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INDUCTION OF BENZOQUINONE FORMATION BY ACTIVATED CARBON IN *LITHOSPERMUM ERYTHRORHIZON* CELL SUSPENSION CULTURES

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Key Word Index—*Lithospermum erythrorhizon*; Boraginaceae; plant cell culture; activated carbon; biosynthesis; echinofuran; shikonin; benzoquinone; naphthoquinone.

Abstract—Cultured cells of *Lithospermum erythrorhizon* which were capable of producing red naphthoquinone (shikonin) derivatives on Linsmaier–Skoog's agar medium stopped synthesizing these compounds when grown in liquid medium without agar. However, when the liquid medium was supplemented with a small amount of activated carbon, the cells produced a new orange benzoquinone derivative, echinofuran B, which may be considered an abnormal metabolite of geranylquinol, the key intermediate in the biosynthesis of shikonin. A similar effect of activated carbon was also observed with a variant cell line incapable of producing shikonin derivatives even on the agar medium. By contrast, the callus cultures grown on the agar medium as well as the dried roots of the intact plant were found to contain a small amount of echinofuran C, another new benzoquinone related to echinofuran B, in addition to shikonin derivatives.

INTRODUCTION

Although the two culture lines, M-18 and C-144, of *Lithospermum erythrorhizon* grown on Linsmaier–Skoog's agar medium [1] produced large amounts of shikonin (**1**) derivatives [2–5], which are the red naphthoquinone pigments found in the root bark, they failed to produce these compounds when grown as a cell suspension culture in the same medium without agar. However, the addition of a small amount of agar powder to the liquid medium induced the formation of shikonin derivatives in the cultured cells [6]. We now report that the addition of a small amount of activated carbon to the liquid medium induces the formation of a new orange benzoquinone derivative (echinofuran B, **2**) in the cultured cells. This compound is a congener of echinofuran C (**3**), a compound that we have isolated as a minor orange pigment from the shikonin-producing callus cultures as well as from the roots of *L. erythrorhizon*.

RESULTS AND DISCUSSION

Effect of activated carbon on quinone formation in the cell suspension culture

When naphthoquinone-producng *Lithospermum* callus cultures (M-18) cultured on Linsmaier–Skoog basal agar medium containing 10^{-6} M indole-3-acetic acid (IAA) and 10^{-5} M kinetin were transferred to liquid medium containing the same plant growth regulators, no shikonin derivatives were produced in the cell suspension cultures at any growth stage during the subsequent transfer generations unless the cells were returned to the original agar medium. However, it was found that the addition of activated carbon to the liquid medium at the beginning of suspension culture caused the formation of an unknown orange pigment instead of shikonin derivatives. A similar effect of activated carbon was observed in the cell suspension culture of strain C-144.

The orange pigment extracted from a mixture of harvested cells and activated carbon was identified as a benzoquinone derivative (**2**). The formation of **2** was observed when the dose of activated carbon was within a range from 30 to 500 mg per 30 ml of the liquid medium inoculated with 500 mg of fresh cells; the yield of **2** after 3 weeks of cultivation was highest (4.2 mg/flask, 2.1% of dry wt of cells) at 150 mg of activated carbon, although the cell growth was reduced to *ca* 50% of the control culture. The addition of activated carbon to the liquid medium either before or after autoclaving was equally effective in inducing the synthesis of **2** in the cultured cells. The time course for the production of **2** by the suspension cultures is shown in Fig. 1.

Activated carbon also induced the formation of **2** in the cell suspension cultures of the other culture strains(B-17 and M-130) which lack the ability to synthesize shikonin derivatives even on the agar medium. Furthermore, activated carbon stimulated the formation of **2** in a cell suspension culture whose shikonin biosynthesis was completely inhibited by the addition of a synthetic auxin, 2,4-D, to the medium [2].

