Lanostane-Type Triterpenoids from *Diospyros discolor*

Chiy-Rong CHEN,^a Chao-Wen CHENG,^b Min-Hsiung PAN,^c Yun-Wen LIAO,^b Chih-Ying TZENG,^b and Chi-I CHANG^{*,b}

^aInstitute of Molecular Biology, National Chung Hsing University; Taichung 402, Taiwan; ^bGraduate Institute of Biotechnology, National Pingtung University of Science and Technology; Pingtung 912, Taiwan; and ^cDepartment of Seafood Science, National Kaohsiung Marine University; Kaohsiung 811, Taiwan.

Received November 7, 2006; accepted March 11, 2007

Four new lanostane-type triterpenes, 24-ethyl-3 β -methoxylanost-9(11)-en-25-ol (1), 3 β -methoxy-24-methylenelanost-9(11)-en-25-ol (2), 3 β -methoxy-25-methyl-24-methylenelanost-9(11)-en-21-ol (3) and 3 β -methoxy-24-methyl-25-dien-24-ol (4) together with three known triterpenes, betulinaldehyde, betulinic acid methyl ester, and ursaldehyde have been isolated from the methanol extract of the twigs of *Diospyros discolor*. The structures of those new compounds were elucidated by spectroscopic methods.

Key words *Diospyros discolor*; triterpene; 24-ethyl-3 β -methoxylanost-9(11)-en-25-ol; 3 β -methoxy-24-methylenelanost-9(11)-en-25-ol; 3 β -methoxy-25-methyl-24-methylenelanost-9(11)-en-21-ol; 3 β -methoxy-24-methyl-25-dien-24-ol

Plants in the *Diospyros* genus (Ebenacea) are well documented as a rich sources of naphthoquinones and triterpenes, which have been found to exhibit ichthyotoxic,^{1,2)} antimicrobial,^{2–4)} and antitumor activities.^{4,5)} Thirteen species of this genus are indigenous to Taiwan. Several species, including fruits of *D. discolor* WILLD.,⁶⁾ leaves of *D. kaki* THUNB.,⁷⁾ barks and stems of *D. eriantha* CHAMP.,^{8,9)} stems of *D. morrisiana* HANCE.,^{10–12)} fruits of *D. ferrea*,¹³⁾ and bark, root, fruits, leaves, and twigs of *D. maritima* BLUME.^{1–5,14–22)} have been studied for their chemical constituents, resulting in the isolation and structure elucidation of various triterpenes, lignans, steroids, benzoquinones, and naphthoquinone. Previously, we had reported on the isolation of some new naphthoquinones^{15,16)} and triterpenes from stems of *D. maritima*.^{5,17–21)} As a part of our continuing interest in secondary metabolites from the *Diospyros* plants, we investigated the chemical principles of the twigs of *D. discolor* and isolated four new lanostane-type triterpenes: 24-ethyl-3 β -methoxylanost-9(11)-en-25-ol (1), 3 β -methoxy-24-methylenelanost-9(11)-en-25-ol (2), 3 β -methoxy-25-methyl-24-methylenelanost-9(11)-en-21-ol (3) and 3 β -methoxy-24-methyl-25-dien-24-ol (4); in addition to three known triterpenes, betulinaldehyde,²³⁾ betulinic acid methyl ester,²⁴⁾ and ursaldehyde.²⁵⁾ In this paper, we report the extraction, isolation, purification, and structural elucidation of four new

lanostane-type triterpenes on the basis of extensive spectroscopic analysis, including 1D and 2D NMR experiments.

