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A New Squalene-type Triterpene from the Woods of *Eurycoma longifolia*

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A new squalene-type triterpene with cytostatic activity, named as longilene peroxide, was isolated from the woods of *Eurycoma longifolia*. The solution and solid forms were elucidated by spectroscopic and X-ray analysis.

Cytostatic principles in woods and roots of *Eurycoma longifolia* (Simaroubaceae) have been found to be some types of terpenes, such as squalene-,[1] tirucallane-type[2] triterpenes, and quassinoids.[3] In a preliminary paper,[1] we reported the isolation and structure determination of a unique squalene-type triterpene, eurylene, with two tetrahydrofuran-rings from the woods. In further evaluation of extracts of *E. longifolia* for cytostatic activity, a new squalene-type triterpene characterized by eight asymmetric carbons and three tetrahydrofuran rings, which possesses different stereochemistry from teurilene,[4] was isolated. Here we report about the structure and conformation in solid and solution state of newly isolated compound, named as longilene peroxide (**1**).

The woods of *E. longifolia* collected in Indonesia were extracted with methanol, and chromatographic purification of chloroform soluble fraction showing cytotoxicity furnished longilene peroxide (**1**).

Longilene peroxide (**1**),[5] colorless needles, mp 142-143 °C, showing the molecular formula, $C_{30}H_{52}O_8$, appeared to contain a peroxide moiety by its positive color reaction.[6] The NMR data of **1** suggested the structure of similar triterpene ether to teurilene with three tetrahydrofuran rings, but with different asymmetry from teurilene[4] due to the negative optical rotation, $[\alpha]_D^{25}$ -23.0° (c 0.44, $CHCl_3$).

Crystallographic analysis of **1** was achieved to determine the exact stereostructure and to obtain detailed information on the conformation of the molecule. Longilene peroxide crystallized from n-hexane-ethyl acetate solution in monoclinic crystals of space group $P2_1$ with lattice constants a=14.506(8), b=11.986(7), c=8.984(5) Å, Z=2, V=1560 Å^3 and D_X=1.151 g cm^{-3}. X-Ray diffraction intensities of 2920 reflections were measured on a Philips PW 1100 diffractometer which were above the 2σ(I) level out of 3490 in the 2θ range of 6° through 156°, using graphite-monochromated CuKα radiation, μ=6.3 cm^{-1}. The crystal structure was determined by the direct method using the MULTAN program[7] and refined by the block-diagonal-matrix least-squares method[8] to an R value of 0.059. The perspective view of the molecule with relative configuration is given in Fig. 2. Longilene peroxide showed a rigid conformation in solid state

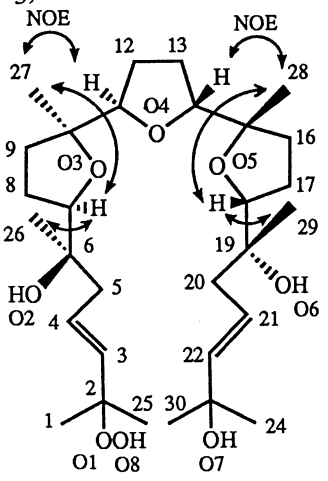

Fig. 1. Structure of longilene peroxide.

with four intramolecular hydrogen bonds (Table 1). One of them, between HO8 and O7 was very strong, so this restricted the flexibility of two terminal branches of the molecule.

In the ^{1}H-NMR spectrum in $CDCl_3$, since the presence of four intramolecular hydrogen bonds involving three hydroxy protons (δ 3.27, 5.05, and 5.26) and one hydroperoxy proton (δ 10.57) were suggested, the solution form was also considered to be similar one to solid state. NOE enhancements observed by NOESYPH spectrum also corroborated the above results (Figs. 1 and 2).

Longilene peroxide showed potent cytostatic activity more than teurylene and eurylene.[9] The molecular conformation with less mobility may be involved in the activity.

