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Three novel diepoxy tetrahydrochromones from agarwood artificially produced by intentional wounding

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Abstract—Three novel diepoxy tetrahydrochromones, oxidoagarochromones A (**1**), B (**2**), and C (**3**), were isolated from agarwood artificially produced by intentional wounding of *Aquilaria crassna*. Inductive production of these compounds was also confirmed at the early stage of wounding in *A. sinensis* and *A. crassna*. These diepoxy tetrahydrochromones would play an important role in understanding the biosynthesis of chromone derivatives in agarwood.

Agarwood, which is a resin-deposited part of the trunk of *Aquilaria* species (Thymelaeaceae), has been used as an incense as well as traditional sedative, analgesic, and digestive medicine in East Asia. Phytochemical analyses of commercial agarwood and its oil have revealed sesquiterpenes[1–4] and chromone derivatives[5–8] as their constituents. With increased consumption of agarwood in recent years, over-exploitation of agarwood in Southeast Asian forest caused depletion of the natural resources. Although agarwood is supposed to be formed by decay, wounding, etc. of the wood of *Aquilaria* species, mystery surrounds the process of resin formation, and efforts in planting agarwood-producing trees seem to have not yet resulted in successful production of agarwood. To clarify the mechanism of the resin formation in agarwood, studies on the biosynthetic pathways of the resin constituents are required. In a previous letter, we examined withered wood of *A. sinensis* Gilg, which is supposed to be at an early stage of agarwood formation, and reported the isolation of eleven chromone derivatives.[9] In this report, we describe the isolation and structure elucidation of three novel diepoxy tetrahydrochromones from intentionally injured *Aquilaria* wood, and inductive production of these compounds by wounding of *Aquilaria* trees.

Wood pieces of intentionally injured *A. crassna* Pierre ex Lecomte obtained in Vietnam in December 2001 (70 g) were extracted with AcOEt at room temperature for a week. The AcOEt extract (698 mg) was chromatographed on ODS with 50% aq to 100% MeOH to afford 12 fractions (fr. 1–7, 50%; fr. 8–11, 67%; fr. 12, 100%). Fraction 7 was successively separated by silica gel column chromatography with $CHCl_3$–MeOH = 19:1, and with hexane–AcOEt = 1:2, then by gel permeation chromatography (GPC)[10] with $CHCl_3$ to afford oxidoagarochromone A (**1**, 4 mg) and B (**2**, 4 mg). Fraction 4 was subjected to silica gel column chromatography ($CHCl_3$–MeOH = 19:1), and GPC ($CHCl_3$) to give oxidoagarochromone C (**3**, 8 mg).

Oxidoagarochromone A (**1**) was obtained as a brown gum and the molecular formula was determined to be $C_{17}H_{14}O_4$ by HRFABMS [*m*/*z* 283.0976 $[M+H]^+$ (calcd for $C_{17}H_{15}O_4$: 283.0970)].[11] The ^{13}C NMR spectrum (Table 1), showing the signals of two methylene groups (δ_C 32.9 and 35.3), a phenyl group (δ_C 126.8, 128.2, 128.6, and 139.2), and a carbonyl group (δ_C 177.6), was similar to that of flindersiachromone (**4**),[6] except that, instead of the aromatic carbons of the chromone system, it had four methine carbons (δ_C 46.5, 46.9,

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Table 1. NMR spectra of compounds **1**–**4**

