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Phytochemistry 66 (2005) 2729–2733
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

# Unusual cyclolanostanes from leaves of *Pandanus boninensis*

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Received 19 April 2005; received in revised form 23 June 2005
Available online 14 October 2005

**Abstract**

Two unusual triterpenoids, (24*S*)-24-methyl-25,32-cyclo-5α-lanosta-9(11)-en-3β-ol and (24*S*)-24-methyl-25,32-cyclo-cycloartane-3β-ol, were isolated from leaves of *Pandanus boninensis* along with known triterpenoids and lignans. Their structures were established on the basis of spectroscopic methods and X-ray analysis.


*Keywords:* *Pandanus boninensis*; Pandanaceae; Leaf; Triterpenoid; 25,32-Cyclolanostane, 25,32-Cyclo-cycloartane; Lignan

## 1. Introduction

Plants of the genus *Pandanus* comprise about 500–600 species and are distributed mainly in tropical and subtropical regions. On the constituents of pandanus plants, pyrrolidine-type alkaloids from *Pandanus amaryllifolius* (Takayama et al., 2000), essential oils from *Pandanus latifolius* (MacLeod and Pieris, 1982), lignans and ionones (Takayama et al., 1999) and essential oils (Vahirua-Lechat et al., 1996) from *Pandanus tectorius,* lignans and benzofurans from *Pandanus odoratissimus* (Jong and Chau, 1998), as well as 4-hydroxybenzoic acid from *Pandanus odorus* (Peungvicha et al., 1998), have previously been characterized. *Pandanus boninensis* Warb. is indigenous to Bonin island in Japan, and has been used as roadside trees with the fruits used as food (Toyoda, 2003). However no phytochemical study of the plant has been studied to date. In this paper, we have isolated two novel triterpenoids from leaves of *P. boninensis* where structures were elucidated as (24*S*)-24-methyl-25,32-cyclo-5α-lanosta-9(11)-en-3β-ol (**1**) and (24*S*)-24-methyl-25,32-cyclo-cycloartane-3β-ol (**2**), respectively.

## 2. Results and discussion

After repeated column chromatography and HPLC separations of the $CHCl_3$-soluble part of a MeOH extract, compounds **1** and **2** were isolated together with known triterpenoids, 24,24-dimethyl-5α-lanosta-9(ll),25-dien-3β-ol (**3**) (Chakravarty et al., 1996), 24-methylenecycloartane-3β-ol (**4**) (Yoshida et al., 1989), and known lignans, (+)-sesamin (Pelter et al., 1982), (+)-kobusin (Iida et al., 1982), (+)-pinoresinol (Miyazawa et al., 1992), (+)-eudesmin (Iida et al., 1982), and salicifoliol (Gonzalez et al., 1989). Identification of known compounds was achieved by comparisons with previously reported spectroscopic data. Compound **1** exhibited a molecular formula of $C_{32}H_{54}O$ by high resolution EI-MS and had IR absorption at 3445 $cm^{-1}$ due to hydroxyl group. The $^1H$ NMR spectrum of **1**, analyzed with the aid of 2D NMR studies (COSY and NOESY), showed the presence of eight tertiary methyls, a secondary methyl ($\delta$ 0.86, *d*, $J = 6.8$ Hz), an olefinic proton ($\delta$ 5.22, m), and a methine proton geminal to hydroxyl group ($\delta$ 3.22, *dd*, $J = 11.7$, 4.4 Hz). In addition,

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doi:10.1016/j.phytochem.2005.08.019

Table 1
$^{13}C$ NMR spectroscopic data of compounds **1** and **2** [150 MHz, $CDCl_3$, $\delta$ (ppm)]

