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Anthrasesamones from roots of *Sesamum indicum*

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Abstract

Three anthraquinones, named anthrasesamones A, B and C, were isolated from the roots of *Sesamum indicum*, and their respective structures were determined to be 1-hydroxy-2-(4-methylpent-3-enyl)anthraquinone, 1,4-dihydroxy-2-(4-methylpent-3-enyl)-anthraquinone and 2-chloro-1,4-dihydroxy-3-(4-methylpent-3-enyl)anthraquinone on the basis of spectroscopic evidence. Two known anthraquinones were also isolated for the first time from *S. indicum* roots and characterized as 2-(4-methylpent-3-enyl)anthraquinone and (*E*)-2-(4-methylpenta-1,3-dienyl)anthraquinone. Anthrasesamone C is a rare chlorinated anthraquinone in higher plants.

Keywords: Sesamum indicum; Pedaliaceae; Sesame root; Anthraquinones; Chlorinated quinone; Anthrasesamones

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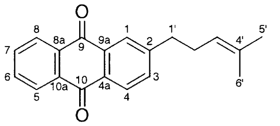
1. Introduction

The number of natural organochlorine compounds in marine and terrestrial plants, bacteria, fungi, lichens, insects, marine animals, some higher animals and a few mammals has grown to more than 1500 and continues to increase up to the present (Gribble, 1996). However, natural chlorine-containing compounds in higher plants are relatively rare, and only limited knowledge of their biosynthetic pathway in higher plants, in particular the metabolism and precursor for chlorination, is available (Engvild, 1986; Gribble, 1992).



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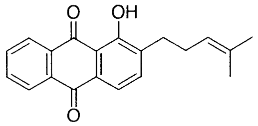

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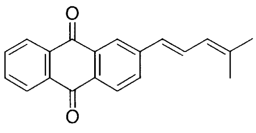

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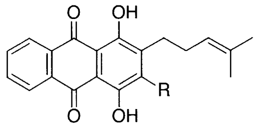

4 R = H
5 R = Cl

Sesamum indicum L. (Pedaliaceae) is one of the most important crops throughout the world. Recently, we found that the roots of *S. indicum* produce a chlorinated antifungal naphthoquinone chlorosesamone (Hasan et al., 2000) and its structurally related metabolites, hydroxysesamone and 2,3-epoxysesamone (Hasan et al., 2001). 2,3-Epoxysesamone was also isolated from the root bark of *S. angolense* (Potterat et al., 1987) and from the hairy roots of *S. indicum* (Ogasawara et al., 1993). Moreover, hairy root cultures of *S. indicum* produce two unusual anthraquinones having C_6 side chains, 2-(4-methylpent-3-enyl)anthraquinone (**1**) and 2-(4-methylpenta-1,3-dienyl)anthraquinone (**2**), but the presence of anthraquinones in the roots of *S. indicum* has not yet been shown (Ogasawara et al., 1993). We therefore undertook this experiment to clarify the production of such anthraquinones by *S. indicum* roots, and found five compounds including a new chlorine-containing anthraquinone (**5**). We here report the identification of two known anthraquinones (**1** and **2**) and the isolation and structures of three new anthraquinones, named anthrasesamones A (**3**), B (**4**) and C (**5**).

2. Results and discussion

Five compounds (**1–5**) were isolated as described in the Experimental section from the hexane-soluble fraction of

