Studies on Agalwood (Jinkō). X. Structures of 2-(2-Phenylethyl)chromone Derivatives

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Three new kinds of phenylethylchromone derivatives, called AH_{17}, AH_{20} and AH_{23}, were isolated from acetone and pyridine extracts of agalwood (Jinkō) from Kalimantan. The structures of AH_{17} and AH_{23} were characterized as 5α,6β,7β-trihydroxy-8α-methoxy-2-(2-phenylethyl)-5,6,7,8-tetrahydrochromone and 5α,6β,7β,8α-tetrahydroxy-2-[2-(2-hydroxyphenyl)ethyl]5,6,7,8-tetrahydrochromone, respectively. AH_{20} was found to be a trimer formed by the ether-linkage made of 2-(2-phenylethyl)chromone and 2 mol of agarotetrol at $C_{5,8'}$ and $C_{6,5'}$.

Keywords 2-(2-phenylethyl)chromone; agalwood; Aquilariaceae; ^{1}H-NMR; ^{13}C-NMR; NOE

In previous papers of this series[1] it was reported that acetone and pyridine extracts of agalwood (Jinkō) from Kalimantan contained various 2-(2-phenylethyl)chromone derivatives such as hydroxylates, methoxylates and hydrogenates, and the dimers and trimers of a 2-(2-phenylethyl)-chromone unit, formed by C–C or ether-linkage.

This paper deals with the isolation and characterization of three new additional minor constituents from the acetone and pyridine extracts, tentatively called H_{17}, AH_{20} and AH_{23}. The procedure of isolation is described in the experimental section.

AH_{17} (**1**), a white powder, $C_{18}H_{20}O_6$, $[\alpha]_D$ +1.94°, exhibited absorption bands due to a phenylethyl group and a trisubstituted γ-pyrone ring in the infrared (IR) and ultraviolet (UV) spectra. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **1** showed four methine proton signals due to the cyclohexenyl moiety together with those of a methoxy and three hydroxy protons. These protons were assigned based on comparison with the data for agarotetrol as shown in Table I. Acetylation of **1** afforded a triacetate (**3**), the ^{1}H-NMR spectrum of which showed acetylation shifts of C_5, C_6 and C_7-H, but no shift of C_8. Therefore, the methoxy group was assigned to be linked at C_8. This was also supported by the C_8 signal at δ 70.17 which showed a low field shift of about 3 ppm, compared with that of agarotetrol in the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of **1**.

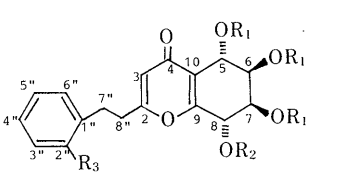
Accordingly, AH_{17} was suggested to be 5α,6β,7β-trihydroxy-8α-methoxy-2-(2-phenylethyl)chromone, **1**.

AH_{23} (**4**), colorless needles, $C_{17}H_{18}O_7$, mp 143—145 °C, was also thought to be a 2-(2-phenylethyl)-5,6,7,8-tetrahydrochromone derivative based on the UV and IR spectra. The ^{1}H-NMR spectrum of **4** showed that the signal pattern of the four methine protons attached to the cyclohexenyl ring was analogous to that of agalotetrol, and their chemical shifts and coupling constants were in fairly good accord with those of **2**, as shown in Table I. The peracetate (**5**) derived from **4** by treatment with acetic anhydride and pyridine showed the presence of five acetoxy groups at δ 2.06, 2.09, 2.09, 2.17 and 2.34 ppm. The signal at δ 2.34

TABLE I. ^{1}H-NMR Spectral Data for AH_{17}, AH_{23} and AH_{20} (δ in DMSO-d_6)

