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HYDROPEROXYSESQUITERPENE AND LIGNAN CONSTITUENTS OF *MAGNOLIA KOBUS*

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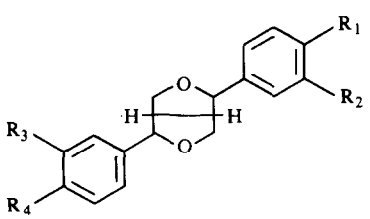
Key Word Index – *Magnolia kobus*; Magnoliaceae; lignans; kobusin; epieudesmin; sesquiterpenes; 9-oxonerolidol; hydroperoxides; kobusimin A and B; ^{13}C NMR.

Abstract—Two new hydroperoxysesquiterpenes, kobusimin A and B, have been isolated from the leaves of *Magnolia kobus*.

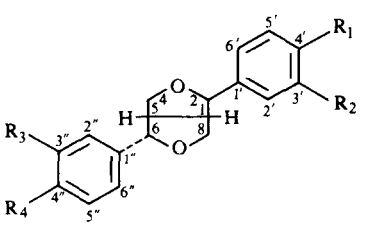
INTRODUCTION

The essential oil of *Magnolia kobus* (Japanese name: Kobusi), a valuable Japanese decorative plant, contains many terpenes [1]. Three lignans, sesamin, kobusin and lirioresinol B dimethyl ether have been found in its leaves [2] and seeds [3].

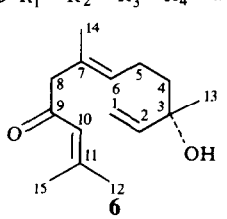
In connection with our interest in biologically active sesquiterpenes, we have re-investigated the chemical constituents of *M. kobus* and hereby report the isolation of two hydroperoxysesquiterpenes, kobusimin A (**7**) and kobusimin B (**8**), along with five known lignans [sesamin (**1**), kobusin (**2**), eudesmin (**3**) [4], epieudesmin (**4**) [4] and phillygenin (**5**) [5]] and the known sesquiterpene [9-oxonerolidol (**6**) [6]].



1 R_1, R_2 = —OCH_2O— R_3, R_4 = —OCH_2O—
2 R_1, R_2 = —OCH_2O— $R_3 = R_4$ = OMe
3 $R_1 = R_2 = R_3 = R_4$ = OMe



4 $R_1 = R_2 = R_3 = R_4$ = OMe
5 R_1 = OH $R_2 = R_3 = R_4$ = OMe



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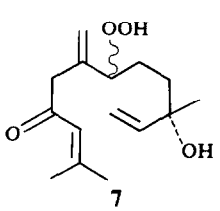

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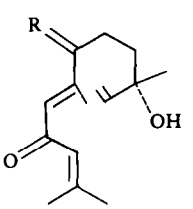
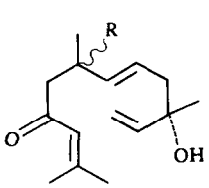


7a R = O
7b R = H, OH



8 R = OOH
8a R = OH

Table 1. ^{13}C NMR data (ppm) for eudesmin (**3**), epieudesmin (**4**) and phillygenin (**5**)

Carbon no.	**3**	**4**	**5**
1	54.0 *d*	54.4 *d*	54.4 *d*
5		50.1 *d*	50.1 *d*
2	85.5 *d*	87.4 *d*	87.5 *d*
6		81.9 *d*	81.9 *d*
4	71.5 *t*	69.6 *d*	69.5 *d*
8		70.9 *t*	70.9 *t*
1′	133.3 *s*	133.5 *s*	132.8 *s*
1″		130.7 *s*	130.7 *s*
5′	110.9 *d*	110.9 *d*	114.0 *d*
5″			110.9 *d*

Run in $CDCl_3$ at 25 MHz; *s*, singlet; *d*, doublet; *t*, triplet. Assignment establishment by frequency off-resonance decoupling.

The position of the phenolic hydroxyl group in phillygenin (**5**) was deduced by comparison of its ^{13}C NMR spectral data with those of eudesmin (**3**) and epieudesmin (**4**) (Table 1) on the basis of Nishibe's report [7]. Only the characterization of the new sesquiterpenes is described here.