Compound 1 gave a positive Liebermann–Burchard test and displayed a molecular ion peak at m/z 486.4422 in its HR-ESI-MS, suggesting a molecular formula of C₃₃H₅₈O₂. The IR spectrum showed the presence of hydroxyl (3438 cm^{−1}) and double bond (1635, 875 cm^{−1}) functionalities. The ¹H-NMR spectrum of 1 (Table 1) indicated the presence of seven tertiary methyls [δ_H 0.62, 0.72, 0.78, 0.95, 1.02 (3H each, s), 1.16 (3H $\times$ 2, s)], one secondary methyl [δ_H 0.88 (3H, d, J =6.4 Hz)], a ethyl [δ_H 1.22 m, 0.94 (3H, t, J =7.2 Hz)], a methoxyl [δ_H 3.35 (3H, s)], one axial-oriented methoxymethine [δ_H 2.62 (1H, dd, J =4.0, 11.2 Hz)], and a typical H-11 proton of lanosta-9(11)-ene [δ_H 5.19 (1H, br d, J =6.0 Hz)].²⁶⁾ The ¹³C-NMR spectrum of 1 revealed 33 carbon signals, which were identified by the assistance of a DEPT experiment as nine methyls, one methoxy carbon, ten sp^3 methylenes, five secondary sp^3 carbonyl carbons, one oxygenated sp^3 methine, five sp^3 quaternary carbons, and two coupled sp^2 olefinic carbons (δ_C 115.0, 148.9). The MS spectrum of 1 show [M]⁺ at m/z 486 accompanied with the major fragment ions at m/z 468 [M–H₂O]⁺, 453 [M–H₂O–CH₃]⁺, and 327 [M–C₁₀H₂₁O (side chain)–2H]⁺. In addition, the major fragment ions at m/z 255, 241 and 229 supported that the double bond located at C-9 and C-11.²⁷⁾ From the above evidences, compound 1 was considered as lanosta-9(11)-ene triterpene with a C₁₀H₂₁O side chain. Detailed comparison of the ¹H- and ¹³C-NMR data of 1 with those of the known lanostane-type triterpene, 25,26,27-trinor-3 β -methoxylanost-9(11)-en-24-oic acid, isolated from the stem bark of *Pinus luchuensis*,²⁸⁾ shows that the signals of the tetracyclic part were very similar to each other, except for the signals of C-20–C-27, C-31, and C-32 in the side chain part. A down-field singlet signal of methyl [δ_H 1.16 (3H $\times$ 2, s)] and the HMBC correlations between H-21 (δ_H 0.88) and C-17 (δ_C 51.0), C-20 (δ_C 37.2), and C-22 (δ_C 36.7); H-32 (δ_H 0.94) and C-24 (δ_C 52.3) and C-31 (δ_C 24.0); H-26 (δ_H 1.16) and C-24 and C-25 (δ_C 74.5) supported that a hydroxyl attached on C-25 and a ethyl [δ_H 1.22 (2H, m), 0.94 (3H, t, J =7.2 Hz)] attached on C-24, respectively. Complete ¹H and ¹³C chemical shifts were established by ¹H–¹H COSY, HMQC, HMBC and NOESY spectra. Thus, the structure of

* To whom correspondence should be addressed. e-mail: changchii@mail.npust.edu.tw

Table 1. ^1H -NMR Data for **1**–**4** (400 MHz in CDCl_3)

