Phytochemistry, Vol. 23, No. 2, pp. 313–318, 1984.
Printed in Great Britain.

0031–9422/84 $3.00 + 0.00

BIOSYNTHESIS OF NAPHTHOQUINONES AND ANTHRAQUINONES IN *STREPTOCARPUS DUNNII* CELL CULTURES*

KENICHIRO INOUE, SHINICHI UEDA, HIDEKAZU NAYESHIRO, NOBUHARU MORITOME and HIROYUKI INOUYE†

Faculty of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto 606, Japan

(*Received* 8 *June* 1983)

Key Word Index—*Streptocarpus dunnii*; Gesneriaceae; dunnione-type naphthoquinones; anthraquinones; biosynthesis; prenylation mechanism.

Abstract—Administration of ^{13}C- and ^{2}H-labelled precursors to *Streptocarpus dunnii* cell cultures demonstrated that the naphthoquinones formed through a unique prenylation mode are biosynthesized via 4-(2′-carboxyphenyl)-4-oxobutanoic acid, 1,4-dihydroxy-2-naphthoic acid, lawsone and lawsone 2-prenyl ether, and that the anthraquinones are biosynthesized through prenylation of 2-carboxy-4-oxo-1-tetralone at the carboxy-bearing carbon atom to form 2-carboxy-2-prenyl-4-oxo-1-tetralone, or through ipso attack of the prenyl group on the corresponding carbon atom of 1,4-dihydroxy-2-naphthoic acid.

INTRODUCTION

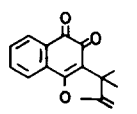
Dunnione (**1**) was isolated from the leaves of *Streptocarpus dunnii* Mast., while 1-hydroxy-2-hydroxymethylanthraquinone (**2**) was obtained from the roots [1–3]. In addition to these quinones, we recently isolated five dunnione congeners, α-dunnione (**3**), dehydrodunnione (**4**), streptocarpone (**5**), 7-hydroxydunnione (**6**) and 8-hydroxydunnione (**7**), as well as one anthraquinone, 1-hydroxy-2-methylanthraquinone (**8**), from the mature plants, plantlets and cultured cells co-existing with half-differentiated plantlets [4, 5].

All these quinones are presumed to be formed through 4-(2′-carboxyphenyl)-4-oxobutanoic acid (OSB)‡ (**9**); and the incorporation of the latter into 2 has actually been demonstrated [3]. As subjects for biosynthetic studies, these quinones provide intriguing materials because of the structural feature of the naphthoquinones with an inverted prenyl group and of the co-occurrence of such naphthoquinones with anthraquinones which seem to be closely related to the rubiaceous anthraquinones. To elucidate the biosynthetic pathways of both of these interesting quinone groups, we have studied the incorporation of stable isotope-labelled precursors into quinones by cell cultures of *S. dunnii*.

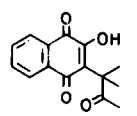
RESULTS AND DISCUSSION

Cell suspension cultures were obtained by subculture of the callus tissues of *S. dunnii* in Linsmaier–Skoog (L–S) liquid medium supplemented with 10^{-5} M indole-3-acetic acid (IAA) and 10^{-6} M kinetin. The culture consisted of undifferentiated cells with half-differentiated plantlets and contained dunnione (**1**), α-dunnione (**3**), dehydrodunnione (**4**), 8-hydroxydunnione (**7**), 1-hydroxy-2-hydroxymethylanthraquinone (**2**) and 1-hydroxy-2-methylanthraquinone (**8**) [4, 5].

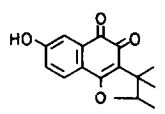
In the first experiment, 4-(2′-[2′-*carboxy*-^{13}C]carboxyphenyl)-4-oxobutanoic acid (**9**) [6, 7] was administered to cell cultures which had been shaken at 25° in the dark for 1 month after transfer. After incubation under the same conditions for a further 2 weeks, the cultures were extracted with benzene. The extract was then subjected to column chromatography on silica gel using benzene as



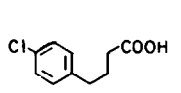
4



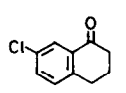
5



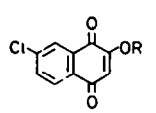
6



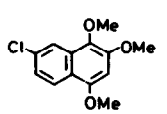
18



19



20 R=H
21 R=Me



22

*Part 20 in the series "Quinones and Related Compounds in Higher Plants". Part of this study was reported as a preliminary communication in (1982) *J. Chem. Soc. Chem. Commun.* 993. For Part 19, see Inoue, K., Shiobara, Y., Nayeshiro, H., Inouye, H., Wilson, G. and Zenk, M. H. (1984) *Phytochemistry* **23**, 307.

†To whom correspondence should be addressed.