| No. | 1 | 2 |
|---|---|---|
| 1 | 36.1 | 32.0 |
| 2 | 27.8 | 30.4 |
| 3 | 78.9 | 78.9 |
| 4 | 39.1 | 40.5 |
| 5 | 52.5 | 47.1 |
| 6 | 21.4 | 21.1 |
| 7 | 28.1 | 26.0 |
| 8 | 41.8 | 48.0 |
| 9 | 148.5 | 20.0 |
| 10 | 39.4 | 26.1 |
| 11 | 115.0 | 26.5 |
| 12 | 37.1 | 32.9 |
| 13 | 44.3 | 45.3 |
| 14 | 47.0 | 48.8 |
| 15 | 33.9 | 35.6 |
| 16 | 27.8 | 28.2 |
| 17 | 51.0 | 52.4 |
| 18 | 14.4 | 18.0 |
| 19 | 22.3 | 29.9 |
| 20 | 36.6 | 36.6 |
| 21 | 18.5 | 18.4 |
| 22 | 33.5[a] | 33.6 |
| 23 | 33.6[a] | 33.6 |
| 24 | 23.6 | 23.6 |
| 25 | 19.8 | 19.8 |
| 26 | 22.4 | 22.4 |
| 27 | 22.8 | 22.8 |
| 28 | 28.2 | 25.5 |
| 29 | 15.7 | 14.0 |
| 30 | 18.5 | 19.3 |
| 31 | 19.6 | 19.6 |
| 32 | 27.1 | 27.2 |

[a] Assignments may be interchangeable in each vertical column.

characteristic non-equivalent protons of a cyclopropyl methylene group ($\delta$ 0.05 and 0.09, each *d*, $J = 4.0$ Hz) were observed. The $^{13}C$ NMR spectrum of **1** (Table 1) showed 32 carbons. The multiplicities of each carbon were made by HMQC experiments, which revealed the presence of nine methyls, ten methylenes, six methines, and seven quaternary carbon atoms. In the EI-MS, compound **1** exhibited important and prominent fragments at *m/z* 439 ($M^+ - CH_3$, base peak) and 313 [$M^+ - C_{10}H_{19}$(side-chain moiety) $- 2H$] which are characteristic fragmentation patterns of Δ9(11)-lanostane-type triterpenoids (Lakshmi et al., 1989; Chakravarty et al., 1996). In addition, the proton and carbon chemical shifts ascribable to A, B, C, and D rings in **1** were very similar to those of **3**. Thus the cyclopropyl methylene group in **1** is present in the side-chain and its structure was deduced from following 2D-NMR spectroscopic studies. Accordingly, **1** showed prominent correlation peaks between cyclopropane protons ($\delta$ 0.05 and 0.09) and three methyl carbons [$\delta$ 19.6 (C-31), 22.4 (C-26), and 22.8 (C-27)] in the HMBC spectrum as well as correlation peaks between cyclopropane protons and three methyl groups [$\delta$ 1.03 ($H_3$-31), 1.06 ($H_3$-27), and 1.09 ($H_3$-26)] in the NOESY spectrum (Fig. 1). Hence, the cyclopropane ring in **1** is located at C-32 and three methyl groups are connected to C-24 and terminal C-25, respectively. The signal at $\delta$ 3.22 was assigned as 3α-H due to its coupling constants (*dd*, $J = 11.7$, 4.4 Hz) and showed HMBC correlations with C-4, C-28 and C-29 ($\delta$ 39.1, 28.2, and 15.7, respectively). Based on the above evidence, the structure of **1** was deduced as 24-methyl-25,32-cyclolanosta-9(11)-en-3β-ol (**1**). The final structure of **1** was unequivocally established by X-ray analysis of its acetate (**1a**). Acetylation of **1** with $Ac_2O$-pyridine afforded an acetate (**1a**), quantitatively. Single crystals of **1a** were subjected to X-ray diffraction analysis. Fig. 2 indicates an ORTEP drawing of **1a** which revealed the relative structure of **1** including a novel 25, 32-cyclopropane ring in the side-chain. Based on the evidence and the biogenetic grounds as well as precise spectroscopic comparisons of **1** and analogous lanostane-type triterpenoids, the structure of **1** was established as (24*S*)-24-methyl-25,32-cyclo-5α-lanosta-9(11)-en-3β-ol.