Position	1	2	3	4
1	1.39 m, 1.82 m	1.41 m, 1.83 m	1.39 m, 1.82 m	1.38 m, 1.82 m
2	1.44 m, 1.88 m	1.44 m, 1.93 m	1.47 m, 1.92 m	1.46 m, 1.91 m
3	2.62 dd (4.0, 11.2)	2.63 dd (4.0, 11.2)	2.62 dd (4.0, 11.2)	2.62 dd (4.0, 11.2)
5	0.87 m	0.89 m	0.86 m	0.87 m
6	1.43 m, 1.62 m	1.46 m, 1.67 m	1.49 m, 1.68 m	1.44 m, 1.66 m
7	1.30 m, 1.65 m	1.32 m, 1.66 m	1.27 m, 1.66 m	1.28 m, 1.66 m
8	2.18 m	2.18 m	2.18 m	2.16 m
11	5.19 br d (6.0)	5.20 br d (6.0)	5.20 br d (6.0)	5.19 br d (6.0)
12	1.92 m, 2.04 m	1.92 m, 2.08 m	1.90 m, 2.08 m	1.89 m, 2.05 m
15	1.36 m	1.36 m	1.40 m	1.34 m
16	1.30 m, 1.88 m	1.30 m, 1.60 m	1.36 m, 1.92 m	1.32 m, 1.88 m
17	1.62 m	1.65 m	1.94 m	1.60 m
18	0.62 s	0.63 s	0.66 s	0.62 s
19	1.02 s	1.02 s	1.02 s	1.02 s
20	1.36 m	1.41 m	1.52 m	1.37 m
21	0.88 d (6.4)	0.90 d (6.4)	3.62 br d (10.4), 3.74 br d (10.4)	0.85 d (6.0)
22	1.04 m, 1.54 m	1.28 m, 1.64 m	1.60 m	0.92 m, 1.33 m
23	1.02 m, 1.58 m	1.95 m, 2.19 m	1.88 m, 2.06 m	1.46 m, 1.62 m
24	1.04 m			
26	1.16 s	1.33 s	1.04 s	4.80 br s, 4.94 br s
27	1.16 s	1.33 s	1.04 s	1.72 s
28	0.95 s	0.95 s	0.95 s	0.94 s
29	0.78 s	0.78 s	0.78 s	0.77 s
30	0.72 s	0.72 s	0.74 s	0.71 s
31	1.22 m	4.75 br d (0.8), 5.07 br s	4.68 br d (0.8), 4.84 br s	1.29 s
32	0.94 t (7.2)		1.04 s	
–OMe	3.35 s	3.35 s	3.35 s	3.35 s

1 was determined as 24-ethyl-3 β -methoxylanost-9(11)-en-25-ol.

The HR-EI-MS of **2** showed a molecular ion peak at m/z 470.4106, which was corresponded to the molecular formula, $\text{C}_{32}\text{H}_{54}\text{O}_2$, and indicated six degrees of unsaturation. The IR spectrum displayed absorptions for hydroxyl (3470 cm^{-1}) and terminal double bond (3070 , 1653 , 901 cm^{-1}) functionalities. The ^1H - and ^{13}C -NMR spectra of **2** (Tables 1, 2) revealed the presence of seven tertiary methyls [δ_{H} 0.63, 0.72, 0.78, 0.95, 1.02 (3H each, s), 1.33 (3H $\times$ 2, s)], a secondary methyl [δ_{H} 0.90 (3H, d, $J=6.4\text{ Hz}$)], a terminal double bond [δ_{H} 4.75 (1H, br d, $J=0.8\text{ Hz}$), 5.07 (1H, br s); δ_{C} 106.9 (t), 157.1 (s)], and a trisubstituted double bond [δ_{H} 5.20 (1H, d, $J=6.0\text{ Hz}$); δ_{C} 115.0 (d), 148.9 (s)]. The above NMR characteristics coupled by the unambiguous comparison of ^{13}C -NMR data corresponding to **1** and **2** revealed that **2** was structurally identical to **1** with the same lanost-9(11)-ene skeleton. The only difference was in the signals of C-20, C-22–C-27 and C-31 of the side chain part. A downfield singlet signal of methyls, H-26 and H-27 (δ_{H} 1.33) showed long range coupling to C-24 (δ_{C} 157.1) and C-25 (δ_{C} 73.8) in the HMBC experiment, thus suggesting a hydroxyl group attached on C-25 and neighboring to the terminal double [δ_{C} 157.0 (s), 106.9 (t)]. Additionally, one of the germinal protons $\text{H}_{\text{a}}\text{-31}$ (δ_{H} 5.07) showed NOESY correlations with H-26 and H-27, while two germinal protons, $\text{H}_{\text{a}}\text{-31}$ and $\text{H}_{\text{b}}\text{-31}$ (δ_{H} 4.75), exhibited HMBC correlations with C-23 (δ_{C} 28.2), C-24 (δ_{C} 157.1), and C-25 (δ_{C} 73.8), hence the side chain structure was assigned as $\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_2\text{C}(\text{CH}_2)\text{C}(\text{CH}_3)_2\text{OH}$. The base fragment ion at m/z 327 [$\text{M}-\text{C}_9\text{H}_{17}\text{O}$ (side chain)–2H] $^+$ in the MS spectrum of **2** further confirmed the proposed structure. Consequently, compound **2** was elucidated to be 3 β -methoxy-24-methylenelanost-9(11)-en-25-ol.

