DOI: 10.1002/ejoc.200700558

Non-Phenolic Dicinnamamides from *Pholiota Spumosa*: Isolation, Synthesis and Antitumour Activity[‡]

Marco Clericuzio,*[a] Silvia Tabasso,[b] Juan A. Garbarino,[c] Marisa Piovano,[c] Venera Cardile,[d] Alessandra Russo,[e] and Giovanni Vidari[f]

Keywords: Natural products / Cinnamic acid / Amides / Structure elucidation / Total synthesis / Prostate cancer

Two new amides derived from cinnamic acid, namely, (*R*)-2-hydroxyputrescine dicinnamamide (**4**) and pholiotic acid {(2*R*)-2-[(*S*)-3-hydroxy-3-methylglutaryloxy]putrescine dicinnamamide} (**5**), in addition to the known compound maytenine (N^1,N^8-dicinnamoyl spermidine) (**3**) were isolated from the fruiting bodies of the Basidiomycete *Pholiota spumosa*. The absolute configuration of **4** was established as (*R*) by its total synthesis starting from (*S*)-dimethylmalate, whereas that of **5** was determined by conversion into the known compound methyl (*S*)-4-[(*S*)-1-(1-naphthalen-1-yl)-ethylcarbamoyl]-3-hydroxy-3-methylbutanoate (**14**). Compounds **3** and **5** exhibited an inhibitory effect on cell growth of the androgen-insensitive DU-145 prostate cancer cells, which suggests that these fungal polyamine conjugates, like other polyamine analogues, might have chemotherapeutic potential against androgen-independent prostatic cancer.
(© Wiley-VCH Verlag GmbH & Co. KGaA, 69451 Weinheim, Germany, 2007)

Introduction

Cinnamic acid amides **1** and **2** (often referred to with the trivial name "dicinnamides"), recently isolated by us from the fruiting bodies of the Basidiomycete *Pholiota spumosa* (Fr.) Sing. (Strophariaceae),[1] belong to a new class of fungal metabolites. In fact **1** and **2**, as well as related compounds **3**–**5** reported in this paper, are the first examples of cinnamic acid amides occurring in the fungal kingdom. Several strictly related metabolites are common constituents of green plants (Angiospermae);[2–5] however, most of them bear oxygenated substituents on the aromatic rings, which results in derivatives of the coumaric, caffeic and ferulic acids (hydroxycinnamoyl acid amides, HCA). Both mono- and diamides of these phenylpropanoic acids have been reported, and the amino moieties are derived from polyamines, such as putrescine[2] and spermidine,[6] or from decarboxylated amino acids such as tryptamine.[3,7] The phenolic groups of plant amides are of primary importance for their noteworthy biological roles, which include, among others, the regulation of flowering mechanisms.[4,8] Moreover, some of these compounds are good inhibitors of α-glucosidase[9] and some have been studied as potential hypocholesterolemic agents.[10]

Amides of aromatic acids different from cinnamic and hydroxycinnamic acids are equally rarely represented among fungal metabolites. Interestingly, Steglich and coworkers[11] isolated a benzamide, named pistillarin [N^1,N^8-bis(3,4-dihydroxy)benzoylspermidine], from the fruiting bodies of *Clavariadelphus* and *Ramaria* (mushrooms belonging to the order Aphyllophorales s.l., Basidiomycetes), which contains phenolic substituents like plant amides.

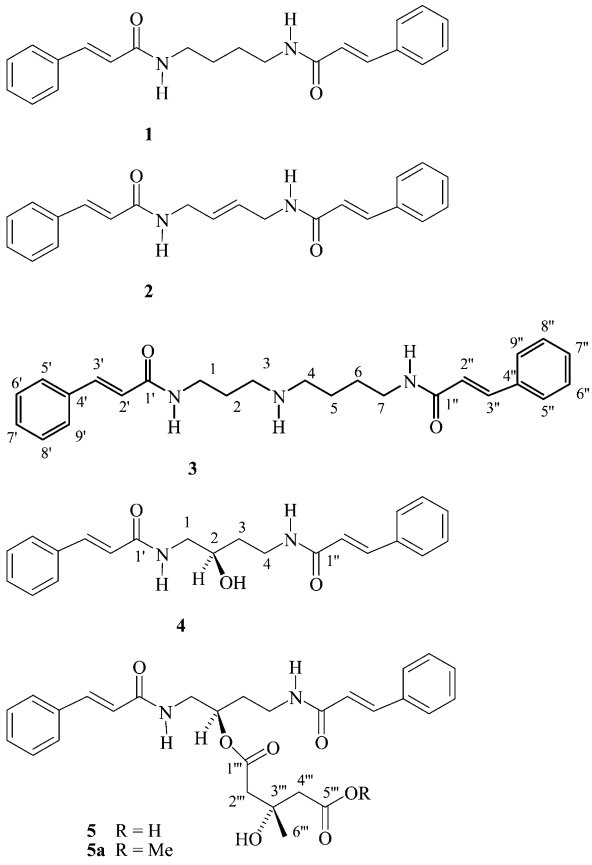
The biological role of the non-phenolic fungal diamides is still largely unknown; dicinnamide **1** seems to be devoid of most bioactivities showed by plants HCA,[9] although we found that **1** inhibits the vitality of human prostate cancer cells.[12] Therefore, in this work we describe the antigrowth activity of compounds **3** and **5** against androgen-dependent LNCaP prostate cancer cells and androgen-insensitive DU-145 prostate cancer cells. Several biochemical parameters, such as cell vitality (MTT assay), cell membrane integrity (lactate dehydrogenase release) and genomic DNA fragmentation (COMET assay) were tested.

[‡] Fungal Metabolites, 53 (part 52: G. Gilardoni, M. Clericuzio, S. Tosi, G. Zanoni, G. Vidari, *J. Nat. Prod.* **2007**, *70*, 137–139); Studies on Chilean Fungi, 5 (part 4: ref.[12]). This work is part of the doctoral thesis of S. T.

[a] Dipartimento di Scienze Ambientali e della Vita, Università del Piemonte Orientale "A. Avogadro",
via Bellini 25/G, 15100 Alessandria, Italy
Fax: +39-0131360390
E-mail: marco.clericuzio@mfn.unipmn.it
[b] Dipartimento di Chimica Generale ed Organica Applicata, Università di Torino,
via P. Giuria 7, 10125 Torino, Italy
[c] Departamento de Quimica, Universidad Técnica F. S. Maria, Casilla 110-V, Valparaiso, Chile
[d] Dipartimento di Scienze Fisiologiche, Università di Catania, Viale A. Doria 6, 95125 Catania, Italy
[e] Dipartimento di Chimica Biologica, Chimica Medica e Biologia Molecolare, Università di Catania,
Viale A. Doria 6, 95125 Catania, Italy
[f] Dipartimento di Chimica Organica, Università di Pavia,
via Taramelli 10, 27100 Pavia, Italy

Supporting information for this article is available on the WWW under http://www.eurjoc.org/ or from the author.

 © 2007 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim



Results and Discussion

The fruiting bodies of *Pholiota spumosa* were soaked in EtOH, which afforded a slightly better yield of extraction than acetone, previously employed.[1] Separation of the crude extract with an RP-18 column afforded three dicinnamamides (**3**–**5**), which eluted before dicinnamide **1**. The spots of these compounds were strongly UV absorptive on TLC plates but remained colourless upon exposure to sulfoaldehydo reagents. The occurrence of dicinnamamides in the various chromatographic fractions could be easily confirmed by analysis of the ^{1}H NMR spectra, and their presence was obvious even in mixtures; in fact, the two doublets at about δ = 6.6 ppm, which showed the typical coupling constant of ca. 16 Hz for the *trans* cinnamic systems (together with two aromatic broad multiplets at about δ = 7.4 and 7.5 ppm) were good markers of their presence. Noteworthy, dicinnamamides **3**–**5** were partly or completely lost on silica gel columns.