RESULTS AND DISCUSSION

IR and UV spectra and specific rotation of kobusimin A (**7**) (see Experimental) indicated that it contained an α,β-unsaturated ketone moiety and that it could be a derivative of 3*S*-(+)-9-oxonerolidol (**6**). The ^1H NMR spectrum in $CDCl_3$ had peaks at δ 4.28 (1H, *br t*, $J = 6$ Hz), 5.0, 5.24 (2H, each *s*, C-14 H) and 10.76 (1H, *s*, OOH, D_2O exchangeable) together with peaks due to allylic protons (δ 4.90–5.92). The ^{13}C NMR spectrum (Table 2) showed 15 peaks of which three were assignable to oxygenated carbons and were located at 200.3 (C-9), 72.7 (C-3) and 89.5 ppm (C-6). The last of these had a chemical shift characteristic of a carbon carrying a hydroperoxy group [8, 9]. An intense ferrous thiocyanate test further supported the presence of a hydroperoxy group [10].

Table 2. ^{13}C NMR data (ppm) for 9-oxonerolidol (**6**) and kobusimin A (**7**)

Carbon no.	**6**	**7**
1	111.4 *t*	111.8 *t*
2	144.7 *d*	144.3 *d*
3	73.1 *s*	72.7 *s*
4	41.8 *t*	38.0 *t*
5	23.0 *t*	25.1 *t*
6	122.6 *d*	89.5 *d*
7	129.4 *s*	141.0 *s*
8	55.1 *t*	45.6 *t*
9	198.8 *s*	200.3 *s*
10	129.1 *d*	123.0 *d*
11	155.3 *s*	158.4 *s*
12	27.6 *q*	27.8 *q*
13	27.8 *q*	27.8 *q*
14	16.5 *q*	120.2 *t*
15	20.7 *q*	21.2 *q*

Run in $CDCl_3$ at 25 MHz; *s*, singlet; *d*, doublet; *t*, triplet; *q*, quartet. Assignment establishment by frequency off-resonance decoupling.

In order to obtain further chemical evidence for an allylic hydroperoxy group, kobusimin A was converted by acetic anhydride and pyridine to the conjugated diketone (**7a**) [UV(MeOH): 290 nm; IR($CHCl_3$) cm^{-1}: 1680, 1650, 1620]. Furthermore, reduction of **7** with triphenylphosphine gave the conjugated enonediol **7b** [IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3620, 3420, 1685, 1620; UV λ_{max}^{MeOH} nm: 272 (sh), 278, 293 (sh) ^1H NMR($CDCl_3$): δ 1.34 (3H, C-13Me), 1.90, 1.96 (9H, C-12, 14, 15Me)]. These observations confirmed the presence of the hydroperoxy group at C-6 except for its configuration.

The second new compound, kobusimin B (**8**) also gave a positive ferrous thiocyanate test. However, it was very labile, and was converted during purification with 10% silver nitrate impregnated Si gel TLC to a diol, deoxykobusimin B (**8a**), which had an ^1H NMR spectrum (see Experimental) similar to that of 3*S*-(+)-3,7-dimethyl-1,5-octadiene-3,7-diol [11] except for the presence of an isopentenone moiety. Therefore, the structure of deoxykobusimin B is assumed to be **8a** except for the stereochemistry of the diol and the geometry of the 5,6-double bond. Kobusimin B should therefore be **8**.

In order to interrelate **6**–**8**, the photo-oxygenation of 9-oxonerolidol (**6**) in methanol using visible light and methylene blue as sensitizer was carried out, and gave kobusimin A (**7**) and B (**8**) in a ratio of *ca* 10 : 1 and a 65% yield. **8** was purified by reduction with triphenyl phospine to give deoxykobusimin B (**8a**).

Addition of singlet-oxygen in the ene reaction is assumed to occur via a *cis* cyclic mechanism, probably perpendicularly to the 6,7-double bond from both sides of the 9-oxonerolidol plane to afford **7** and **8** [12]. Consequently, the configuration of the hydroperoxy groups in kobusimin A and B is not known.

EXPERIMENTAL

Mps are uncorr. ^1H NMR (100 MHz) and ^{13}C NMR (25 MHz): $CDCl_3$, δ units relative to TMS. MS (20 or 70 eV) direct insertion. IR: $CHCl_3$. $[\alpha]_D$: $CHCl_3$ or CCl_4. UV: MeOH. Spots were detected by UV (254 nm) and spraying with 10% H_2SO_4 and then heating at 100° or with ferrous thiocyanate reagent.

Extraction and separation. The MeOH extract of fresh leaves (3.3 kg) of *M. kobus* D.C. collected in July, 1980 at our University, was divided into *n*-hexane and $CHCl_3$-soluble fractions (23 g). The residue was chromatographed over a column of Si gel (400 g) using C_6H_6 with gradually increasing proportions of EtOAc as eluant.

First fraction (C_6H_6–EtOAc, 20 : 1) gave sesamin (**1**, 1.8 g), kobusin (**2**, 1.5 g), 9-oxonerolidol (**6**, 0.5 g), kobusimin A (**7**, 0.1 g) and kobusimin B (**8**, 0.05 g). The second fraction (5 : 1) gave eudesmin (**3**, 1.7 g), epieudesmin (**4**, 0.1 g) and phillygenin (**5**, 1.2 g).

