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Novel Antimalarial Guaiane-type Sesquiterpenoids from *Nardostachys chinensis* Roots

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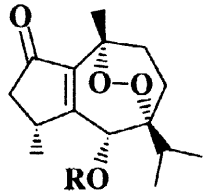
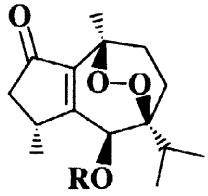
Abstract: Three guaiane-type sesquiterpenoids, nardoperoxide (**1a**), isonardoperoxide (**2a**) and nardoxide (**3**), were isolated from *Nardostachys chinensis* roots. Their structures were elucidated by spectral means. Among them, two endoperoxides, nardoperoxide (**1a**) and isonardoperoxide (**2a**), showed strong antimalarial activity against *Plasmodium falciparum* malaria (EC_{50} 1.5×10^{-6} and 6.0×10^{-7} M, respectively).

The crude drug "Kanshoko", the rhizomes and roots of *Nardostachys chinensis* Batalin and its relatives, *N. grandiflora* DC. and *N. jatamansi* DC. (Valerianaceae), has been employed as a sedative and an analgesic in Oriental medicines. It is well known to be rich in terpenoids.[1-3] In the course of our search for new types of antimalarial compounds from plants, nardosinone (**4**),[2] a major constituent of *N. chinensis*, was found to show antimalarial activity against *Plasmodium falciparum* malaria (EC_{50} 4.5×10^{-6} M). This finding prompted us to investigate other antimalarial compounds from this plant. Malaria is a parasitic disease, which significantly threatens human health worldwide. A number of medicines such as chloroquine and quinine are available for treatment of malaria, but the rapid development of drug resistance is a serious problem. Medicinal agents based on novel mechanisms of action are, therefore, required to overcome emergence of resistance and to control an ever-increasing number of epidemics caused by the malaria parasite. We report herein three novel guaiane-type sesquiterpenoids, nardoperoxide (**1a**), isonardoperoxide (**2a**) and nardoxide (**3**), from *N. chinensis* roots and their strong antimalarial activities. Although a variety of sesquiterpenoids have so far been isolated from the present plant, this is the first isolation of sesquiterpenoids of the guaiane-type, and they show a characteristic feature to have a cyclopentenone bearing double bond between C-1 and C-5.

Ethyl acetate-soluble oil (230 g) obtained from methanol extracts of *N. chinensis* (3.0 kg) was fractionated over silica gel. Fraction eluted with hexane-ethyl acetate (8:2) afforded nardoperoxide (**1a**) (163 mg), isonardoperoxide (**2a**) (354 mg) and nardoxide (**3**) (30 mg).



1a: R=H
1b: R=Bz



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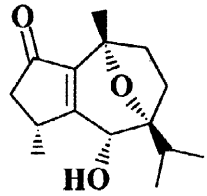
2b: R=Bz



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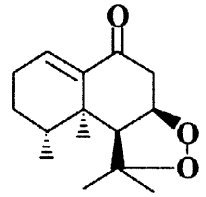

4

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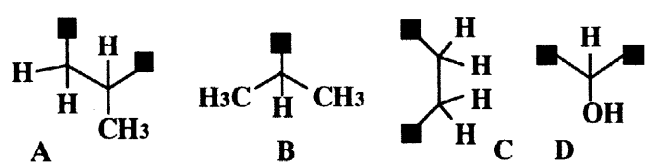
Table 1. ^1H and ^{13}C NMR Data for Nardoperoxide (**1a**), Isonardoperoxide (**2a**), and Nardoxide (**3**) in $CDCl_3$.