Although the addition of activated carbon to the medium is known to promote embryogenesis or organogenesis in callus cultures as well as in anther cultures [7–15], the present study demonstrates for the first time that activated carbon brings about a significant change in the secondary metabolism. Since the benzoquinone was never observed in the cells inoculated into a liquid medium that was previously shaken overnight with activated carbon (30–500 mg/30 ml) and then filtered through a sintered glass filter, its formation could not be

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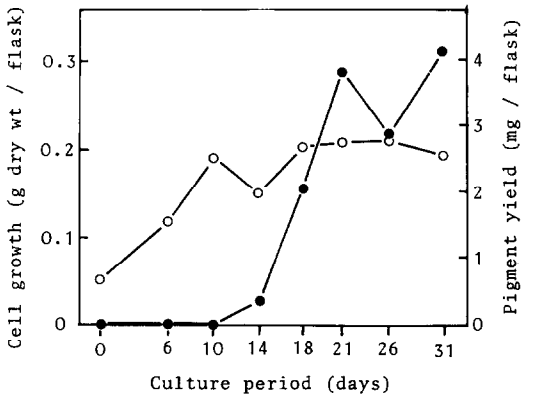


Fig. 1. Time course of cell growth (O——O) and benzoquinone formation (●——●) in the *Lithospermum* cell suspension culture grown in a liquid medium (30 ml) containing activated carbon (150 mg).

due to the release of an impurity from activated carbon, nor due to the removal of an inhibitory component of the fresh medium. Therefore, it is possible that activated carbon may adsorb an inhibitor of secondary metabolism released from the cultured cells into the liquid medium.

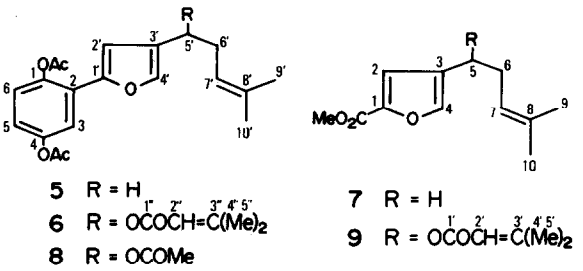
Examination of callus cultures and roots for the presence of benzoquinones

In order to ascertain if echinofuran B (**2**) or any other related benzoquinone was present either in the original callus tissues grown on agar medium without activated carbon or in the intact roots of *L. erythrorhizon*, the chloroform extracts of these materials were subjected to chemical examination. TLC analysis failed to detect the presence of **2**, but it revealed the presence of a small amount of a yellow pigment besides the ordinary red shikonin pigments in both materials. This unknown pigment was found to be a new benzoquinone, echinofuran C (**3**) (see below), which is considered to be a derivative of **2**.

Chemical structures of echinofuran B and echinofuran C

Echinofuran B (**2**), $C_{16}H_{16}O_3$, was isolated as an orange oil exhibiting UV absorption maxima (EtOH) at 259 and 453 nm (log ε 4.20 and 3.70, respectively) and IR absorption bands ($CHCl_3$) at 1648, 1632, 1590, and 1560 cm^{-1}. Its ^1H NMR spectrum ($CDCl_3$) showed in the aromatic proton region four singlets (δ 6.73, 6.75, 7.35 and 7.36) and a broad singlet (δ 7.02). These spectral data suggested that **2** was a benzoquinone related to echinofuran (**4**), a compound which was isolated from the callus tissues of another boraginaceous plant, *Echium lycopsis* [16].

The presence of the 1,4-benzoquinone moiety was confirmed by the following method: **2** was treated with Zn–Ac_2O–pyridine to give a leucodiacetate (**5**) whose ^1H NMR spectrum exhibited a typical ABX pattern (δ 6.99, 1H, *dd*, J = 2.5 and 9.0 Hz; 7.12, 1H, *d*, J = 9.0 Hz; 7.52, 1H, *d*, J = 2.5 Hz) which was superimposable on that of the leucodiacetate (**8**) of **4**, indicating that **5** was a 1,4-quinol diacetate with an alkyl substituent and, therefore, that **2** was a monosubstituted benzoquinone.