‡2-Succinylbenzoic acid (OSB) is the trivial name for 4-(2′-carboxyphenyl)-4-oxobutanoic acid. Other trivial names employed throughout this paper are 2-carboxy-4-oxo-1-tetralone (COT) for 2-carboxy-2,3-dihydro-1,4-naphthoquinone and 2-prenyl-2-carboxy-4-oxo-1-tetralone (prenyl-COT) for 2-carboxy-2,3,-dihydro-2-prenyl-1,4-napthoquinone.

eluant to give dunnione (**1**), 1-hydroxy-2-hydroxymethylanthraquinone (**2**), α-dunnione (**3**), 8-hydroxydunnione (**7**) and 1-hydroxy-2-methylanthraquinone (**8**). In the ^{13}C NMR spectra of the naphthoquinones (**1**, **3** and **7**), the relevant signals assignable to C-1 (δ 180.9, 178.1 and 180.2, respectively) were remarkably enriched, whereas in the spectrum of the anthraquinone (**2**), the signal due to C-10 (δ 181.4) was prominently enriched. The enrichment factors of ^{13}C of these quinones calculated on the basis of the mass spectra were in the range 8–20%, indicating a high incorporation of [^{13}C]OSB (**9**) into these quinones (Table 1) [8]. Thus the effectiveness of OSB (**9**) as a precursor of both groups of quinones was established as expected.

The incorporation of ^{13}C into C-10 of both anthraquinones as opposed to C-9 as in the case of the rubiaceous anthraquinones having similar structures suggested the following two possible biosynthetic pathways for their formation: (i) Prenylation at C-2 of COT (**10**) to form prenyl-COT (**11**) which is then converted via catalponone (**12**) to 2-prenyl-1,4-naphthohydroquinone (**13**). (ii) Prenylation at C-2 of 1,4-dihydroxy-2-naphthoic acid (**14**) by ipso attack of the prenyl group to form 2-prenyl-1,4-naphthohydroquinone (**13**). The former prenylation mode is observed in the biosynthesis of the quinone congeners of *Catalpa ovata* [9], whereas the latter is seen in the formation of menaquinone by *Mycobacterium phlei*, etc. [10–12]. On the other hand, the incorporation of ^{13}C into C-1 of the naphthoquinones strongly suggested that these quinones were biosynthesized via 2-hydroxy-3-(1, 1-dimethylallyl)-1,4-naphthoquinone (**15**) formed by a Claisen-type rearrangement of lawsone 2-prenyl ether (**16**). As an argument for this pathway, the incorporations of [^{14}C]OSB and [^{14}C]-1,4-dihydroxy-2-naphthoic acid (**14**) into lawsone (**17**) had already been demonstrated in *Impatiens balsamina* [13, 14]. It had also been suggested that **17** could be formed through the oxidation and concomitant decarboxylation of COT(**10**) [15].

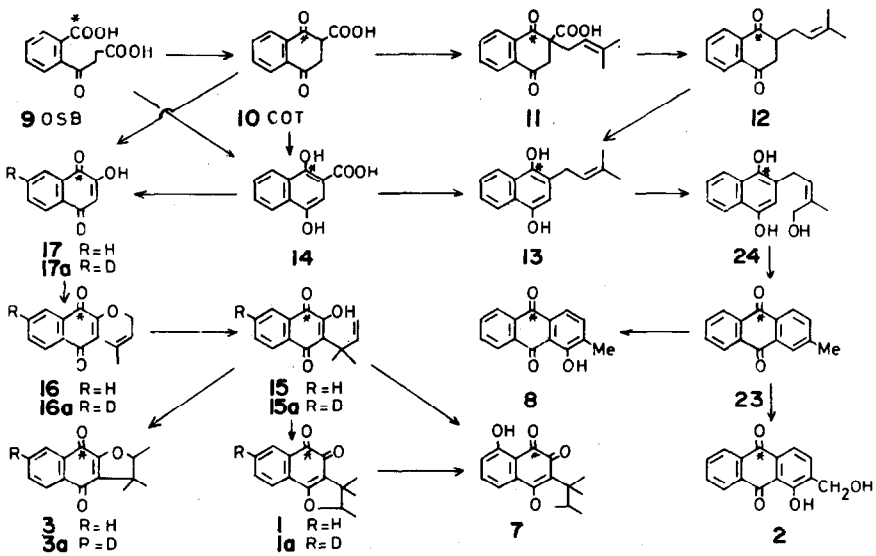
We attempted to demonstrate the intermediacy of lawsone (**17**) and its prenyl ether (**16**) in the biosynthesis of the above-described naphthoquinones by administration experiments using [7-^2H]-labelled **17** and **16**. These compounds were synthesized in the following way: 4-(4-chlorophenyl)butanoic acid (**18**) as its acid chloride was subjected to Friedel–Crafts reaction to yield 7-chloro-1-tetralone (**19**), which was then converted to 7-chlorolawsone (**20**) by oxidation with oxygen and potassium tertiary butoxide in tertiary butanol. Treatment of **20** with methanol and hydrochloric acid gave the 2-methyl ether **21**, which, on reduction with hydrochloric acid–stannous chloride, followed by methylation with dimethyl sulphate–sodium hydroxide, yielded 7-chloro-1,2,4-trimethoxynaphthalene (**22**). On replacement of chlorine by ^2H through reduction with NaB^2H_4–$PdCl_2$ [16] followed by demethylation with BBr_3, the latter gave [7-^2H]lawsone (**17a**). The ^1H NMR spectrum of this compound contained one less proton signal than that of unlabelled **17**. Furthermore, the enrichment factor derived from its mass spectrum was 94% excess. Finally, [7-^2H]lawsone (**17a**) gave [7-^2H]lawsone 2-prenyl ether (**16a**) through reaction with dimethylallyl bromide in the presence of potassium carbonate.