Compound **3** exhibited a molecular formula of $\text{C}_{33}\text{H}_{56}\text{O}_2$ from the molecular ion at m/z [M] $^+$ 484.4270 by HR-EI-MS, indicating the presence of six degrees of unsaturation. The IR spectrum revealed absorption bands at 3466 cm^{-1} (hydroxyl) and terminal double bond (3040 , 1632 , 890 cm^{-1}) functionalities. The ^1H -NMR spectrum of **3** (Table 1) showed resonances for eight methyls [δ_{H} 0.66, 0.74, 0.78, 0.95, 1.02 (3H each, s), 1.04 (3H $\times$ 3, s)], a hydroxymethylene group attached to a tertiary carbon [δ_{H} 3.62 (1H, br d, $J=10.4\text{ Hz}$), 3.74 (1H, br d, $J=10.4\text{ Hz}$)], an axial methine proton bearing a methoxy [δ_{H} 2.62 (1H, dd, $J=4.0$, 11.2 Hz)], two germinal olefinic protons [δ_{H} 4.68 (1H, br d, $J=0.8\text{ Hz}$), 4.84 (br s)], and a trisubstituted double bond [δ_{H} 5.20 (1H, d, $J=6.0\text{ Hz}$)]. A comparison of the ^1H - and ^{13}C -NMR spectra between **2** and **3** (Table 2) suggested that **3** was a lanost-9(11)-ene triterpene with a different side chain. The oxygenated methylene H-21 (δ_{H} 3.62, 3.74) exhibited the HMBC correlations with C-17 (δ_{C} 45.0), C-20 (δ_{C} 43.3), and C-22 (δ_{C} 30.1) suggesting a primary alcohol located on C-21. The singlet signal of three chemically equivalent methyls [δ_{H} 1.04 (3H $\times$ 3, s, H-26, H-27, H-32)] observed in the ^1H -NMR spectrum of **3**, which showed the NOESY correlations with H-31 (δ_{H} 4.68, 4.84) and HMBC correlations with C-24 (δ_{C} 158.8) led us to elucidate the structure of the side chain as $\text{CH}(\text{CH}_2\text{OH})\text{CH}_2\text{CH}_2\text{C}(\text{CH}_2)\text{C}(\text{CH}_3)_3$. ^1H - ^1H COSY, HMQC, and HMBC analysis, together with the fragment ion at m/z 327 [M –side chain–2H] $^+$ further confirmed the assigned structure. The stereochemistry of C-20 was assigned as *R* configuration due to the NOESY correlations between H-18 (δ_{H} 0.66) and H-20 (δ_{H} 1.52); and H-12 (δ_{H} 1.90) and H-21 (δ_{H} 3.62, 3.74). Thus, compound **3** was accordingly determined as 3 β -methoxy-25-methyl-24-methylenelanost-9(11)-en-21-ol.