5 R = H
6 R = OCOCH=C(Me)$_2$
8 R = OCOMe

7 R = H
9 R = OCOCH=C(Me)$_2$

The ^1H NMR spectrum of **2** exhibited two singlets (δ 1.60 and 1.70) assigned to *gem.*-methyl groups on a double bond, a quartet (δ 2.26, 2H, J = 7.0 Hz) due to allyl methylene protons, a triplet (δ 2.48, 2H, J = 7.0 Hz) due to methylene protons adjacent to the allyl methylene and a broad triplet (δ 5.14, 1H, J = 7.0 Hz) for a vinyl proton. These signals were quite similar to those of the side chain of deoxyshikonin isolated from the roots of *L. erythrorhizon* and *Macrotomia euchroma* [17], demonstrating that the aliphatic side chain of **2** was a 4-methyl-3-pentenyl group. The presence of the 1,4-benzoquinone and 4-methyl-3-pentenyl moieties was confirmed by the ^{13}C NMR data of **2** (Table 1).

The ^{13}C NMR data also suggested that the remaining part (C_4H_2O) of the molecule was a furan ring. This was confirmed by a positive response of **2** to Ehrlich's test. On the basis of the ^1H NMR data of **5**, which showed two one-proton singlets (δ 6.58 and 7.24) assigned to the β- and α-protons of the furan ring, respectively, **2** was clearly

Table 1. ^{13}C NMR chemical shifts of echinofurans B and C (25.05 MHz, $CDCl_3$, TMS as internal standard)

Carbon No.	Echinofuran B (**2**)	Echinofuran C (**3**)
1	185.47 *s**	185.15 *s**
2	133.69 *s*†	133.53 *s*†
3	125.13 *d*	125.69 *d*
4	187.24 *s**	187.13 *s**
5	136.47 *d*‡	136.53 *d*
6	136.56 *d*‡	136.53 *d*
1′	146.08 *s*	146.40 *s*
2′	120.29 *d*§	117.77 *d*§
3′	129.09 *s*†	129.17 *s*†
4′	142.17 *d*	143.01 *d*
5′	28.34 *t*	67.11 *d*
6′	24.99 *t*	33.69 *t*
7′	123.34 *d*§	118.47 *d*§
8′	132.67 *s*†	135.22 *s*†
9′	17.77 *q*	17.98 *q*
10′	25.64 *q*	25.74 *q*
1″		165.75 *s*
2″		116.00 *d*
3″		157.27 *s*
4″		20.28 *q*
5″		27.40 *q*

*, †, ‡, § Data with the same sign within each column may be reversed.

substituted at C-2 and C-4. In order to clarify the substitution pattern of the furan ring, **2** was oxidized with alkaline hydrogen peroxide, followed by methylation with ethereal diazomethane according to the method described by Inouye *et al.* [16] to give a methyl ester (**7**). The ^{1}H NMR spectrum of **7** exhibited two singlets of furanoid protons at δ 7.18 and 7.53 in addition to signals assigned to a carbomethoxyl group and a 4-methyl-3-pentenyl group. Through the use of the published ^{1}H NMR data on the oxidation product of **4** and its derivatives [16], the oxidation product of **2** was assigned structure **7**. Thus, echinofuran B must have the structure shown by formula **2**.

Echinofuran C (**3**), $C_{21}H_{22}O_5$, $[\alpha]_D^{25}$ −60.0° (*c* 0.90, $CHCl_3$), was obtained as an orange oil exhibiting UV absorption maxima (EtOH) at 253 and 438 nm (log ε 4.31 and 3.62, respectively) and IR absorption bands ($CHCl_3$) at 1715, 1642, 1590 and 1562 cm^{-1}. Its ^{1}H NMR spectrum ($CDCl_3$) showed in the aromatic proton region four one-proton singlets (δ 6.73, 6.75, 7.47 and 7.54) and a broad singlet (δ 7.02). These spectral data as well as the ^{13}C NMR data (Table 1) suggested that **3** was a benzoquinone related to **2** and **4**.

The presence of a monosubstituted 1,4-benzoquinone was confirmed by the ^{1}H NMR spectrum of the leucodiacetate (**6**) of **3** which showed an ABX pattern (δ 7.01, *dd*, J = 2.5 and 7.0 Hz; 7.13, *d*, J = 9.0 Hz; 7.52, *d*, J = 2.5 Hz) typical for a monosubstituted 1,4-quinol diacetate as in the case of **5** and **8**.