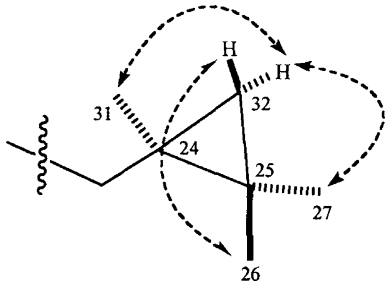
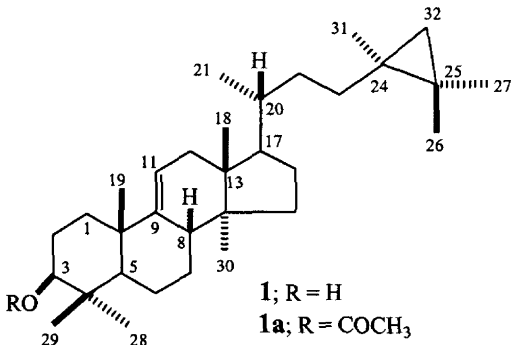
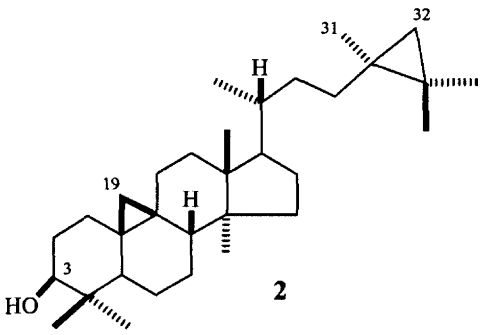



Fig. 1. Selected NOESY correlations of **1** and **2**.



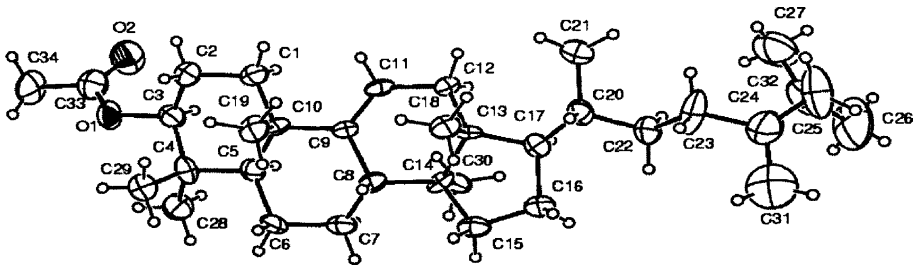



Fig. 2. The ORTEP depiction of **1a**. Anisotropic ellipsoids are represented by a 30% probability level.

Compound **2** exhibited a molecular formula of $C_{32}H_{54}O$ as revealed molecules by its high resolution EI-MS and had IR absorption at 3430 $cm^{-1}$ due to the hydroxyl group. The $^1H$ NMR spectrum of **2**, analyzed with aid of 2D NMR spectroscopic studies showed the presence of seven tertiary methyls, a secondary methyl ($\delta$ 0.85, *d*, $J = 6.6$ Hz), and a methine proton geminal to hydroxyl group ($\delta$ 3.29, *dd*, $J = 11.4$, 4.2 Hz). In addition, two pairs of cyclopropyl methylene protons ($\delta$ 0.05 and 0.09, each *d*, $J = 3.8$ Hz; $\delta$ 0.33 and 0.56, each *d*, $J = 4.2$ Hz) were present. The $^{13}C$ NMR spectrum of **2** (Table 1) showed 32 carbons consisting of eight methyls, twelve methylenes, five methines, and seven quaternary carbon atoms. In the EI-MS, compound **2** showed important and prominent fragment at *m/z* 315 [$M^+ - C_{10}H_9$(side-chain moiety)] which is a characteristic fragmentation pattern of cycloartane-type triterpenoids (Yoshida et al., 1989; Yano et al., 1992). A precise comparison of the $^1H$ and $^{13}C$ NMR spectra of **1** and **2** revealed that the side-chain moiety in **2** is same with **1**. However, the signal due to an olefinic proton (H-11) in **1** was absent in **2**, being replaced by a cyclopropyl methylene group ($\delta$ 0.33 and 0.56). In addition, the proton and carbon chemical shifts ascribable to A, B, C, and D rings in **2** were essentially same with those of compound **4** and cyclolartane-3β-ol (Milon et al., 1989). On the basis of the above evidence as well as the precise spectroscopic comparisons of **2** and analogous cycloartane-type triterpenoids, the structure of **2** was concluded to be (24*S*)-24-methyl-25,32-cyclo-cycloartane-3β-ol (**2**). The occurrence of triterpenoids having a cyclopropane ring in the side-chain such as **1** and **2** is very rare in nature, because only one homocyclotirucallane, named sinetirucallol, has so far been isolated from *Spiranthes sinensis* (Lin et al., 2001) to the best our knowledge. Further, co-occurrence of compounds **1**–**4** in the same plant is interesting from a biogenetic viewpoint.