Table 2. ^{13}C -NMR Data for **1**–**4** (100 MHz in CDCl_3)

Position	1	2	3	4
1	36.2 t	36.2 t	36.2 t	36.2 t
2	22.8 t	22.8 t	22.7 t	22.8 t
3	88.8 d	88.8 d	88.8 d	88.8 d
4	39.2 s	39.3 s	39.3 s	39.2 s
5	53.2 d	53.2 d	53.2 d	53.2 d
6	21.5 t	21.5 t	21.5 t	21.5 t
7	28.4 t	28.4 t	28.4 t	28.5 t
8	42.3 d	42.0 d	42.1 d	42.0 d
9	148.9 s	148.9 s	149.2 s	148.9 s
10	39.6 s	39.7 s	39.7 s	39.6 s
11	115.0 d	115.0 d	114.7 d	115.0 d
12	37.3 t	37.3 t	36.7 t	37.3 t
13	44.5 s	44.6 s	44.3 s	44.5 s
14	47.3 s	47.3 s	47.3 s	47.2 s
15	34.1 t	34.1 t	34.0 t	34.2 t
16	28.3 t	28.3 t	27.8 t	28.2 t
17	51.0 d	51.1 d	45.0 d	51.0 d
18	14.6 q	14.6 q	14.9 q	14.6 q
19	22.5 q	22.5 q	22.5 q	22.5 q
20	37.2 d	36.6 d	43.3 d	36.3 d
21	18.7 q	18.7 q	62.8 t	18.7 q
22	36.7 t	36.1 t	30.1 t	30.2 t
23	27.5 t	28.2 t	28.4 t	37.0 t
24	52.3 d	157.1 s	158.8 s	75.8 s
25	74.5 s	73.8 s	36.5 s	150.6 s
26	27.6 q	29.5 q	29.6 q	109.8 t
27	27.7 q	29.5 q	29.6 q	19.7 q
28	28.5 q	28.5 q	28.5 q	28.5 q
29	16.7 q	16.7 q	16.7 q	16.7 q
30	18.7 q	18.7 q	18.7 q	18.7 q
31	24.0 t	106.9 t	106.3 t	28.1 q
32	14.0 q			
–OMe	57.8 q	57.8 q	57.8 q	57.8 q

The molecular formula of **4** was assigned as $\text{C}_{32}\text{H}_{54}\text{O}_2$, on the basis of the HR-EI-MS ($[\text{M}]^+ m/z$ 470.4103), ^{13}C -NMR and DEPT spectra. Analysis of the IR spectrum of **4** suggested that it contains 3486 cm^{-1} (hydroxyl), and a terminal double bond ($3050, 1652, 895\text{ cm}^{-1}$). Both the ^1H - and ^{13}C -NMR data (Tables 1, 2) of **4** were identical to those of **2**, with respect to the side chain part. The ^1H - and ^{13}C -NMR spectra of **4** revealed an isopropenyl group [δ_{H} 1.72 (3H, s), 4.80 (1H, brs), 4.94 (1H, brs); δ_{C} 19.7 (q), 109.8.0 (t), 150.6 (s)], a downfield methyl [δ_{H} 1.29 (3H, s)] attached to an oxygenated carbon C-24 (δ_{C} 75.8). From the observations of the HMBC experiment, the correlations between H-26 (δ_{H} 4.80, 4.94) and C-24, C-25 (δ_{C} 150.6), and C-27 (δ_{C} 19.7); H-27 (δ_{H} 1.72) and C-24, C-25, and C-26 (δ_{C} 109.8); H-31 (δ_{H} 1.29) and C-23 (δ_{C} 37.0), C-24, and C-25 suggested the structure of the side chain was $\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_2\text{C}(\text{CH}_3)(\text{OH})\text{C}(\text{CH}_2)\text{CH}_3$, which was also supported by the fragment ion peaks of EI-MS spectrum at m/z 327 [M –side chain– 2H^+]. Thus, compound **4** was elucidated to be 3 β -methoxy-24-methylstan-9(11),25-dien-24-ol.