[7-^2H]-**17a** was administered to cell cultures grown under the same conditions as those used for the administration of OSB (**9**). In this case, however, **17a** was administered to the cell cultures at half the dose level used for OSB because of its acute toxicity to the cells, at the OSB dose level. After shaking for 2 weeks, the cultures were extracted with benzene. In addition to quinones **1**, **2**, **3**, **7** and **8**, the extract newly afforded 2-methylanthraquinone (**23**). The UV spectrum of **23** suggested an anthraquinone structure [17], and the ^1H NMR spectrum showed a singlet (δ 2.54) of an aromatic methyl group, an A_2B_2-type signal (δ 7.77–7.82 and 8.29–8.33) of four aromatic protons, doublets (δ 7.64 and 8.25, J = 7.5 Hz) of two aromatic protons located in the *ortho* position and a broad singlet (δ 8.11) of a proton at a peri position to a carbonyl group. Thus this substance was assumed to be 2-methylanthraquinone (**23**). This was confirmed by comparison with an authentic specimen.

α-Dunnione (**3a**), the principal naphthoquinone, showed in the ^2H NMR spectrum a singlet (δ 7.71) due to ^2H on C-7. The enrichment factors of ^2H in naphthoquinones **1**, **3** and **7** calculated from their mass spectra [18] unambiguously demonstrated the intermediacy of lawsone (**17**) in the biosynthesis of these naphthoquinones (Table 1). As was expected, lawsone (**17a**) was not incorporated into anthraquinones **23**, **2** and **8**, which were presumed to be formed after OSB (**9**) or COT (**10**) in a different way from those of the naphthoquinones. The overflow production of quinone **23** observed in this experiment can be explained by redirection of endogenous 2-prenyl-1,4-naphthohydroquinone (**13**) into anthraquinone biosynthesis as a result of feedback control caused by administration of lawsone (**17a**). Quinone **23**

Table 1. Specific incorporation ratios (%) of ^{13}C- or ^2H-labelled precursors into the quinones of the *S. dunnii* cell culture

Quinones	Precursors		
	[^{13}C]OSB (**9**)	[^2H]Lawsone (**17a**)	[^2H]Lawsone 2-prenyl ether (**16a**)
α-Dunnione (**3**) or **3a**	19.48	24.04	65.27
Dunnione (**1**) or **1a**	11.58	17.51	58.17
8-Hydroxydunnione (**7**) or **7a**	7.66	0.84	2.20
1-Hydroxy-2-methylanthraquinone (**8**)	9.50	0	0
1-Hydroxy-2-hydroxy-methylanthraquinone (**2**)	20.70	0	0
2-Methylanthraquinone (**23**)	—	0	0
2-Hydroxy-3-(1,1-dimethylallyl)-1,4-naphthoquinone (**15a**)	—	—	82.26



Scheme 1. Pathways for the biosynthesis of quinones in *S. dunnii* cell cultures.

seems to be the first anthraquinone formed through the cyclization of a derivative of 2-prenyl-1,4-naphthohydroquinone (**13**), most probably 2-(3-hydroxymethyl-2-butenyl)-1,4-naphthohydroquinone (**24**). It is noteworthy that considering the specific incorporation ratios of ^{13}C into the succeeding anthraquinones **2** and **8**, they seem to be formed via different routes after **23**.

Next, [7-^{2}H]lawsone 2-prenyl ether (**16a**), a possible intermediate subsequent to **17**, was administered to the cell cultures under the same conditions as those for the administration of OSB (**9**). On usual work-up, the benzene extract of cell cultures gave 2-hydroxy-3-(1,1-dimethylallyl)-1,4-naphthoquinone (**15a**) along with **1**, **2**, **3**, **7**, **8** and **23**. Substance **15a**, $C_{15}{}^2HH_{13}O_3$, showed UV and visible absorptions at 252, 276, 325 and 491 nm (log ε 4.42, 4.41, 3.63 and 3.12) as well as IR absorptions at 3240, 1660, 1630, 1610 and 1580 cm^{-1}, suggesting a lapachol-type naphthoquinone. In the ^{1}H NMR spectrum, it showed a singlet (δ 1.57) of a *gem.*-dimethyl group, double-doublets (δ 4.97, J = 1.0, 10.5 Hz and δ 5.00, J = 1.0, 17.5 Hz) of terminal methylene protons and a double-doublet (δ 6.29, J = 10.5, 17.5 Hz) of an olefinic proton. Furthermore, it indicated in the aromatic region broad doublets (δ 7.75 and 8.08, J = 8.0 Hz) due to two *ortho*-positioned protons, of which the one at δ 7.75 was assignable to a proton peri-positioned to a quinone carbonyl group, a broad singlet (δ 8.05) due to a proton peri-positioned to another quinone carbonyl group and situated on the carbon adjacent to the deuterium-bearing carbon atom and finally a singlet (δ 7.83) due to a hydroxy proton. On the basis of these data together with the high incorporation of ^{2}H of **16a** into the corresponding position, the structure **15a** was assigned to this naphthoquinone. This was verified by comparison with the Claisen rearrangement product of lawsone 2-prenyl ether (**16**).