The ^{1}H NMR spectrum of **3** showed two singlets (δ 1.60 and 1.70) assigned to *gem.*-methyl groups on a double bond, two one-proton triplets (δ 2.56, J = 7.0 Hz; 2.58, J = 7.0 Hz) due to allylic methylene protons, a broad triplet (δ 5.08, J = 7.0 Hz) for a vinyl proton and a triplet (δ 5.77, J = 7.0 Hz) assignable to a proton on an acyloxy-bearing carbon. The pattern of these signals resembled that of the acyloxy-carrying C_6-side chain of **4** and acylated shikonin. The acyloxy group was assigned as 3,3-dimethylacryloyloxy on the basis of the ^{1}H NMR signals (δ 1.90, 3H, *s*; 2.17, 3H, *s*; 5.69, 1H, *s*, *br*) which were identical with those of 3,3-dimethylacrylshikonin. This was supported by GLC analysis of the alkaline hydrolysate of **3** as well as by the major fragment peak $[M-100]^+$ [18] in the mass spectrum of **3**. Thus, **3** was shown to have a 1-(3,3-dimethylacryloyloxy)-4-methyl-3-pentenyl group.

The presence of a furan ring was indicated by a positive response of **3** to Ehrlich's test as in the case of **2**. The ^{1}H NMR spectrum of **6** exhibited two one-proton singlets (δ 6.72 and 7.44) which were assigned to β- and α-protons of a furan ring, showing that **3** was a furan substituted at C-2 and C-4. The oxidation of **3** with alkaline hydrogen peroxide followed by methylation with ethereal diazomethane gave a methyl ester (**9**). The ^{1}H NMR spectrum of **9** showed two one-proton singlets assigned to the furanoid protons at δ 7.18 and 7.53 in addition to a carbomethoxyl and a 1-(3,3-dimethylacryloyloxy)-4-methyl-3-pentenyl group. These NMR data indicated, as in the case of **2** and **4** [16], that the oxidation product of **3** was **9**. Therefore, echinofuran C was shown to have the structure shown by formula **3**.

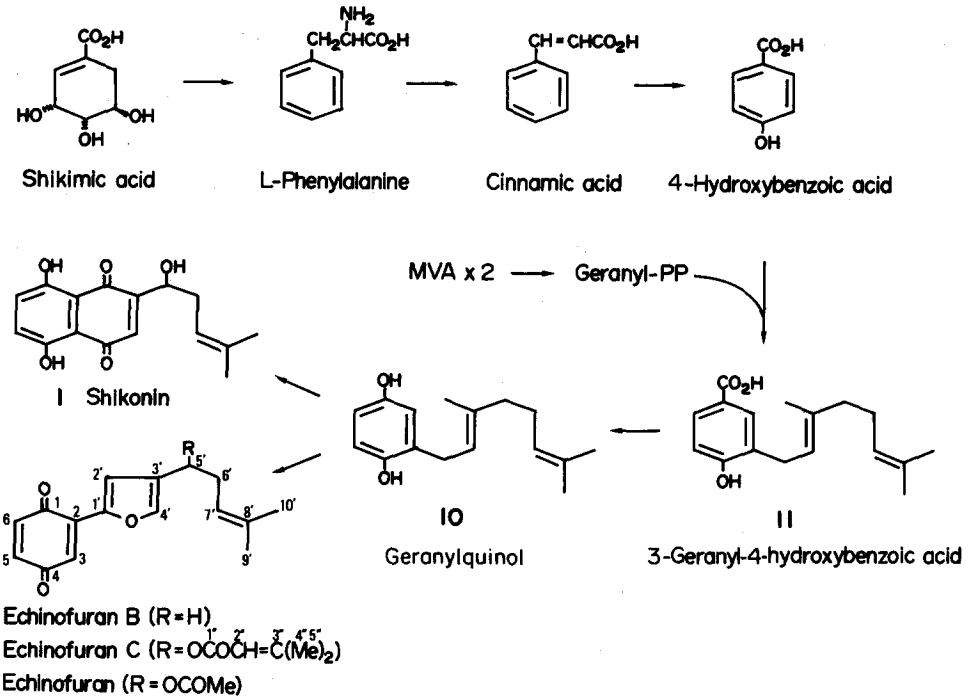
The chemical analyses described above demonstrated that the pigment accumulated by the *Lithospermum* cell cultures in the presence of activated carbon is a benzoquinone (**2**) having a furan ring in the side chain. It turned out that this unexpected metabolite is a congener of echinofuran (**4**) isolated from *Echium lycopsis* callus cultures as well as of echinofuran C (**3**) found in the callus cultures and roots of *L. erythrorhizon*. The structures of these compounds suggest that they are derived from geranylquinol (**10**), the key intermediate of shikonin biosynthesis [19], through the unusual formation of a furan ring in the side chain (Scheme 1). This type of abnormal metabolism may take place occasionally even in the intact plant under certain physiological conditions, resulting in the production of **3**. For the cultured cells in which the biosynthesis of shikonin is repressed possibly at one of the steps in the conversion of 3-geranyl-4-hydroxybenzoic acid (**11**) to **10** [19], the addition of activated carbon to the liquid medium would activate the reaction leading to **10** by an unknown mechanism, although **10** could not be metabolized further to **1** but to **2** via an otherwise hidden pathway. The fact that the cultures treated with activated carbon always produce **2** instead of **3** or **4** suggests that the reactions responsible for the synthesis of **2** are quite specific.