Experimental

General Experimental Procedures Optical rotations were measured using a JASCO DIP-180 digital spectropolarimeter. UV spectra were measured in MeOH on a Shimadzu UV-1601PC spectrophotometer. IR spectra were recorded on a Nicolet 510P FT-IR spectrometer. NMR spectra were recorded in CDCl_3 at room temperature on a Varian Mercury plus 400 NMR spectrometer, and the solvent resonance was used as internal shift reference (TMS as standard). The 2D NMR spectra were recorded using standard pulse sequences. EI-MS and HR-EI-MS were recorded on a Finnigan TSQ-

700 and a JEOL SX-102A spectrometer, respectively. TLC was performed using silica gel 60 F_{254} plates (Merck). CC was performed on silica gel (230–400 mesh ASTM, Merck). HPLC was performed using a Lichrosorb silica gel 60 ($5\text{ }\mu\text{m}$) column ($250\times 10\text{ mm}$).

Plant Material The twigs of *D. discolor* were collected in Ping-Tung, Taiwan, in July, 2004. The plant material was identified by Prof. Sheng-Zehn Yang, Department of Forestry, National Pingtung University of Science and Technology. A voucher specimen (no. 8517) has been deposited at the Herbarium of this same institution.

Extraction and Isolation Air-dried pieces of the twigs of *D. discolor* (21 kg) were extracted three times with methanol (60 l) at room temperature (7 d each). The MeOH extract was evaporated *in vacuo* to afford a black residue, which was suspended in H_2O (2 l) and then partitioned sequentially using EtOAc and *n*-BuOH (11 $\times$ 3). The EtOAc fraction (503 g) was chromatographed over silica gel, using mixtures of *n*-hexane and EtOAc of increasing polarity as eluents. Twelve fractions were collected as follows: 1 [4 l, *n*-hexane–EtOAc (19:1)], 2 [3 l, *n*-hexane–EtOAc (9:1)], 3 [3 l, *n*-hexane–EtOAc (8:2)], 4 [4 l, *n*-hexane–EtOAc (7:3)], 5 [4 l, *n*-hexane–EtOAc (6:4)], 6 [3 l, *n*-hexane–EtOAc (5:5)], 7 [(3 l, *n*-hexane–EtOAc (4:6)], 8 [(4 l, *n*-hexane–EtOAc (3:7)], 9 [(3 l, *n*-hexane–EtOAc (2:8)], 10 [(3 l, *n*-hexane–EtOAc (1:9)], 11 [(3 l, *n*-hexane–EtOAc (1:19)], 12 [(7 l, EtOAc). Fraction 4 was further chromatographed on a silica gel column ($5\times 45\text{ cm}$), eluted with CH_2Cl_2 –EtOAc (100:1) to yield nine fractions (800 ml each), 4A–I. HPLC of fraction 4G with *n*-hexane– CH_2Cl_2 –EtOAc (20:12:1) as eluent, 2 ml/min, afforded **4** (5 mg, $t_{\text{R}}=19.2\text{ min}$). HPLC of fraction 4H with *n*-hexane–EtOAc (7:3) as eluent, 2 ml/min, afforded **3** (8 mg, $t_{\text{R}}=18.5\text{ min}$). HPLC of fraction 4I with CH_2Cl_2 –EtOAc (40:1) as eluent, 2 ml/min, afforded **1** (3 mg, $t_{\text{R}}=17.2\text{ min}$) and **2** (4 mg, $t_{\text{R}}=21.8\text{ min}$), respectively. Fraction 5 was further chromatographed on a silica gel column ($5\times 45\text{ cm}$) eluted with CH_2Cl_2 –EtOAc (40:1) to generate ten fractions (each 800 ml), 5A–J. HPLC of fraction 5F with *n*-hexane– CH_2Cl_2 –EtOAc (16:6:1) as eluent, 2 ml/min, afforded betulinolaldehyde (12 mg, $t_{\text{R}}=20.6\text{ min}$). HPLC of fraction 5G with *n*-hexane–EtOAc (3:1) as eluent, 2 ml/min, afforded betulinic acid methyl ester (6 mg, $t_{\text{R}}=17.2\text{ min}$) and ursaldehyde (8 mg, $t_{\text{R}}=23.2\text{ min}$), respectively.

