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METABOLIC RELATION BETWEEN NAPHTHALENE DERIVATIVES IN *JUGLANS*

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Key Word Index —*Juglans regia*; Juglandaceae; walnut; biosynthesis; 4-oxo-α-tetralone; β-hydrojuglone; 1,4-naphthoquinone; juglone; 4-hydroxy-1-naphthalenyl-β-D-glucoside; 4,8-dihydroxy-1-naphthalenyl-β-D-glucoside.

Abstract It has been shown by labelling experiments that 4-oxo-α-tetralone and β-hydrojuglone may be involved in the biosynthesis of naphthoquinols in *Juglans regia*. Experiments on the metabolic role of aglycones and glucosides are discussed and a hypothetical scheme for these biosynthetic relationships is presented.

INTRODUCTION

4-(2′-Carboxyphenyl)-4-oxobutyric acid, i.e. *o*-succinoylbenzoic acid [1] (**1**) (Scheme 1), is known to be an intermediate in the biosynthesis of bacterial menaquinones and plant quinones [1–8]. The reactions leading from (**1**) to various quinones (e.g. **6** and **11**), however, are a matter of discussion. 2-Carboxy-4-oxo-α-tetralone (COT, **2**) [6, 7] and 1,4-dihydroxy-2-carboxynaphthalene (**3**) [2, 8–12] are possible intermediates in this sequence.

While (**2**) is reasonably assumed to be the product of a Dieckmann type cyclization of (**1**) [9], a possible role in quinone biosynthesis is also attributed to (**3**). This suggestion is based on the observation that (**3**) has been isolated from the culture broth of an *E. coli* mutant unable to produce menaquinones [10]. Moreover, a radioactively labelled sample of (**3**) has been very efficiently incorporated into plant naphthoquinones [8] and an enzyme system from *E. coli* requiring ATP and Coenzyme A was shown to mediate the conversion of (**1**) to (**3**) [11]. (**3**) has also been demonstrated to act as a substrate of 1,4-dihydroxy-2-naphthoate: octaprenyltransferase, an enzyme detected in crude extracts of *E. coli* [12].

Based on the observation, however, that tetralone derivatives which are also derived from (**1**) are encountered in *Catalpa* plants and that prenylation of these compounds occurs at a prearomatic stage [6, 7], it has been suggested [7] that (**3**) is an artifact which might be formed by enolization of (**2**).

In an attempt to contribute to the solution of some of the above contradictions, some new experiments on naphthoquinol biosynthesis were undertaken.

RESULTS AND DISCUSSION

Among those quinones which are derived from shikimic acid and *o*-succinoylbenzoic acid, juglone (**11**) is unusual because it is synthesized within *Juglans regia* plants via at least one symmetrical intermediate [13–16] such as 1,4-naphthoquinone (**6**) [8]. Whereas the origin of (**6**) from (**1**) seems to be obvious, the pathway may involve either metabolites (**2**), (**4**) and (**5**), or (**2**), (**3**) and (**5**) or all six. Support for pathway (**2**) → (**3**) → (**5**) has recently been presented [8]. While we failed to isolate (**4**) from *Juglans*, experiments listed in Table 1 indicate that 4-oxo-α-tetralone (**4**) is present in *Juglans* and is derived from (**1**); carrier material of (**4**) reisolated from a homogenate of a *Juglans* plant infused with ^{14}C-labelled (**1**) turned out to be radioactive. After derivatisation and repeated recrystallization, the specific activity remained constant (Table 1).

We had to take into account, however, that if (**2**) was present (**4**) might have been an artifact derived by decarboxylation of (**2**) during extraction. If we were able to show, however, that β-hydrojuglone (**8**) was present in the plant, an obvious precursor of (**8**) would be (**4**) and therefore support the view that (**4**) is a genuine natural product rather than an artifact from (**2**). (**8**) has recently been shown to occur in *Lomatia* (Proteaceae) plants [17]. From the same homogenate (see above), β-hydrojuglone carrier was also reisolated and turned out to be labelled. After derivatization and repeated recrystallization the specific activity remained constant (Table 1). (**4**) and (**8**) may therefore be involved in the biosynthesis of naphthalene derivatives in *Juglans* plants.