Compound **3** was the first dicinnamamide to elute. Its MS (ESI+) spectrum showed an $[M + 1]^+$ pseudomolecular ion at m/z = 406, which is indicative of an odd molecular weight of 405 and suggests the presence of a third nitrogen atom in comparison to the structures of **1** and **2**. By means of NMR spectroscopic analysis, **3** was unequivocally assigned the structure N^1,N^8-dicinnamoylspermidine, which is a compound known by the name maytenine and previously isolated from the roots and bark of the Amazonian tree *Maytenus chuchuasha*.[13] Maytenine shows important bioactivities, such as antipyretic and vasodilatation properties,[14] which have stimulated much synthetic work.[15] To the best of our knowledge, compound **3** is the only example of a non-phenolic cinnamamide found in plants. In the Experimental Section we report the complete NMR spectra of maytenine (**3**) in two different solvents (CD_3OD and [D_8]-THF/D_2O), whereas in the original paper[13] the NMR spectroscopic data were reported in $CDCl_3$, in which **3** is scarcely soluble.

The second dicinnamamide to elute was **4**, and it was more difficult to obtain in pure form. In fact, it constantly coeluted with dicinnamamide **5** on RP-18 columns; however, it could be separated by employing Sephadex LH-20 columns or, alternatively, by silica gel column chromatography after methylation of **5** to **5a** (see Experimental Section for details). Compound **4** was obtained in small amounts (about 2 mg per gram of crude extract) as a fine powder that stuck to glassware, and it is partly soluble only in 95% aqueous MeOH. The molecular formula $C_{22}H_{24}N_2O_3$ was derived from the HRMS (ESI+) spectrum, which exhibited a pseudomolecular ion peak at 387.16807 corresponding to $C_{22}H_{24}N_2O_3Na$. In comparison to those of **1**, the NMR spectra of **4** showed the presence of two nonequivalent cinnamic units along with an additional secondary alcoholic resonance at δ = 69.2 ppm in the ^{13}C NMR spectrum and at δ = 3.79 ppm in the ^{1}H NMR spectrum (see Tables 1 and 2). Compound **4** thus corresponded to N^1,N^4-dicinnamamoyl 2-hydroxyputrescine, and it was found to be the dextrorotatory enantiomer. Be-

Table 1. ^{1}H NMR (400 MHz, CD_3OD) chemical shifts (in ppm) of compounds **4** and **5**.

Proton	**4**	**5**
1	3.48 (m), 3.43 (m)	3.64 (dd, J = 13.6, 4.0 Hz), 3.45 (m)
2	3.79 (m)	5.11 (m)
3	1.78 (m), 1.65 (m)	1.90 (m)
4	3.40 (m), 3.30 (m)	3.40 (m)
2′, 2′′	6.65 (d, J = 16.0 Hz), 6.59 (d, J = 16.0 Hz)	6.63 (d, J = 15.8 Hz), 6.60 (d, J = 15.8 Hz)
3′, 3′′	7.50 (d, J = 16.0 Hz), 7.48 (d, J = 16.0 Hz)	7.57 (d, J = 15.8 Hz), 7.49 (d, J = 15.8 Hz)
5′, 9′, 5′′, 9′′	7.41–7.35 (m)	7.41–7.34 (m)
6′, 7′, 8′, 6′′, 7′′, 8′′	7.57–7.49 (m)	7.56–7.50 (m)
2′′′	–	2.70 (d, J = 13.8 Hz)
4′′′	–	2.56 (d, J = 14.5 Hz)
6′′′	–	1.40 (s)

 © 2007 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

cause crystals suitable for X-ray analysis could not be obtained, the absolute configuration of (+)-(**4**) was established on the basis of a homochiral sample prepared by total synthesis.

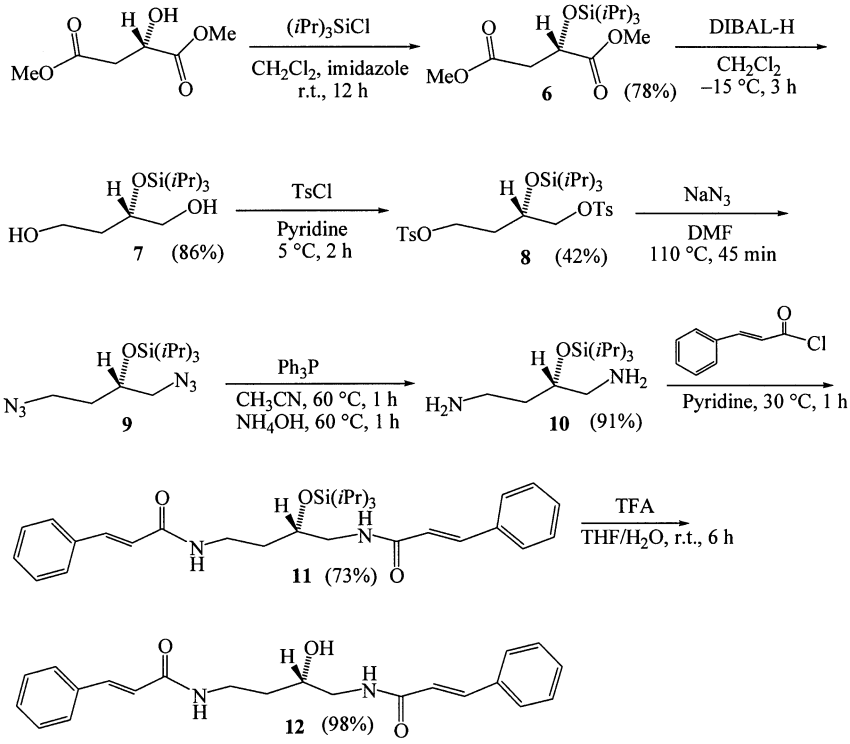
Table 2. ^{13}C NMR (100 MHz, CD_3OD) chemical shifts (in ppm) of compounds **4** and **5**.

Carbon	**4**	**5**
1	46.7 (t)	43.1 (t)
2	69.2 (d)	72.2 (d)
3	35.4 (t)	32.4 (t)
4	37.4 (t)	36.8 (t)
1′, 1′′	169.0 (s), 168.8 (s)	168.7 (s), 168.4 (s)
2′, 2′′	121.8 (d), 121.1 (d)	121.6 (d), 121.5 (d)
3′, 3′′	141.9 (d), 141.7 (d)	141.9 (d), 141.5 (d)
4′, 4′′	136.2 (2×d)	136.1 (d), 136.0 (d)
5′, 9′, 5′′, 9′′	129.9 (4×d)	129.7 (4×d)
6′, 8′, 6′′, 8′′	130.8 (4×d)	130.7 (2×d), 130.6 (2×d)
7′, 7′′	129.9 (2×d)	128.7 (d), 128.6 (d)
1′′′	–	172.3 (s)
2′′′	–	46.6 (t)
3′′′	–	70.9 (s)
4′′′	–	46.8 (t)
5′′′	–	175.2[a] (s)
6′′′	–	27.9 (q)

[a] Low intensity.