	AH_{17} (**1**)	AH_{23} (**4**)	AH_{20} (**6**)		
			Unit A	Unit B	Unit C
5-H	4.32 dd (J=8.1, 6.6)	4.31 d (J=7.3)	5.31 d (J=8.3)	4.87 d (J=7.7)	
6-H	3.74 ddd (J=8.1, 5.9, 2.1)	3.84 dd (J=7.3, 2.0)	4.36 dd (J=8.3, 2.1)	4.96 dd (J=7.7, 1.9)	7.46 d (6′-H) (J=9.3)
7-H	3.93 ddd (J=3.8, 3.7, 2.1)	3.75 dd (J=3.8, 2.0)	3.91 dd (J=3.2, 2.1)	3.98 dd (J=3.7, 1.9)	7.88 d (7′-H) (J=9.3)
8-H	4.14 d (J=3.7)	4.49 d (J=3.8)	4.58 d (J=3.2)	4.60 d (J=3.7)	
5-OH	5.83 d (J=6.6)				
6-OH	5.18 d (J=5.9)				
7-OH	5.09 d (J=3.8)				
3-H	6.10 s	6.09 s	6.12 s, 6.16 s, 6.20 s[a]		
CH_2CH_2	2.87 m ($C_{7''}$) 2.96 m ($C_{8''}$)	2.81 m ($C_{7''}$) 2.88 m ($C_{8''}$)	2.62, 2.68, 2.86, 2.94[a] (each m, 2H), 3.00 (m, 4H)		
C_6H_5	7.24 m (1H) 7.26 m (4H)	6.71 dt (4″-H) 6.80 dd (3″-H) 7.02 dt (5″-H) 7.08 dd (6″-H) (each J=7.5, 1.5)	7.02 (m, 2H), 7.20, 7.27 (m, 13H)[a]		
CH_3O	3.36 s				

a) Individual assignments of units A, B and C are difficult.



1 : $R_1=R_3=H$, $R_2=CH_3$
2 : $R_1=R_2=R_3=H$
3 : $R_1=CH_3CO$, $R_2=CH_3$, $R_3=H$
4 : $R_1=R_2=H$, $R_3=OH$
5 : $R_1=R_2=CH_3CO$, $R_3=CH_3COO$

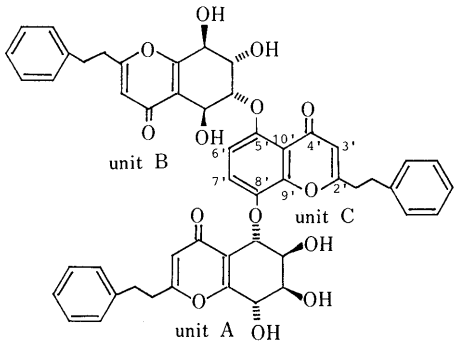


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Chart 1

TABLE II. ^{13}C-NMR Spectral Data for AH_{17}, AH_{23} and AH_{20}[a]

Carbon	AH_{17}	AH_{23}	AH_{20}		
			Unit A	Unit B	Unit C
2,2′	168.20	168.30	167.82, 167.82, 167.97[b]		
3,3′	113.54	112.34	112.75, 112.95, 110.17[b]		
4,4′	179.60	178.38	177.94, 177.96, 178.31[b]		
5,5′	71.37	68.32	77.87	66.97	151.77
6,6′	72.91	70.59	68.62	82.88	113.63
7,7′	75.47	72.59	72.86	70.33	123.02
8,8′	70.17	64.61	64.35	63.39	144.47
9,9′	169.29	163.00	159.58, 161.75[b]		149.51
10,10′	120.53	120.64	120.34, 121.69[b]		117.98
1″	140.43	125.96	139.76, 139.88, 139.96[b]		
2″	128.83	155.00	128.31	128.31	128.31
3″	128.56	114.79	128.21	128.21	128.21
4″	126.63	127.23	126.01, 126.10, 126.17[b]		
5″	128.56	118.81	128.21	128.21	128.21
6″	128.83	129.60	128.31	128.31	128.31
7″	35.15	26.72	34.02, 34.08, 34.21[b]		
8″	32.74	32.24	31.62, 31.62, 31.76[b]		
CH_3O	58.93				

a) Assignments were based on the results of ^{1}H-^{13}C-COSY. b) Individual assignments of units A, B and C are difficult.

was assigned to the acetoxyl function linked to the phenyl group because it is further downfield than the other acetoxy groups. The observed values of the phenylethyl carbon signals in the ^{13}C-NMR spectrum were in close agreement with those values of AH_{2b}, the 1′-hydroxyl derivative of isoagarotetrol taking the solution effect into account.[1c]

Accordingly, AH_{23} was characterized as 5α,6β,7β,8α-tetrahydroxy-2-[2-(2-hydroxyphenyl)ethyl]5,6,7,8-tetrahydrochromone, **4**.