Sesamin (**1**). Mp 121–125°. $[\alpha]_D$ +69.2° ($CHCl_3$; *c* 0.68). MW 354 (MS). $C_{20}H_{28}O_6$. UV λ_{max}^{MeOH} nm: 236, 286. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1610, 1505, 1490, 1445, 1250. ^1H NMR: δ 3.04 (2H, *m*, C-1, 5H), 3.84 (2H, *dd*, $J = 4$, 9 Hz, C-4, 8H), 4.2 (2H, *dd*, $J = 7$, 9 Hz, C-4, 8H), 4.7 (2H, *d*, $J = 4$ Hz, C-2, 6H), 5.94 (2H, *s*, $-OCH_2O-$), 6.78–6.84 (ar.H).

Kobusin (**2**). Colourless oil. $[\alpha]_D$ +58.6° ($CHCl_3$; *c* 3.33). MW 370 (MS). $C_{21}H_{22}O_6$. UV λ_{max}^{MeOH} nm: 232, 284. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1610, 1595, 1505, 1490, 1445, 1250. ^1H NMR: δ 3.08 (2H, *m*, C-1, 5H), 3.84–3.94 (2H, *m*, C-4, 8H), 3.84, 3.86 (2 × OMe), 4.24 (2H, *dd*, $J = 7$, 9 Hz, C-4, 8H), 4.72 (2H, *d*, $J = 4$ Hz, C-2, 6H), 5.92 ($-OCH_2O-$), 6.76–6.84 (ar.H).

Eudesmin (**3**). Mp 98–100°. $[\alpha]_D$ +61.0° ($CHCl_3$; *c* 0.4). MW 386 (MS). $C_{22}H_{26}H_6$. UV λ_{max}^{MeOH} nm: 232, 279. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1610, 1595, 1510, 1465, 1260, 1140. ^1H NMR: δ 3.12 (2H, *m*, C-1, 5H), 3.86–3.96 (2H, *m*, C-4, 8H), 3.86, 3.9 (4 × OMe), 4.26 (2H, *dd*, $J = 7$, 9 Hz, C-4, 8H), 4.76 (2H, *d*, $J = 4$ Hz, C-2, 6H), 6.84–6.92 (ar.H). ^{13}C NMR: Table 1.

Epieudesmin (**4**). Mp 122–125°. $[\alpha]_D$ +113.3° ($CHCl_3$; *c* 0.38). MW 386 (MS). $C_{22}H_{26}O_6$. UV λ_{max}^{MeOH} nm: 231, 279. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1610, 1595, 1510, 1470, 1265, 1160, 1140. ^1H NMR: δ 2.94 (1H, *m*, C-5H), 3.2–3.4 (2H, *m*, C-1, 4H), 3.7–4.2 (3H, *m*, C-4, 8H), 3.85, 3.86, 3.88, (3 × OMe), 4.44 (1H, *d*, $J = 7$ Hz, C-6H), 4.85 (1H, *d*, $J = 5$ Hz, C-2H), 6.8–6.92 (ar.H). ^{13}C NMR: Table 1.

Phillygenin (**5**). Mp 135–136°. $[\alpha]_D$ +91.6° ($CHCl_3$; *c* 0.5). MW 372 (MS). Calc. for $C_{21}H_{24}O_6$: C, 67.25; H, 6.33. Found: C, 67.73; H, 6.50%. UV λ_{max}^{MeOH} nm (log ϵ): 231 (4.46), 280 (3.80). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3575 (OH), 1615, 1595, 1515, 1470, 1275. ^1H NMR: δ 2.90 (1H, *m*, C-5H), 3.2–3.4 (2H, *m*, C-1, 4H), 3.6–4.2 (3H, *m*, C-4, 8H), 3.84, 3.88 (2 × OMe), 4.42 (1H, *d*, $J = 7$ Hz, C-6H), 4.84 (1H, *d*, $J = 5$ Hz, C-2H), 5.56 (1H, *s*, OH). ^{13}C NMR: Table 1.

3S-(+)-9-*Oxonerolidol* (**6**). Colourless oil. $[\alpha]_D$ +18.6° (CCl_4; *c* 1.6). UV λ_{max}^{MeOH} nm: 241. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 3500, 1680, 1620. MS *m/z*: 236 (M^+, $C_{15}H_{24}O_2$), 218, 203. ^1H NMR: δ 1.28 (3H, *s*, C-13Me), 1.6 (3H, *s*, C-14Me), 1.88 (3H, *s*, C-12Me), 2.12 (3H, *s*, C-15Me), 3.0 (2H, *s*, C-8H), 4.9–6.0 (3H, *m*, CH_2=CH–, $J = 17$, 10, 3 Hz), 5.12 (1H, *m*, C-6H), 6.04 (1H, *br s*, C-10H). ^{13}C NMR: Table 2.