In the ^{2}H NMR spectra of **1a** and **3a** isolated in this administration experiment, signals due to the introduced ^{2}H at the 7-position were observed at δ 7.59 and 7.71, respectively, and in the ^{1}H NMR spectra of these compounds, a decrease in the intensity of the corresponding proton signals was observed. However, no notable deformation due to the introduced ^{2}H was observed in either the ^{1}H NMR or the ^{2}H NMR spectrum. On the other hand, the specific incorporation ratios of ^{2}H into **1a**, **3a**, **7** and **15a** calculated on the basis of the mass spectra were in accord with these NMR spectral findings (Table 1).

The overflow production of **15a** caused by administration of **16a** might be partly ascribed to a spontaneous non-enzymatic rearrangement, because shaking **16** in the same medium as that used for the incubation of the cells yielded **15**. However, it is worth mentioning that the precursorship of **16**, ranked in the same level as **17**, was demonstrated by dilution analysis after administration of [^{14}C]OSB (**9**) to the *S. dunnii* cell culture (unpublished data).

The biosynthetic pathways of both groups of quinones in *S. dunnii* elucidated through this work can therefore be summarized as follows (Scheme 1). (i) Anthraquinones **2** and **8**, etc. are formed from shikimate, OSB (**9**), 2-prenyl-1,4-naphthohydroquinone (**13**) and a cyclization product, 2-methylanthraquinone (**23**), while the key intermediate (**13**), in turn, is derived from OSB either via COT (**10**), prenyl-COT (**11**) and catalponone (**12**), or via 1,4-dihydroxy-2-naphthoic acid (**14**). (ii) Naphthoquinones **1**, **3** and **7** are biosynthesized via 1,4-dihydroxy-2-naphthoic acid (**14**), lawsone (**17**), lawsone 2-prenyl ether (**16**) and the Claisen-type rearrangement product of the latter, 2-hydroxy-3-(1,1-dimethylallyl)-1,4-naphthoquinone (**15**), which would be the key intermediate for all the naphthoquinones in this plant.

In the experiments with [^{2}H]-labelled **17** and **16**, the specific incorporation ratios of the two compounds into **7** were much lower than those into **1** and **3**. Furthermore, they were even lower than the value of [^{13}C]OSB (**9**) into **7**. These facts strongly suggest that 8-hydroxydunnione (**7**) is formed from **1** through stereoselective epoxidation of the 7,8-double bond and subsequent ring fission with an NIH shift [19]. In the same way, formation of 7-

hydroxydunnione (6), a minor constituent of the mature *S. dunnii* plant, would be explained by ring opening of the same epoxide in the opposite direction.

EXPERIMENTAL

General procedures. Mps: uncorr; ^{1}H NMR (200 MHz) and ^{13}C NMR (50.1 MHz): TMS as int. standard; ^{2}H NMR (30.6 MHz): $CDCl_3$ (δ 7.26) as int. standard. MS: direct inlet, 70 eV; TLC: silica gel GF_{254} (Merck); prep. TLC: PF_{254} (Merck). Spots were visualized under UV irradiation. CC: silica gel AR (Mallinckrodt) and charcoal (Wako).

Feeding experiment with [^{13}C]*OSB* (1). Cultured cells of *S. dunnii* grown on Linsmaier–Skoog (L–S) agar medium supplemented with IAA (1×10^{-6} M) and kinetin (1×10^{-5} M) were subcultured every 2 weeks. After 22 passages, the cells were transferred to a liquid medium (170 ml × 5) of the same composition as described above and incubated on a reciprocating shaker at 25° in the dark for 1 month. The plant material consisted of undifferentiated cells and half-differentiated plantlets in the ratio 4:6. A soln of [^{13}C]OSB (enrichment factor 90%, 50 mg) in sterile H_2O (5 ml) containing Tween 80 soln (0.5 ml, 1 drop/100 ml) was fed to the cell cultures and incubated for a further 2 weeks under the same conditions. The medium (870 ml) was shaken with C_6H_6 (500 ml × 3) and the cultured cells including plantlets (94 g) were soaked in the same solvent (500 ml × 3) at room temp. for 3 days. The extracts were combined and concd *in vacuo* to give a residue (115.4 mg), which was subjected to CC on silica gel (10 g) with C_6H_6–EtOAc (99:1) as eluant, collecting 50 ml fractions. Fraction 4, on concn, gave a residue, which was recrystallized from MeOH to give 1-hydroxy-2-methylanthraquinone (**8**). (3.1 mg) as yellow needles; mp 183–184°; MS *m/z* (rel. int.): 239 $[M+1]^+$ (27.3), 238 $[M]^+$ (100). Each of fractions 5, 9 and 12 was concd *in vacuo* to give a residue, which was recrystallized from Et_2O–petrol to give α-dunnione (**3**) (10 mg) as yellow plates [mp 109–110°; MS *m/z* (rel. int.: 243 $[M+1]^+$ (29.4), 242 $[M]^+$ (46.1)], dunnione (**1**) (5.2 mg) as red needles [mp 96–97°; MS *m/z* (rel. int.): 243 $[M+1]^+$ (18.2), 242 $[M]^+$ (46.1)] and 8-hydroxydunnione (**7**) (6.3 mg) as red needles [mp 151–152°; MS *m/z* (rel. int.): 259 $[M+1]^+$ (10.6), 258 $[M]^+$ (52.4)]. Combined fractions 13–15, on concn, gave a residue, which was recrystallized from MeOH to give 1-hydroxy-2-hydroxymethylanthraquinone (**2**) (30.3 mg) as yellow needles, mp 214–215°; MS *m/z* (rel. int.): 255 $[M+1]^+$ (16.5), 254 $[M]^+$ (100).