	4[6]	**1**		**2**		**3**		
	^{13}C[a]	^{13}C[a]	^{1}H[a]	^{13}C[a]	^{1}H[a]	^{13}C[a]	^{1}H[a]	^{1}H[b]
2	168.3	168.1		168.2		168.2		
3	110.2	114.2	6.18 (1H, s)	114.2	6.16 (1H, s)	114.2	6.15 (1H, s)	6.15 (1H, s)
4	178.0	177.5		177.5		177.6		
4a	123.8	120.8		120.8		120.8		
5	124.9	46.9	4.35 (1H, d, J = 3.3 Hz)	46.9	4.35 (1H, d, J = 3.4 Hz)	46.9	4.34 (1H, d, J = 3.3 Hz)	4.11 (1H, d, J = 3.7 Hz)
6	125.7	46.5	3.84 (1H, t, J = 3.3 Hz)	46.4	3.84 (1H, t, J = 3.4 Hz)	46.4	3.84 (1H, t. J = 3.3 Hz)	3.87 (1H, dd, J = 3.7, 2.8 Hz)
7	133.4	48.7	3.98 (1H, t, J = 3.3 Hz)	48.6	3.98 (1H, t, J = 3.4 Hz)	48.6	3.98 (1H, t, J = 3.3 Hz)	4.06 (1H, dd, J = 3.7, 2.8 Hz)
8	117.8	47.7	3.82 (1H, d, J = 3.3 Hz)	47.7	3.83 (1H, d, J = 3.4 Hz)	47.7	3.84 (1H, d, J = 3.3 Hz)	3.92 (1H, d, J = 3.7 Hz)
8a	156.5	161.1		161.1		161.1		
1′	139.7	139.2		131.2		132.4		
2′	128.6	128.2	7.19 (1H, d, J = 8.3 Hz)	129.2	7.09 (1H, d, J = 8.8 Hz)	114.3	6.76 (1H, d, J = 2.2 Hz)	6.76 (1H, d, J = 2.1 Hz)
3′	128.3	128.8	7.31 (1H, m)	114.1	6.84 (1H, d, J = 8.8 Hz)	145.8		
4′	126.5	126.8	7.24 (1H, t, J = 7.3 Hz)	158.4		145.4		
5′	128.3	128.8	7.31 (1H, m)	114.1	6.84 (1H, d, J = 8.8 Hz)	110.8	6.77 (1H, d, J = 8.3 Hz)	6.83 (1H, d, J = 8.2 Hz)
6′	128.6	128.2	7.19 (1H, d, J = 8.3 Hz)	129.2	7.09 (1H, d, J = 8.8 Hz)	119.7	6.63 (1H, dd, J = 8.3, 2.2 Hz)	6.66 (1H, dd, J = 8.2, 2.1 Hz)
7′	32.9	32.9	2.99 (2H, m)	32.0	2.94 (2H, m)	32.2	2.90 (2H, m)	
8′	36.0	35.3	2.87 (2H, t, J = 7.6 Hz)	35.6	2.83 (2H, m)	35.4	2.83 (2H, m)	2.91 (4H, m)
4′-OMe				55.3	3.79 (3H, s)	56.0	3.87 (3H, s)	3.80 (3H, s)
3′-OH							5.65 (1H, s)	

[a] In $CDCl_3$.
[b] In acetone-d_6.