Kobusimin A (**7**). Colourless oil. Positive ferrous thiocyanate test. $[\alpha]_D$ +9.7° (CCl_4; *c* 0.35). UV λ_{max}^{MeOH} nm: 242. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3620, 3300, 1680, 1620, 1600, 1120, 1050, 1000, 925. MS *m/z*: 268 (no M^+, $C_{15}H_{24}O_4$), 250, 234, 216. ^1H NMR: δ 1.26 (3H, *s*, C-13Me), 1.92 (3H, *s*, C-12Me), 2.14 (3H, *s*, C-15Me), 3.18 (2H, *s*, C-8H), 4.28 (1H, *br t*, $J = 6$ Hz, C-6H), 5.0, 5.24 (2H, each *s*, C-14H), 4.9–5.92 (3H, *m*, CH_2=CH–), 6.05 (1H, *br s*, C-10H), 10.76 (1H, *s*, OOH). ^{13}C NMR: Table 2.

3,7,11 - *Trimethyl* - 6,9 - *dioxo* - 1,7,10 - *triene* - 3 - *ol* (**7a**). 20 mg of kobusimin A (**7**) was treated with Ac_2O (1 ml) and pyridine (1 ml) at room temp. overnight. Ice was then added to the reaction mixture, which was treated in the usual manner. The residue was passed through a column of Si gel (3 g $CHCl_3$) to give an oil (**7a**, 5 mg). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3620, 3500, 1680, 1650, 1620, UV λ_{max}^{MeOH} nm: 290. MS *m/z*: 250 (M^+, $C_{15}H_{22}O_3$). ^1H NMR: δ 1.36 (3H, *s*, C-13Me), 1.52 (3H, *s*, C-14Me), 1.9 (3H, *s*, C-12Me), 2.05 (3H, *s*, C-15Me).

3,7,11 - *Trimethyl* - 9 - *oxo* - 1,7,10 - *triene* - 3,6 - *diol* (**7b**). 30 mg of kobusimin A (**7**) was dissolved in MeOH (8 ml), and the soln stirred with 1.5 eq. (45 mg) of Ph_3P for 2 hr. The reaction mixture was evaporated to dryness and the residue chromatographed on Si gel using $CHCl_3$–MeOH (10 : 1) as eluent to afford an oil (**7b**, 10 mg). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3620, 3420, 1685, 1620. UV λ_{max}^{MeOH} nm: 272 (sh), 278, 293 (sh). MS *m/z*: 252 (no M^+), 234 ($M^+ - H_2O$), 216 ($M^+ - 2 \times H_2O$). ^1H NMR: δ 1.34 (3H, *s*, C-13Me), 1.9, 1.96 (9H, each *s*, C-12, 14, 15Me), 5.06–6.2 (3H, *m*, CH_2=CH–), 5.1 (1H, *m*, C-6H), 6.04 (2H, *m*, C-8, 10H).

Deoxykobusimin B (**8a**). Colourless oil. UV λ_{max}^{MeOH} nm: 238. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 3500, 1675, 1615. MS *m/z*: 253 ($M^+ + 1$, $C_{15}H_{24}O_3$), 235, 217. ^1H NMR: δ 1.26, 1.30 (6H, each *s*, C-13, 14Me), 1.92 (3H, *s*, C-12Me), 2.2 (3H, *s*, C-15Me), 2.68 (2H, *br s*, C-8H), 4.92–6.08 (3H, *m*, CH_2=CH–), 5.64 (2H, *br s*, C-6, 7H), 6.04 (1H, *br s*, C-10H).

Photo-oxygenation of 9-oxonerolidol (**6**). 80 mg of 9-oxonerolidol (**6**) and 8 mg of methylene blue were dissolved in MeOH (20 ml) and placed in a Pyrex tube connected to an O_2 source. The reaction tube was equipped with a 100 W high-pressure Hg lamp, bubbling gently with O_2 at 15°. After 3 hr, the reaction mixture was worked-up by evaporation of the solvent and the residue purified by prep. TLC (Si gel) to give 52 mg of kobusimin A (**7**) and 5 mg of crude kobusimin B (**8**), which was reduced with Ph_3P to provide deoxykobusimin B (**8a**, 2 mg). The resulting kobusimin A (**7**) and deoxykobusimin B (**8a**) was identical by IR and ^1H NMR comparison.

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