Both (*S*)- and (*R*)-hydroxyputrescine were previously synthesized from commercial (*S*)-and (*R*)-dimethylmalate[16] by diborane reduction of the corresponding hydroxy unprotected primary diamides. In contrast, the direct reduction of *O*-TBDMS protected (*S*)-hydroxydisuccinamide to the corresponding diamine, envisaged as an advanced precursor of dicinnamamide **12**, was unfeasible by us. In fact, the diborane reduction gave mainly the N^1-monoamine instead of the desired diamine, whereas upon exposure to $LiAlH_4$ the silyl ether protection was lost and extensive racemization occurred (data not shown). A different synthetic approach was therefore conceived (Scheme 1), which was based on the reduction of diazide **9** prepared from the Staudinger reaction[17] of ditosylate **8**. In principle, **8** appeared to be available by tosylation of diol **7**, which in turn was readily achievable from the reduction of *O*-TIPS protected (*S*)-dimethylmalate **6** with DIBAL-H by a procedure outlined by Goti et al.[18]

The reduction of **6** gave diol **7** in good yields (86%); however, its subsequent bistosylation was marred by the co-occurring intramolecular S_N2 reaction of the intermediate monotosylate, which led to the TIPS derivative of 3-hydroxytetrahydrofuran. Under optimized experimental conditions, specifically, excess TsCl and pyridine as the base instead of NEt_3 at 5 °C, the yield of **8** could be raised to about 42%. In the following conversion of ditosylate **8** into diazide **9**,[17,19] the solvent proved to exert a major role, as the azidation was slow and largely incomplete after 16 h in 95% aqueous MeCN at 70 °C, whereas it went to completion in 45 min in DMF at 110 °C. Subsequently, diazide **9** was immediately treated with PPh_3 to afford diamine (*S*)-(+)-**10** (91% overall yield from **8**), which was then acylated with freshly prepared cinnamoyl chloride. Exposure of diamide **11** to aqueous TFA at room temperature resulted in smooth cleavage of the hydroxy protecting group to yield (*S*)-hydroxyputrescine-1,4-dicinnamamide (**12**). The synthetic sample was found to be identical to natural com-



Scheme 1. Synthetic route to (*S*)-2-hydroxyputrescine 1,4-dicinnamamide (**12**) from dimethyl (*S*)-malate. Yields are in brackets.

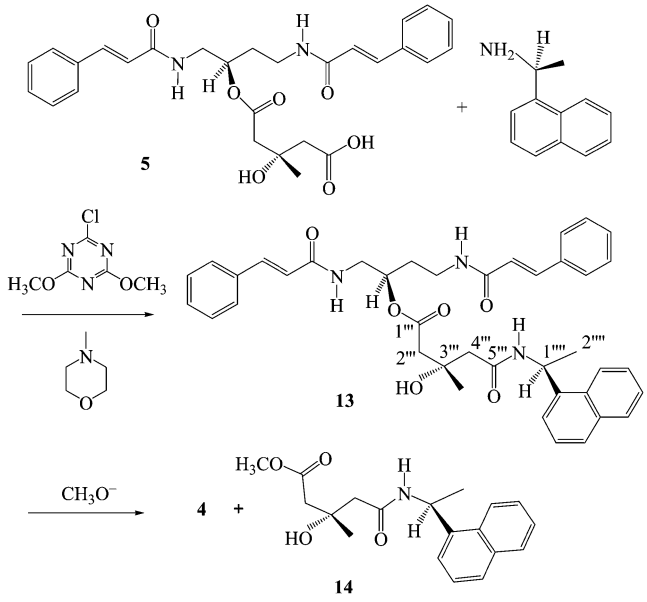
 © 2007 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

pound **4** [^{1}H and ^{13}C NMR spectra, low- and high-resolution MS (ESI+), TLC mobility under different chromatographic conditions] except for the sign of the optical rotation, which was negative for (*S*)-**12**; as a consequence, natural product **4** was assigned the (*R*) configuration.

In nature, (+)-2-hydroxyputrescine has been isolated from *Pseudomonas* bacteria,[20] and it was assigned the (*S*) configuration as the corresponding (+)-dihydrochloride.[16] Biosynthetically, it derives from putrescine.[20] Two HCA derivatives of 2-hydroxyputrescine are reported in the literature,[21] in which the amino moiety is derived from the (*S*)-enantiomer. However, the unique polyamine-derived amino acid hypusine, contained in the eukaryotic translation initiation factor 5A (eIF5A), is derived from the (*R*) enantiomer of 2-hydroxyputrescine.[22]

The last dicinnamamide to elute was **5** and it showed a more complex ^{13}C NMR spectrum than those of **3** and **4**, and two additional C=O resonances were found at δ = 172.3 and 175.2 ppm in CD_3OD. The MS (ESI) spectrum of **5** gave a clear pseudomolecular ion at *m*/*z* = 507 in the negative ionization mode, which corresponds to *MW* = 508. A typical IR broad band around 3000 cm^{-1} suggested that one of the additional carbonyl groups belonged to a carboxylic acid functionality, whereas the second carbonyl group was assigned to an ester moiety on the basis of the observed downfield acylation shift of the secondary alcohol resonance from δ = 3.8 ppm in the ^{1}H NMR spectrum of dicinnamamide **4** to δ = 5.1 ppm in the spectrum of **5**. This proton correlated, in the HSQC spectrum, with a methine oxygenated carbon at δ = 72.2 ppm. The additional presence of a quaternary carbon resonance at δ = 70.9 ppm along with a singlet at δ = 1.4 ppm for the methyl group and two AB_q signals at δ = 2.5 and 2.7 ppm in the ^{1}H NMR spectrum firmly established that **5** corresponded to the ester of **4** with 3-hydroxy-3-methyl glutaric acid (HMGA). This acidic moiety was found in a small group of biosynthetically unrelated fungal metabolites, among which the most representative are a few lanostane triterpenes, like fasciculol E, previously isolated by us from the same mushroom (*Ph. spumosa*)[1] or the hebelomic acids A, B, E and F isolated from *Hebeloma senescens*.[23] We gave compound **5** the trivial name pholiotic acid.

To assign the absolute configuration of the HMGA moiety in **5**, we followed our general procedure first used for hebelomic acid A.[23] To this purpose, pholiotic acid (**5**) was condensed with (*S*)-1-(1-naphthyl)ethylamine according to the Kaminsky protocol[24] to yield triamide **13**, which was transesterified with NaOMe to afford 2-hydroxyputrescine 1,4-dicinnamamide and glutaramide **14** (Scheme 2). The former was found to be identical to natural product **4**, including the sign and magnitude of the optical rotation. This indicated that the absolute configuration at C-2 in pholiotic acid (**5**) is (*R*), as in dicinnamamide **4**, and in agreement with straightforward biosynthetic considerations. Moreover, compound **14** was identical in all respects to an authentic sample prepared from hebelomic acid A, including the optical rotation;[23] the stereocentre of HMGA in **5** was therefore assigned the (*S*) absolute configuration. Noteworthy, all the HMGA moieties occurring as esters in different fungal metabolites show this same absolute configuration, irrespective of the structures of the alcoholic counterparts. This observation seems to indicate that the enzyme(s) catalyzing the esterification step are rather substrate-aspecific, but completely enantioselective as to the recognition of the two enantiotopic carboxylic groups of HMGA. Nothing is known so far on the biochemistry of these HMGA derivatives.



Scheme 2. Absolute stereochemistry determination of the HMGA moiety of pholiotic acid (**5**).

Antitumour Activity

The polyamines putrescine, spermidine and spermine are organic cations found in all mammalian cells and are required for cell proliferation. Polyamine analogues inhibit cell growth and kill cancer cells both in tissue culture as well as in experimental animal models.[25–29] Polyamines are important for prostate cancer cell function.