24-Ethyl-3 β -methoxystan-9(11)-en-25-ol (**1**): Amorphous, white powder; $[\alpha]_{\text{D}}^{25} +45.2^\circ$ ($c=0.3$, CHCl_3); IR (KBr) ν_{max} 3438, 2933, 2868, 1635, 1456, 1376, 1363, 1101, 981, 875, 741 cm^{-1} ; ^1H - and ^{13}C -NMR data, see Tables 1 and 2; EI-MS m/z 486 [$\text{M}]^+$ (1), 468 (10), 453 (25), 421 (30), 327 (27), 173 (19), 119 (24), 95 (43), 83 (46), 69 (71), 55 (100); HR-EI-MS m/z 486.4422 (Calcd for $\text{C}_{33}\text{H}_{58}\text{O}_2$ 486.4439).

3 β -Methoxy-24-methylstan-9(11)-en-25-ol (**2**): Amorphous, white powder; $[\alpha]_{\text{D}}^{25} +92.1^\circ$ ($c=0.2$, CHCl_3); IR (KBr) ν_{max} 3470, 3070, 2939, 2864, 1653, 1456, 1376, 1363, 1076, 901 cm^{-1} ; ^1H - and ^{13}C -NMR data, see Tables 1 and 2; EI-MS m/z 470 [$\text{M}]^+$ (1), 452 (18), 437 (14), 405 (16), 328 (23), 327 (100), 295 (24), 255 (7), 241 (8), 159 (9), 173 (31), 95 (6); HR-EI-MS m/z 470.4106 (Calcd for $\text{C}_{32}\text{H}_{54}\text{O}_2$ 470.4126).

3 β -Methoxy-25-methyl-24-methylstan-9(11)-en-21-ol (**3**): Amorphous, white powder; $[\alpha]_{\text{D}}^{25} +90.6^\circ$ ($c=0.5$, CHCl_3); IR (KBr) ν_{max} 3466, 3040, 2940, 2868, 2356, 2326, 1735, 1632, 1464, 1376, 1360, 1186, 1104, 1033, 890, 741 cm^{-1} ; ^1H - and ^{13}C -NMR data, see Tables 1 and 2; EI-MS m/z 484 [$\text{M}]^+$ (39), 469 (100), 438 (37), 437 (93), 427 (67), 419 (33), 328 (28), 327 (79), 295 (15), 173 (21), 159 (18); HR-EI-MS m/z 484.4270 (Calcd for $\text{C}_{33}\text{H}_{56}\text{O}_2$ 484.4282).

3 β -Methoxy-24-methylstan-9(11),25-dien-24-ol (**4**): Amorphous, white powder; $[\alpha]_{\text{D}}^{25} +82.1^\circ$ ($c=0.3$, CHCl_3); IR (KBr) ν_{max} 3486, 3050, 2937, 2848, 2356, 2326, 1652, 1456, 1376, 1371, 1078, 895, 668 cm^{-1} ; ^1H - and ^{13}C -NMR data, see Tables 1 and 2; EI-MS m/z 470 [$\text{M}]^+$ (1), 452 (20), 437 (15), 405 (16), 328 (25), 327 (100), 295 (28), 215 (18), 173 (52), 159 (27), 107 (31), 95 (46); HR-EI-MS m/z 470.4103 (Calcd for $\text{C}_{32}\text{H}_{54}\text{O}_2$ 470.4126).

Acknowledgements This research was supported by grants from the National Science Council of the Republic of China (NSC 95-2313-B-020-018). We thank Ms. Shou-Ling Huang and Ms. Shu-Yun Sun for the NMR data acquisition and HR-EI-MS measurement in the Instrumentation Center of the College of Science, National Taiwan University.

References

- 1) Higa M., Noha N., Yokaryo H., Ogiyama K., Yogi S., *Chem. Pharm. Bull.*, **50**, 590–593 (2002).
- 2) Higa M., Ogiyama K., Yogi S., *Chem. Pharm. Bull.*, **46**, 1189–1193 (1998).
- 3) Khan M. R., Timi D., *Fitoterapia*, **70**, 194–196 (1999).