Synthesis of [7-^{2}H]*lawsone* (**17**) *and its 2-prenyl ether* (**16**). (i) *Preparation of 7-chloro-1-tetralone* (**19**): 4-(4-chlorophenyl)-butanoic acid (**18**) (10 g) was suspended in $SOCl_2$ (5 ml) and refluxed for 1 hr. After evapn of the excess $SOCl_2$, the residue was dissolved in dry CS_2 (100 ml). Pulverized $AlCl_3$ (8.0 g) was added to this soln at once under vigorous stirring and refluxed for 3 hr. The reaction was poured into a mixture of conc. HCl and ice, and extracted with $CHCl_3$ (200 ml × 3). The $CHCl_3$ layer was washed with H_2O, dried and concd *in vacuo* to give a residue, which was purified by elution through a charcoal (15 g) column with MeOH. After removal of the solvent, the residue was recrystallized from H_2O–EtOH to give **19** (5.4 g) as colourless plates. Mp 94–95°; UV λ_{max}^{MeOH} nm (log ε): 245 (4.01), 305 (3.30); IR ν_{max}^{KBr} cm^{-1}: 1670, 1590; ^{1}H NMR ($CDCl_3$): δ 1.97–2.98 (6H, *m*, H-2, 2′, 3, 3′, 4, 4′), 7.03 (1H, *d*, *J* = 8.0 Hz, H-5), 7.28 (1H, *dd*, *J* = 2.0, 8.0 Hz, H-6), 7.83 (1H, *d*, *J* = 2.0 Hz, H-8). (Found: C, 66.39; H, 4.95; Cl, 19.53. Calc. for $C_{10}H_9OCl$: C, 66.50; H, 5.02; Cl, 19.63%.)

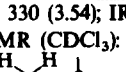
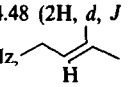
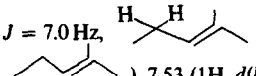
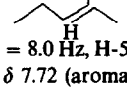
(ii) *7-Chlorolawsone* (**20**): To a soln of K (2.7 g) in dry tertiary BuOH (83 ml) was added a soln of **19** (5.3 g) in dry tertiary BuOH (70 ml) with continuous stirring. The mixture was then stirred for 4 hr at room temp. under a stream of O_2, after which it was poured into H_2O (150 ml) and acidified with 4 M HCl followed by extraction with Et_2O (150 ml × 3). The Et_2O layer was washed with H_2O and then shaken with 5% Na_2CO_3 (200 ml × 3). The Na_2CO_3 layer was acidified with 4 M HCl and extracted with Et_2O (150 ml × 3). The combined extracts were washed with H_2O, dried and concd *in vacuo* to give a yellow residue (2.14 g), which, on recrystallization from HOAc, yielded **20** (1.8 g) as yellow needles. Mp 214–215°; UV λ_{max}^{MeOH} nm (log ε): 237 (4.29), 242 (4.27), 255 (inf.) (4.16), 264 (inf.) (4.26), 272 (4.33), 281 (inf.) (4.16), 325 (3.81), 460 (3.40); IR ν_{max}^{KBr} cm^{-1}: 3150, 1670, 1635, 1580; ^{1}H NMR (DMSO-d_6): δ 6.04 (1H, *s*, –OH), 7.70–7.83 (4H, *m*, H-3, 5, 6, 8). (Found: C, 57.47; H, 2.45; Cl, 16.89. $C_{10}H_5O_3Cl$ requires: C, 57.58; H, 2.42; Cl, 17.00%.)

(iii) *7-Chlorolawsone 2-methyl ether* (**21**): Conc. HCl (0.1 ml) was added to a suspension of **20** (1.0 g) in MeOH (70 ml) and refluxed for 3 hr. After being cooled, the resulting yellow crystals were collected by filtration and recrystallized from MeOH to give **21** (1.0 g) as yellow needles. Mp 229–230°; UV λ_{max}^{MeOH} nm (log ε): 248 (4.37), 253 (4.38), 280.5 (4.17), 327 (3.52); IR ν_{max}^{KBr} cm^{-1}: 1680, 1640, 1610, 1580; ^{1}H NMR ($CDCl_3$): δ 3.82 (3H, *s*, –OMe), 6.04 (1H, *s*, H-3), 7.50 (1H, *dd*, *J* = 2.0, 8.0 Hz, H-6), 7.87 (1H, *d*, *J* = 8.0 Hz, H-5), 7.92 (1H, *d*, *J* = 2.0 Hz, H-8). (Found: C, 59.31; H, 2.90; Cl, 15.97. $C_{11}H_7O_3Cl$ requires: C, 59.35; H, 3.17; Cl 15.93%).