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fresh roots of *S. indicum* collected in Kagawa, Japan. The HR-EIMS and ^{13}C NMR spectral data (Table 2) for compound **1** showed its molecular formula to be $C_{20}H_{18}O_2$. A 2-substituted anthraquinone ring was deduced from 14 ^{13}C resonances (C-1–C-10a) (Berger and Castonguay, 1978) and five aromatic proton signals at δ_H 7.58, 7.77, 8.10, 8.20 and 8.29 (Table 1), as well as the IR absorption band at 1676 cm^{-1} and UV absorption bands at 258, 277 and 330 nm (Thomson, 1971). The ^{1}H NMR spectrum of **1** exhibited two methyl broad singlets at δ_H 1.52 and 1.66, two methylene signals at δ_H 2.36 and 2.79 and a vinyl methine signal at δ_H 5.13, suggesting the presence of a 4-methylpent-3-enyl group in **1**. The structure of **1** was unambiguously substantiated by synthesis of **1** (Eckert and Amos, 1982), and synthetic **1** was identical with the isolated **1**. Consequently, compound **1** was identified as 2-(4-methylpent-3-enyl)anthraquinone. Full assignments of ^{1}H and ^{13}C NMR signals of **1**, some of which were ambiguous previously, were confirmed by analysis of DEPT, DIFNOE, ^{1}H–^{1}H COSY, ^{13}C–^{1}H COSY, HMQC and LR-HETCOR (HMBC and COLOC) data as shown in Tables 1 and 2. Compound **2** had the molecular formula $C_{20}H_{16}O_2$ (HR-EIMS and ^{13}C NMR data). The 14 ^{13}C resonances caused by C-1 to C-10a resembled those of **1**, suggesting that **2** has the same structure as **1**, except for the side chain. The ^{1}H NMR and ^{1}H–^{1}H COSY data indicated that **2** contains a conjugated diene moiety in its side chain. Thus, compound **2** was identified as (*E*)-2-(4-methylpenta-1,3-dienyl)anthraquinone. This is the first report of the occurrence of anthraquinones **1** and **2** in the roots of *S. indicum*.

Table 2
^{13}C NMR spectral data for anthraquinones **1**, **3**, **4** and **5** in $CDCl_3$

C	**1**	**3**	**4**	**5**
1	127.0	160.9	157.3	156.8
2	149.6	138.8	144.8	142.2
3	134.5	136.6	128.3	133.07
4	127.4	119.2	157.8	154.2
4a	131.5	131.3	111.1	111.0
5	127.1[a]	127.2	126.9[b]	127.11[a]
6	134.0[a]	134.5	134.3[b]	134.7[a]
7	133.9[a]	133.9	134.2[b]	134.6[a]
8	127.2[a]	126.8	127.0[b]	127.09[a]
8a	133.61[a]	133.3	133.6[b]	133.12[a]
9	183.5	189.0	187.2[b]	186.7[a]
9a	133.4	115.3	112.0	110.6
10	183.1	182.4	186.3[b]	186.6[a]
10a	133.63[a]	133.8	133.7[b]	133.5[a]
1′	36.3	30.3	30.5	28.1
2′	29.4	27.4	27.1	26.4
3′	122.6	123.2	122.9	122.8
4′	133.1	132.8	133.2	133.2
5′	25.7	25.6	25.7	25.7
6′	17.7	17.6	17.7	17.6

[a] Assignments may be interchanged.
[b] Assignment of a C_4 linkage may be interchanged by another one.

Anthrasesamone A (**3**) had the molecular formula $C_{20}H_{18}O_3$ (HR-EIMS and ^{13}C NMR data). The IR spectrum of **3** exhibited two characteristic carbonyl absorptions of chelated and non-chelated anthraquinones at 1631 and 1672 cm^{-1}, respectively (Thomson, 1971). Moreover, the ^{1}H NMR spectrum of **3** revealed a chelated hydroxy proton signal at δ_H 12.95 and two aromatic methine doublets at δ_H 7.47 and 7.72, suggesting that **3** is a 1-hydroxy analogue of **1**. The NMR spectroscopic assignments of the two methyl groups (C-5′ and C-6′) were established by the DIFNOE data. The attachment of the side chain and hydroxyl group to the anthraquinone ring was demonstrated by the LR-HETCOR experiments (H-1′/C-1, C-2, C-3; 1-OH/C-1, C-2, C-9a). Therefore, the structure for anthrasesamone A (**3**) was established as 1-hydroxy-2-(4-methylpent-3-enyl)anthraquinone.