Prostate cancer, a disease still globally widespread, usually progresses from an androgen-dependent to an independent stage, which makes antiandrogen therapy ineffective and leads to an increase in metastatic potential and incurable malignancy.[30] Cytotoxic chemotherapy is used to control and treat prostate cancer at this stage, but it remains relatively unselective and highly toxic to normal tissues. Therefore, there has been increasing interest in the use of structural polyamine analogues as prostate cancer chemotherapeutic agents, as a variety of analogues have been synthesized and shown to be active against several prostate cancer cells.[31,32] Our previous study demonstrated that putrescine-1,4-dicinnamamide (**1**), isolated from the same fungus (*Pholiota spumosa*),[1] inhibits the cell growth of DU-145 cancer cells,[12] as it was also found for some synthetic polyamine analogues.[31,32]

 © 2007 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

We have now investigated the responses of the human prostate cancer cell lines LNCaP and DU-145 to maytenine (**3**) and pholiotic acid (**5**). These cell lines were chosen because they represent a spectrum of androgen-dependent and androgen-independent prostate cancers, respectively. The results, summarized in Figure 1, show that compounds **3** and **5**, used at nontoxic concentrations in normal cells (data not shown), exhibited a significant ($p < 0.001$) and a dose-dependent inhibitory effect only on DU-145 cell growth. In fact, the vitality was 87 and 91% of LNCaP cells exposed to 50 μM of compounds **3** and **5**, respectively. In contrast, other studies have evidenced that DU-145, a well-characterized androgen-independent human prostate cancer cell line, was more sensitive to cell growth inhibition induced by polyamine analogues than androgen-sensitive LNCaP prostate cancer cells, and that the latter cancer cells maintained the largest total polyamine content.[31]

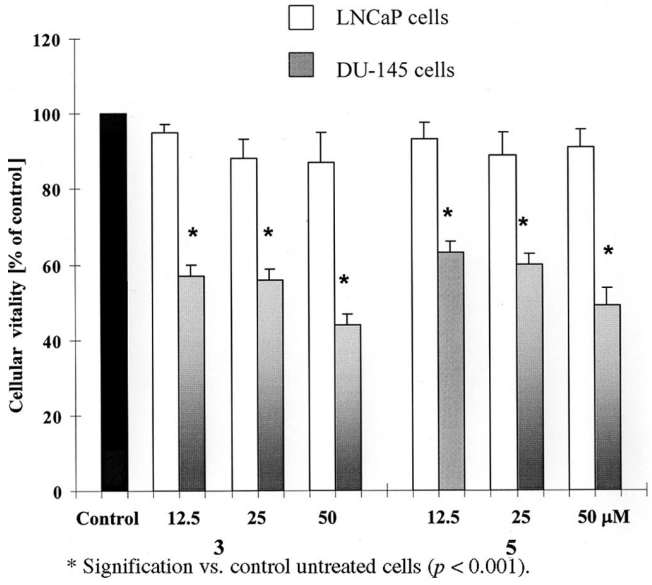


Figure 1. Effect of compounds **3** and **5**, at different concentrations for 72 h, on LNCaP and DU-145 cell growth. The stock solution of the compounds was prepared in DMSO and the final concentration of this solvent was kept constant at 0.25%. Control cultures received DMSO alone. Reported values are the mean ± SD of three experiments performed in quadruplicate.

Necrosis results in a disruption of cytoplasmic membrane and the necrotic cells release cytoplasmic LDH and other cytotoxic substances into the medium. We therefore examined the membrane permeability of the treated cells and the existence of LDH in their culture medium. No statistically significant increase in LDH release was observed in DU-145 cells treated with compounds **3** and **5** even at the highest concentration, specifically, 50 μM (Table 3). The tested compounds were also inactive on LNCaP cells in this assay (Table 4).

Nuclear DNA fragmentation was analyzed by using the COMET assay, a sensitive method for the detection of DNA strand breaks in individual cells; this is a versatile tool that is highly effective in human biomonitoring of natural compounds. Quantification of the COMET data is re-

Table 3. Lactate dehydrogenase (LDH) release in LNCaP and DU-145 cells: untreated and treated with compounds **3** and **5** at different concentrations for 72 h. The stock solution of the compounds was prepared in DMSO and the final concentration of this solvent was kept constant at 0.25%. Control cultures received DMSO alone. The results are expressed as percentage of LDH released into the cell medium with respect to total LDH. Each value represents the mean ± SD of three experiments, performed in quadruplicate.

Treatments	% LDH released
LNCaP cells	
Control	6.9 ± 0.7
3	
12.5 μM	7.3 ± 0.1
25 μM	6.4 ± 0.9
50 μM	7.5 ± 0.8
5	
12.5 μM	4.9 ± 0.9
25 μM	6.3 ± 0.7
50 μM	7.5 ± 0.6
DU-145 cells	
Control	4.6 ± 0.3
3	
12.5 μM	4.4 ± 0.5
25 μM	5.4 ± 0.5
50 μM	5.7 ± 0.9
5	
12.5 μM	3.4 ± 0.6
25 μM	4.4 ± 0.5
50 μM	5.8 ± 0.9

Table 4. Comet assay of genomic DNA in LNCaP and DU-145 cells: untreated and treated with compounds **3** and **5** at different concentrations for 72 h. The stock solution of the compounds was prepared in DMSO and the final concentration of this solvent was kept constant at 0.25%. Control cultures received DMSO alone. Reported values are the mean ± SD of three experiments performed in quadruplicate.

Treatments	TDNA[a]	TMOM[b]
LNCaP cells		
Control	15 ± 3.5	97 ± 5.7
3		
12.5 μM	17 ± 5.3	105 ± 16
25 μM	17 ± 3.4	99 ± 7
50 μM	18 ± 2.3	103 ± 13
5		
12.5 μM	16 ± 7.3	95 ± 11
25 μM	14 ± 4.1	111 ± 9
50 μM	13 ± 2.7	100 ± 14
DU-145 cells		
Control	21 ± 5	96 ± 7.7
3		
12.5 μM	86 ± 15[c]	996 ± 15[c]
25 μM	101 ± 13[c]	1112 ± 39[c]
50 μM	198 ± 19[c]	2009 ± 36[c]
5		
12.5 μM	88 ± 14[c]	989 ± 18[c]
25 μM	111 ± 13[c]	1123 ± 27[c]
50 μM	176 ± 17[c]	1889 ± 36[c]

[a] TDNA is the percentage of the fragmented DNA. [b] TMOM is the tail moment expressed as the product of TD (distance between head and tail) and TDNA. [c] Significant vs. control untreated cells ($p < 0.001$).

 © 2007 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

ported as TDNA and TMOM values in Table 4. The results clearly evidence DNA damage only in DU-145 cells exposed to compounds **3** and **5** for 72 h and drastic increases in both the TDNA and TMOM values at 25 and 50 μM concentrations are also observed. These results seem to confirm an apoptotic cell death, as recent literature data[33] indicate that only comets with high values of TMOM (tail moments) and TDNA (distance between head and tail of the comet) can be related to apoptosis.

In summary, these data suggest that compounds **3** (maytenine) and **5** (pholiotic acid), which are fungal metabolites belonging to the class of polyamine conjugates, may have chemotherapeutic potential in the case where prostatic cancer has become androgen independent, which justifies further investigations in order to discover new biological mechanisms.