Table 1. The distances of intramolecular H-bondings

From	To	Distances/Å
HO7	O2	2.03 (5)
HO2	O6	2.07 (5)
HO6	O4	1.89 (5)
HO8	O7	1.79 (4)

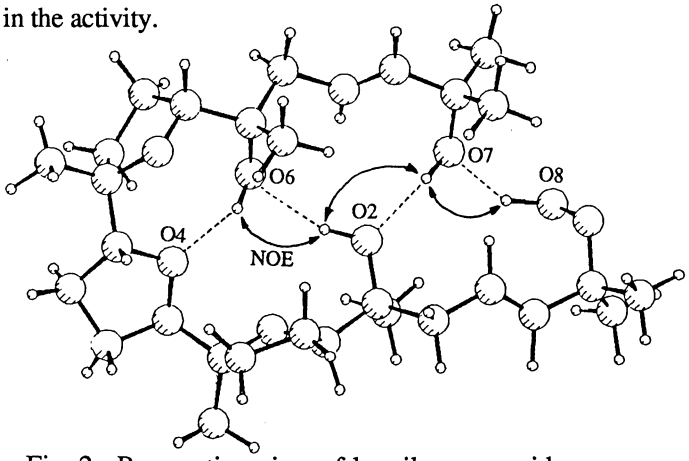

Fig. 2. Perspective view of longilene peroxide.

References

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5) ^{1}H-NMR data of **1** ($CDCl_3$, 500 MHz) δ: 1.09 and 1.11 (each 3H, s, H-27 or 28)), 1.21, 1.22, and 1.38 (each 3H, s, H-26 or 1 or 25), 1.29 (6H, s, H-29 and H-24 or 28), 1.33 (3H, s, H-24 or 28), 1.49 (4H, m, H-9, 16, 12 and 13), 1.79 (1H, dd, J=13.2, 8.6, H-5), 1.88 (3H, m, H-20, 17, and 8), 2.00 - 2.11 (6H, m, H-12, 13, 17, 18, 9, and 16), 2.20 (2H, dd, J=13.4, 7.0, H-5 and 20), 3.27 (1H, s, 7-OH), 3.73 (2H, m, H-7 and 18), 4.12 (2H, m, H-11 and 14), 5.05 (1H, d, J=1.4, 6-OH), 5.26 (1H, s, 2-OH), 5.45 (1H, d, J=15.7, H-3), 5.63 (1H, d, J=15.6, H-22), 5.78 (1H, ddd, J=15.2, 7.4, 1.1, H-21), 5.83 (1H, ddd, J=15.4, 6.4, 2.2, H-4), 10.57 (1H, d, J=2.8, OOH). ^{13}C-NMR data of **1** ($CDCl_3$, 100 MHz) δ: 23.6 (C-27 or 28), 24.3 (C-29), 24.3 (C-1 or 25), 24.3×2 (C-26 and C-27 or 28), 25.2 (C-17 or 8), 25.8 (C-17 or 8), 26.8 (C-1 or 25), 29.4 (C-12 or 13), 29.6 (C-24 or 30), 29.7 (C-12 or 13), 29.8 (C-24 or 30), 29.9 (C-9 or 16), 30.2 (C-9 or 16), 40.9 (C-20), 41.3 (C-5), 70.6 (C-23), 73.8 (C-19), 73.9 (C-6), 81.1 (C-2), 84.1 (C-18), 85.1 (C-10 or 15), 85.1 (C-11 or 14), 85.5 (C-7), 85.6 (C-10 or 15), 85.8 (C-11 or 14), 122.3 (C-21), 125.9 (C-4), 137.1 (C-3), 141.2 (C-22). IR (KBr) cm^{-1}: 3350, 2980, 1460, 1375, 1090, 1070. EI-MS *m/z* (%); 488 (M^+-H_2O_2-H_2O, 2, Calcd 488.3502 for $C_{30}H_{48}O_5$, Found 488.3502), 325 (26), 264 (25), 237 (43), 209 (48), 153 (86), 127 (100).
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9) Longilene peroxide exhibits the cytotoxic activity: IC_{50} 5.3 μg/ml against KB cells.

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