AH_{20} (**6**), a white powder, $[\alpha]_D$ −27.83°, showed a molecular ion at *m/z* 882 in the field desorption-mass spectrum (FD-MS) giving the molecular formula $C_{51}H_{46}O_{14}$. The IR and UV spectra of **6** exhibited strong absorption maxima due to a γ-pyrone ring. The ^{1}H-NMR spectrum showed the presence of three proton signals at δ 6.12, 6.16 and 6.20, and three sets of phenylethyl groups, indicating the tri-2-(2-phenylethyl)-chromone derivative for **6**, considering molecular weight. Two units of the trimer, units A and B, appeared to be 5,6,7,8-tetrahydroxyphenylethylchromone derivatives based on the presence of two sets of four methine proton signals which were assigned by ^{1}H selective irradiation. Unit A was characterized as agarotetrol linked at C_5 by the ether bond to another monomeric unit (unit C), based on the four vicinal methine protons which showed analogous chemical shifts and coupling systems to those of AH_{10}, AH_{15} and AH_{18}.[1d,e] Likewise, unit B was suggested to be agarotetrol formed an ether-linkage at C_6 to unit C because of the low field displacement of 6-H, about 0.6 ppm compared with that of unit A (Table I). It appears that the structure of unit C is 5,8-dialkoxy-2-(2-phenylethyl)chromone, based on the two doublet proton signals assumed to be located at the C_6 and C_7 positions of the chromone ring. The structure was supported by the ^{13}C-NMR spectrum, indicating a similar sequence of the carbon signals in the chromone ring to those of AH_7, 5,8-dihydroxy-2-(2-phenylethyl)chromone (Table II).[1f] Their assignments were confirmed by the analyses of ^{1}H–^{13}C correlation spectroscopy (COSY). In order to determine the positions of two ether-linkages among units A, B and C, **6** was subjected to measurement of the nuclear Overhauser effect (NOE) difference values. Irradiation of 5-H at δ 5.31 resulted in an appreciable NOE increase of the 7′-H at δ 7.88, and NOE was detected between the 7-H at δ 3.98 and 6′-H at δ 7.46. Therefore, it was found that units A and B were linked to unit C by ether bonds at $C_{5,8'}$ and $C_{6,5'}$ positions, respectively.

Accordingly, AH_{20} was characterized as the trimer **6**.

AH_{17} is the first example of the methoxy function in the hexenyl ring of the polyoxy 2-(2-phenylethyl)chromone derivatives, agarotetrol and isoagarotetrol.[1b] It is said that the cyclohexenyl moiety of agarotetrol has 5e′-OH group in contrast to its 5-O-acetate, in which the 5-OH group is a′ configuration because of the intramolecular hydrogen bonding and the steric and electrostatic repulsions between the pyrone carbonyl and the 5-OAc function.[2] But the interconversion of 5e′-OH and 5a′-OAc did not accompany in the cyclohexenyl moiety of **1** and **3** as shown in the ^{1}H-NMR spectra. Therefore, the conformational change in the cyclohexenyl moiety of 2-(2-phenylethyl)-5,6,7,8-tetrahydrochromones is thought to depend delicately on the other substituent groups at C_6, C_7 and C_8 positions together with the relationship between the γ-pyrone carbonyl and the 5-OH function. The hydroxylation at C_2 position of the phenylethyl group as shown in AH_{23} has also been found in the structures of AH_9[1f] and AH_{2b}.[1c] AH_{20} is specific for the ether-linkage at $C_{6,5'}$ and $C_{5,8'}$ between each unit of the two agarotetrols and 2-(2-phenylethyl)-chromone.

Experimental

Melting points were determined on a micro melting point apparatus (Yanagimoto) and are uncorrected. The UV spectra were obtained in MeOH with a Shimadzu UV-200s spectrometer, and IR spectra (in KBr disks and $CHCl_3$) with a Shimadzu IR 27G spectrometer. The ^1H (300.0 MHz) and ^{13}C (75.4 MHz) NMR spectra were taken on a Varian XL-300 spectrometer in dimethyl sulfoxide-d_6 (DMSO-d_6) and $CDCl_3$ solutions. Chemical shifts are given in δ (ppm) with tetramethylsilane as an internal standard (s, singlet; d, doublet; t, triplet; dd, double doublet; ddd, double double doublet; m, multiplet; br, broad).

Column chromatographies were performed on Kieselgel 60 (70—230 mesh, Merck), Kiesel 60 silanisiert (70—230 mesh, Merck), Sephadex LH-20 (Pharmacia Fine Chemicals), and LiChroprep Rp-8 (40—63 mesh) pre-packed column (Merck).