Experimental Section

General Conditions: Optical rotations were determined with a Perkin–Elmer 241 polarimeter. Melting points were determined with a Stuart Scientific SMP3 apparatus. IR spectra were recorded with an FTIR Perkin–Elmer BX spectrometer. The NMR experiments were performed with a Bruker Avance 400 NMR spectrometer; the ^{1}H and ^{13}C chemical shifts are reported relative to residual solvent signals: CD_3OD: 3.31 (^{1}H), 49.0 (^{13}C); $CDCl_3$: 7.26 (^{1}H), 77.10 (^{13}C); DMSO: 2.50 (^{1}H), 39.5 (^{13}C); THF: 3.74, 1.88 (^{1}H), 68.7, 25.7 (^{13}C); CD_3CN: 1.94 (^{1}H), 118.2, 1.3 (^{13}C). The abbreviation s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and br. = broad are used throughout. MS (ESI) experiments were carried out both in positive and in negative ion mode by using an ion-trap Thermo Finnigan LCQ Duo Thermoquest spectrometer equipped with the Xcalibur software. High-resolution ESI mass spectra were determined with an FT-ICR Apex II Mass Spectrometer of Bruker Daltonics. UV spectra were recorded with a UNICAM 8700 UV/Vis Spectrophotometer, and CD spectra were recorded with a Jasco J-710 spectropolarimeter. Thin-layer chromatography was performed on silica gel F_{254} sheets (direct phase TLC) or RP-18 F_{254} sheets (reverse-phase TLC). The compounds were visualized under UV light (254 and 366 nm) and by spraying with sulfoanisaldehyde, phosphomolybdic acid or ninhydrin solutions, followed by heating. Medium-pressure liquid chromatography (MPLC) was performed with Fluka silica gel 100 C-18 (15–35 μm, fully endcapped) or Merck-VWR LiChrospher RP-18 (12 μm). Lipophilic Sephadex LH-20 (25–100 μm), purchased from Sigma–Aldrich, was employed for gel-filtration liquid chromatography. Gas chromatographic analysis was performed with a Perkin–Elmer Autosystem apparatus (Agilent Technologies) equipped with a dimethylpolysiloxane capillary column (25 m long, 0.20 mm i.d., 0.33 μm film thickness). All glassware used in the synthesis of compounds **12** and **14** were dried in an oven at 100 °C overnight and assembled under a stream of dry N_2.

Fungal Material, Extraction and Isolation: Fruiting bodies of *Ph. spumosa* (1.2 kg) were collected in the Reserva Nacional Forestal Lago Peñuelas (Valparaiso, Chile) and identified by one of us (M. C.). A voucher specimen was deposited at the University F. S. Maria of Valparaiso. Immediately after collection, the mushrooms were frozen at –20 °C; eventually they were minced whilst frozen and then extracted with EtOH, and the solution was allowed to reach r.t. The solution thus obtained was filtered and then concentrated under reduced pressure at 35 °C; the solid residue (5 g) was separated by MPLC (flow 7 mL min^{-1}, pressure 100 psi, λ = 245 nm) on an RP-18 reverse-phase column (particle size: 15–35 μm) by using a H_2O/MeOH gradient (from 7:3 to 1:3). Nineteen fractions were collected. The most polar fractions (A_{1-3}) contained a mixture of styryl–pyrones, whereas fasciculol E[1] was eluted in A_4 and A_5. Fractions A_6 and A_7 contained maytenine (**3**), which after a second separation with Sephadex LH-20 (MeOH/EtOAc, 7:3) was obtained in pure form (40 mg). (*R*)-2-Hydroxyputrescine 1,4-dicinnamamide (**4**) was eluted in fractions A_{8-13}, together with pholiotic acid (**5**) and putrescine 1,4-dicinnamamide (**1**), which was obtained pure (70 mg) in fractions A_{14} and A_{15}. Fractions A_{8-13} were subsequently purified by gel-permeation chromatography on a Sephadex LH-20 column, eluted with MeOH, which allowed separation of **4** from acid **5**. Coeluting dicinnamamides **4** and **1** (20 mg) were finally purified by an RP-18 column (particle size:12 μm), eluted with a gradient mixture of H_2O/MeOH/MeCN (3:2:1 to 1:1:1 + 0.5% HCOOH). This allowed alcohol **4** to be obtained in an almost pure form (6 mg). The LH-20 fractions containing pholiotic acid (**5**; 60 mg) were submitted to a final purification with an RP-18 column (particle size: 12 μ) and eluted with a mixture of H_2O/MeOH/MeCN (1:1:1 to 1:2:1 + 0.5% HCOOH); 28 mg of **5** were obtained in this way. In fractions A_{16} and A_{17}, dicinnamamide **1** and small amounts of (*E*)-2,3-dehydroputrescine 1,4-dicinnamamide (**2**) coeluted.

***N*^{1},*N*^{8}-Dicinnamoylspermidine (Maytenine; 3):** Yellow waxy solid. ^{1}H NMR (400 MHz, CD_3OD): δ = 7.59–7.48 (m, 6 H, Ar), 7.46 (d, *J* = 16.0 Hz, 2 H, 3′-H and 3′′-H), 7.34–7.27 (m, 4 H, Ar), 6.58 (d, *J* = 16.0 Hz, 2 H, 2′-H and 2′′-H), 3.41 (m, 2 H, 1-H), 3.30 (m, 2 H, 7-H), 3.02 (m, 4 H, 3-H and 4-H), 1.94 (m, 2 H, 2-H), 1.71 (m, 2 H, 5-H), 1.48 (m, 2 H, 6-H) ppm. ^{13}C NMR (100 MHz, CD_3OD): δ = 169.5 (s, C-1′ or C-1′′), 168.8 (s, C-1′′ or C-1′), 142.3 (d, C-3′ or C-3′′), 141.8 (d, C-3′′ or 3′), 136.1 (s, C-4′ or C-4′′), 136.0 (s, C-4′′ or C-4′), 130.9 (d, 2 C, C-7′ and C-7′′), 130.0 (d, 4 C, C-6′, C-6′′, C-8′, C-8′′), 128.9 (d, 2 C, C-5′ and C-5′′ or C-9′ and C-9′′), 128.8 (d, 2 C, C-9′ and C-9′′ or C-5′ and C-5′′), 121.7 (d, C-2′ or C-2′′), 121.3 (d, C-2′′ or C-2′), 48.1 (t, C-4), 46.2 (t, C-3), 39.6 (t, C-7), 37.1 (t, C-1), 27.7 (t, C-2), 27.5 (t, C-6), 24.6 (t, C-5) ppm. ^{1}H NMR (400 MHz, [D_8]THF + 5% D_2O): δ = 8.21 (m, 2 H, amidic N-*H*), 7.34–7.32 (m, 4 H, Ar), 7.30 (d, *J* = 15.0 Hz, 1 H, 3′-H or 3′′-H), 7.28 (d, *J* = 15.0 Hz, 1 H, 3′′-H or 3′-H), 7.12–7.05 (m, 4 H, Ar), 6.48 (d, *J* = 15.0 Hz, 1 H, 2′-H or 2′′-H), 6.46 (d, *J* = 15.0 Hz, 1 H, 2′′-H or 2′-H), 3.34 (br. t, 2 H, 1-H), 3.26 (t, *J* = 6.4 Hz, 2 H, 7-H), 2.99 (m, 4 H, 3-H and 4-H), 1.99 (m, 2 H, 2-H), 1.79 (m, 2 H, 5-H), 1.59 (m, 2 H, 6-H) ppm. ^{13}C NMR (100 MHz, [D_8]THF + 5% D_2O): δ = 168.2 (s, C-1′ or C-1′′), 167.7 (s, C-1′′ or C-1′), 141.2 (d, C-3′ or C-3′′), 140.8 (d, C-3′′ or C-3′), 136.8 (s, C-4′ or C-4′′), 136.6 (s, C-4′′ or C-4′), 130.5 (d, Ar), 130.4 (d, Ar), 129.9 (d, Ar), 129.8 (d, Ar), 129.1 (d, Ar), 129.0 (d, Ar), 121.9 (d, C-2′ or C-2′′), 121.5 (d, C-2′′ or C-2′), 48.5 (t, C-4), 46.5 (t, C-3), 39.9 (t, C-7), 37.7 (t, C-1), 27.6 (t, 2 C, C-2 and C-6), 24.8 (t, C-5) ppm. IR (thin film): $\tilde{\nu}$ = 3300, 3273, 2930, 2891, 1670, 1657, 1614, 1515, 1224, 979 cm^{-1}. MS (ESI+): *m*/*z* = 406 [M + H]$^+$, 833 [2M + Na]$^+$. MS (ESI–, NEt_3 added): *m*/*z* = 404.45 [M – H]$^-$. HRMS (ESI+): calcd. for $C_{25}H_{32}N_3O_2^+$ [M + H]$^+$ 406.24890; found 406.24864.

