J. Braz. Chem. Soc., Vol. 15, No. 5, 742-747, 2004.
Printed in Brazil - ©2004 Sociedade Brasileira de Química
0103 - 5053 $6.00+0.00

Article

Isoprenoid Compounds from *Euphorbia portlandica.* X-ray Structure of Lupeportlandol, a New Lupane Triterpene

Ana M. Madureira[a], Maria Teresa Duarte[b], Maria Fátima M. Piedade[b,c] José R. Ascenso[b] and Maria-José U. Ferreira[*,a]

[a] *Centro de Estudos de Ciências Farmacêuticas, Faculdade de Farmácia da Universidade de Lisboa, Av. das Forças Armadas, 1600-083 Lisboa, Portugal*

[b] *Centro de Química Estrutural, Instituto Superior Técnico, Av. Rovisco Pais, 1096 Lisboa, Portugal*

[c] *Departamento de Química e Bioquímica, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal*

O estudo fitoquímico dos extratos de Me_2CO da planta inteira e seca de *Euphorbia portlandica* levou ao isolamento de um novo álcool triterpênico pentacíclico, com o esqueleto do lupano, designado por lupeportlandol. A sua estrutura foi estabelecida como 3α-hidroxi-19αH-lup-20(29)-eno. Foram também isolados os já conhecidos triterpeno pentacíclico glutinol e o esteróide β-sitostenona. A caracterização do novo composto e do seu derivado acetilado foi baseada em métodos espectroscópicos e numa análise de difração de raio-X. O acetato de lupeportlandol mostrou-se inativo em ensaios de citotoxicidade *in vitro* contra três linhagens de células tumorais humanas: MCF-7 (câncer da mama), NCI-H460 (câncer do pulmão) e SF-268 (câncer do SNC).

Phytochemical survey of the Me_2CO extracts of the whole dried plant *Euphorbia portlandica* led to the isolation of a new pentacyclic triterpene alcohol, with the lupane skeleton, named lupeportlandol. Its structure was established as 3α-hydroxy-19αH-lup-20(29)-ene. The known pentacyclic triterpene glutinol and the steroid β-sitostenone were also isolated. The characterization of the new compound and its acetylated derivative was based on spectroscopic methods and an X-ray diffraction analysis. Lupeportlandol acetate was inactive in cytotoxicity assays *in vitro* against three human tumor cell lines: MCF-7 (breast cancer), NCI-H460 (non-small cell lung cancer) and SF-268 (CNS cancer).

Keywords: *Euphorbia portlandica*, triterpenes, steroids, lupane, X-ray diffraction

Introduction

Euphorbia portlandica L., from Euphorbiaceae family, is frequently found in the coast of Portugal, mainly in sand and rocks near the sea. *Euphorbia* species have been used in the traditional medicine for treatment of cancers, tumors and warts for hundred of years.[1] They are characterized by the existence of a latex very rich in isoprenic compounds, whose major constituents are tetra and pentacyclic triterpenes that revealed a wide spectrum of biological activities.[2-5] Among pentacyclic triterpenes, lupane derivatives have demonstrated antiviral activity [6-8] as well as anti-proliferative activity against various tumor cell lines.[9-11]

Previous studies on this species have afforded rearranged jatrophane-type diterpenes, which have been found to be effective modulators of multidrug resistance in tumor cells.[12] The present paper reports the isolation and structure determination of a new pentacyclic triterpene alcohol with the lupane skeleton (**1**), as well as the isolation of the known compounds glutinol (**2**) and β-sitostenone (**3**) from the Me_2CO extracts of the whole dried plant *Euphorbia portlandica*. The evaluation of the acetylated derivative of compound **1** (**1a**) for its *in vitro* effect on the growth of three human cancer cell lines is also reported.