(iv) *7-Chloro-1, 2, 4-trimethoxynaphthalene* (**22**): To a suspension of **21** (1.0 g) in EtOH (15 ml), a soln of $SnCl_2$ (1.8 g) in conc. HCl (1.8 ml) was added and the soln was stirred for 1 hr at room temp. The reaction mixture was poured into H_2O (100 ml) and the resultant ppt. was collected by filtration and washed with H_2O. After being dried, the ppt. was suspended in Me_2SO_4 (5.3 ml) under N_2 and then 50% KOH (17 ml) was added dropwise to the suspension over a period of 30 min with continuous stirring. The mixture, after 1 hr of refluxing, was poured into H_2O (100 ml) and extracted with $CHCl_3$ (50 ml × 3). The $CHCl_3$ layer was washed with H_2O, dried and concd *in vacuo* to afford a residue, which was recrystallized from MeOH to give **22** (860 mg) as colourless needles. Mp 79–80°; UV λ_{max}^{MeOH} nm (log ε): 225 (4.49), 248.5 (4.78), 313 (3.80), 355 (3.62); IR ν_{max}^{KBr} cm^{-1}: 1616, 1590, 1580; ^{1}H NMR ($CDCl_3$): δ 3.83, 3.89, 3.90 (each 3 H, *s*, –OMe), 6.46 (1 H, *s*, H-3), 7.07 (1 H, *dd*, *J* = 2.0, 8.0 Hz, H-6), 7.82 (1H, *d*, *J* = 2.0 Hz, H-8), 7.88 (1 H, *d*, *J* = 8.0 Hz, H-5). (Found: C, 61.84; H, 5.15; Cl, 14.01. $C_{13}H_{13}O_3Cl$ requires: C, 61.79; H, 5.19; Cl, 14.03%.)

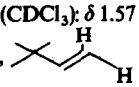
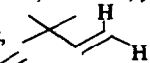
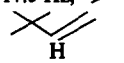
(v) [7-^{2}H]*Lawsone* (**17a**): $PdCl_2$ (849 mg) was added to an ice-cold soln of **22** (604 mg) in MeOH (16 ml) under Ar. $NaBH_4$ (1.0 g) was then added to the stirred suspension during 30 min and stirring was continued for a further 1 hr at room temp. The reaction was then poured into dilute HCl (100 ml) and extracted with $CHCl_3$ (70 ml × 3). The $CHCl_3$ layer was washed successively with brine and H_2O, dried and concd *in vacuo* to give a residue (52 mg), which was subjected to prep. TLC (C_6H_6–EtOAc, 4:1). From the main band around R_f 0.65, a colourless oily product was obtained. ^{1}H NMR ($CDCl_3$): δ 3.83, 3.87, 3.90 (each 3H, *s*, –OMe), 6.84 (1 H, *s*, H-3), 7.00–7.45 (2H, *m*, H-6, 7), 7.78–8.05 (2H, *m*, H-5, 8). This product was dissolved in dry CH_2Cl_2 (44 ml) without further purification. In an Ar atmosphere, BBr_3 (1.25 ml) was added to the soln and stirred for 2 hr at room temp. The reaction was poured into ice-H_2O and extracted with Et_2O (50 ml × 3). The combined extracts were washed with H_2O, dried and concd *in vacuo* to give an orange residue, which was recrystallized from HOAc to give lawsone (**17**) (393 mg) as orange-yellow needles. Mp 192° (dec.); UV λ_{max}^{MeOH} nm (log ε): 243 (4.12), 249 (4.16), 274 (4.10), 333 (3.38); IR ν_{max}^{KBr} cm^{-1}: 1670, 1630, 1590, 1575; ^{1}H NMR (DMSO-d_6): δ 6.05 (1H, *s*, –OH), 7.53–7.93 (5H, *m*, H-3, 5, 6, 7, 8); MS *m/z*: 174 $[M]^+$. (Found: C, 69.15; H, 3.40. Calc. for $C_{10}H_6O_3$: C, 68.97; H, 3.47%.) Work-up of **22** (600 mg) with NaB^2H_4–$PdCl_2$ in CH_3OD in the same way as described above gave [7-^{2}H]lawsone (**17a**)

(380 mg). Mp 192° (dec.); ^{1}H NMR (DMSO-d_6): δ 6.05 (1H, *s*, –OH), 7.57–7.83 (4H, *m*, H-3, 5, 6, 8); ^{2}H NMR (DMSO): δ 7.68 (^{2}H-7); MS *m/z*: 175 $[M]^+$ ($C_{10}{}^2H_1H_5O_3$).

