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

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[1-^{13}C]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 K$_2$ 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 succinic aldehyde (Weische *et al.*, 1987), thus opening the naphthoquinone pathway. The availability of [1-^{13}C]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-^{14}C-labelled shikimic acid (see above) (Zenk and Leistner, 1967).
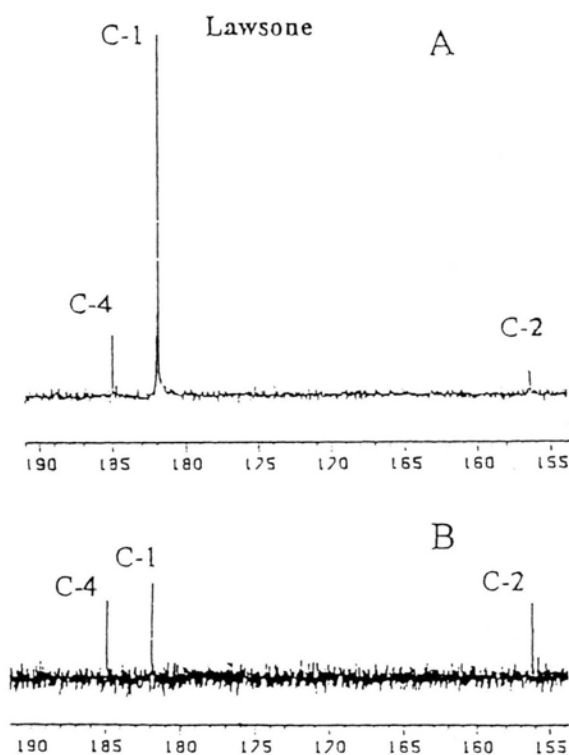

Fig. 1. Proton-decoupled ^{13}C NMR partial spectra of lawsone. A: after feeding experiment with [1-^{13}C]o-succinylbenzoic acid; B: unlabelled reference.

[1-^{13}C]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. balsamina*, 3 weeks old (about 5 g dwt; 17 cm in height). After 48 h, lawsone was isolated as described previously (Zenk and Leistner, 1967). The yield of lawsone was 37.7 µmol ($\varepsilon = 2.88 \times 10^6$ cm^2/mol at 330 nm in MeOH). Incorporation of the ^{13}C-labelled precursor was 14% (calculated from mass spectral data; Finnigan MAT quadrupole SSQ 700). The NMR spectrum (Dawson *et al.*, 1989) (CDCl$_3$; Bruker AM, 360 MHz) showed that the carbonyl function at C-1 of lawsone was strongly labelled, but that at C-4 (Fig. 1) only minimally, thereby demonstrating that o-succinylbenzoic acid is asymmetrically incorporated into lawsone. 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-^{13}C-labelled carboxyl group of o-succinylbenzoic acid, feeding of this potential precursor to *Juglans regia* seedlings was conducted.

Juglone

Fig. 2. Proton-decoupled ^{13}C NMR partial spectra of juglone. A: after feeding experiment with [1-^{13}C]o-succinylbenzoic acid; B: unlabelled reference.