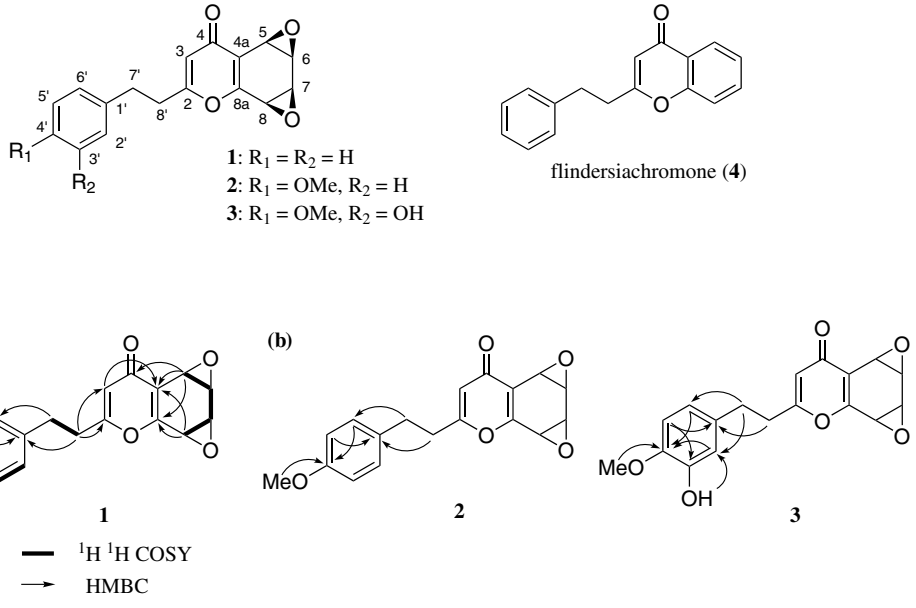

Figure 1. Selected 2D NMR correlations: (a) HMBC and ^{1}H ^{1}H COSY in compound **1**; (b) HMBC in the phenylethyl part of compounds **2** and **3**.

47.7, and 48.7). From the ^{1}H NMR, ^{1}H ^{1}H COSY, and HMQC spectra (Table 1 and Fig. 1a), these four carbons were concluded to form a series of consecutive methines [δ_C 46.9, δ_H 4.35 (1H, d, J = 3.3 Hz); δ_C 46.5, δ_H 3.84 (1H, t, J = 3.3 Hz); δ_C 48.7, δ_H 3.98 (1H, t, J = 3.3 Hz), and δ_C 47.7, δ_H 3.82 (1H, d, J = 3.3 Hz)]. In the HMBC spectrum (Fig. 1a), the methine proton at δ_H 4.35, which was located at one end of the consecutive

methines, showed correlation peaks with the carbonyl carbon (δ_C 177.6) and two olefinic carbons [δ_C 120.8 (C-4a) and 161.1 (C-8a)], whereas the methine proton at δ_H 3.82, which was located at the other end of the methines, correlated with the latter two olefinic carbons (δ_C 120.8 and 161.1). These correlations indicate that these methines form a part of a tetrasubstituted tetrahydrochromone system. From its molecular formula ($C_{17}H_{14}O_4$), the substituents on these four methines should be two oxygens, that is, **1** has a 5,6:7,8-diepoxy-tetrahydrochromone structure. This was supported by the carbon chemical shifts of the methines appearing at higher field than normal oxymethines.[12] Thus, the structure was determined to be 5,6:7,8-diepoxy-2-(2-phenylethyl)-5,6,7,8-tetrahydrochromone.

Oxidoagarochromone B (**2**) was obtained as pale yellow prisms (mp 142–144 °C) with the molecular formula of $C_{18}H_{16}O_5$ [*m/z* 313.1068 $[M+H]^+$ (calcd for $C_{18}H_{17}O_5$: 313.1076)].[13] The ^1H and ^{13}C NMR spectra (Table 1) were very similar to those of compound **1**, except for the phenyl group part and the presence of an additional methoxy group (δ_H 3.79). Its ^1H NMR spectrum (Table 1) showed the presence of an A_2B_2 coupling system [δ_H 6.84 and 7.09 (each, 2H, d, J = 8.8 Hz)] as the aromatic moiety and the HMBC correlations located the methoxy group at the *para*-position of the phenyl ring. Consequently, the structure of **2** was elucidated to be 5,6:7,8-diepoxy-2-[2-(4-methoxyphenyl)ethyl]-5,6,7,8-tetrahydrochromone.