(vi) [7-^{2}H]*Lawsone 2-prenyl ether* (**16a**): Dimethylallyl bromide (540 mg) and K_2CO_3 (630 mg) were added to a suspension of **17** (315 mg) in Me_2CO (10 ml) and the mixture was refluxed for 3 hr with vigorous stirring. After standing overnight at room temp., the resulting ppt. was filtered off and washed with Me_2CO. The combined filtrate and washings were concd *in vacuo* to give a residue, which was subjected to CC on silica gel (30 g) with C_6H_6–EtOAc (19:1) as eluant, collecting 50 ml fractions. The residue obtained from fraction 5, on recrystallization from Et_2O, gave **16** as yellow needles (74 mg). Mp 150–151°; UV λ_{max}^{MeOH} nm (log ε): 242.5 (4.31), 248.5 (4.33), 277 (4.19), 330 (3.54); IR ν_{max}^{KBr} cm^{-1}: 1680, 1650, 1600, 1590, 1570; ^{1}H NMR ($CDCl_3$): δ 1.72 (6H, *s*, *gem.*-Me), 4.48 (2H, *d*, *J* = 6.0 Hz, ), 5.72 (1H, *t*(*br*), *J* = 6.0 Hz, ), 6.02 (1H, *s*, H-3), 7.45–7.67 (2H, *m*, H-6, 7), 7.80–8.00 (2H, *m*, H-5, 8). (Found: C, 74.38; H, 5.47. Calc. for $C_{15}H_{14}O_3$: C, 74.37; H, 5.82%.) [7-^{2}H]Lawsone (**17a**) (315 mg) was prenylated in the same way as described above to give [7-^{2}H]lawsone 2-prenyl ether (**16a**) (75 mg). Mp 150–151°; ^{1}H NMR ($CDCl_3$): δ 1.75 (6H, *s*, *gem.*-Me), 4.48 (2H, *d*, *J* = 7.0 Hz, ), 5.72 (1H, *t*, *J* = 7.0 Hz, ), 7.53 (1H, *d*(*br*), *J* = 8.0 Hz, H-6), 7.90 (1H, *d*, *J* = 8.0 Hz, H-5), 7.93 (1H, *d*, *J* = 2.0 Hz, H-8); ^{2}H NMR ($CDCl_3$): δ 7.72 (aromatic ^{2}H-7); MS *m/z*: 243 $[M]^+$ ($C_{15}{}^2H_1H_{13}O_3$).

Feeding expt with [7-^{2}H]*lawsone* (**17a**). To suspension cultures (170 ml × 5) of *S. dunnii* incubated for 2 months under the same conditions as in the case of the [^{13}C]OSB feeding expt, a suspension of [7-^{2}H]lawsone (**17a**) (enrichment factor 94% excess, 25 mg) in 70% EtOH (4 ml) and sterile H_2O (2 ml) containing Tween 80 (1 ml, 1 drop/100 ml) was fed and incubated for a further 2 weeks. The medium (860 ml) and the cells (116 g) were extracted with C_6H_6 in the usual way. The extracts were concd *in vacuo* to give a residue, which was subjected to prep. TLC (C_6H_6–EtOAc, 4:1, 3 developments) to give **8** (R_f 0.58, 2.0 mg), **3a** [R_f 0.54; 12.7 mg; mp 109–110°; MS *m/z* (rel. int.): 243 $[M]^+$ (14.5), 242 $[M]^+$ (55.2)] **1a** [R_f 0.35; 0.7 mg; mp 96–97°; MS *m/z* (rel. int.): 243 $[M+1]^+$ (12.2), 242 $[M]^+$ (49.8%)], **7** [R_f 0.28; 6.5 mg; mp 151–152°; MS *m/z* (rel. int.): 259 $[M+1]^+$ (16.5), 258 $[M]^+$ (63.9%)] and **2** [R_f 0.21, 21.3 mg). In addition, a band around R_f 0.56 gave pale-yellow needles (2.5 mg); mp 177° (sublimes); UV λ_{max}^{MeOH} nm (log ε): 255 (4.65), 265 (4.32), 274 (4.24), 324 (3.66); IR ν_{max}^{KBr} cm^{-1}: 1670, 1590. This substance was identified as 2-methylanthraquinone (**23**) (IR and ^{1}H NMR).

Feeding expt with [7-^{2}H]*lawsone 2-prenyl ether* (**16a**). To suspension cultures of *S. dunnii* incubated for 1 month under the same conditions as above, a suspension of [7-^{2}H]lawsone 2-prenyl ether (**16a**) (enrichment factor 94% excess, 50 mg) in 70% EtOH (5 ml) was fed and incubated for a further 2 weeks. The medium (870 ml) and the cells (62 g) were extracted with C_6H_6 (in total 1000 ml × 3) in the usual way. The extracts were concd *in vacuo* and the resultant residue was subjected to prep. TLC (C_6H_6–EtOAc, 9:1, 3 developments) to give **8** (R_f 0.58, 2.0 mg), **3** [R_f 0.54; 12.2 mg; MS *m/z* (rel. int.): 243 $[M+1]^+$ (68.5), 242 $[M]^+$ (33.5)], **1** [R_f 0.35; 5.5 mg; MS *m/z* (rel. int.): 243 $[M+1]^+$ (36.5), 242 $[M]^+$ (23.4)], **7** [R_f 0.28; 3.7 mg; MS *m/z* (rel. int.): 259 $[M+1]^+$ (24.8), 258 $[M]^+$ (99.7)]; **2** (R_f 0.21, 16.5 mg) and **23** (R_f 0.56, 2.3 mg). Compounds **1**, **3** and **7** also showed the signal of ^{2}H-7. In addition to the above described compounds, a band around R_f 0.55 gave 2-hydroxy-3-(1,1-dimethylallyl)-1,4-[7-^{2}H]naphthoquinone (**15a**) (3.1 mg) as yellow needles (from MeOH–H_2O). Mp 69–70°; MS *m/z* (rel. int.): 243 $[M+1]^+$ (71.4), 242 $[M]^+$ (14.8). This substance was identified with an authentic sample synthesized by Claisen rearrangement of lawsone 2-prenyl ether (**16**) (mmp, IR). Otherwise unreacted starting material (8.0 mg) was recovered from the band around R_f 0.57.