(*R*)-(+)-2-Hydroxyputrescine 1,4-Dicinnamamide (4): Microcrystalline powder, $[\alpha]_D^{22}$ = +3.0 (*c* = 0.5, MeOH). ^{1}H and ^{13}C NMR (CD_3OD), see Tables 1 and 2, respectively. UV (MeOH): λ (ε, L mol^{-1} cm^{-1}) = 275.0 (10600), 204.5 (9200) nm. CD (MeOH): λ ($\Delta\varepsilon$) = 285.0 (+0.26), 256.0 (–0.33) nm. MS (ESI+): *m*/*z* = 365.2 [M + H]$^+$, 387.2 [M + Na]$^+$. HRMS (ESI+): calcd. for $C_{22}H_{24}N_2O_3Na^+$ 387.16791; found 387.16807.

 © 2007 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

Pholiotic Acid {(2*R*)-2-[(*S*)-3-Hydroxy-3-methylglutaryloxy]putrescine Dicinnamamide; 5}: Waxy solid. $[a]_D^{22}$ = +18.5 (*c* = 0.4, MeOH). ^{1}H and ^{13}C NMR (CD_3OD), see Tables 1 and 2, respectively. ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 8.40 (m, 1 H, N*H*), 8.11 (br. t, 1 H, N*H*), 7.55–7.51 (m, 4 H, Ar), 7.49–7.40 (m, 8 H, Ar, 3′-H and 3′′-H), 6.67 (d, *J* = 14.6 Hz, 1 H, 2′-H or 2′′-H), 6.58 (d, *J* = 14.6 Hz, 1 H, 2′′-H or 2′-H), 4.89 (m, 1 H, 2-H), 3.45 (m, 2 H, 1-H), 3.21 (m, 2 H, 4-H), 2.57 (AB_q, 2 H, 2′′′-H or 4′′′-H), 2.41 (AB_q, 2 H, 4′′′-H or 2′′′-H), 1.73 (m, 2 H, 3-H), 1.27 (s, 3 H, 6′′′-H) ppm. ^{13}C NMR (100 MHz, [D_6]DMSO): δ = 173.0 (s, C-5′′′), 170.4 (s, C-1′′′), 165.3 (s, C-1′ or C-1′′), 165.0 (s, C-1′′ or C-1′), 139.0 (d, C-3′ or C-3′′), 138.6 (d, C-3′′ or C-3′), 135.0 (s, 2 C, C-4′ and C-4′′), 129.5 (d, 4 C, Ar), 129.0 (d, 2 C, Ar), 127.6 (d, 4 C, Ar), 122.3 (d, C-2′ or C-2′′), 122.1 (d, C-2′′ or C-2′), 70.8 (d, C-2), 69.2 (s, C-3′′′), 46.0 (t, 2 C, C-2′′′ and C-4′′′), 41.3 (t, C-1), 35.3 (t, C-4), 31.3 (t, C-3), 27.6 (q, C-6′′′) ppm. IR (thin film): $\tilde{\nu}$ = 3700–2400 (br., CO*OH* stretching), 3308, 3077, 2924, 2851, 1723, 1714, 1659, 1620, 1614, 1556, 1449, 1343, 1226, 1079, 1028, 978 cm^{-1}. UV (MeOH): λ (ε, L mol^{-1} cm^{-1}): 274.5 (9000), 216.5 (6800) nm. CD (MeOH): λ ($\Delta\varepsilon$) = 331.0 (+0.06), 268.5 (+0.29), 220.0 (–0.47) nm. MS (ESI–): *m*/*z* = 507.4 [M – H]$^-$. MS (ESI+, HCOOH added): *m*/*z* = 509.6 [M + H]$^+$, 531.2 [M + Na]$^+$. MS (EI): *m*/*z* (%) = 448 (1) [M – CH_3COOH]$^+$, 407 (4), 406 (5), 405 (3), 259 (10), 199 (65), 131 (100), 103 (71), 77 (46). HRMS (ESI+): calcd. for $C_{28}H_{31}N_2O_7^-$ [M – H]$^-$ 507.21367; found 507.21325.

Methyl Pholiotate (5a): An ethereal solution of CH_2N_2 (0.4 mL) was added dropwise to pholiotic acid (**5**; 8 mg) in MeOH (0.5 mL). The resulting solution was evaporated under vacuum, and the residue was chromatographed on a silica gel column (benzene/EtOAc, 2:1) to afford methyl pholiotate (**5a**; 4 mg) as a colourless oil. $[a]_D^{22}$ = +4.0 (*c* = 0.2, MeOH). ^{1}H NMR (400 MHz, CD_3OD): δ = 7.60–7.48 (m, 4 H, Ar), 7.41–7.34 (m, 8 H, Ar, 3′-H and 3′′-H), 6.61 (d, *J* = 15.8 Hz, 2 H, 2′-H and 2′′-H), 5.14 (m, 1 H, 2-H), 3.68 (m, 1 H, 1a-H), 3.64 (s, 3 H, COOCH_3), 3.45 (m, 1 H, 1b-H), 3.39 (m, 2 H, 4-H), 2.74 (AB_q, 2 H, 2′′′-H or 4′′′-H), 2.70 (AB_q, 2 H, 4′′′-H or 2′′′-H), 1.89 (m, 2 H, 3-H), 1.41 (s, 3 H, 6′′′-H) ppm. ^{13}C NMR (100 MHz, CD_3OD): δ = 173.2 (s, C-5′′′), 172.4 (s, C-1′′′), 169.1 (s, C-1′ or C-1′′), 168.9 (s, C-1′′ or C-1′), 142.3 (d, C-3′ or C-3′′), 142.1 (d, C-3′′ or C-3′), 136.3 (d, 2 C, C-4′ and C-4′′), 130.1 (d, 4 C, Ar), 129.0 (d, 2 C, Ar), 122.4 (d, 4 C, Ar), 121.7 (d, C-2′ or C-2′′), 121.6 (d, C-2′′ or C-2′), 72.3 (d, C-2), 71.1 (s, C-3′′′), 52.1 (q, COOCH_3), 46.6 (t, C-2′′′ or C-4′′′), 46.3 (t, C-4′′′ or C-2′′′), 43.4 (t, C-1), 37.1 (t, C-4), 32.5 (t, C-3), 26.1 (q, C-6′′′) ppm. MS (ESI+): *m*/*z* = 523 [M + H]$^+$, 545 [M + Na]$^+$.