EXPERIMENTAL

Plant material and method of culture. L. erythrorhizon callus cultures were derived from germinating seed on Linsmaier–Skoog's agar medium [1] containing 10^{-6} M 2,4-D and 10^{-5} M kinetin, and subcultured on basal agar medium containing 10^{-6} M IAA and 10^{-5} M kinetin at 1 month intervals for 10 years at 25° in the dark. The cell suspension cultures were agitated in 100 ml conical flasks containing 30 ml medium in a reciprocal shaker at a speed of 100 strokes/min at 25° in the dark, and were subcultured for over 1 year at 3 week intervals in liquid medium containing the same growth regulators as those of the agar medium. Activated carbon ('Shirasagi', Takeda Chemical Industries, Ltd., Japan; 30, 150, 300 and 500 mg per 30 ml medium) was added to the liquid medium usually before autoclaving.

Isolation of echinofuran B (**2**). A mixture of activated C and the cultured cells (34 g dry wt) obtained by filtration of the cell suspension cultures supplemented with activated C (150 mg per 30 ml medium), which had been agitated for 3 weeks at 25° in the dark, was homogenized in a mortar with C_6H_6 (200 ml). The C_6H_6 extract was subjected to CC (silica gel, hexane–C_6H_6, 1:1) to isolate **2** (130 mg) as an orange oil. $UV\lambda_{max}^{EtOH}$ nm (log ε): 259 (4.20) and 453 (3.70); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2890, 2820, 1648, 1632, 1590, 1560, 1085, 895 and 845; ^{1}H NMR ($CDCl_3$, 60 MHz): δ 1.60 and 1.70 (3H each, *s*, 9′- and 10′-H), 2.26 (2H, *q*, J = 7.0 Hz, 6′-H), 2.48 (2H, *t*, J = 7.0 Hz, 5′-H), 5.14 (1H, *t* (*br*), J = 7.0 Hz, 7′-H), 6.73 (1H, *s*, 5- or 6-H), 6.75 (1H, *s*, 6- or 5-H), 7.02 (1H, *s* (*br*), 3-H), 7.35 (1H, *s*, 2′- or 4′-H) and 7.36 (1H, *s*, 4′- or 2′-H). (Found: $[M]^+$, 256.1087; $C_{16}H_{16}O_3$ requires: $[M]^+$, 256.1099.) On Ehrlich's test, **2** gave an orange colour which changed to blue upon heating in a boiling water bath.