Experimental

General experimental procedures

Melting points were determined on a Kofler apparatus and are not corrected. Optical rotations were obtained using a Perkin-Elmer 241-MC polarimeter. IR spectra were

* e-mail: mjuferreira@ff.ul.pt

determined on a Perkin-Elmer 1310 instrument. The NMR spectra were recorded on a Varian Unity-300 NMR spectrometer (^{1}H 300 MHz; ^{13}C 75.4 MHz), with TMS as internal standard and $CDCl_3$ as solvent. MS were taken on a Kratos MS25RF spectrometer (70 eV) and HRMS on a Finnigan FT/MS 2001-DT. Column chromatography was carried out on SiO_2 (Merck 9385). TLC was performed on precoated SiO_2 F_{254} plates (Merck 5554 and 5744) and visualized under UV light and by spraying with sulphuric acid-acetic acid-water (1:20:4) followed by heating. HPLC was carried out on a Merck-Hitachi instrument, with UV detection, using a Merck LiChrospher 100 RP-18 (10 μm, 250 x 10 mm) column. The purity of the isolated compounds was monitored by means of analytical TLC and HPLC or GC being the latter analyses performed on a Hewlet Packard-5890 with a HP-17 column (10 m x 0.53 mm x 2.0 μm, 50 % PhMe silicone), isothermally, at 270 ºC, with He as carrier gas (20 mL min^{-1}) and injection and detection temperature 300 ºC; cholesterol acetate was used as an internal standard.

Plant material

Euphorbia portlandica was collected in the west coast of Portugal near the beach of Vale Furado, Nazaré, in September 1997 and identified by Dr. Teresa Vasconcelos of Instituto Superior de Agronomia, University of Lisboa. A voucher specimen (nº 248) has been deposited at the herbarium (LISI) of Instituto Superior de Agronomia.

Extraction and isolation

The air-dried whole powdered plant (4.8 Kg) of *Euphorbia portlandica* was extracted, by maceration, with acetone (7 x 8 L) at room temperature. Evaporation of the solvent (under *vacuum*, 40 ºC) from the crude extract gave a residue (367 g), which was suspended on a MeOH/H_2O solution (1:1, 2 L) and extracted with *n*-hexane (3 x 1 L). The *n*-hexane extract was dried (Na_2SO_4) and evaporated (40 ºC), yielding a residue (170 g) that was submitted to column chromatography on SiO_2 (1.2 Kg), using mixtures of *n*-hexane/EtOAc of increasing polarity (100:0 to 0:100) and EtOAc/MeOH (75:25) as eluting solvents yielding eight crude fractions after TLC and GC control. The more apolar crude frations (*n*-hexane/EtOAc; 100:0 to 92.5:7.5) contained mainly waxes and were discarded. The residue (27 g) of the crude fraction A (*n*-hexane/EtOAc; 92.5:7.5) was subjected to column chromatography on SiO_2 (1 Kg) using *n*-hexane/EtOAc (100:0 to 75:25); eleven fractions eluted with *n*-hexane/EtOAc (96:4 to 95:5), after combined (1.5 g) and concentrated, crystallized from the elution solvent affording 80 mg of pure compound **2** (R_f: 0.36, CH_2Cl_2; GC R_t: 1.73). The mother liquor (1.3 g) were subjected to column chromatography (130 g) with *n*-hexane/CH_2Cl_2 (100:0 to 0:100) and CH_2Cl_2/EtOAc (50:50 and 0:100); the residue (120 mg) of fractions eluted with *n*-hexane/CH_2Cl_2, 80:20 to 77.5:22.5, after being subjected to crystallization with acetone, yielded respectively 10 mg of **1** (R_f: 0.48, CH_2Cl_2; GC R_t: 1.91) and 180 mg of **2**. Purification of the mother liquor residue of **1**, by preparative TLC (2 x *n*-hexane/CH_2Cl_2, 50:50), yielded 26 mg of **1**. The crude fraction B (*n*-hexane/EtOAc, 92.5:7.5 to 90:10) was submitted to three successive column chromatographies, both with mixtures of *n*-hexane/CH_2Cl_2 as above and with *n*-hexane/Me_2CO (0:100 to 90:10), furnishing a product (65 mg; *n*-hexane/Me_2CO; 96:4), that was further purified by preparative TLC (2 x CH_2Cl_2), affording 40 mg of a product that was pooled to the fractions eluted with *n*-hexane/Me_2CO, 95:5 (20 mg) and subjected to reverse phase HPLC (MeOH/H_2O, 95:5; 5 mL min^{-1}) yielding 10 mg of **3** (HPLC R_t: 51 min; GC R_t: 1.86; R_f: 0.26, CH_2Cl_2).