The specific incorporation of (**1**) into the aromatic and quinonoid moieties of glucosides (**7**) and (**10**) has also been demonstrated (Table 2). It was shown that after hydrolysis radioactivity resided in aglycones only, whereas the glucose was inactive (Table 2). This indicates that the ^{14}C-labelled precursor was not extensively degraded and then incorporated into (**7**) and (**10**), but that incorporation took place specifically as has previously been demonstrated in the case of free 1,4-naphthoquinone (**6**) and juglone (**11**) [8].

The co-occurrence of aglycones and glucosides raised the question as to the sequence of hydroxylation and glucosylation. Glucoside (**7**) may be hydroxylated to yield (**10**) or else (**5**) may be hydroxylated and then glucosylation may take place to yield (**10**). Simultaneous application to *Juglans* plants and to callus cultures [18] of 6T-D-Glucose and 1,4-^{14}C-1,4-naphthoquinone

Table 1. Results of reisolation experiments. Application of 4-(2′-carboxyphenyl)-4-oxobutyrate-[2-^{14}C] (**1**) (0.8 μCi) to *Juglans regia* and reisolation of 1,4-naphthoquinone (**6**), 4-oxo-α-tetralone (**4**) and β-hydrojuglone (**8**) from a crude extract of this plant

Compound	Sp. act. (dpm/μM) Exp. 1	Total act. (dpm) Exp. 1	Incorp. rate (%) Experiment 1	2
Juglone (**11**)	3173	344.544	19.4	25.8
1,4-Naphthoquinone (**6**)	1745*	22 000	1.25	2.25
4-Oxo-α-tetralone (**4**)	146.8†	2664	0.15	0.15
p-Nitrophenyl-hydrazone [21] of (**4**), recrystallizations				
1.	155.5		–	–
2.	140.2			
3.	144.8			
β-Hydrojuglone (**8**)	1369‡	11 544	0.65	1.15
p-Nitrophenyl-hydrazone [21] of (**8**), recrystallizations				
1.	1503	—		
2.	1421			
3.	1414			

* 2.0 mg, † 2.3 mg, ‡ 2.5 mg carrier. Data corrected for loss during purification.

Table 2. Incorporation of 4(2′-carboxyphenyl)-4-oxobutyrate-[2-^{14}C] (**1**) into 1,4-naphthoquinone (**6**), juglone (**11**), 4-Hydroxy-1-naphthalenyl-β-D-glucoside (**7**) and 4,8-Dihydroxy-1-naphthalenyl-β-D-glucoside (**10**)

Compound	Sp. act. (dpm/μM)	Amount isolated (μM)	Incorp. rate (%)
1,4-Naphthoquinone (**6**)	5210.0	19.2*	1.69
Juglone (**11**)	5071.6	60.7	5.21
4-Hydroxy-1-naphthalenyl-β-D-glucoside (**7**)	5124.0	8.7†	0.72
Products of hydrolysis			
(A) 1,4-Naphthoquinone	5005.9	8.5	0.72
(B) Glucose	0	8.5	0.0
4,8-Dihydroxy-1-naphthalenyl-β-D-glucoside (**10**)	5851.6	20.1	1.99
Products of hydrolysis			
(A) Juglone	6161.0	21.2	1.99
(B) Glucose	0	21.2	0

* 3.04 mg, † 2.8 mg carrier.