Oxidoagarochromone C (**3**) was obtained as a pale yellow crystalline solid (mp 79–82 °C) with the molecular formula of $C_{18}H_{16}O_6$ [*m/z* 329.1031 $[M+H]^+$ (calcd for $C_{18}H_{17}O_6$: 329.1025)].[14] The ^{13}C NMR spectrum (Table 1) was very similar to that of compound **2**, except for the phenyl part. The ^1H NMR spectrum (Table 1) showed the presence of a hydroxy signal at δ_H 5.65, a methoxy group at δ_H 3.87, and a set of 1,3,4-substituted phenyl proton system at δ_H 6.63 (1H, dd, J = 8.3, 2.2 Hz), δ_H 6.76 (1H, d, J = 2.2 Hz), and δ_H 6.77 (1H, d, J = 8.3 Hz). The HMBC correlations shown in Figure 1b indicated the positions of the hydroxy and methoxy groups at C′-3 and C′-4, respectively. Thus, the structure of **3** was determined to be 5,6:7,8-diepoxy-2-[2-(3-hydroxy-4-methoxyphenyl)ethyl]-5,6,7,8-tetrahydrochromone.

Compounds **1**–**3** were considered to have the same relative stereochemistry, because of the similar coupling constants of H-5, 6, 7, and 8 in their ^1H NMR. The coupling constants of the methine protons of **3** in acetone-d_6 ($J_{5,6} = J_{7,8}$ = 3.7 Hz, $J_{6,7}$ = 2.8 Hz) were in good agreement with those of *syn*-naphthalene-1,2:3,4-dioxide ($J_{1,2} = J_{3,4}$ = 3.6 Hz, $J_{6,7}$ = 3.0 Hz) rather than those of *anti*-naphthalene-1,2:3,4-dioxide ($J_{1,2} = J_{3,4}$ = 4.2 Hz, $J_{6,7}$ = 1.8 Hz).[15] Therefore, these diepoxides were concluded to have *syn* configuration. These compounds showed small optical rotation. Their optical purity and absolute stereochemistry will be examined in the future.

The inductive nature of the formation of the diepoxy compounds was examined by a wounding experiment using trees of *A. sinensis* and *A. crassna* grown in the greenhouse of the Experimental Station for Medicinal Plants, Graduate School of Pharmaceutical Sciences, Kyoto University. The trunks and branches were impaled by stainless steel pins and the resulted resinous parts were collected and extracted with AcOEt.[16] HPLC analyses[17] of the AcOEt extracts showed that compounds **1**–**3** are present only in the resinous part, not in the intact part, of the wounded trees (Fig. 2). No other known chromone derivative was detectable in the extracts. The identity of the peaks was confirmed by isolation of the compounds. The AcOEt extract obtained from the resinous part of wounded *A. sinensis* was subjected to silica gel column chromatography (hexane–acetone = 3:2) and GPC ($CHCl_3$) to obtain oxidoagarochromones A (**1**, 23 mg), B (**2**, 68 mg), and C (**3**, 95 mg) as the major constituents.

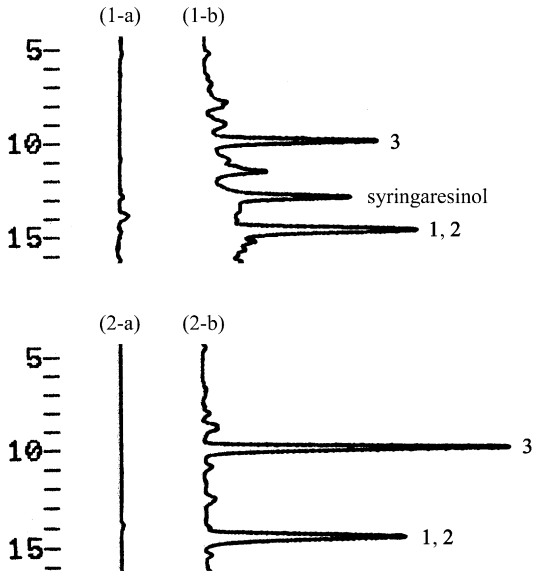

Figure 2. HPLC analyses: (1-a) intact and (1-b) resinous part (50 days after wounding) of *A. sinensis*; (2-a) intact and (2-b) resinous part (135 days after wounding) of *A. crassna*.