Anthrasesamone B (**4**) had the molecular formula $C_{20}H_{18}O_4$ (HR-EIMS and ^{13}C NMR data). The ^{1}H

Table 1
^{1}H NMR spectral data for anthraquinones **1**, **3**, **4** and **5** in $CDCl_3$

H	**1**	**3**	**4**	**5**
1	8.10 *br.d* (1.8)			
3	7.58 *br.dd* (7.9, 1.8)	7.47 *br.d* (7.7)	7.13 *br.s*	
4	8.20 *d* (7.9)	7.72 *d* (7.7)		
5	8.29 *m*	8.25 *m*	8.32[a] *m*	8.34 *m*
6	7.77 *m*	7.76 *m*	7.80 *m*	7.83 *m*
7	7.77 *m*	7.76 *m*	7.80 *m*	7.83 *m*
8	8.29 *m*	8.27 *m*	8.34[a] *m*	8.34 *m*
1′	2.79 *t*-like (7.6)[b]	2.75 *t*-like (7.6)[b]	2.75 *t*-like (7.7)[b]	2.97 *t*-like (8.0)[b]
2′	2.36 *br.dt* (7.1, 7.6)	2.33 *br.dt* (7.1, 7.6)	2.35 *br.dt* (7.1, 7.7)	2.30 *br.dt* (7.3, 8.0)
3′	5.13 *tqq* (7.1, 1.4, 1.4)	5.17 *tqq* (7.1, 1.4, 1.4)	5.17 *tqq* (7.1, 1.4, 1.4)	5.25 *tqq* (7.3, 1.4, 1.4)
5′	1.66 *br.s*	1.66 *br.s*	1.68 *br.s*	1.68 *br.s*
6′	1.52 *br.s*	1.54 *br.s*	1.58 *br.s*	1.59 *br.s*
1-OH		12.95 *br.s*	13.40 *br.s*	13.48 *s*
4-OH			12.97 *s*	13.66 *s*

[a] Assignments may be interchanged.
[b] Mean values of chemical shifts and *J* values.

NMR and IR spectra of **4**, respectively, showed an aromatic methine singlet at δ_H 7.13 and two chelated hydroxy proton signals at δ_H 12.97 and 13.40 and only a chelated carbonyl absorption at 1624 cm^{-1}, suggesting that **4** is a 1,4-dihydroxy analogue of **1**. The structure for anthrasesamone B (**4**) was definitely determined to be 1,4-dihydroxy-2-(4-methylpent-3-enyl)anthraquinone.

Anthrasesamone C (**5**) had the molecular formula $C_{20}H_{17}O_4Cl$ (HR-EIMS and ^{13}C NMR data). The EIMS spectrum of **5** showed an isotopic peak (M+2) at *m*/*z* 358 about one third the intensity of the molecular ion peak (*m*/*z* 356). The ^1H and ^{13}C NMR spectral data for **5** were very similar to those for **4**, except for the absence of the H-3 aromatic methine signal at δ_H 7.13 observed in **4**, suggesting that **5** is a monochlorinated derivative of **4**. The structure for anthrasesamone C (**5**) was established as 2-chloro-1,4-dihydroxy-3-(4-methylpent-3-enyl)anthraquinone. Anthrasesamone C (**5**) is a novel chlorine-containing anthraquinone, which is rare in higher plants (Engvild, 1986; Gribble, 1992; Thomson, 1971, 1997).

Five anthraquinones **1–5** have the same carbon skeleton, an anthraquinone ring plus a C_6 side chain. Recently, we isolated and characterized a chlorine-containing naphthoquinone chlorosesamone (Hasan et al., 2000) and its related naphthoquinones (Hasan et al., 2001), which carry a naphthoquinone ring and a dimethylallyl (C_5) side chain, produced by *S. indicum* roots. Some anthraquinones produced by plants are biosynthesized from prenylnaphthoquinones (Herbert, 1989; Inoue et al., 1984; Han et al., 2002). Furthermore, shikonin having a naphthoquinone ring and a C_6 side chain is formed from 4-hydroxybenzoic acid and geranyl diphosphate (Herbert, 1989; Li et al., 1998). These findings therefore suggest that 2-(4-methylpent-3-enyl)anthraquinones (**1**) is biosynthesized through the formation of the geranylnaphthoquinone intermediate and the subsequent cyclization of its naphthoquinone intermediate to generate the third aromatic ring. The anthraquinone **1** would be the common intermediate of the other anthraquinones **2–5**.