*Lupeportlandol (3α-hydroxy-19αH-lup-20(29)-ene) (**1**)*. White needles, mp 212-214 ºC (Me_2CO); $[\alpha]_D^{25} = +44°$ ($CHCl_3$, *c* 0.10); HRMS, *m/z*: 426.38552 [M]$^+$, (426.38561 Calc. for $C_{30}H_{50}O$); IR (KBr), ν_{max}/cm^{-1}: 3405, 3104, 2944, 2919, 2866, 1640, 1456, 1381, 1082, 996, 884; ^{1}H NMR and ^{13}C NMR see Tables 1 and 2; EIMS, *m/z* (rel. int.): 426 [M]$^+$ (6), 411[M - CH_3]$^+$ (2), 408 [M - $H_2$0]$^+$ (1), 393 [M-CH_3 - $H_2$0]$^+$ (2), 315 (7), 218 (23), 207 (87), 189 (100), 175 (24), 161 (24), 149 (18), 147 (24), 135 (47), 121 (34), 107 (39), 95 (41), 81 (48), 69 (48), 55 (42), 43 (45).

*Lupeportlandol acetate (3α-acetoxy-19αH-lup-20(29)-ene (**1a**)*. Compound **1** (26 mg) was acetylated with Ac_2O-pyridine 1:1 at room temperature, overnight. The usual workup gave 25 mg of **1a** (R_f: 0.77, CH_2Cl_2): White needles, mp 230-232 ºC (Me_2CO); $[\alpha]_D^{25} = +25°$ ($CHCl_3$, *c* 0.40); IR (KBr), ν_{max}/cm^{-1}: 2939, 2858, 1733, 1645, 1453, 1373, 1243, 1180, 1140, 1103, 1056, 1036, 1017, 983, 965, 941, 883; ^{1}H NMR and ^{13}C NMR see Tables 1 and 2; EIMS, *m/z* (rel. int.): 408 [M - HOAc]$^+$ (2), 393 [M - HOAc - CH_3]$^+$ (2), 218 (5), 202 (8), 189 (88), 136 (42), 135 (55), 121 (100), 119 (49), 109 (90), 107 (70), 55 (43), 43 (25).

X-ray crystallographic analysis

Appropriate crystals of **1** for X-ray diffraction analysis were obtained by recrystallization from Me_2CO. The crystal data were collected using a Turbo CAD4 diffractometer using graphite monochromated Cu radiation. A summary of the crystal data and refinement conditions is presented in Table 3. During data collection,

Table 1. ^{1}H NMR data of **1** and **1a** ($CDCl_3$, δ in ppm, J in Hz)

H	**1**	**1a**
3β	3.39 br s ($W_{1/2}$ = 7)	4.72 br s ($W_{1/2}$ = 7)
19α	2.54 m ($W_{1/2}$ = 30)	2.55 m ($W_{1/2}$ = 30)
Me-23	0.93 s	0.88 s
Me-24	0.83 s	0.84 s
Me-25	0.86 s	0.87 s
Me-26	1.06 s	1.06 s
Me-27	0.94 s	0.96 s
Me-28	0.91 s	0.92 s
29a	4.72 br s	4.72 br s
29b	4.63 br s	4.63 br s
Me-30	1.69 s	1.69 s
3-OAc	—	2.08 s

Table 2. ^{13}C NMR data of compounds **1** and **1a** ($CDCl_3$, δ in ppm)

Carbon	**1** (δ_C)	**1a** (δ_C)	DEPT	Carbon	**1**(δ_C)	**1a** (δ_C)	DEPT
1	33.3	33.9	CH_2	16	36.9	36.9	CH_2
2	25.3	27.8	CH_2	17	41.8	41.8	C
3	76.3	78.4	CH	18	50.6	50.7	CH
4	37.5	37.2	C	19	44.8	44.8	CH
5	49.1	50.3	CH	20	150.6	150.6	C
6	18.3	18.2	CH_2	21	30.6	30.6	CH_2
7	34.0	34.0	CH_2	22	41.4	41.4	CH_2
8	41.1	41.2	C	23	28.2	27.9	CH_3
9	50.4	50.3	CH	24	22.1	21.8	CH_3
10	37.3	37.2	C	25	15.9	16.0	CH_3
11	20.7	20.7	CH_2	26	15.9	15.9	CH_3
12	25.2	25.2	CH_2	27	14.5	14.6	CH_3
13	34.7	34.8	CH	28	20.6	20.6	CH_3
14	43.1	43.2	C	29	108.9	108.9	CH_2
15	27.5	27.6	CH_2	30	25.1	25.2	CH_3

the intensity of three standard reflections was monitored every hour of X-ray exposure time showing no significant decay. The structure was solved and refined using the WinGX package.[13] The program used to solve the structure was SIR97,[14] and to refine was SHELXL 97.[15] All hydrogens were found except the O-H hydrogen that was placed in calculated position riding on a carrier atom with isotropic displacement parameter amounting 1.5 times the value of the equivalent isotropic displacement parameter of the carrier atom. Absolute structure configuration was confirmed by the Flack parameter (see Table 3). Graphics were done with ORTEP-3 for Windows version 1.076,[16] included in WinGX system.

