Benzophenone Participation in Xanthone Biosynthesis *(Gentianaceae)*†

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THE suggestion that oxidative coupling may be involved in xanthone biosynthesis[1] has been further developed by *in vitro* oxidation studies on benzophenones[2] and also by the reported co-occurrence of maclurin (2,3′,4,4′,6-pentahydroxybenzophenone) with 1,3,5,6- and 1,3,6,7-tetrahydroxyxanthone in *Symphonia globulifera* L.[3] We have studied the incorporation of ^{14}C and ^{3}H labelled compounds into the xanthones produced by *Gentiana lutea* and find that a benzophenone is indeed a precursor.

A re-examination of an extract of fresh *Gentiana lutea* rhizome (4·7 kg.) has revealed the presence of 1,3,7-trihydroxyxanthone (II) (gentisein, 0·002%), 1,7-dihydroxy-3-methoxyxanthone (gentisin),[4] and 1,3-dihydroxy-7-methoxyxanthone (isogentisin, 0·014%),[5] 1-hydroxy-3,7-dimethoxyxanthone (0·01%), and 1,3,7-trimethoxyxanthone (0·002%). Gas chromatographic examination of the methylated phenolic root-extract indicated the presence of 6-hydroxy-2,3′,4-trimethoxybenzophenone and quantitative measurements showed that the rhizome contained 2,3′,4,6-tetrahydroxybenzophenone (I) to the extent of 0·0003%. The Scheme illustrates the inter-relationship suggested by the presence of these compounds in *Gentiana lutea*.

Substantiation of this scheme has been obtained by the use of ^{14}C- and ^{3}H-labelled precursors and their incorporation into the xanthones which were isolated. On the basis of the hydroxylation pattern associated with the benzophenone (I) it is to be expected that it, and the xanthones (II) derived from it, should have been elaborated from "acetate" and "shikimate" as indicated in the Scheme. This is indeed the case as phenylalanine is incorporated solely into ring B and the bridgehead (carbonyl) carbon. Conversely, sodium acetate was found to be primarily associated with ring A, an observation previously reported by Floss and Rettig.[6] In a typical experiment, 150—250 g. of rhizome, cut into discs, was incubated in water (pH 6·4) containing the labelled precursor for 3 days. The integrity of this method for incorporation was realised by comparison with a wick-feeding experiment on an intact plant (4 weeks). The xanthones isolated by t.l.c., *i.e.* gentisein and a gentisin–isogentisin mixture, were combined and demethylated to give gentisein only. Fusion of gentisein with sodium hydroxide–potassium hydroxide[6] and isolation of the products gave phloroglucinol (III) and Gentisic acid (IV). The Table lists the values obtained from the incorporation of anticipated precursors.

The failure of phloroglucinol to be incorporated as an intact unit suggests the participation of a polyketide by attachment to the phenylalanine (shikimate)-derived B-ring system followed by cyclisation to the benzophenone (a somewhat similar sequence has been suggested for flavanoid biosynthesis[7]).

The high incorporation of sodium [2-^{14}C]acetate enabled a radiochemical analysis for 2,3′,4,6-tetrahydroxybenzophenone (I) to be undertaken. Isolation of an impure benzophenone fraction from a tissue-culture incubation experiment and dilution with inactive pure benzophenone followed by co-crystallisation produced an active product

Precursor		% Incorp.	Specific activity (d.p.m. μmole^{-1}) Gentisein (II)	Phloroglucinol (III)	Gentisic acid (IV)
0·05 mc [U-^{14}C]-l-Phenylalanine	W.F.	0·044	454	—	—
0·05 mc [U-^{14}C]-l-Phenylalanine	T.C.	0·044	242	3	238
0·1 mc (±)-[3-^{14}C]Phenylalanine	T.C.	0·047*	3806	42	3954
0·1 mc Sodium[2-^{14}C]acetate	T.C.	0·53	506	341	127
0·6 μc [2,4,6-^{14}C]Phloroglucinol†	T.C.	0	0	0	0
7 μc 2,3′,4,6-Tetrahydroxy[5′-^{3}H]benzophenone‡	T.C.	0·009	170	—	—
			671§	—	—

T.C. = Tissue-culture incubation, W.F. = Wick-feeding incorporation.

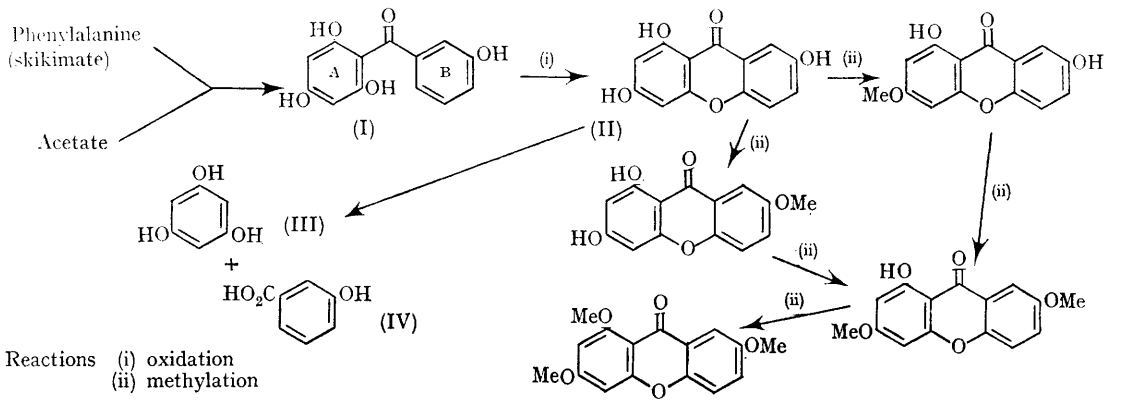
* Corrected for incorporation of 1-isomer only.

† L. Patschke, W. Bartz, and H. Grisebach, *Z. Naturforsch.*, 1964, **19b**, 1110.

‡ Prepared by T_2O–H_2PtCl_4 replacement of H by T (J. L. Garnett and R. J. Hodges, *Chem. Comm.*, 1967, 1001), followed by equilibration in alkaline solution to constant activity.

§ Isolated as a mixture of gentisin and isogentisin.

† Part of these results was presented at the Annual General Meeting of the Chemical Society, Dublin, April 1968.



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which gave the activity associated with the naturally occurring benzophenone as 449 d.p.m. By comparison with the specific activity of gentisein isolated from the same source, the concentration of 2,3′,4,6-tetrahydroxybenzophenone (I) in rhizome (215 g.) was 1·2 mg. (*i.e.* 0·0005%).

A feeding of the tritiated benzophenone into *G. lutea* and its conversion to gentisein and a mixture of gentisin and isogentisin substantiates the role played by the benzophenone and the use of oxidative coupling, followed by methylation in this case, for the formation of the xanthones produced by *G. lutea*.

These findings confirm that plant-derived xanthones can be produced *in vivo* by oxidative coupling of hydroxylated benzophenones whereby the xanthone ring system is produced directly. An alternative process where the hydroxylation pattern of the benzophenone allows a dienone intermediate to be produced, which subsequently rearranges to a xanthone[8] (analogous to the tetrahydroisoquinoline–aporphine alkaloid inter-conversions), still remains of a speculative nature.

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