Reductive acetylation of echinofuran B (**2**). A mixture of **2** (50 mg), Zn powder (50 mg), Ac_2O (1.5 ml) and pyridine (1.5 ml) was allowed to stand at room temp. for 2 hr. Ice-H_2O was poured onto the mixture and the whole soln was extracted with Et_2O (15 ml × 3). The Et_2O layer was washed with 1 M HCl, 5% $NaHCO_3$ and H_2O successively, dried (Na_2SO_4) and concd *in vacuo*. The crude product was purified by prep. TLC (C_6H_6–EtOAc, 9:1, R_f 0.40) to give **5** (30 mg) as a colourless oil. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2920, 1755, 1615, 1490, 1370, and 1170; ^{1}H NMR ($CDCl_3$, 60 MHz): δ 1.61 and 1.72 (3H each, *s* (*br*), 9′- and 10′-H), 2.27 (2H, *q*, J = 7.0 Hz, 6′-H), 2.33 and 2.38 (3H each, *s*, 1- and 4-OAc), 2.48 (2H, *t*, J = 7.0 Hz, 5′-H), 5.16 (1H, *t* (*br*), J = 7.0 Hz, 7′-H), 6.58 (1H, *s*, 2′-H), 6.99 (1H, *dd*, J = 2.5 and



Scheme 1.

9.0 Hz, 5-H), 7.12 (1H, *d*, J = 9.0 Hz, 6-H), 7.24 (1H, *s*, 4'-H) and 7.52 (1H, *d*, J = 2.5 Hz, 3-H). (Found: $[M]^+$, 342.1458; $C_{20}H_{22}O_5$ requires: $[M]^+$, 342.1467.)

Oxidation of **2** *with alkaline* H_2O_2. To a soln of **2** (25 mg) in MeOH (0.5 ml) was added a mixture of 30% H_2O_2 (1.5 ml) and 10% NaOH (1.5 ml) under ice-cooling. The mixture was stirred for 1 hr, neutralized with 0.1 M HCl and extracted with Et_2O (10 ml × 3). The Et_2O extract was washed with H_2O, dried (Na_2SO_4) and concd *in vacuo*. The residue was methylated with ethereal CH_2N_2 and subjected to prep. TLC (silica gel, C_6H_6–EtOAc, 4:1, R_f 0.75) to give **7** (7.5 mg) as a colourless oil. UV λ_{max}^{MeOH} nm (log ε): 220 (3.67) and 260 (3.96). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1720, 1604, 1508, 1436, 1316, 1104 and 916. ^1H NMR ($CDCl_3$, 60 MHz): δ 1.60 and 1.71 (3H each, *s* (*br*), 9- and 10-H), 2.39 (4H, *t*, J = 5.0 Hz, 5- and 6-H), 3.89 (3H, *s*, 1-COOMe), 5.17 (1H, *br*, 7-H), 7.07 (1H, *s*, 2-H) and 7.34 (1H, *s*, 4-H). (Found: $[M]^+$, 208.1095; $C_{12}H_{16}O_3$ requires: $[M]^+$, 208.1099.)

Isolation of echinofuran C (**3**). *Lithospermum* callus tissues (100 g fr. wt) grown on Linsmaier–Skoog basal agar medium containing 10^{-6} M IAA and 10^{-5} M kinetin were extracted in a mortar with $CHCl_3$. The $CHCl_3$ extract was washed with H_2O, dried (Na_2SO_4) and concd *in vacuo* to yield a dark red oil. The oil (250 mg) was dissolved in Et_2O (200 ml) and washed with 1 M KOH (250 ml × 3) to remove the red naphthoquinones into the alkaline layer. The Et_2O layer was washed with H_2O, dried (Na_2SO_4) and concd *in vacuo*. The resulting oil (50 mg) was subjected to CC (silica gel, $CHCl_3$–C_6H_6, 1:50) to give echinofuran C (**3**) (20 mg) as an orange oil. $[\alpha]_D^{25}$ − 60.0° (*c* 0.90; $CHCl_3$); UV λ_{max}^{EtOH} nm (log ε): 253 (4.31) and 438 (3.62); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2890, 2820, 1715, 1642 (*br*), 1590, 1562, 1280, 1085, 915, 900 and 845; ^1H NMR ($CDCl_3$, 60 MHz): δ 1.60 and 1.70 (3H each, *s*, 9'- and 10'-H), 1.90 and 2.17 (3H each, *s*, 5''- and 4''-H), 2.56 and 2.58 (1H each, *t*, J = 7.0 Hz, 6'-H), 5.08 (1H, *t*, J = 7.0 Hz, 7'-H), 5.69 (1H, *s* (*br*), 2''-H), 5.77(1H, *t*, J = 7.0 Hz, 5'-H), 6.73 (1H, *s*, 5- or 6H), 6.75 (1H, *s*, 6- or 5-H), 7.02 (1H, *s*(*br*), 3-H), 7.47 (1H, *s*, 2'- or 4'-H) and 7.54 (1H, *s*, 4'- or 2'-H). (Found: $[M]^+$, 354.1474; $C_{21}H_{22}O_5$ requires: $[M]^+$, 354.1467.) On Ehrlich's test, **3** gave an orange colour which changed to green upon heating in a boiling water bath.