The diepoxy compounds isolated from intentionally wounded wood of *Aquilaria* species were not detected in the intact wood and have not been reported from natural agarwood. This suggests that these compounds are specifically produced by wounding or that they are accumulated only at the early stage of agarwood formation and gradually converted to chromones or other phenolic compounds. Biosynthesis of oxidoagarochromones is under examination, and further investigation on the relationship between these new diepoxy tetrahydrochromones and known chromone derivatives would provide important information to understand the biosynthesis of chromone derivatives in agarwood.

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10. GPC was carried out using LC-918 recycling HPLC system equipped with JAIGEL-1H and -2H (each 20×600 mm) (Japan Analytical Industry) tandemly connected.
11. Compound **1**: Brown gum; $[\alpha]_D^{25}$ −1.3 (MeOH, *c* 0.35); HRFABMS *m/z* 283.0976 $[M+H]^+$ (calcd for $C_{17}H_{15}O_4$; 283.0970); UV λ_{max} (log ε) (MeOH) 254 (4.11), 208 (4.39) nm; CD (*c* 2.8×10^{-3}, MeOH) $\Delta\varepsilon^{25}$ +1.3 (256 nm) (positive maximum), −0.9 (294 nm) (negative maximum); IR ν_{max} (KBr) 1658, 1620, 1466, 1404, 1192 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$ and acetone-d_6) and ^{13}C NMR (125 MHz, $CDCl_3$): Table 1.
12. Pretsch, E.; Seibl, J.; Simon, W.; Clerc, T. *Tables of Spectral Data for Structure Determination of Organic Compounds*, 2nd ed.; Springer: New York, 1989, pp C170–C173.
13. Compound **2**: Pale yellow prisms; mp 142–144 °C; $[\alpha]_D^{25}$ +0.3 (*c* 0.31, MeOH); HRFABMS *m/z* 313.1068 $[M+H]^+$ (calcd for $C_{18}H_{17}O_5$; 313.1076); UV λ_{max} (log ε) (MeOH) 254 (4.06), 223 (4.33), 203 (4.37) nm; CD (*c* 3.1×10^{-3}, MeOH) $\Delta\varepsilon^{25}$ −2.3 (257 nm) (negative maximum), +2.4 (295 nm) (positive maximum); IR ν_{max} (KBr) 1658, 1620, 1512, 1462, 1404, 1246, 1192 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$): Table 1.
14. Compound **3**: Pale yellow crystalline solid; mp 69–72 °C; $[\alpha]_D^{25}$ −11.8 (*c* 0.59, $CHCl_3$); HRFABMS *m/z* 329.1031 $[M+H]^+$ (calcd for $C_{18}H_{17}O_6$; 329.1025); UV λ_{max} (log ε) (MeOH) 254 (3.97), 207 (4.42) nm; CD (*c* 3.3×10^{-3}, MeOH) $\Delta\varepsilon^{25}$ −2.8 (256 nm) (negative maximum), +2.9 (294 nm) (positive maximum); IR ν_{max} (KBr) 3217, 1659, 1612, 1512, 1466, 1408, 1273, 1242, 1196, 1130 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$): Table 1.
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16. For HPLC analyses, the resinous and intact parts of *A. sinensis* and *A. crassna* (0.4–0.6 g) were extracted with AcOEt overnight at room temperature. For isolation of compounds, the resinous parts of *A. sinensis* (610 g) collected 50 days after wounding were extracted three times with AcOEt overnight at room temperature to give 5.0 g of the extract.
17. Each extract was dissolved in MeOH, then passed through a Sep-Pak® cartridge, and the eluate was concentrated to dryness. The residue was dissolved in MeOH at 1 mg/ml and analyzed by HPLC. Conditions: column, YMC-Pack ODS/R, 10 μm, 120 Å, 4.6×250 mm I.D.; detection, 254 nm; solvent, MeOH–water [37% (0 min) to 100% (30 min) MeOH]; flow rate, 1 ml/min.