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 ($\varepsilon = 3.68 \times 10^6$ cm^2/mol at 477 nm in MeOH). As calculated from mass spectral data, 10.5% incorporation of the ^{13}C-labelled precursor was achieved. The NMR spectrum (Bowden *et al.*, 1979) (CDCl$_3$) 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, lawsone and juglone, differing only in their hydroxylation pattern (position 2 *vs.* 5) are formed from different intermediates.
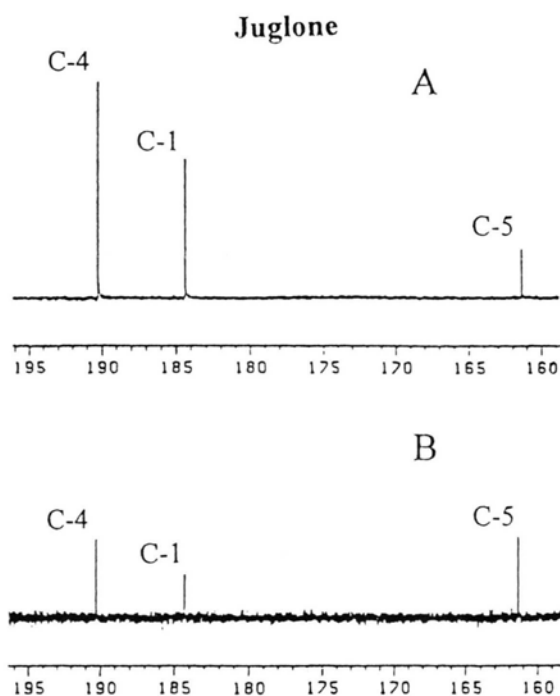
Fig. 3. Late steps of the biosynthetic pathway to juglone and lawsone (● = ^{13}C label).
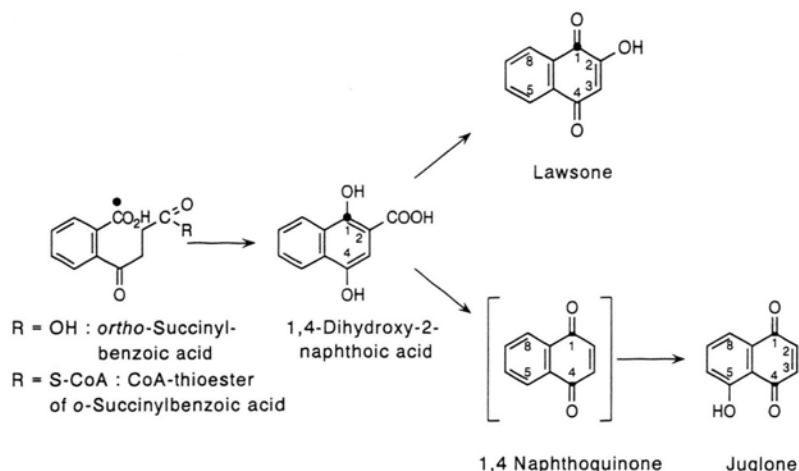
Bentley R. and Meganathan R. (1987), in: *Escherichia coli* and *Salmonella typhimurium*. Cellular and Molecular Biology (F. C. Neidhart, ed.). American Society of Microbiology, Washington D.C., pp. 512–520.

Bowden B. F., Cameron D. W., Crossley M. J., Feutrill G. I., Griffiths P. G. and Kelly D. P. (1979), ^{13}C NMR studies of 1,4-naphthoquinones and 9,10-anthraquinones. Aust. J. Chem. **32**, 769–777.

Dansette P. and Azerad R. (1970), New intermediates in naphthoquinone and menaquinone biosynthesis. Biochem. Biophys. Res. Commun. **40**, 1090–1095.

Dawson B. A., Girard M., Kindack D., Fillion J. and Awang D. V. C. (1989), ^{13}C NMR of lapachol and some related naphthoquinones. Magn. Reson. Chem. **27**, 1176–1177.

Inoue K., Ueda S., Shiobara Y. and Inouye H. (1977), 2-Carboxy-4-hydroxy-α-tetralone, a precursor for catalponol biosynthesis. Phytochemistry **16**, 1689–1694.

Inoue K., Shiobara Y., Nayeshiro H., Inouye H., Wilson G. and Zenk M. H. (1979), Site of prenylation in anthraquinone biosynthesis in cell cultures of *Galicum mollugo*. J. Chem. Soc. Chem. Commun., 957–959.

Inouye H. and Leistner E. (1988), in: The Chemistry of Quinoid Compounds, **Vol. II** (S. Patai and Z. Rapoport, eds.). John Wiley & Sons Ltd., New York, pp. 1293–1349.

Kolkmann R. and Leistner E. (1987a), Synthesis, analysis and characterization of the coenzyme A esters of *o*-succinylbenzoic acid, an intermediate in vitamin K_2 biosynthesis. Z. Naturforsch. **42c**, 542–552.

Kolkmann R. and Leistner E. (1987b), 4-(2′-Carboxyphenyl)-4-oxobutyryl coenzyme A ester, an intermediate in vitamin K_2 biosynthesis. Z. Naturforsch. **42c**, 1207–1214.

Leistner E. and Zenk M. H. (1968), Zur Biogenese von 5-Hydroxy-1,4-naphthochinon (Juglon) in *Juglans regia* L. Z. Naturforsch. **23b**, 259–268.

Müller W.-U. and Leistner E. (1978a), Metabolic relation between naphthalene derivatives in *Juglans*. Phytochemistry **17**, 1735–1738.

Müller W.-U. and Leistner E. (1978b), Aglycones and glycosides of oxygenated naphthalenes and a glycosyltransferase from *Juglans*. Phytochemistry **17**, 1739–1742.

Weische A., Garvert W. and Leistner E. (1987), Biosynthesis of *o*-succinylbenzoic acid II. Properties of *o*-succinylbenzoic acid synthase, an enzyme involved in vitamin K_2 biosynthesis. Arch. Biochem. Biophys. **256**, 223–231.

Zenk M. H. and Leistner E. (1967), On the mode of incorporation of shikimic acid into 2-hydroxy-1,4-naphthoquinone (lawsone). Z. Naturforsch. **22b**, 460.