Biosynthesis of Quinones of *Streptocarpus dunnii* Cell Cultures

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Three unusually prenylated naphthoquinones (**1**), (**5**), and (**6**) of *Streptocarpus dunnii* are found to be biosynthesised *via* (**2**), (**8**), (**9**), and the Claisen type rearrangement product (**10**) of the latter, while two anthraquinones (**3**) and (**4**) of the same plant are shown to be formed in a prenylation mode different from that of the structurally related anthraquinones of Rubiaceous plants.

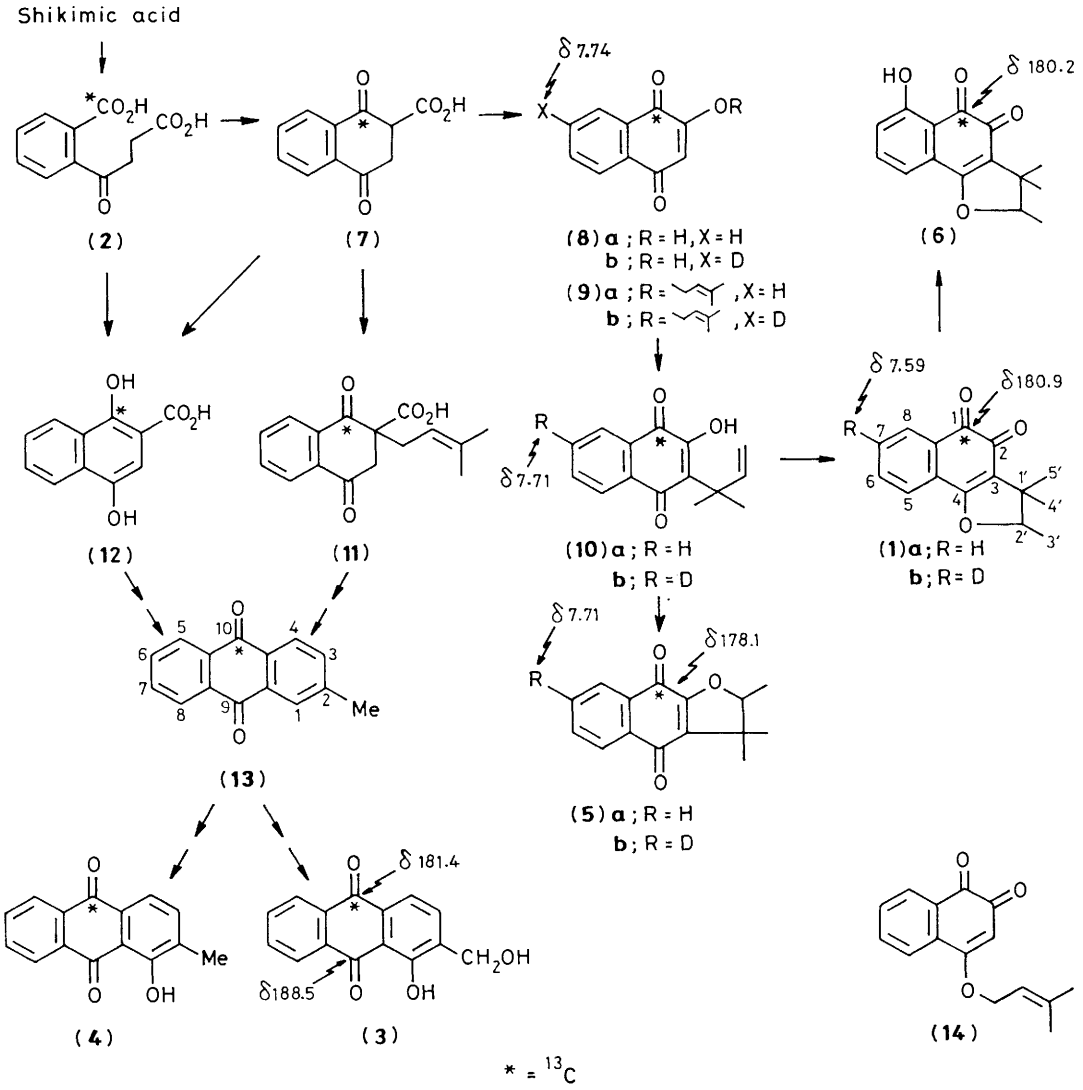
We have reported the isolation of six dunnione (**1**) type naphthoquinones and two anthraquinones from *Streptocarpus dunnii* Mast. (Gesneriaceae) and its cell cultures.[1] These naphthoquinones feature an unusual binding mode of the prenyl group to the quinone nucleus, which probably stems from 4-(2-carboxyphenyl)-4-oxobutanoic acid (**2**) already known as a precursor of one of the anthraquinones (**3**).[2] These anthraquinones are structurally congeneric to those of Rubiaceous plants.[3]

We therefore studied the biosynthesis of these quinones by feeding experiments with ^{13}C and ^{2}H labelled precursors. *S. dunnii* cell suspension cultures coexisting with half differentiated plantlets were used as plant material. They were incubated in Linsmaier–Skoog medium supplemented with 10^{-5} M indoleacetic acid and 10^{-6} M kinetin at 25 °C in the dark for one month after subculturing.