Dimethyl (*S*)-(–)-2-(Triisopropylsilyloxy)butandioate (6): Triisopropylsilyl chloride (3.86 g, 20 mmol) and imidazole (0.64 g, 9.5 mmol) were added to dimethyl (*S*)-malate (1.5 g, 9.2 mmol) in DMF (10 mL). The reaction mixture was stirred at r.t. for 27 h under a N_2 atmosphere. At the end, H_2O (10 mL) was added, and the mixture was extracted with CH_2Cl_2 (2 × 10 mL). The organic layer was dried with anhydrous Na_2SO_4, and the solvent was removed under vacuum. The residue (5.6 g) was chromatographed on a silica gel column deactivated with Et_3N (toluene/CH_2Cl_2, 3:1) to afford the protected dimethyl malate **6** (2.28 g, 78%) as a colourless viscous liquid. $[a]_D^{22}$ = –12.8 (*c* = 1.9, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.72 (t, *J* = 6.2 Hz, 1 H, 2-H), 3.71 (s, 3 H, OCH_3), 3.65 (s, 3 H, OCH_3), 2.77 (d, *J* = 6.0 Hz, 1 H, 3a-H), 2.74 (d, *J* = 6.0 Hz, 1 H, 3b-H), 1.13 [m, 3 H, 3 × C*H*(CH_3)$_2$], 1.06 [br., 18 H, 3 × CH(CH_3)$_2$] ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.3 (s, C-1), 171.3 (s, C-4), 70.1 (d, C-2), 52.6 (q, OCH_3), 52.3 (q, OCH_3), 41.1 (t, C-3), 18.5 [q, 6 C, 3 × CH(CH_3)$_2$], 13.2 [br., 3 C, 3 × *C*H(CH_3)$_2$] ppm. MS (ESI+): *m*/*z* = 341.2 [M + Na]$^+$.

(*S*)-2-(Triisopropylsilyloxy)butane-1,4-diol (7): A solution of DIBAL-H (1 M in CH_2Cl_2, 31.2 mL, 5.2 equiv.) was added dropwise to compound **6** (1.9 g, 6.0 mmol) in CH_2Cl_2 (5 mL) under a N_2 flow at –15 °C, and the disappearance of **6** was monitored by GC. After 3 h, the reaction mixture was quenched by adding 1-propanol (6 mL) and H_2O (3 mL) and then partitioned between an aqueous saturated solution of NH_4Cl (10 mL) and 2-butanone (20 mL). The organic layer was washed with H_2O (5 mL) and dried with anhydrous Na_2SO_4; the solvent was evaporated under vacuum to afford pure diol **7** (1.34 g, 86%) as a viscous liquid. $[a]_D^{22}$ = –7.3 (*c* = 4.2, MeOH). ^{1}H NMR (400 MHz, CD_3CN): δ = 4.12 (m, 1 H, 2-H), 3.80 (m, 2 H, 1-H), 3.70 (m, 2 H, 4-H), 1.93 (m, 2 H, 3-H), 1.30 [m, 3 H, 3 × C*H*(CH_3)$_2$], 1.16 [br., 18 H, 3 × CH(CH_3)$_2$] ppm. ^{13}C NMR (100 MHz, CD_3CN): δ = 72.0 (t, C-1), 66.5 (d, C-2), 58.9 (t, C-4), 38.2 (t, C-3), 18.5 [q, 6 C, 3 × CH(*C*H_3)$_2$], 13.2 [d, 3 C, 3 × *C*H(CH_3)$_2$] ppm. MS (ESI+): *m*/*z* = 285.2 [M + Na]$^+$.

(*S*)-2-(Triisopropylsilyloxy)butane-1,4-diol Ditosylate (8): Compound **7** (1.34 g, 5.1 mmol) in dry MeCN (3 mL) was added dropwise to *p*-toluenesulfonyl chloride (5 g, 26 mmol) in CH_2Cl_2 (6 mL) to which dry pyridine (1.5 mL) had been previously added. The reaction mixture was stirred for 1 h at 5 °C, followed by an additional hour at r.t., and then quenched by adding H_2O (10 mL). The aqueous phase was extracted with CH_2Cl_2 (2 × 10 mL). The organic layers were collected, dried with anhydrous Na_2SO_4 and the solvents evaporated under vacuum. The resulting residue (2.15 g) was chromatographed on a silica gel column deactivated with Et_3N (petroleum ether/CH_2Cl_2, 8.5:1.5 to 3:7) to afford **8** (1.2 g, 42%) as a colourless viscous liquid. $[a]_D^{22}$ = –8.6 (*c* = 1.1, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.74 (m, 4 H, Ar), 7.36–7.32 (m, 4 H, Ar), 4.08 (m, 3 H, 1a-H, 2-H and 4a-H), 3.87 (m, 1 H, 4b-H), 3.82 (m, 1 H, 1b-H), 2.45 (s, 3 H, Ar-CH_3), 2.44 (s, 3 H, Ar-CH_3), 1.83 (m, 2 H, 3-H), 0.92 [m, 3 H, 3 × C*H*(CH_3)$_2$], 0.88 [br., 18 H, 3 × CH(CH_3)$_2$] ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 145.2 (s, Ar), 145.0 (s, Ar), 132.9 (s, Ar), 132.7 (s, Ar), 130.0 (d, 2 C, Ar), 129.9 (d, 2 C, Ar), 128.1 (d, 2 C, Ar), 128.0 (d, 2 C, Ar), 72.1 (t, C-1), 66.9 (d, C-2), 66.3 (t, C-4), 33.5 (t, C-3), 27.7 (q, 2 C, 2 × Ar-*C*H_3), 18.0 [q, 6 C, 3 × CH(*C*H_3)$_2$], 12.4 [br., 3 C, 3 × *C*H(CH_3)$_2$] ppm. MS (ESI+): *m*/*z* = 593.16 [M + Na]$^+$, 571.26 [M + H]$^+$.

(*S*)-(1,4-Diazidobutyl-2-oxy)triisopropylsilane (9): Solid NaN_3 (1.40 g, 21.6 mmol) was added portionwise to compound **8** (1.2 g, 2.16 mmol) in DMF (6 mL), and the reaction mixture was stirred at 110 °C for 45 min. H_2O (10 mL) was added, and the mixture was extracted with EtOAc (2 × 10 mL). The collected organic layers were dried with anhydrous Na_2SO_4, and the solvent was evaporated under vacuum at r.t. to yield diazide **9** (0.64 g, 95%), which was immediately used in the next reaction.

(*S*)-(+)-2-(Triisopropylsilyloxy)butane-1,4-diamine (10): PPh_3 (1.47 g, 5.61 mmol) dissolved in MeCN (5 mL) was added to a solution of freshly prepared diazide **9** (0.64 g, 2.04 mmol) in MeCN (5 mL) whilst stirring, and the resulting mixture was kept at 60 °C for 1 h. Subsequently, H_2O (3 mL) and 30% NH_4OH (7 mL) were added and stirring was continued at 60 °C for an additional hour. The reaction mixture was cooled to r.t. and then was extracted with EtOAc; the organic layer was dried with Na_2SO_4, and the solvent was evaporated under vacuum. The residue was chromatographed on an RP-18 column (H_2O/MeOH, 2:3 to 1:9 + 1% acetic acid) to yield **10** (0.48 g, 91%) as a viscous liquid. $[a]_D^{22}$ = +1.7 (*c* = 0.33, MeOH). ^{1}H NMR (400 MHz, CD_3OD): δ = 4.15 (m, 1 H, 2-H), 2.91 (m, 1 H, 1a-H), 2.87 (m, 1 H, 1b-H), 2.82 (m, 1 H, 4a-H), 2.79 (m, 1 H, 4b-H), 1.86 (m, 1 H, 3a-H), 1.80 (m, 1 H, 3b-H), 1.15 [m, 3 H, 3 × C*H*(CH_3)$_2$], 1.10 [d, *J* = 6.6 Hz, 18 H, 3 × CH(CH_3)$_2$]

 © 2007 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

ppm. ^{13}C NMR (100 MHz, CD_3OD): δ = 71.4 (d, C-2), 51.9 (t, C-1), 40.1 (t, C-3), 40.0 (t, C-4), 18.5 [q, 6 C, 3 × CH(CH_3)$_2$], 13.4 [d, 3 C, 3 × CH(CH$_3$)$_2$] ppm. MS (ESI+): *m*/*z* = 261 [M + H]$^+$. HRMS (ESI+): calcd. for $C_{13}H_{33}N_2OSi^+$ 261.23567; found 261.23545.