## 3. Experimental

### 3.1. General

Mps are uncorr. $^1H$ and $^{13}C$ NMR spectra were measured on a JEOL JNM-ECA 600SN ($^1H$ at 600 MHz and $^{13}C$ NMR at 150 MHz) spectrometer with $CDCl_3$ as a solvent and TMS as an int. standard. El- and HR EI-MS spectra were obtained with a JEOL JMS-700T spectrometer. IR were measured with JASCO FT/IR-410 spectrometer with KBr disks. Optical rotations were measured on a JASCO DIP-140 polarimeter. Preparative HPLC was performed on a JAI LC-908 instrument using JAIODS-120T column with a differential refractometer.

### 3.2. Plant material

Leaves of *Pandanus boninensis* Warb. were collected in February 2002 in the Bonin island, Japan and a voucher specimen (No. 119) has been deposited at the Herbarium of the Faculty of Pharmaceutical Sciences, Setsunan University.

### 3.3. Extraction and isolation

The crushed leaves (800 g) were extracted with MeOH ($3 \times 61$) at room temperature. The MeOH solution was evaporated in vacuo to give an extract (86.0 g). The MeOH extract (85.0 g) was next suspended with $H_2O$ and the aqueous suspension extracted with $CHCl_3$($3 \times 0.81$). The resulting $CHC1_3$ extract (16.7 g) was subjected to silica gel chromatography with $CHCl_3$–MeOH containing an increasing MeOH concentration to give 10 fractions (A–J). Fraction C (0.48 g) containing **1**, **2**, and other constituents was further purified by repeated preparative HPLC separation eluting with $CH_3CN$–$CHCl_3$ (3:1) to afford **1** (78 mg), **2** (13 mg), **3** (4.2 mg), and **4** (3.0 mg), respectively. Fractions containing lignans (Fractions F–H) were further separated by HPLC to afford (+)-sesamin (1.8 mg), (+)-kobusin (2.0 mg), (+)-pinoresinol (27 mg), (+)-eudesmin (23 mg), and salicifoliol (2.0 mg), respectively.

### 3.4. (24S)-24-methyl-25,32-cyclo-5α-lanosta-9(11)-en-3β-ol (1)

Colorless fine crystals, m.p. 211–212° (MeOH); $[\alpha]_D^{20} + 69.6°$ (*c* 1.00, $CHCl_3$); IR $\nu_{max}$ $cm^{-1}$: 3445, 2940, 1455, 1370, 1040; El- and high resolution EI-MS *m/z* (rel. int.): 454.4178 ($M^+$, $C_{32}H_{54}O$ requires 454.4174, 57), 439 (100), 421 (68), 314 (30), 313.2533 ($M^+$-side-chain-2H, $C_{22}H_{33}O$ requires 313.2531, 74), 273 (25); $^1$HNMR $\delta$ 0.05 (1H, *d*, $J = 4.0$ Hz) and 0.09 (1H, *d*, $J = 4.0$ Hz) ($H_2$-32), 0.64 (3H, *s*, $H_3$-18), 0.74(3H, *s*, $H_3$-30), 0.82 (3H, *s*, $H_3$-29), 0.86, (3H, *d*, $J = 6.8$ Hz, $H_3$-21), 0.99 (3H, *s*, $H_3$-28), 1.03 (3H, *s*, $H_3$-31), 1.05 (3H, *s*, $H_3$-19), 1.06 (3H, *s*, $H_3$-

27), 1.09(3H, *s*, $H_3$-26), 3.22 (1H, *dd*, $J = 11.7$, 4.4 Hz, H-3α), 5.22 (IH, *m*, H-11). For $^{13}C$ NMR spectroscopic data, see Table 1.