[2-*carboxy*-^{13}C]-4-(2-Carboxyphenyl)-4-oxobutanoic acid (**2**)[3] (50 mg, 86.5 atom %) was fed to the cultures (5 × 170 ml medium) and incubated under the same conditions for an additional 2 weeks. The combined benzene extracts of the cells and plantlets as well as the medium were chromatographed on silica gel to give dunnione (**1a**) (5.2 mg), 1-hydroxy-2-hydroxymethylanthraquinone (**3**) (30.3 mg), 1-hydroxy-2-methylanthraquinone (**4**) (3.1 mg), α-dunnione (**5a**) (10.0 mg), and 8-hydroxydunnione (**6**) (6.3 mg). The ^{13}C n.m.r. spectra of these quinones indicated that the label was introduced into the 1-position for naphthoquinones and the 10-position for anthraquinones. The enrichment factors of ^{13}C in the quinones (**1a**), (**3**), (**4**), (**5a**), and (**6**) calculated on the basis of the mass spectra were 11.6, 20.7, 9.5, 19.5, and 7.7 atom % excess, respectively. Therefore, it was verified that all these quinones were formed *via* (**2**). The location of the introduced label in the quinones led to the following conclusions. (i) The naphthoquinones are biosynthesised *via* 2-carboxy-2,3-dihydro-1,4-naphthoquinone (**7**) and lawsone (**8a**);[4] the latter is prenylated at the C-2 position to form the ether (**9a**) followed by a Claisen-type rearrangement to afford the naphthoquinone (**10a**), which is further converted into (**1a**), (**5a**), and (**6**). (ii) The anthraquinones are not formed *via* the C-3 prenylation as in Rubiaceous plants;[3] they are formed *via* (**7**) and the prenylation product (**11**),[5] as for the naphthoquinone congeners of *Catalpa ovata*, or *via* the naphthoic acid (**12**),[6] as for the menaquinones of *Mycobacterium phlei*.

The intermediacy of lawsone (**8**) in the biosynthesis of naphthoquinones was proved in the following way. Synthesised [7-^{2}H]-lawsone (**8b**)† (25 mg, 94.0 atom %) was administered to the cultures (5 × 170 ml) under the conditions described above, and after two weeks incubation, the usual constituents (**1b**) (0.7 mg), (**3**) (21.3 mg), (**4**) (2.0 mg), (**5b**) (12.7 mg), and (**6**) (6.5 mg) along with tectoquinone (**13**) (1.0 mg) were isolated. The mass spectra of these compounds indicated that the label was incorporated into naphthoquinones (**1b**), (**5b**), and (**6**) with enrichment factors of 17.5, 24.1, and 0.8 atom % excess, respectively, and no label was introduced into the anthraquinones (**3**), (**4**), and (**13**). The ^{2}H n.m.r. spectrum of (**5b**) showed that the label was on C-7, establishing the intermediacy of (**8**). The unusual production of (**13**), which may be caused by a feedback control from the

† The synthesis of [7-^{2}H]-lawsone (**8b**) starting from 4-(4-chlorophenyl)butanoic acid will be reported elsewhere.



Scheme 1

addition of (**8**), supported the intermediacy of (**13**) in the biosynthesis of (**3**) and (**4**).

The intermediacy of lawsone 2-prenyl ether (**9**) was then examined. The [7-$^2H_1$]-ether (**9b**) (50 mg) derived from [7-$^2H_1$]-lawsone (**8b**) was fed to the cultures (5 × 170 ml) and the quinones were isolated as before. In this experiment, besides the constituents (**1b**) (5.5 mg), (**3**) (16.5 mg), (**4**) (2.0 mg), (**5b**) (12.2 mg), (**6**) (3.7 mg), and (**13**) (2.3 mg), the intermediate (**10b**) (31 mg) was also obtained. Its structure was established by an alternative synthesis. The enrichment factors of ^2H in the quinones (**1b**), (**5b**), and (**6**) were 58.2, 65.3, and 2.2 atom % excess, respectively, whereas the value for (**10b**) was 82.6 atom % excess. This unusually high value and the high yield of (**10b**) can be explained by the overflow production caused by the excessive addition of the precursor (**9b**). The label in the quinones (**1b**), (**5b**), and (**10b**) was shown to be at C-7 from their ^2H n.m.r. spectra. The above results unequivocally demonstrate that naphthoquinones of the cell cultures of *S. dunnii* are biosynthesised *via* (**8**), (**9**), and (**10**). The production of the intermediate (**10**) on addition of (**9**) excludes the possibility that the quinones are formed *via* direct prenylation of (**8**) in an inverted mode, or *via* the Claisen-type rearrangement of 1,2-naphthoquinone 4-prenyl ether (**14**), which might be formed from (**8**).

On the basis of these results, the biosynthetic pathway shown in Scheme 1 is proposed for quinones of cell cultures of *S. dunnii*. The extremely low enrichment factors for 8-hydroxydunnione (**6**) compared to those of the other quinones in both ^2H feeding experiments can be attributed to the NIH shift[7] during the hydroxylation of (**1**).

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