Position No.	**1a** ^1H [a]	**1a** ^{13}C [b]	**2a** ^1H [a]	**2a** ^{13}C [b]	**3** ^1H [a]	**3** ^{13}C [b]
1	–	144.7 (s)	–	143.6 (s)	–	144.2 (s)
2	–	204.8 (s)	–	204.9 (s)	–	205.4 (s)
3 α	2.16 (dd, *J* =2.7, 18.5)	45.9 (t)	2.10 (dd, *J* =1.8, 18.7)	45.5 (t)	2.04 (dd, *J* =2.5, 18.8)	45.1 (t)
β	2.71 (dd, *J* =6.5, 18.5)		2.80 (dd, *J* =6.7, 18.7)		2.64 (dd, *J* =6.3, 18.8)	
4	2.83 (ddq, *J* =2.7, 6.5, 6.7)	36.6 (d)	3.17 (d-quint, *J* =1.7, 7.1)	32.6 (d)	2.86 (ddq, *J* =2.5, 6.3, 7.3)	38.5 (d)
5	–	177.7 (s)	–	178.8 (s)	–	172.6 (s)
6	4.18 (br.s)	72.5 (d)	4.35 (br.s)	69.2 (d)	3.89 (br.s)	70.8 (d)
7	–	83.9 (s)	–	83.4 (s)	–	87.4 (s)
8 α	1.94 (dd, *J* =8.4, 14.0)	20.2 (t)	1.06 (ddd, *J* =8.3, 9.9, 13.6)	20.1 (t)	1.89 (dd, *J* =9.7, 13.2)	20.0 (t)
β	1.00 (ddd, *J* =8.2, 10.2, 14.0)		1.96 (dd, *J* =7.5, 13.6)		1.05 (td, *J* =8.7,13.2)	
9 α	2.07 (ddd, *J* =8.4, 10.2, 14.0)	28.3 (t)	1.78 (dd, *J* =8.3, 13.5)	28.6 (t)	1.55 (ddd, *J* =8.7, 9.7, 11.4)	28.5 (t)
β	1.75 (dd, *J* =8.2, 14.0)		2.08 (ddd, *J* =7.5, 9.9, 13.5)		1.75 (dd, *J* =8.7, 11.4)	
10	–	79.3 (s)	–	78.9 (s)	–	79.8 (s)
11	2.35 (sept, *J* =6.8)	31.5 (d)	2.36 (sept, *J* =6.9)	31.9 (d)	2.38 (sept, *J* =6.9)	36.0 (d)
12	0.91 (3H, d, *J* =6.8)	15.7 (q)	0.93 (3H, d, *J* =6.9)	15.8 (q)	0.95 (3H, d, *J* =6.9)	15.4 (q)
13	0.92 (3H, d, *J* =6.8)	17.7 (q)	0.94 (3H, d, *J* =6.9)	17.8 (q)	0.96 (3H, d, *J* =6.9)	17.3 (q)
14	1.40 (3H, d, *J* =6.7)	22.4 (q)	1.23 (3H, d, *J* =7.1)	22.6 (q)	1.36 (3H, d, *J* =7.3)	25.6 (q)
15	1.64 (3H, s)	20.2 (q)	1.63 (3H, s)	18.9 (q)	1.63 (3H, s)	20.0 (q)

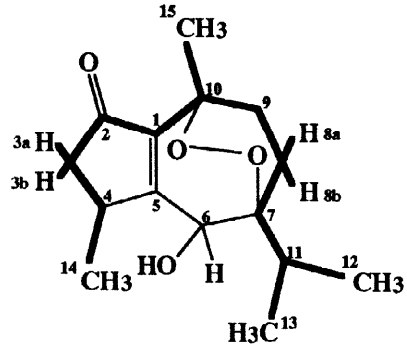
[a] Recorded at 300 MHz. Splitting patterns and coupling constants (Hz) are in parentheses. s: singlet, d: doublet, t: triplet, q: quartet, quint: quintet, sept: septet.

[b] Recorded at 75 MHz. $CDCl_3$ as internal standard (δ 77.1). Multiplicity was based on the DEPT spectra. q: primary carbon, t: secondary carbon, d: tertiary carbon, s: quaternary carbon.