Table 3. T/^{14}C ratios in metabolites (**1**) to (**4**) and its products of hydrolysis after application of a mixture of 6T-D-glucose and 1,4-naphthoquinone-[1,4-^{14}C] to *Juglans regia* plants. Average of two experiments. Essentially the same observations were made with a *Juglans* callus culture. Figures in parentheses are T/^{14}C ratios (%) relative to the feeding solution (100 %)

		Isotope	Total act. (μCi)	T/^{14}C ratio (%)	Incorp. [%] ^{14}C only
Precursor	6T-D Glucose	T	491.0	118.9	
	1,4-^{14}C-1,4 Naphthoquinone	^{14}C	41.3		
Products	1,4-Naphthoquinone (**6**)	T	0.20	0.19	2.5
		^{14}C	1.07	(0.16)	
	Juglone (**11**)	T	0.67	0.29	
		^{14}C	2.31	(0.24)	5.6
	4-Hydroxy-1-naphthalenyl-	T	5.27	5.43	
	β-D-glucoside (**7**)	^{14}C	0.97	(4.57)	2.35
	1,4-Naphthoquinone obtained	T	–	0.015	
	after hydrolysis of (**7**)	^{14}C	–	(0.013)	–
	Glucose obtained after	T		560.3	
	hydrolysis of (**7**)	^{14}C	–	(471.2)	–
	4,8-Dihydroxy-1-naphthalenyl-	T	6.20	12.4	
	β-D-glucoside (**10**)	^{14}C	0.50	(10.4)	1.21
	Juglone obtained by hydrolysis	T	–	0.029	
	of (**10**)	^{14}C	–	(0.024)	
	Glucose obtained by hydrolysis	T		810.2	
	of (**10**)	^{14}C	–	(681.4)	–

showed that as expected, (7) was labelled with tritium and ^{14}C ($T/^{14}C$-ratio 5.43, Table 3). If this glucoside was hydroxylated to yield (10) the $T/^{14}C$ ratios of both (7) and (10) would have to be identical. This, however, was not observed. The $T/^{14}C$ ratios of (7) and (10) differed significantly (Table 3).

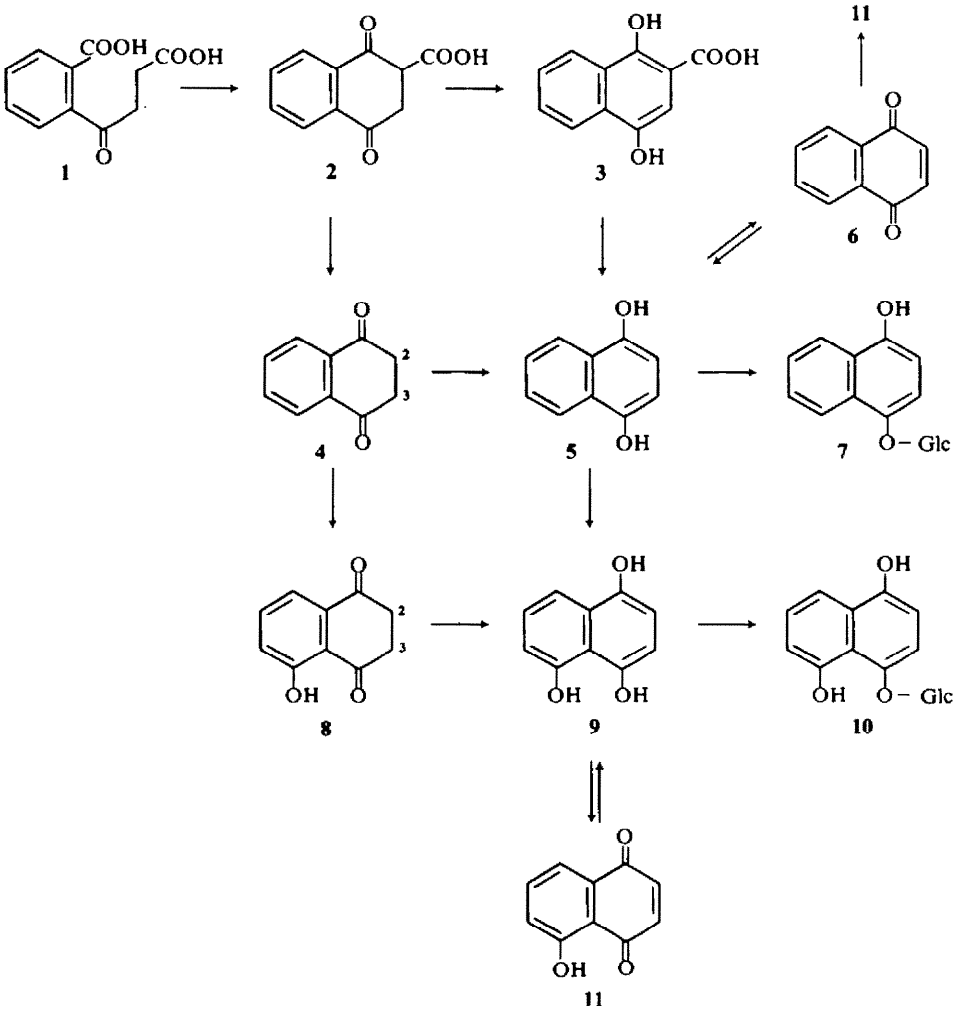
From this experiment the following conclusions are drawn: (1) Whereas in indole alkaloid [19] and in coumarin biosynthesis [20] glycosides are obligatory intermediates, this does not apply to the metabolism of naphthalene derivatives in *Juglans* where aglycones (4) and (5) are likely to be hydroxylated. (2) An *O*-glycosylation process requires hydroxylated rather than quinonoid compounds to be present. Since 1,4-naphthoquinone-[1,4-^{14}C] had been applied to the plant material and since incorporation of the labelled quinone into the glucoside had been observed, reduction of (6) to (5) is an essential process. This justifies hydroquinones (5) and (9) to be inserted into the hypothetical Scheme (1). (3) Since hydroxylation of glucoside (7) does not take place, aglycones (5) and (9) have to be true natural products which are neither derived by enolization of (4) and (8) nor by hydrolysis of (7) and (10) during the isolation procedure. This is in agreement with previous observations [18].

