Different Mode of Incorporation of o-Succinylbenzoic Acid into the Naphthoquinones Juglone and Lawsone in Higher Plants

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[1-13C]o-Succinylbenzoic acid is incorporated to approximately 50% into each of the keto groups of the quinone ring of juglone in Juglans regia. The same labelled precursor is transformed into the keto group of position 1 in lawsone, the pigment of Impatiens balsamina, suggesting two different intermediates in the biosynthesis of these simple 2- or 5-hydroxy-1,4-naphthoquinones.

The biosynthesis of certain higher plant naphthoquinones became clearer when it could be shown that all seven carbon atoms of shikimic acid were incorporated in toto into the naphthoquinones juglone of Juglans regia and lawsone of Impatiens balsamina (Zenk and Leistner, 1967; Leistner and Zenk, 1968). Careful degradation studies of both naphthoquinones showed that in lawsone the carboxyl group of shikimic acid was incorporated into the carbonyl groups of C-1 and/or C-4, while that same carboxyl group of shikimic acid was equally distributed between C-1 and C-4 of juglone. Due to the symmetry of phthalic acid formed in the chemical degradation of the labelled lawsone, it could not be decided whether the carboxyl group of shikimic acid was incorporated into lawsone symmetrically (C-1 and C-4) or asymmetrically (C-1 or C-4). Based on an ingenious proposition of Dansette and Azerad (1970), o-succinylbenzoic acid and 1,4-dihydroxy-2-naphthoic acid were assumed precursors in the formation of naphthoquinones. These intermediates were later indeed recognized as true precursors of several naphthoquinones of the vitamin K2 type in microorganisms (Bentley and Meganathan, 1987; Inouye and Leistner, 1988) with o-succinylbenzoic acid being the crucial metabolite formed from isochorismate and thiamine pyrophosphate activated succinyl aldehyde (Weische et al., 1987), thus opening the naphthoquinone pathway. The availability of [1-13C]o-succinylbenzoic acid (Inoue et al., 1979) made it possible to investigate the mode of incorporation of this precursor molecule into lawsone and juglone by NMR techniques and to determine whether a symmetrical intermediate has to be postulated in the biosynthesis of one or both naphthoquinones. This question could not be answered for lawsone using classical degradation studies which involve U-14C-labelled shikimic acid (see above) (Zenk and Leistner, 1967).

![Fig. 1. Proton-decoupled 13C NMR partial spectra of lawsone. A: after feeding experiment with [1-13C]o-succinylbenzoic acid; B: unlabelled reference.](image-url)

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[1-13C]O-Succinylbenzoic acid (Inoue et al., 1979) (13.6 μmol) was dissolved in 2 ml of water and fed through cut stems to two plants of I. balsaminna, 3 weeks old (about 5 g dwt; 17 cm in height). After 48 h, lawson was isolated as described previously (Zenk and Leistner, 1967). The yield of lawson was 37.7 μmol (ε = 2.88×10^6 cm^2/mol at 330 nm in MeOH). Incorporation of the 13C-labelled precursor was 14% (calculated from mass spectral data; Finnigan MAT quadrupole SSQ 700). The NMR spectrum (Dawson et al., 1989) (CDCl3; Bruker AM, 360 MHz) showed that the carbonyl function at C-1 of lawson was strongly labelled, but that at C-4 (Fig. 1) only minimally, thereby demonstrating that o-succinylbenzoic acid is asymmetrically incorporated into lawson. To confirm the symmetrical incorporation of the carboxyl group of shikimic acid into both keto groups (C-1 and C-4) of juglone, which should be equivalent to the 1,3C-labelled carboxyl group of o-succinylbenzoic acid, feeding of this potential precursor to Juglans regia seedlings was conducted.

Again, 13.6 μmol of precursor were fed in 2 ml of water to one 11 weeks old plant (2.0 g dwt; 15 cm in height). After a 48 h feeding period, the plant was worked up and juglone isolated as described (Leistner and Zenk, 1968). The yield of juglone was 70.8 μmol (ε = 3.68×10^6 cm^2/mol at 477 nm in MeOH). As calculated from mass spectral data, 10.5% incorporation of the 13C-labelled precursor was achieved. The NMR spectrum (Bowden et al., 1979) (CDCl3) showed that both keto groups at C-1 and C-4 of juglone were labelled to approximately 50% each (Fig. 2), thus confirming previous results which suggested that a symmetrical molecule has to be an intermediate in the formation of juglone (Leistner and Zenk, 1968).

As depicted in Fig. 3, o-succinylbenzoic acid is obviously transformed as the CoA thioester (Kolkmann and Leistner, 1987a; Kolkmann and Leistner, 1987b) into 1,4-dihydroxy-2-naphthoic acid which can in turn either be oxidatively decarboxylated to yield 2-hydroxy-1,4-naphthoquinone (lawsone) or decarboxylated to yield a symmetrical intermediate such as 1,4-naphthoquinone or naphthohydroquinone. The former has been shown to be an excellent precursor of juglone (Leistner and Zenk, 1968). Specific hydroxylation at position 5 would yield juglone. Indeed, 1,4-naphthoquinone and the glucoside of its hydroquinone have been isolated from various Juglans plants and cell suspension cultures (Müller and Leistner, 1978b). It cannot be excluded, however, that hydrated naphthalene derivatives, such as 4-oxo-α-tetralone and, in addition, β-hydrojuglone, may also be involved in juglone biosynthesis (Müller and Leistner, 1978a; Inoue et al., 1977).

It is remarkable, however, that both naphthoquinones, lawson and juglone, differing only in their hydroxylation pattern (position 2 vs. 5) are formed from different intermediates.

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Fig. 3. Late steps of the biosynthetic pathway to juglone and lawsone (● = 13C label).