The molecular formula $C_{15}H_{22}O_4$ indicated for isonardoperoxide (**2a**), $[\alpha]_D$ +6.8° (c 0.38, MeOH), a yellowish oil, was established by a molecular ion peak at *m/z* 266 $[M]^+$ in its EI mass spectrum and analyses of its ^1H and ^{13}C NMR spectra (Table 1). The ^{13}C NMR spectrum displayed the presence of four methyl, three methylene, two methine, one oxymethine and two quaternary carbons bearing an oxygen atom as well as a tetrasubstituted double bond and one carbonyl group in the molecule. The H-H COSY spectrum showing four sets of proton spin systems gave the segments A - D. The COLOC spectrum showed cross peaks at C-2 (δ 204.9)/H-3a and H-3b (δ 2.10 and 2.80), proposing a linkage of the carbonyl carbon (C-2) with the methylene (C-3) of the segment A. Moreover, C-H long range couplings at C-7 (δ 83.4)/H-12 and H-13 (δ 0.93 and 0.94), C-11 (δ 31.9)/H-8a and H-8b (δ 1.06 and 1.96), C-10 (δ 78.9)/H-8b (δ 1.96) and C-9 (δ 28.6)/H-15 (δ 1.63) indicated connections of the segments B, C and a methyl group. Correlations between

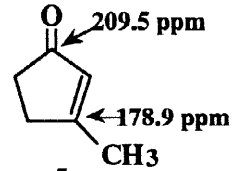


C-1 (δ 143.6) and H-15 (δ 1.63) and H-3a (δ 2.10) revealed that the carbonyl carbon (C-2) is linked to C-10 through the olefinic carbon at C-1. The C-5 carbon signal (δ 178.8) resonated at a lower position than that of the ordinary olefinic carbon, and it is well matched with the β-carbon (δ 178.9) of 3-methyl-2-cyclopentenone (**5**),[4] indicating the presence of an α,β-unsaturated ketone group on the five-membered ring. The carbon sequence deduced above, along with its molecular formula and the segment D, suggested isonardoperoxide (**2a**) to be a sesquiterpenoid having a guaiane skeleton with an endoperoxide bridge.



Planar structure of **2a**. Bold bond lines were confirmed by COLOC spectrum.

In the EI mass spectrum, nardoperoxide (**1a**), $[\alpha]_D$ +31.0° (c 0.33, MeOH), mp 129-130 °C, showed the same molecular ion peak (*m/z* 266 $[M]^+$) and fragmentation patterns as those of isonardoperoxide (**2a**). In addition, the ^{13}C and ^1H NMR spectra of nardoperoxide (**1a**) closely resembled those of isonardoperoxide (**2a**). The former (**1a**) is clearly different from the latter (**2a**) only in the chemical shifts of the ^1H NMR signals due to H-4 and H-14. These facts indicated both the compounds (**1a** and **2a**) to have the same structures except for the stereochemistry.



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For clarification of the relative stereochemistry of nardoperoxide (**1a**) and isonardoperoxide (**2a**), NOE difference spectra were analyzed. As for nardoperoxide (**1a**) and isonardoperoxide (**2a**), distinct NOE between H-6 on the carbon next to the hydroxyl group and H-8a suggested that the hydroxyl group on C-6 and the endoperoxide bridge are oriented on the same side of the seven-membered ring. The H-14 signal (δ 1.40) of nardoperoxide (**1a**) was observed at a lower field than that of isonardoperoxide (**2a**) (δ 1.23), while the H-4 signal (δ 2.83) of nardoperoxide (**1a**) resonated at higher field than that of isonardoperoxide (**2a**) (δ 3.17). The differences in the ^1H NMR signals of the two compounds (**1a** and **2a**) may be due to the anisotropic effect of the hydroxyl group on C-6, implying the stereochemistries of H-4, H-14 and the C-6 hydroxyl group as represented in the formulas **1a** and **2a**. These results were supported by NOE experiment. Irradiation of H-6 of isonardoperoxide (**2a**) enhanced H-14 signal, while no NOE was detected in H-14 by saturation of H-6 in nardoperoxide (**1a**).