3. Experimental

3.1. General experimental procedures

NMR spectra were recorded with a Jeol JNM-A400 FT NMR spectrometer at 400 MHz for ^1H and 100.4 MHz for ^{13}C. All NMR chemical shifts were referenced to $CDCl_3$ (δ_H 7.24, δ_C 77.0). Mass spectra were obtained with a Jeol JMS-SX102AQQ hybrid mass spectrometer. UV–vis spectra were measured with a Shimadzu UV-1600 UV–visible spectrophotometer, and IR spectra with a Jasco FT/IR-7000 spectrometer. Silica gel 60 (70–230 mesh, Nacalai Tesque) and Cosmosil 140C_{18}-PREP (Nacalai Tesque) were used for CC.

3.2. Plant material

Roots of *Sesamum indicum* L. were collected in September 2000 from fields in Kagawa, Japan after harvesting mature seeds. The harvested seeds are maintained at the Laboratory of Biofunctional Phytochemistry, Department of Biochemistry and Food Science, Faculty of Agriculture, Kagawa University, Japan.

3.3. Extraction and isolation of anthraquinones

Fresh roots of *S. indicum* (11.5 kg) were treated twice with MeOH at room temp. The combined MeOH extracts were evaporated under reduced pressure, and the aq. soln. obtained (3 l) was partitioned with hexane (1.5 l×2) to give hexane-soluble fraction (17.6 g). Half of this fraction (8.8 g) was subjected to CC on silica gel using stepwise elution with Me_2CO–hexane. A portion (1.0 g) of the fraction eluted with 5% Me_2CO in hexane (5.1 g) was purified by reversed phase CC (Cosmosil 140C_{18}-PRER; EtOH) to afford 80 fractions. Fractions 32–52 (424 mg) were further purified by prep. HPLC (Cosmosil 5C_{18}-MS, 250×10 mm; 254 nm) with MeOH–HOAc (100:0.1; 1.5 ml min^{-1}) as solvent to give **1** (3.2 mg), **2** (0.5 mg), **3** (25.4 mg), **4** (4.8 mg) and **5** (4.7 mg).

3.4. Anthrasesamone A (3)

Yellow solid. UV λ_{max} (EtOH) nm (log ε): 228 (4.26), 248 (4.50), 255 (4.51), 281 *sh* (4.10), 328 (3.51), 394 *sh* (3.79), 414 (3.87), 435 *sh* (3.76). IR ν_{max} (KBr) cm^{-1}: 2973, 2911, 2852, 1672, 1631, 1591, 1474, 1438, 1362, 1326, 1294, 1264, 1233. For ^1H and ^{13}C NMR spectral data see Tables 1 and 2. EIMS (probe) 70 eV, *m*/*z* (rel. int.): 306 $[M]^+$ (40), 238 (100). HR-EIMS *m*/*z*: 306.1255; $C_{20}H_{18}O_3$ requires 306.1256.

3.5. Anthrasesamone B (4)

Red solid. UV λ_{max} (EtOH) nm (log ε): 229 *sh* (4.29), 251 (4.60), 256 *sh* (4.56), 286 (3.99), 328 *sh* (3.48), 459 *sh* (3.94), 484 (4.01), 502 *sh* (3.86), 517 (3.83). IR ν_{max} (KBr) cm^{-1}: 2969, 2931, 2866, 1624, 1590, 1431, 1415, 1367, 1346, 1307, 1270, 1251, 1237. For ^1H and ^{13}C NMR spectral data see Tables 1 and 2. EIMS (probe) 70 eV, *m*/*z* (rel. int.): 322 $[M]^+$ (76), 254 (100). HR-EIMS *m*/*z*: 322.1206; $C_{20}H_{18}O_4$ requires 322.1205.