Cancer cell growth assay

The *in vitro* effect of compound **1a** on the growth of human tumor cell lines MCF-7 (breast cancer), NCI-H460 (non-small cell lung cancer) and SF-268 (CNS cancer) was performed according to established protocols.[17-19] Doxorubicin was used as the positive control.

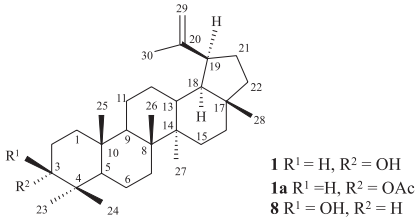
Table 3. Crystal data and structure refinement for lupeportlandol (**1**)

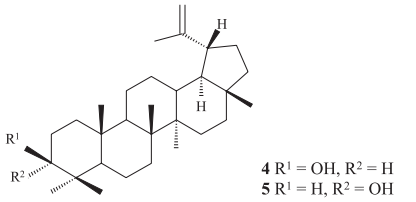
Empirical formula	$C_{30}H_{50}O$
Formula weight	426.70
Temperature	293 (2) K
Wavelength	1.54180 Å
Crystal system	Monoclinic
Space group	P21
Unit cell dimensions	a = 7.574 (2) Å b = 16.563 (3) Å c = 10.139 (2) Å β = 98.15 (2) deg.
Volume	1259.1(5) Å^3
Z	2
Calculated density	1.126 Mg/m^3
Absorption coefficient	0.482 mm^{-1}
F(000)	476
Crystal size	0.5 x 0.3 x 0.2 mm
Theta range for data collection	4.41 to 66.89 deg.
Limiting indices	-1 < = h < =9; -1 < = < = 19; -12 < = l < = 12
Reflections collected / unique	3079 / 2486 [R (int) = 0.0272]
Completeness to theta = 66.89	99.9 %
Absorption correction	None
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2486 / 1 / 477
Goodness-of-fit on F^2	1.053
Final R indices [I > 2 sigma (I)]	R1 = 0.0318; wR2 = 0.0845
R indices (all data)	R1 = 0.0333; wR2 = 0.0862
Absolute structure parameter	-0.2 (4)
Extinction coefficient	0.0222(14)
Largest diff. peak and hole	0.144 and - 0.126 e. Å^{-3}

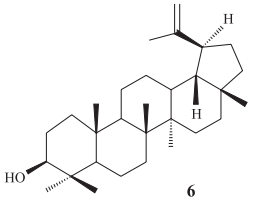
Results and Discussion

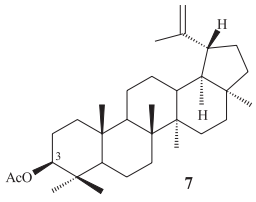
The acetone extract of the whole dried plant *Euphorbia portlandica* was partitioned between a MeOH/H_2O solution and hexane. Fractionation of the hexane extract yielded a new pentacyclic triterpene alcohol with a lupane skeleton, named lupeportlandol, which structure was established as 3α-hydroxy-19αH-lup-20(29)-ene (**1**). The known pentacyclic triterpene glutinol (**2**) and the steroid β-sitostenone (**3**) were also isolated and identified.

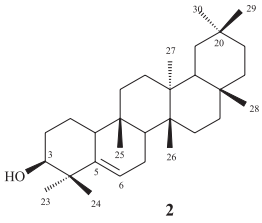
Compound **1** showed a molecular ion, in the HREIMS, at *m/z* 426.38552 indicating the molecular formula $C_{30}H_{50}O$ (calc. 426.38561). The IR spectrum of **1** showed absorption bands for a hydroxyl group (3405 cm^{-1}) and an exocyclic methylene group (3104, 1640, 884 cm^{-1}). The EI-MS spectrum showed the molecular ion peak at *m/z* 426, the common fragments of pentacyclic triterpenes and a base peak at *m/z* 189 (Figure 1). This fragment may arise from the cleavage between C-8/C-14 and C-12/C-13 bonds with H-transfer and suggests the lupane or hopane skeleton for **1**.[20, 21] The ^{1}H NMR spectrum of **1** (Table 1) showed the presence of six tertiary methyl groups (δ 0.83, 0.86, 0.91, 0.93, 0.94, and 1.06) and one methyl group attached to an sp^2 carbon at δ 1.69. The latter methyl group and the