A possible biosynthetic relation between metabolites (6, 7, 10, 11) isolated from *Juglans* plants, likely intermediates (3, 4, 8) in juglone (11) biosynthesis and an assumed metabolic product (2) of *o*-succinoylbenzoic acid (1) is depicted in Scheme 1.

EXPERIMENTAL

Plant material. The plant material has been described elsewhere [18].

Isolation of metabolites. The isolation of compounds (1) to (4) has been previously described [18]. 4-Oxo-α-tetralone (4) and β-hydrojuglone (8) were reisolated from a crude extract of *Juglans* regia by the following procedure. The plant material was cut into pieces and kept in Et_2O (peroxide free) at room temp. for 12 hr. The Et_2O was filtered, carrier materials were added (Table 1), the extract concd and submitted to TLC (Si gel). C_6H_6–$CHCl_3$, 8:2 (R_f (11) 0.43; (6) 0.35; (4) 0.19; (8) 0.27). (6) Was rechromatographed by TLC (Si gel) using the following systems: C_6H_6, R_f 0.42; C_6H_6–petrol, 2:3 and after drying the plate C_6H_6, R_f 0.33. (4) Was rechromatographed by TLC (Si gel): EtOAc–$CHCl_3$–HCOOH–H_2O, 82:8:5:5, R_f 0.70; $CHCl_3$, R_f 0.42. (8) Was rechromatographed by TLC (Si gel): $CHCl_3$,



Scheme 1. Possible biosynthetic relation between metabolites (6, 7, 10, 11) isolated from *Juglans* plants, likely intermediates (3, 4, 8) in juglone (11) biosynthesis and an assumed metabolic product (2) of *o*-succinoylbenzoic acid (1).

R_f 0.47; C_6H_6–Et_2O, 9:1, R_f 0.69; EtOH. R_f 0·87. Before prepn of the hydrazones further amounts of carrier materials were added.

Synthesis of (**4**) *and* (**8**). (**8**) has been synthesized as described by Thomson [17]. (**4**) was prepared according to a private communication of Professor Inouye, Kyoto, Japan.

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