- 4) Gu J. Q., Graf T. N., Lee D., Chai H. B., Mi Q., Kardono L. B. S., Setyowati F. M., Ismail R., Riswan S., Farnsworth N. R., Cordell G. A., *J. Nat. Prod.*, **67**, 1156—1161 (2004).
- 5) Kuo Y. H., Chang C. I., Li S. Y., Chou C. J., Chen C. F., Kuo Y. H., Lee K. H., *Planta Med.*, **63**, 363—365 (1997).
- 6) Lin S. R., *J. Taiwan Pharm. Assoc.*, **30**, 132—135 (1978).
- 7) Chou C. J., *Ann. Rep. Nat. Res. Inst. Chin. Med.*, **1984**, 117—140 (1984).
- 8) Chen C. C., Yu H. J., Huang Y. L., *Chin. Pharm. J.*, **44**, 229—233 (1992).
- 9) Chen C. C., Yu H. J., Ou J. C., Pan T. M., *J. Chin. Chem. Soc.*, **41**, 195—198 (1994).
- 10) Lee T. J., Shih T. S., Lin Y. M., Chen F. C., *Formosan Sci.*, **38**, 147—151 (1984).
- 11) Chen H. C., Lin Y. M., Shih T. S., Chen F. C., *Formosan Sci.*, **41**, 46—52 (1987).
- 12) Chen H. K., Chen K. J., Lin Y. S., Chen F. C., *Formosan Sci.*, **44**, 63—71 (1991).
- 13) Kuo Y. H., Li S. Y., Shen C. C., Yang L. M., Huang H. C., Liao W. B., Chang C. I., Kuo Y. H., Chen C. F., *Chin. Pharm. J.*, **49**, 207—216 (1997).
- 14) Tezuka M., Takahashi C., Kuroyanagi M., Satake M., Yoshihira K., Natori S., *Phytochemistry*, **12**, 175—183 (1973).
- 15) Kuo Y. H., Chang C. I., Kuo Y. H., *J. Chin. Chem. Soc.*, **43**, 511—514 (1996).
- 16) Kuo Y. H., Chang C. I., Kuo Y. H., Huang S. L., *J. Chin. Chem. Soc.*, **45**, 111—113 (1998).
- 17) Kuo Y. H., Chang C. I., Kuo Y. H., *Phytochemistry*, **46**, 1135—1137 (1997).
- 18) Kuo Y. H., Chang C. I., Kuo Y. H., *Chem. Pharm. Bull.*, **45**, 1221—1222 (1997).
- 19) Chang C. I., Kuo Y. H., *Chem. Pharm. Bull.*, **46**, 1627—1629 (1998).
- 20) Chang C. I., Kuo Y. H., *J. Nat. Prod.*, **62**, 309—310 (1999).
- 21) Kuo Y. H., Chang C. I., *Chem. Pharm. Bull.*, **48**, 1211—1214 (2000).
- 22) Kuo Y. H., Huang S. L., Chang C. I., *Phytochemistry*, **49**, 2505—2507 (1998).
- 23) Hata K., Hori K., Takahashi S., *J. Nat. Prod.*, **65**, 645—648 (2002).
- 24) Gonzalez A. G., Amaro J., Fraga B. M., Luis J. G., *Phytochemistry*, **22**, 1828—1830 (1983).
- 25) Hota R. K., Bapuji M., *Phytochemistry*, **35**, 1073—1074 (1994).
- 26) Chakravarty A. K., Das B., Masuda K., Ageta H., *Phytochemistry*, **42**, 1109—1113 (1996).
- 27) Budzikiewicz H., Wilson J. M., Djerassi C., *J. Am. Chem. Soc.*, **85**, 3688—3699 (1963).
- 28) Wada S., Tanaka R., *J. Nat. Prod.*, **63**, 1055—1057 (2000).