Claisen rearrangement of lawsone 2-prenyl ether (**16**). **16** (360 mg) was dissolved in dry EtOH (20 ml) and refluxed for 8 hr. After evapn of the solvent, the residue (360 mg) was subjected to prep. TLC (C_6H_6–EtOAc, 9:1) to give a minor band around R_f 0.42 and major one around R_f 0.50. The minor band gave, on usual work-up, the rearrangement product **15**, which was recrystallized from MeOH–H_2O to yield yellow needles (9.0 mg). Mp 69–70°; ^{1}H NMR ($CDCl_3$): δ 1.57 (6H, *s*, *gem.*-Me), 4.67 (1H, *dd*, *J* = 1.0, 10.5 Hz, ), 5.00 (1H, *dd*, *J* = 1.0, 17.5 Hz, ), 6.29 (1H, *dd*, *J* = 10.5, 17.5 Hz, ), 7.61–7.79 (2H, *m*, H-6, 7), 7.84 (1H, *s*, –OH), 8.03–8.09 (2H, *m*, H-5, 8). (Found: C, 74.14; H, 5.75. Calc. for $C_{15}H_{14}O_3$: C, 74.37; H, 5.82%.) The major band gave the unreacted starting material **16**.

Incubation of **16** *in the growth medium.* A suspension of **16** (5 mg) in 70% EtOH (0.5 ml) was added to the same medium (80 ml) used for suspension culturing and shaken under the same conditions as in the administration expt for 2 weeks. The mixture was extracted with C_6H_6 (50 ml × 3), dried and evapd *in vacuo*. The resultant residue gave two spots of **15** (R_f 0.42) and **16** (R_f 0.49) on TLC (C_6H_6–EtOAc, 19:1), whose ratio was shown to be 49:51, respectively.

Acknowledgements—We thank Dr. N. Akimoto, Faculty of Pharmaceutical Sciences, Kyoto University, for measurements of mass spectra and the members of the Microanalytical Centre of Kyoto University for microanalyses. This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan, to which our thanks are also due.

REFERENCES

1. Price, J. R. and Robinson, R. (1939) *J. Chem. Soc.* 1522.
2. Price, J. R. and Robinson, R. (1940) *J. Chem. Soc.* 1493.
3. Stöckigt, J., Srocka, U. and Zenk, M. H. (1973) *Phytochemistry* **12**, 2389.
4. Inoue, K., Nayeshiro, H. and Inouye, H. (1982) *Chem. Pharm. Bull. (Tokyo)* **30**, 2265.
5. Inoue, K., Ueda, S., Nayeshiro, H. and Inouye, H. (1983) *Phytochemistry* **22**, 737.
6. Inouye, H., Ueda, S., Inoue, K., Hayashi, T. and Hibi, T. (1975) *Chem. Pharm. Bull. (Tokyo)* **23**, 2523.
7. Inoue, K., Ueda, S., Nayeshiro, H. and Inouye, H. (1984) *Phytochemistry* (in press).
8. Campbell, I. M., (1974) *Bioorg. Chem.* **3**, 386.
9. Inoue, K., Ueda, S., Shiobara, Y., Kimura, I. and Inouye, H. (1981) *J. Chem. Soc. Perkin Trans.* 1, 1246.
10. Baldwin, R. M., Snyder, C. D. and Rapoport, H. (1973) *J. Am. Chem. Soc.* **95**, 276.
11. Baldwin, R. M., Snyder, C. D. and Rapoport, H. (1974) *Biochemistry* **13**, 1523.
12. Shineberg, B. and Young, I. G. (1976) *Biochemistry* **15**, 2754.
13. Dansette, P. and Azerard, R. (1970) *Biochem. Biophys. Res. Commun.* **40**, 1090.
14. Müller, W.-U. and Leistner, E. (1976) *Phytochemistry* **15**, 407.
15. Inoue, K., Shiobara, Y. and Inouye, H. (1977) *Chem. Pharm. Bull. (Tokyo)* **25**, 1468.
16. Bosin, T. R., Raymond, M. G. and Buckpitt, A. R. (1973)

Tetrahedron Letters 4699.
17. Thomson, R. H. (1971) *Naturally Occurring Quinones*, p. 368. Academic Press, London.
18. Budzikiewicz, H., Djerassi, C. and Williams, D. H. (1964) *Structure Elucidation of Natural Products by Mass Spectrometry*, Vol. 1, p. 34. Holden-Day, San Francisco.
19. Daly, J. W., Jerina, D. M. and Witkop, B. (1972) *Experientia* **28**, 1129.