Isolation of AH_{17}, AH_{20} and AH_{23} The fraction $B^{1g)}$ (650 mg) was subjected to column chromatography ($CHCl_3$–MeOH, 9:1 v/v) to give crude AH_{16} (65 mg), AH_{17} (62 mg) and a residue. AH_{17} (40 mg) was obtained as colorless needles from the crude fraction which was further chromatographed on a column of Sephadex LH-20 (MeOH). A pyridine extract (300 g) from residue-$2^{1a)}$ was refluxed with MeOH to obtain a viscous extract (56.5 g) which on silica gel column chromatography ($CHCl_3$–MeOH–H_2O, 90:10:1 v/v) gave four fractions, fr_1 (8.6 g), fr_2 (11.1 g), fr_3 (7.7 g) and fr_4 (18.5 g). Fr_4 (18.5 g) was chromatographed on Sephadex LH-20 (MeOH) followed by LiChroprep Rp-8 (MeOH–H_2O, 7:3 v/v) to yield AH_{23} fraction (211 mg). AH_{23} (18 mg) was obtained as colorless needles by repeated column chromatography on silica gel ($CHCl_3$–MeOH–H_2O, 8:2:0.2 v/v).

AH_{17} (1) A white powder, (mp 130—135 °C), $[\alpha]_D$ +1.94 (c=1.03, MeOH). UV λ_{max}^{MeOH} nm (ε): 207 (18216), 252 (13678). IR (KBr, cm^{-1}): 3360 (OH), 1658, 1600 (γ-pyrone ring). ^1H- and ^{13}C-NMR: Tables I and II. FD-MS m/z: 332 (M^+), 302 ($M^+ - H_2O$). *Anal.* Calcd for $C_{18}H_{20}O_6 \cdot 2/3H_2O$: C, 62.78; H, 6.24. Found: C, 62.72; H, 6.07.

Acetylation of 1 A mixture of Ac_2O–pyridine (1:1 v/v, 2 ml) and **1** (10.3 mg) was allowed to stand for a day at room temperature, and evaporated to dryness under reduced pressure. The residue was purified by column chromatography (hexane–AcOEt, 2:1 v/v) to give **3** (9.5 mg) as a white powder. $[\alpha]_D$ −104.8° (c=1.03, $CHCl_3$). UV λ_{max}^{MeOH} nm (ε): 202 (21984), 243 (11816). IR ($CHCl_3$) cm^{-1}: 1752 (ester), 1665, 1625 (γ-pyrone ring). ^1H-NMR ($CDCl_3$, 80 MHz): 2.04 (s, 6H, $CH_3CO \times 2$), 2.16 (s, 3H, CH_3CO), 2.86 (m, 4H, CH_2CH_2), 3.59 (s, 3H, CH_3O), 4.45 (d, J=3.3 Hz, 8-H), 5.58 (dd, J=3.3, 2.5 Hz, 7-H), 5.61 (dd, J=8.5, 2.5 Hz, 6-H), 6.11 (d, J=8.5 Hz, 5-H), 6.13 (s, 3-H), 7.25 (m, 5H, aromatic H). Atmospherie pressure ionization-mass spectrum (API-MS) m/z: 459 $[M+H]^+$ (100%).

AH_{23} (4) Colorless needles, mp 143—145 °C. UV λ_{max}^{MeOH} nm (ε): 210 (12846), 246 (9653). IR (KBr) cm^{-1}: 3355 (OH), 1645, 1560 (γ-pyrone ring), 1222, 1095, 1010, 982, 740 (1,2-disubstituted benzene). ^1H- and ^{13}C-NMR: Tables I and II. API-MS m/z: 335.2 $[M+H]^+$ (100%).

Acetylation of 4 **4** (11 mg) was acetylated in the same manner as described for **1** to give an acetate (**5**, 9 mg) as a white powder. (mp 72—74 °C), $[\alpha]_D$ −17.4° (c=1.09, MeOH). UV λ_{max}^{MeOH} nm (ε): 243 (14416). IR ($CHCl_3$) cm^{-1}: 1755 (ester), 1670, 1632 (γ-pyrone ring). ^1H-NMR ($CDCl_3$, 80 MHz): 2.06, 2.09, 2.09, 2.17, 2.34 (each s, CH_3CO), 2.80 (m, 4H, CH_2CH_2), 5.49 (dd, J=7.9, 2.5 Hz, 7-H), 5.50 (dd, J=3.3, 2.5 Hz, 6-H), 5.99 (s, 3-H), 6.11 (d, J=3.3 Hz, 5-H), 6.18 (d, J=7.9 Hz, 8-H), 7.26 (m, 4H, aromatic H). API-MS m/z: 545.2 $[M+H]^+$.

AH_{20} (6) A white powder (mp 143—145 °C), $[\alpha]_D$ −27.83° (c=0.97, MeOH). UV λ_{max}^{MeOH} nm (ε): 212 (50063), 247 (48242). IR (KBr) cm^{-1}: 3320 (OH), 1652, 1595 (γ-pyrone ring). ^1H- and ^{13}C-NMR: Tables I and II. FD-MS m/z: 883 $[M+H]^+$.

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