Echinofuran C (**3**, 460 mg) was also isolated from the dried roots (100 g) of *L. erythrorhizon* and identified (TLC, IR, UV and ^1H NMR) by the method described earlier.

Identification of the acyl moiety of **3** *by GLC*. To 1 mg **3** in MeOH (0.1 ml) was added 2 M NaOH (1 ml). The soln was shaken overnight at 25°, adjusted to pH 3 with 2 M HCl and extracted with Et_2O (5 ml × 3). The Et_2O layer was washed with H_2O, dried (Na_2SO_4) and concd *in vacuo* to give a red oil. GLC analysis of the red oil was performed with a 10% DEGS–1% H_3PO_4 stainless column (2 m × 3 mm). The conditions were as follows: column temp., 100–180° (4°/min); injector temp., 200°; detector temp., 230°; carrier gas, N_2 30 ml/min. The test substance gave a single peak with a R_t (5.9 min) identical with that of an authentic sample of β,β-dimethylacrylic acid.

Reductive acetylation of echinofuran C (**3**). A mixture of **3** (50 mg), Zn powder (50 mg), Ac_2O (1.5 ml) and pyridine (1.5 ml) was treated by the same method as used for the reductive acetylation of **2** and the crude product was purified by a similar procedure as applied to **5** to yield **6** (30 mg) as a colourless oil. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2920, 1755, 1700, 1643, 1615, 1490, 1440, 1365 and 1165; ^1H NMR ($CDCl_3$, 60 MHz): δ 1.63 and 1.70 (3H each, *s* (*br*), 9'- and 10'-H), 1.90 and 2.18 (3H each, *s* (*br*), 4''- and 5''-H), 2.33 and 2.36 (3H each, *s*, 1- and 4-OAc), 2.57 (2H, *br*, 6'-H), 5.10 (1H, *t* (*br*), J = 7.0 Hz, 7'-H), 5.69 (1H, *s* (*br*), 2''-H), 5.77 (1H, *t*, J = 7.0 Hz, 5'-H), 6.72 (1H, *s*, 2'-H), 7.01 (1H, *dd*, J = 2.5 and 9.0 Hz, 5-H), 7.13 (1H, *d*, J = 9.0 Hz, 6-H), 7.44 (1H, *s*, 4'-H) and 7.52 (1H, *d*, J = 2.5 Hz, 3-H). (Found: $[M]^+$, 440.1825; $C_{25}H_{28}O_7$ requires: $[M]^+$, 440.1836.)

Oxidation of **3** *with alkaline* H_2O_2. To a soln of **3** (29 mg) in

MeOH (1 ml) was added a mixture of 30% H_2O_2 (1.5 ml) and 10% NaOH (1.5 ml) under ice-cooling. The mixture was treated as described above to give **9** (9.1 mg) as a colourless oil. UV λ_{max}^{MeOH} nm (log ε): 252 (4.01) and 218 (4.16). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1720, 1650, 1603, 1508, 1438, 1313, 1146 and 916; ^1H NMR ($CDCl_3$, 60 MHz): δ 1.62, 1.69, 1.91 and 2.17 (3H each, *s* (*br*), 9-, 10-, 4'- and 5'-H), 2.55 (2H, *t*, J = 7.0 Hz, 6-H), 3.91 (3H, *s*, 1-COOMe), 5.08 (1H, *br*, 7-H), 5.68 (1H, *s* (*br*), 2'-H), 5.75 (1H, *t*, J = 7.0 Hz, 5-H), 7.18 (1H, *s*, 2-H) and 7.53 (1H, *s*, 4-H). (Found: $[M]^+$, 306.1463; $C_{17}H_{22}O_5$ requires: $[M]^+$, 306.1467.)

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