### 3.5. (24S)-24-methyl-25,32-cyclo-cycloartane-3β-ol (2)

Amorphous powder, $[\alpha]_D^{20} + 38.8°$ (*c* 0.54, $CHCl_3$); IR $\nu_{max}$ $cm^{-1}$: 3430, 2920, 1469, 1375, 1050, 1020; El- and high resolution EI-MS *m/z* (rel. int.): 454.4168 ($M^+$, $C_{32}H_{54}O$ requires 454.4174, 55), 436 (98), 421 (100), 409 (23), 315.2688 ($M^+$-side-chain, $C_{22}H_{35}O$ requires 315.2688, 42), 314 (73); $^1H$ NMR δ 0.05 (1H, *d*, $J = 3.8$ Hz) and 0.09 (1H, *d*, $J = 3.8$ Hz) ($H_2$-32), 0.33 (1H, *d*, $J = 4.2$ Hz) and 0.56 (1H, *d*, $J = 4.2$ Hz) ($H_2$-19), 0.81 (3H, *s*, $H_3$-29), 0.85. (3H, *d*, $J = 6.6$ Hz, $H_3$-21), 0.90 (3H, *s*, $H_3$-30), 0.96 (3H, *s*, $H_3$-18), 0.97 (3H, *s*, $H_3$-28), 1.03 (3H, *s*, $H_3$-31), 1.06 (3H, *s*, $H_3$-27), 1.09 (3H, *s*, $H_3$-26), 3.29 (1H, *dd*, $J = 11.4$, 4.2 Hz, H-3α). For $^{13}C$ NMR spectroscopic data, see Table 1.

### 3.6. Acetylation of compound 1

A solution of **1** (21 mg) in py. (2 ml) and $Ac_2O$ (0.5 ml) was left standing overnight at room temp, poured into ice-water and extracted with $Et_2O$. After usual work-up, the residue was recrystallized with MeOH to give colorless plates (11 mg) of **1a**. M.p. 195–198° (MeOH); IR $\nu_{max}$ $cm^{-1}$: 2940, 1735, 1240, 1031; EI- and high resolution EI-MS *m/z* (rel. int.): 496.4274 ($M^+$, $C_{34}H_{56}O_2$ requires 496.4280, 47), 481 (100), 421 (82), 355 ($M^+$-side-chain-2H, 90), 316 (20); $^1H$ NMR δ 0.05 (IH, *d*, $J = 3.9$ Hz) and 0.09 (IH, *d*, $J = 3.9$ Hz) ($H_2$-32), 0.64 (3H, *s*, $H_3$-18), 0.74 (3H, *s*, $H_3$-30), 0.86 (3H, *d*, $J = 5.9$ Hz, $H_3$-21), 0.87 (3H, *s*, $H_3$-28), 0.89 (3H, *s*, $H_3$-29), 1.03 (3H, *s*, $H_3$-31), 1.06 (3H, *s*, $H_3$-19), 1.07 (3H, *s*, $H_3$-27), 1.09 (3H, *s*, $H_3$-26), 2.05 (3H, *s*, OAc), 4.48 (IH, *dd*, $J = 11.2$, 4.5 Hz, H-3α), 5.22 (1H, *m*, H-11).

### 3.7. X-ray analysis of 1a

Colorless plates, $C_{34}H_{56}O_2$, orthorhombic, space group $P2_12_12_1$, $a = 7.410(2)$ Å, $b = 10.557(2)$ Å, $c = 39.718(4)$ Å, $V = 3107.0(11)$ Å$^3$, $\alpha = \beta = \gamma = 90°$, $Z = 4$, $D_{calc} = 1.062$ g/cm$^{-3}$. The intensity data were collected using graphite monochromated Mo Kα radiation. SHELXTL-NT Version 5.1 (Bruker, 1999) was used for unit cell determination, data collection and data reduction. Total number of data is 6271, while observed data is 1387 with $F_o > 4\,\mathrm{Sig}(F_o)$. The crystal showed no variation in intensities of three check reflections during the course of data collection, Lorentz and polarization corrections were applied. Absorption correction has also been applied. The structure was solved by direct method using the software SHELXTL-NT Ver5.1 inserted in BRUKER AXS diffractometer and refined using SHELXL 97. Non-H atoms were refined anisotropically by full matrix least squares techniques. All H atoms were included at geometrically calculated positions and allowed to ride on their parent atoms with $U_{iso}(H) = 1.5U_{eq}$(parent) for methyl H atoms and $U_{iso} = 1.2U_{eq}$ for all other H atoms. All geometrical calculations were done with PARST to give final $R = 0.056$ ($wR = 0.145$). Fig. 2 shows ORTEP (Burnett and Johnson, 1996) drawing of **1a** which confirmed the whole structure of **1a**. Crystallographic data have been deposited at the Cambridge Crystallographic Data Center (CCDC) (deposition number CCDC 275105). These data can be obtained free of the charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033; e-mail; deposit@ccdc.cam.ac.uk).

## Acknowledgements

We are grateful to Ogasawara branch of the Tokyo Metropolitan Government in Japan for permission to collect the plant material.

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