The EI mass spectrum of nardoxide (**3**), $[\alpha]_D$ -16.2° (c 0.28, MeOH), a colorless oil, indicated a molecular ion peak at *m/z* 250, which differs from those of nardoperoxide (**1a**) and isonardoperoxide (**2a**) by a 16 mass unit. Both the ^1H and ^{13}C NMR spectra of nardoxide (**3**) were similar to those of nardoperoxide (**1a**), demonstrating that nardoxide (**3**) has an ether linkage in its molecule instead of the endoperoxide in nardoperoxide (**1a**). Chemical shifts of H-4 (δ 2.86 ppm) and H-14 (δ 1.36

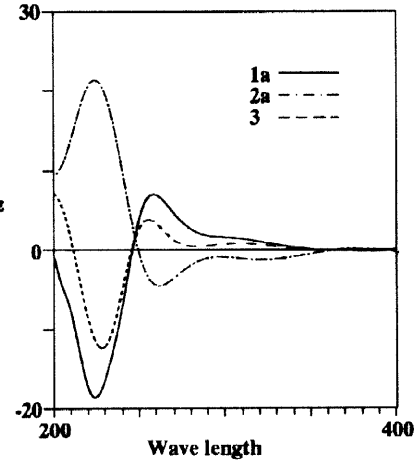

Fig. 1 CD Spectra of **1a**, **2a** and **3**.

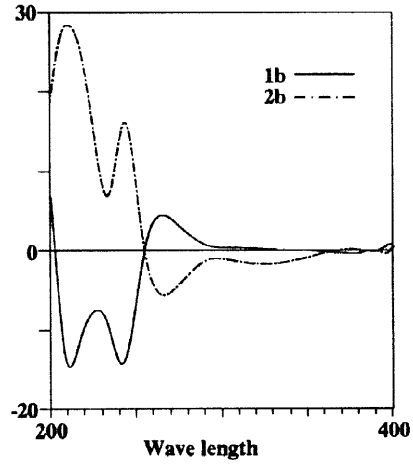

Fig. 2 CD Spectra of **1b** and **2b**.

ppm) of nardoxide (**3**) are almost the same as those of nardoperoxide (**1a**), and nardoxide (**3**) showed NOEs of H-6–H-8a and H-6–H-4. These data, thus, led to the conclusion that the relative configurations of nardoxide (**3**) and nardoperoxide (**1a**) are identical.

Absolute stereochemistry was determined by CD spectra of nardoperoxide (**1a**), isonardoperoxide (**2a**), isonardol (**3**) and their benzoylated derivatives.[5] The CD spectrum of nardoperoxide benzoate (**1b**) showed a negative Cotton effect at 242 nm ($\Delta\varepsilon$ -13.9) due to an interaction of the benzoyl chromophore with the enone group, demonstrating the *R* configuration at the C-6 benzoyl group (Fig. 2). On the other hand, isonardoperoxide benzoate (**2b**) exhibiting an antipodal CD spectrum (243 nm, $\Delta\varepsilon$ +15.8) to the nardoperoxide benzoate (**1b**) indicated the *S* configuration at the C-6 benzoyl group. Moreover, nardoxide (**3**) and nardoperoxide (**1a**) had similar CD spectra including the signs and amplitude of Cotton effects (Fig. 1). Thus, the absolute stereostructures of nardoperoxide (**1a**), isonardoperoxide (**2a**) and isonardol (**3**) were determined.

Antimalarial activities against *P. falciparum* and cytotoxicities against FM3A and KB cells of these compounds were examined. The EC_{50} values were 1.5×10^{-6}, 6.0×10^{-7} and $>4.0 \times 10^{-5}$ M, respectively (Table 2). It is noteworthy that the activities and selectivities of isonardoperoxide (**2a**) are comparable to those of quinine, a clinically used drug. Nardoperoxide (**1a**) and isonardoperoxide (**2a**) must be promising lead compounds of a new type of antimalarial drug.