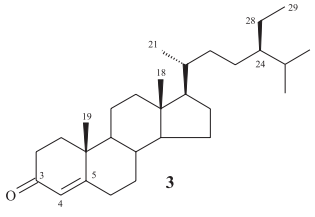












exocyclic methylene protons at δ 4.72 and 4.63, support the existence of an isopropenyl group in the molecule. The proton at δ 3.39 was ascribed to H-3 and must be equatorially (3α-OH) oriented since it appears as a broad singlet ($W_{1/2}$ = 7 Hz), resulting from small couplings to the protons H-2 ($J_{3eq,2ax} \cong J_{3eq,\ 2eq}$) and is slightly downfield shifted ($\Delta\delta \cong 0.20$ ppm) relatively to the normal chemical shift of H-3, axially oriented in common triterpenes, that appears as a double doublet near δ 3.20.[22, 23] The multiplet appearing at δ 2.54 was assigned to H-19.

The ^{13}C NMR spectrum showed signals for 30 carbon atoms with multiplicities assigned by DEPT experiment The low field region of this spectrum shows the olefinic

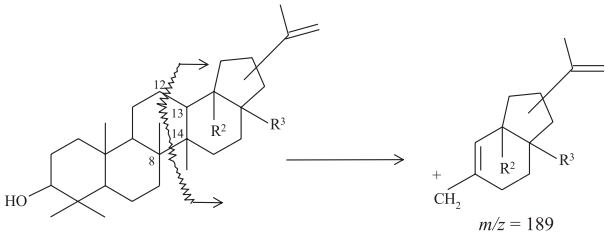


Figure 1. Diagnostic fragment for lupane (R^2 = H; R^3 = CH_3) and hopane (R^2 = CH_3; R^3 = H) triterpenes.

carbons at δ 108.9 (CH_2) and 150.6 (C) assigned to the terminal double bond of the isopropenyl group and one methine carbon at δ 76.3. In the high-field region seven CH_3, ten CH_2, five methines, and five quaternary carbons were identified. Acetylation of **1**, with Ac_2O-pyridine, yielded a monoacetyl derivative **1a** that showed essentially the same ^{1}H NMR spectrum, except for the signal due to H-3 shifted to lower field (δ 4.72 brs) and the acetyl methyl resonance (δ 2.08.)

The above results agree with a lupane skeleton for **1** having an unusual configuration for the hydroxyl group at C-3 (3α-OH) that was further confirmed by the carbon resonances of ring A which were shifted upfield (except C-10), when compared to those reported for lupeol (**4**), whereas paramagnetic shielding is observed for Me-24. [23] However, by comparing the NMR data of **1** with those reported for epilupeol (**5**) significant differences are observed for proton and carbon resonances of rings D and E. [24, 25] A literature survey revealed that the chemical shift of the angular methyl group Me-28 of **1** (δ 0.91), closely resembled those found for lupane derivatives ($\delta \cong 0.90$) with a *cis* D/E ring junction nepehinol (**6**) (H-18β, Me-28β and H-19α)[22,26] and 17-epi-lupenyl acetate (**7**) (H-18α, Me-28α and H-19β) [27] as well as 19αH-lupeol (**8**) [23] but different from lupeol (H-19β; δ 0.79) despite the same *trans* D/E ring junction (H-18α and Me-28β) in both compounds.[23] The stereochemistry of **1** was partially solved by through space proton-proton correlations observed in a NOESY spectrum. Indeed, the correlations between Me-25/Me-26, Me-26/Me-28 and Me-28/ isopropenyl (Figure 2) and the assumption of the same β configuration for both methyl groups at C-10 and C-8, as found in lupeol, provided evidence for a β configuration for both the isopropenyl group and Me-28.

