboxylation of prenyl-COT (8) with 2S configuration.

In the preceding paper[1], we assumed that 1 and α-lapachones would be formed from either 2 or 3. However, in view of the inference that the route COT → 8 → 3 is the main biosynthetic pathway in the callus cultures, it seems probable that 1, 4, 5 and 6 are biosynthesized through several ways via the key intermediate 3. Although further experiments will be required to decide which of both routes 7 → CHT → COT and 7 → COT → CHT is the correct way from 7 to COT and CHT, comparison of the radioactivity of 16 with those of 15 and 13 suggested that the latter is likely to be the main pathway.

From the results mentioned so far, we propose that the biosynthetic routes of a series of naphthoquinone congeners including catalpalactone (1) are shown by heavy lines in Scheme 1. The reversible ones shown by light lines may also occur simultaneously.

References and notes

1) K. Inoue, S. Ueda, Y. Shiobara and H. Inouye, Phytochemistry *16*, 1689 (1977) and references cited therein.

2) H. Inouye, T. Okuda and T. Hayashi, Chem. Pharm. Bull. (Tokyo) *23*, 384 (1975); H. Inouye, T. Hayashi and T. Shingu, *ibid.* *23*, 392 (1975).

3) This substance was also isolated from *Radermachera sinica* Hemsl.; K. Inoue, C. Chen and H. Inouye, unpublished results.

4) Recently, the absolute structure of catalponol (2) was revised by correlation with isocatalponol and on ground of x-ray analysis; K. Inoue, H. Inouye, T. Taga, R. Fujita, K. Osaki and K. Kuriyama, unpublished results. Cf. C. H. Brieskorn and R. Pöhlmann, Arch. d. Pharm. *309*, 829 (1976). The structures shown in Scheme 1 were depicted based on the revised stereostructures.

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