Tetrahedron Letters Vol. 21, pp 621 - 622 0040-4039/80/0208-0621$02.00/0

STEREOCHEMISTRY OF PRENYLATION AND SUBSEQUENT DECARBOXYLATION IN THE BIOSYNTHESIS OF PRENYLNAPHTHOQUINONE CONGENERS IN CALLUS CULTURES OF *CATALPA OVATA*

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Summary: The main biosynthetic route from 4-(2'-carboxyphenyl)-4-oxobutanoic acid (1) *via* 2-carboxy-4-oxo-1-tetralone (COT) to several quinonoids in callus cultures of *Catalpa ovata* was definitely demonstrated to pass stereospecifically through (2S)-prenyl-COT ((2S)-2) and (2R)-catalponone ((2R)-3).

In the preceding paper,[1] we reported that the main biosynthetic pathway for the naphthoquinone congeners produced by callus cultures of *Catalpa ovata* G. Don is likely to pass through 4-(2'-carboxyphenyl)-4-oxobutanoic acid (1), 2-carboxy-4-oxo-1-tetralone (COT), 2-prenyl-COT (2) and catalponone (3).

This paper deals with the elucidation of the precise stereochemical course in the prenylation of COT and the subsequent decarboxylation during the biosynthesis of the naphthoquinone congeners. For this purpose, the dilution analysis was performed with both (2R)- and (2S)-enantiomers of 2-prenyl-COT (2) as well as with those of catalponone (3) after administrating [2'-^{14}C-carboxy]-1 to the callus cultures of *C. ovata*.

Since 2-prenyl-COT (2) was expected to be highly unstable, it was trapped as the stable methyl ester by immediate treatment of the methanolic extracts of the callus cells with diazomethane as reported previously.[1] The two enantiomers of 2-prenyl-COT methyl ester (4), necessary for the present study, were synthesized as follows: Racemic 4 was reduced with $LiAl(OBu^t)_3H$ to give 2-carbomethoxy-2-prenyl-4-oxo-1α-tetralol ((1S,2S)- + (1R,2R)-5) as the major product. The relative configuration at C-1 and C-2 of 5 was inferred from the NMR data of a derivative of 5. The resolution of the acid (6) obtained by alkaline hydrolysis of 5 through quinine salt followed by methylation with diazomethane yielded two enantiomers of 5. Of these two, the (+)-form should have (1S,2S)-, while (-)-form (1R,2R)-configuration, as the *p*-bromobenzoate of the former shows the first Cotton effect at 254.5 nm (exciton chirality rule) and a further positive Cotton effect at 325 nm (aryl ketone rule) in the CD spectrum. Jones oxidation of both enantiomers (5) gave the corresponding (2S)-(-)-2-prenyl-COT methyl ester ((2S)-4), $[\alpha]_D$ -64.2° (MeOH), and (2R)-(+)-2-prenyl-COT methyl ester ((2R)-4), $[\alpha]_D$ +65.5° (MeOH).

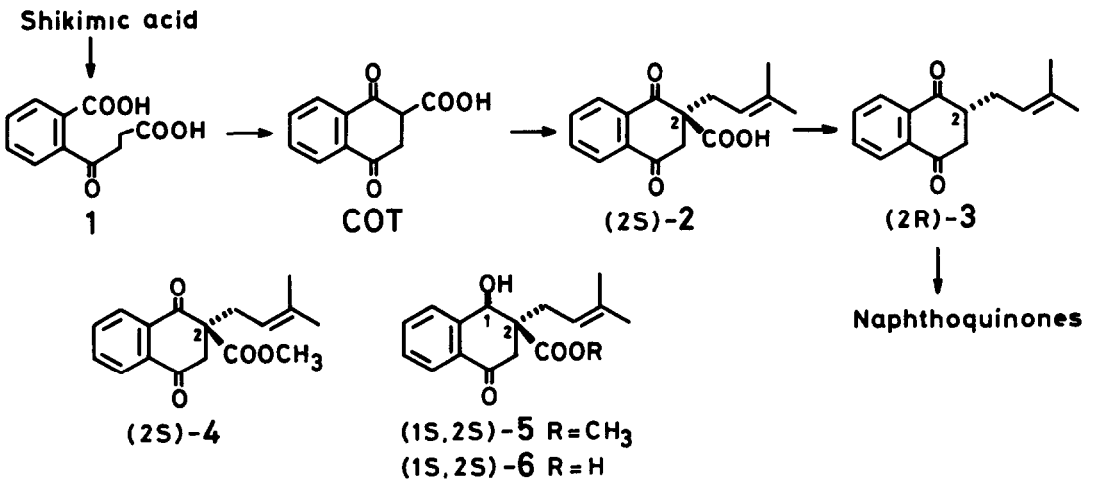
Feeding experiments were carried out twice in the following way: An aqueous solution of [2'-^{14}C-carboxy]-1 was added to the *C. ovata* callus tissues (one month after subculturing) and the cultures were kept at 24° C in the dark for 40 hours. One half of the tissues was homogenized in cold methanol (-10° C) using a glass homogenizer and centrifuged. The resulting supernatant was immediately treated with excess amount of diazomethane. (2S)-(-)-2-prenyl-COT

methyl ester ((2S)-4) was added to one half of this diazomethane-treated solution, while the same amount of the (2R)-(+)-compound ((2R)-4) was added to the other half. The other half of the callus tissues was extracted with benzene. (2S)-(+)-Catalponone ((2S)-3)[1] was added to one half of the benzene extract, while (2R)-(-)-compound ((2R)-3) was added to the other half. These four mixtures were concentrated *in vacuo* and subjected to further purifications. Both enantiomers of 4 were crystallized after conversion to the tetralols, ((1S,2S)-5) and ((1R,2R)-5), by $LiAl(OBu^t)_3H$ reduction, while those of 3 as they were. The incorporation ratios of 1 into the isolated substances (Table 1) indicate that 1 is incorporated into (2S)-2 and (2R)-3 in much higher ratios than into their enantiomers. These results definitely demonstrate that, as expected, COT is prenylated stereospecifically to give (2S)-2 which is then decarboxylated with retention of the configuration giving rise to (2R)-catalponone ((2R)-3), the key intermediate in the biosynthesis of the naphthoquinone congeners.

In view of the co-occurrence of menaquinone-1 in the callus tissues,[1] this pathway may highly suggest the mode of biosynthesis of vitamins K also.

Table 1 Incorporation of [2'-^{14}C-carboxy]-4-(2'-carboxyphenyl)-4-oxobutanoic acid into both enantiomers of prenyl-COT methyl ester (4) and catalponone (3)

Substances trapped	Expt. A	Expt. B
(2S)-prenyl-COT methyl ester ((2S)-4)	0.0084	0.0058
(2R)-prenyl-COT methyl ester ((2R)-4)	0.00035	0.00019
(2R)-catalponone ((2R)-3)	0.045	0.18
(2S)-catalponone ((2S)-3)	0.0022	0.015



References

1 H. Inouye, S. Ueda, K. Inoue, Y. Shiobara and I. Wada, *Tetrahedron Letters*, 1978, 4551.

(Received in Japan 5 November 1979)