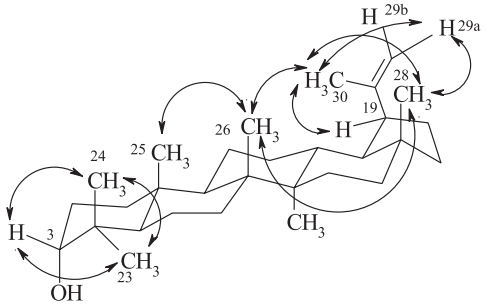


Figure 2. Relevant NOE correlations observed for **1**.

The less clear stereochemical aspects of **1**, as the ambiguity in the stereochemistry of C-18, led to an X-ray diffraction analysis of compound **1**. Figure 3 shows the ORTEP diagram of the molecule establishing that the structure of lupeportlandol is 3α-hydroxy-19αH-lup-20(29)-ene, a new epimer of both compounds **5** (at C-19) and **8** (at C-3). The distances and angles within the molecule, as well

as the determined conformation of the rings, agree with the data from other triterpenic derivatives.[28-31] This data also agree with the conformation found both in 3α,4α-epoxy-D:A-*friedo*-18β,19αH-lupane[30] and 3β-hydroxy-20-oxo-29(20→19)*abeo*lupane[31] where no double bonds were found in the carbon skeleton of the molecule.

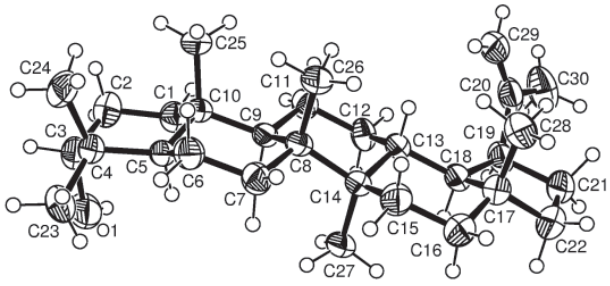


Figure 3. ORTEP diagram of compound **1** with numbering scheme showing the specific stereochemistry at C-18.

Compounds **2** and **3**, respectively identified as glutinol and β-sitostenone, were also isolated. Their physical and spectral data were in agreement with those reported in the literature. [22, 32-34]

The ability of compound **1a** to inhibit the *in vitro* growth of MCF-7, NCI-H460 and SF-268 cell lines was evaluated. It showed to be inactive ($GI_{50} > 100\,\mu M$) against the three cell lines.

Acknowledgments

The authors are grateful to Prof. Maria São José and Dr. Madalena Pedro from Faculdade de Farmácia, University of Porto, Portugal, for the tumor cell growth assays. The authors thank also Dr. Teresa Vasconcelos (ISA, University of Lisbon, Portugal) for identification of the plant and Mr. I. Marques (IST) for low resolution mass spectra.

This work was supported by FCT (POCTI, Quadro Comunitário de Apoio III) and PRODEP.

Supplementary Information

Supplementary crystallographic data for the structure have been deposited at the Cambridge Crystallographic Data Centre no. CCDC 225799. Copies of the data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from Cambridge Crystallographic Data Centre, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax +44 1223 336033; or e-mail: deposit@ccdc.cam.ac.uk).