3.6. Anthrasesamone C (5)

Red solid. UV λ_{max} (EtOH) nm (log ε): 230 *sh* (4.22), 253 (4.58), 258 (4.63), 292 (3.95), 332 *sh* (3.51), 471 sh (3.96), 484 (3.98), 504 *sh* (3.84), 518 (3.79). IR ν_{max} (KBr) cm^{-1}: 2973, 2922, 2855, 1624, 1585, 1450, 1415, 1399, 1345, 1305, 1271, 1242. For ^{1}H and ^{13}C NMR spectral data see Tables 1 and 2. EIMS (probe) 70 eV, m/z (rel. int.): 358 $[M+2]^+$ (10), 356 $[M]^+$ (31), 321 (47), 290 (34), 288 (100). HR-EIMS m/z: 356.0811; $C_{20}H_{17}O_4Cl$ requires 356.0815.

3.7. Synthesis of **1**

A soln. of 1,4-naphthoquinone (0.50 g) and β-myrcene (7-methyl-3-methyleneocta-1,6-diene, 0.86 g) in dry toluene (10 ml) was stirred for 6 days at 50 °C, then was cooled to room temp. A soln. of 1 M KOH in MeOH (5 ml) was added to the reaction mixture, and the whole was stirred vigorously for 90 min at room temp. without sealing. The reaction mixture was diluted with H_2O and treated with EtOAc (25 ml×3). The EtOAc soln. obtained was washed with satd. NaCl and evaporated to dryness. The residue was purified by silica gel CC with EtOAc–hexane (5:95) as solvent to afford **1** (0.86 g) in 94% yield.

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References

Berger, Y., Castonguay, A., 1978. The carbon-13 nuclear magnetic resonance spectra of anthraquinone, eight polyhydroxyanthraquinones and eight polymethoxyanthraquinones. Organic Magnetic Resonance 11, 375–377.

Eckert, R.C., Amos, L.W., 1982. Influence of hydrophilicity on the delignification efficiency of anthraquinone derivatives. J. Wood Chem. Technol. 2, 57–71.

Engvild, K.C., 1986. Chlorine-containing natural compounds in higher plants. Phytochemistry 25, 781–791.

Gribble, G.W., 1992. Naturally occurring organohalogen compounds—a survey. J. Nat. Prod. 55, 1353–1395.

Gribble, G.W., 1996. The diversity of natural organochlorines in living organisms. Pure Appl. Chem. 68, 1699–1712.

Han, Y.S., van der Heijden, R., Lefeber, A.W.M., Erkelens, C., Verpoorte, R., 2002. Biosynthesis of anthraquinones in cell cultures of *Cinchona* 'Robusta' proceeds via the methylerythritol 4-phosphate pathway. Phytochemistry 59, 45–55.

Hasan, A.F.M.F., Begum, S., Furumoto, T., Fukui, H., 2000. A new chlorinated red naphthoquinone from roots of *Sesamum indicum*. Biosci. Biotechnol. Biochem. 64, 873–874.

Hasan, A.F.M.F., Furumoto, T., Begum, S., Fukui, H., 2001. Hydroxysesamone and 2,3-epoxysesamone from roots of *Sesamum indicum*. Phytochemistry 58, 1225–1228.

Herbert, R.B., 1989. The Biosynthesis of Secondary Metabolites, second ed. Chapman and Hall, New York.

Inoue, K., Ueda, S., Nayeshiro, H., Moritome, N., Inouye, H., 1984. Biosynthesis of naphthoquinones and anthraquinones in *Streptocarpus dunnii* cell cultures. Phytochemistry 23, 313–318.

Li, S.M., Hennig, S., Heide, L., 1998. Shikonin: a geranyl diphosphate-derived plant hemiterpenoid formed via the mevalonate pathway. Tetrahedron Lett. 39, 2721–2724.

Ogasawara, T., Chiba, K., Tada, M., 1993. Production in high-yield of a naphthoquinone by a hairy root culture of *Sesamum indicum*. Phytochemistry 33, 1095–1098.

Potterat, O., Stoeckli-Evans, H., Msonthi, J.D., Hostettmann, K., 1987. Two new antifungal naphthoxirene derivatives and their glucosides from *Sesamum angolense* WELW. Helv. Chim. Acta 70, 1551–1557.

Thomson, R.H., 1971. Naturally Occurring Quinones, second ed. Academic Press, London.

Thomson, R.H., 1997. Naturally Occurring Quinones IV, Recent Advances. Blackie Academic & Professional, London.