Table 2. Antimalarial Activities against *P. falciparum* and Cytotoxicities against FM3A and KB cells.

	P. falciparum	FM3A cell		KB cell	
Compounds	EC50 (M)	EC50 (M)	Selectivity[b]	EC50 (M)	Selectivity[b]
nardoperoxide (**1a**)	1.5×10^{-6}	3.4×10^{-5}	23	$>6.4 \times 10^{-5}$ (92%)[a]	>43
isonardoperoxide (**2a**)	6.0×10^{-7}	$>4.5 \times 10^{-5}$ (80%)[a]	>75	1.0×10^{-4}	167
nardoxide (**3**)	$>4.0 \times 10^{-5}$ (86%)[a]	$>4.0 \times 10^{-5}$ (97%)[a]	>1	$>8.0 \times 10^{-5}$ (78%)[a]	>2
nardosinone (**4**)	4.5×10^{-6}	$>6.5 \times 10^{-5}$ (75%)[a]	>14	9.6×10^{-5}	21
chloroquine	1.8×10^{-8}	3.2×10^{-5}	1778	2.3×10^{-5}	1278
quinine	1.1×10^{-7}	1.0×10^{-4}	909	4.6×10^{-5}	418

[a] Growth percent at the concetration indicated. Concentration over 10^{-4} is not significant in the development of the drug.
[b] Selectivity = cytotoxicity / antimalarial activity

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4. Breitmaier, E.; Voelter, W. *Carbon-13 NMR Spectroscopy*; VCH: New York, 1987; p. 218.
5. Benzoylation of **1a** to **1b** and of **2a** to **2b**: A solution of **1a** (1 mg) in dry pyridine (1 ml) was stirred with benzoyl chloride (30 μl) for 12 h at room temperature. The solution was extracted with EtOAc to afford **1b**; EI-MS *m/z*: 369 $[M-1]^+$, 105 (base); ^1H NMR (300 MHz, $CDCl_3$) δ: 8.15 (2H, br. d, *J*=7.5, OBz), 7.58 (1H, tt, *J*=1.4, 7.5, OBz), 7.45 (2H, br. t, *J*=7.5, OBz), 6.02 (1H, s, H-6), 2.89 (1H, ddq, *J*=2.8, 6.9, 6.9, H-4), 2.72 (1H, dd, *J*=6.7, 18.7, H-3), 2.13 (1H, dd, *J*=2.5, 18.7, H-3), 1.69 (3H, s, H-15), 1.12 (1H, ddd, *J*=7.8, 9.9, 13.6, H-8), 1.10 (3H, d, *J*=6.5, H-14), 0.94 (3H, d, *J*=7.4, H-13), 0.84 (3H, d, *J*=6.5, H-12). Transformation of **2a** to **2b** was carried out in the same manner as mentioned above; EI-MS *m/z*: 370 $[M]^+$, 105 (base); ^1H NMR (300 MHz, $CDCl_3$) δ 8.15 (2H, br. d, *J*=7.5, OBz), 7.58 (1H, tt, *J*=1.5, 7.5, OBz), 7.45 (2H, br. t, *J*=7.5, OBz), 6.13 (1H, s, H-6), 2.84 (1H, dq, *J*=1.0, 6.9, H-4), 2.73 (1H, dd, *J*=6.6, 18.5, H-3), 2.08 (1H, dd, *J*=1.2, 18.5, H-3), 1.99 (1H, ddd, *J*=6.5, 13.7, 17.7, H-9), 1.85 (1H, dd, *J*=8.4, 13.7, H-8), 1.66 (3H, s, H-15), 1.33 (3H, d, *J*=6.9, H-14), 1.18 (1H, ddd, *J*=8.7, 10.0, 13.8, H-8), 0.92 (3H, d, *J*=6.9, H-13), 0.90 (3H, d, *J*=6.9, H-12).