References

1. Hartwell, J. L.; *Lloydia* **1969**, *62*, 153.
2. Huang, L.; Chen, C. H.; *Curr. Drug Targets Infect. Disord.* **2002**, *2*, 33.
3. Novotny, L.; Vachalkova, A.; Biggs, D.; *Neoplasma* **2001**, *48*, 241.
4. Tanaka, R.; Kinouchi, Y.; Tokuda, H.; Nishino, H.; Matsunaga, S.; *Planta Med.* **2000**, *66*, 630.
5. Madureira, A. M.; Ascenso, J. R.; Valdeira, L.; Duarte, A.; Frade, J. P.; Freitas, G.; Ferreira, M. J. U.; *Nat. Prod. Res.* **2003**, *17*, 375.
6. Sun, I. C.; Kashiwada, Y.; Morris-Natschke, S. L.; Lee, K. H.; *Curr. Top. Med. Chem.* **2003**, *3*, 155.
7. Hashimoto, F.; Kashiwada, Y.; Cosentino, L. M.; Chen, C. H.; Garrett, P. E.; Lee, K. H.; *Bioorg. Med. Chem.* **1997**, *5*, 2133.
8. Baltina, L. A.; Flekhter, O. B.; Nigmatullina, L. R.; Boreko, E. I.; Pavlova, N. I.; Nikolaeva, S. N.; Savinova, O. V., Tolstikov, G. A.; *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3549.
9. Hata, K.; Hori, K.; Ogasawara, H.; Takahashi, S.; *Toxicol. Lett.* **2003**, *143*, 1.
10. Setzer, W. N.; Setzer, M. C.; *Mini Rev. Med. Chem.* **2003**, *3*, 540.
11. Chaturvedula, V. S. C.; Schilling, J. K.; Johnson, R. K.; Kingston, D. G.; *J. Nat. Prod.* **2003**, *66*, 419.
12. Madureira, A. M.; Gyémánt, N.; Ugocsai, K.; Ascenso, J. R.; Abreu, P. M.; Hohmann, J.; Molnár, J.; Ferreira. M. J.; *Planta Med.* **2004**, *70,* 45-49
13. Farrugia, L. J.; *J. Appl. Crystallogr.* **1999**, *32,* 837.
14. Altomare, A.; Burla, M. C.; Camalli, M; Cascarano, G.; Giacovazzo, C.; Guagliardi, A.; Moliterni, A. G. G.; Polidori, G.; Spagna, R.; *J. Appl. Crystallogr.* **1998**, *32*, 115.
15. Sheldrick, G. M.; *SHELXL97- Program for Refining Crystal Structures*, Univ. of Göttingen, Germany, 1993.
16. Farrugia, L. J.; *J. Appl. Crystallogr.* **1997**, *30,* 565.
17. Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro-Wolff, A.; Gray-Goodrich, M.; Campbell, H.; Mayo, J.; Boyd, M.; *J. Nalt. Cancer Inst.* **1991**, *83*, 757.
18. Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R.; *J. Natl. Cancer Inst.* **1990**, *82*, 1107.
19. Valente, C.; Ferreira, M. J. U.; Abreu, P. M.; Pedro, M.; Cerqueira, F.; Nascimento, M. S. J.; *Planta Med.* **2003**, *69*, 361.
20. Budzikiewick, H.; Wilson, J. M.; Djerassi, C.; *J. Org. Chem.* **1963**, *85*, 3688.
21. Ogunkoya, L.; *Phytochemistry* **1981**, *20*, 121.
22. Akihisa, T.; Shimizu, N.; Kawaguchi, R.; Tamura, T.; Matsumoto, T.; *J. Jpn. Oil Chem. Soc.* **1986**, *35*, 17.
23. Nazir, M.; Ahmad, W.; Kreiser, W.; *Pak. J. Sci. Ind. Res.* **1998**, *41*, 6.
24. Waterman, P. G.; Ampofo, S.; *Phytochemistry* 1985, *12*, 2925.
25. Carvalho, L.; Seita, J.; *Fitoterapia* **1995**, *66*, 273.

26. Ahmad, V. U.; Bano, S.; Mohammad, F. V.; *Planta Med*. **1985**, *6*. 521.
27. Shiojima, K.; Suzuki, H.; Kodera, N.; Kubota, K.; Tsushima, S.; Ageta, H.; Chand, H. C.; Chen, Y. P.; *Chem. Pharm. Bull.* **1994**, *42*, 2193.
28. Ferreira, M. J. U.; Lobo, A. M.; O'Mahoney, C. A.; Williams, D. J.; Wyler, H.; *Helv. Chim. Acta* **1991**, *74,* 1329.
29. Konda, Y.; Iguchi, M.; Harigaya, Y.; Takayanagi, H.; Ogura, H.; Li, X.; Lou, H.; Onda, M.; *Tetrahedron Lett.* **1990**, *37*, 5315.
30. Yokoyama Y.; Moriyama, Y.; Tsuyuki, T.; Takahasi, T.; Iitaka, Y.; *Bull. Chem. Soc. Jpn.* **1980**, *53*, 2971.
31. Chiang, Y.; Kuo, Y.; *J. Org. Chem.* **2002,** *67*, 7656.
32. Tori, M.; Torh, T.; Tachibana, K.; Yamada S.; Tsuyuki, T.; Takahashi, T.; *Bull. Chem. Soc. Jpn.* **1977**, *50*, 469.
33. Oleo, R. S. G.; Torres, L. M. B.; Roque, R. C.; Roque, N. F.; *Magn*. *Reson*. *Chem*. **1994**, *32*, 378.
34. Greca, M. D.; Monaco, P.; Previtera, L.; *J. Nat. Prod*. **1990**, *53*, 1430.

Received: January 5, 2004
Published on the web: September 16, 2004