Cinnamoyl Chloride: To a solution of cinnamic acid (1.3 g, 9 mmol) in CH_2Cl_2 (5 mL) was added oxalyl chloride (3.4 g, 27 mmol). The reaction mixture was stirred at r.t. for 3 h; subsequently, the solvent and excess oxalyl chloride were removed under vacuum. The cinnamoyl chloride thus obtained was employed in the next reaction step with no further purification.

(*S*)-(–)-2-(Triisopropylsilyloxy)putrescine 1,4-Dicinnamamide (11): To freshly prepared cinnamoyl chloride was added dropwise a solution of **10** (0.45 g, 1.73 mmol) in CH_2Cl_2 (5 mL) followed by dry pyridine (1 mL). Whilst stirring, the reaction was kept at 30 °C for 1 h. Subsequently, the solvent was evaporated under vacuum, and the residue was chromatographed on a silica gel column deactivated with Et_3N (toluene/EtOAc, 3:2) to yield dicinnamamide **11** (0.75 g, 73%) as a waxy solid. $[\alpha]_D^{22}$ = –4.6 (*c* = 0.4, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.82 (d, *J* = 15.6 Hz, 1 H, 3′-H or 3′′-H), 7.75 (d, *J* = 15.6 Hz, 1 H, 3′′-H or 3′-H), 7.64–7.60 (m, 6 H, Ar), 7.53–7.39 (m, 4 H, Ar), 7.22 (m, 2 H, 2 × N-*H*), 6.68 (d, *J* = 15.6 Hz, 1 H, 2′-H or 2′′-H), 6.60 (d, *J* = 15.6 Hz, 1 H, 2′′-H or 2′-H), 4.24 (m, 1 H, 2-H), 3.90 (m, 1 H, 1a-H), 3.75 (m, 1 H, 1b-H), 3.63 (m, 1 H, 4a-H), 3.51 (m, 1 H, 4b-H), 2.03 (m, 2 H, 3-H), 1.39 [m, 3 H, 3 × C*H*(CH$_3$)$_2$], 1.18 [br., 18 H, 3 × CH(C*H*$_3$)$_2$] ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 167.6 (s, C-1′or C-1′′), 167.3 (s, C-1′′ or C-1′), 142.4 (d, C-3′ or C-3′′), 141.5 (d, C-3′′ or C-3′), 135.8 (s, C-4′ or C-4′′), 135.4 (s, C-4′′ or C-4′), 130.3 (d, 5 C, Ar), 129.5 (d, 5 C, Ar), 121.6 (d, C-2′ or 2′′), 121.1 (d, C-2′′ or C-2′), 71.0 (d, C-2), 45.1 (t, C-1), 37.0 (t, C-4), 34.2 (t, C-3), 18.9 [q, 6 C, 3 × CH(*C*H$_3$)$_2$], 13.1 [d, 3 C, 3 × *C*H(CH$_3$)$_2$] ppm. MS (ESI+): *m*/*z* = 543.4 [M + Na]$^+$, 521.3 [M + H]$^+$. MS (ESI–): *m*/*z* = 519.1 [M – H]$^-$.

(*S*)-(–)-2-Hydroxyputrescine 1,4-Dicinnamamide (12): To dicinnamamide **11** (0.75 g, 1.44 mmol) was added THF (3 mL), H_2O (1 mL) and CF_3COOH (1 mL) whilst stirring at r.t. The reaction was monitored by silica gel TLC (toluene/EtOAc, 3:7), and it went to completion in 6 h. After neutralization with a saturated aqueous solution of $NaHCO_3$, the volatiles were removed under vacuum, and the residue was chromatographed on a silica gel column (toluene/EtOAc, 1:1 to EtOAc/MeOH, 9:1) to afford compound **12**. Recrystallization from MeOH gave a white microcrystalline powder (0.51 g, 98%). M.p. >190 °C (decomp.). $[\alpha]_D^{22}$ = –2.5 (*c* = 1.6, MeOH). The ^{1}H and ^{13}C NMR spectra in CD_3OD were identical to those of natural product **4** (see Tables 1 and 2, respectively). CD (MeOH): λ ($\Delta\varepsilon$) = 288.0 (–0.28), 258.0 (+0.29) nm. MS (ESI+): *m*/*z* = 365.2 [M + H]$^+$, 387.2 [M + Na]$^+$. HRMS (ESI+): calcd. for $C_{22}H_{24}N_2O_3Na$ [M + Na]$^+$ 387.16791; found 387.16764. HRMS (ESI+): calcd. for $C_{44}H_{48}N_4O_6Na$ [2M + Na]$^+$ 751.34661; found 751.34725.

(*R*)-2-{(*S*)-4-[(*S*)-1-(1-Naphthalen-1-yl)ethylacarbamoyl]-3-hydroxy-3-methylbutanoyloxy}putrescine 1,4-Dicinnamamide (13): To a stirred solution of pholiotic acid (**5**; 20 mg, 39.3 μmol) and 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT; 7 mg, 40 μmol) in MeCN (2 mL), was added dropwise *N*-methylmorpholine (4.4 μL, 40 μmol) whilst the temperature of the solution was kept at about –5 °C. Stirring was continued at 0 °C for 4 h, and the reaction was monitored by silica gel TLC (EtOAc/MeOH, 9:1). Subsequently, to the solution was added a mixture of *N*-methylmorpholine (4.4 μL, 40 μmol) and (*S*)-1-(1-naphthyl)ethylamine (7 mg, 40 μmol) in THF (2 mL). The reaction was stirred for an additional 2 h at 0 °C and for 14 h at r.t. The volatiles were evaporated under vacuum, and the residue was suspended in EtOAc (15 mL). This suspension was washed successively with H_2O (5 mL), a 10% citric acid solution (5 mL), H_2O (5 mL), a saturated $NaHCO_3$ solution (5 mL) and finally H_2O (5 mL). The organic layer was dried (Na_2SO_4), and the solvent was evaporated. The residue was chromatographed on a silica gel column (toluene/EtOAc, 2.5:1 to 1:1) to afford pure triamide **13** (15 mg, 61%) as a waxy solid $[\alpha]_D^{22}$ = +5.0 (*c* = 0.9, MeOH). ^{1}H NMR (400 MHz, CD_3OD): δ = 7.85 (m, 1 H, Ar), 7.76 (d, *J* = 8.0 Hz, 1 H, Ar), 7.51–7.46 (m, 7 H, Ar, 3′-H and 3′′-H), 7.44 (m, 1 H, Ar), 7.42 (m, 1 H, Ar), 7.38–7.34 (m, 8 H, Ar), 6.60 (d, *J* = 15.8 Hz, 1 H, 2′-H or 2′′-H), 6.58 (d, *J* = 15.8 Hz, 1 H, 2′′-H or 2′-H), 5.85 (q, *J* = 6.9 Hz, 1 H, 1′′′′-H), 5.09 (m, 1 H, 2-H), 3.89 (dd, *J* = 8.8 and 3.8 Hz, 1 H, 1a-H), 3.65 (m, 1 H, 1b-H), 3.59 (m, 1 H, 4a-H), 3.43 (m, 1 H, 4b-H), 2.60 (m, 2 H, 2′′′or-H 4′′′-H), 2.59 (m, 2 H, 4′′′-H or 2′′′-H), 1.84 (m, 2 H, 3-H), 1.58 (d, *J* = 6.9 Hz, 3 H, 2′′′′-H), 1.37 (s, 3 H, 6′′′-H) ppm. ^{13}C NMR (100 MHz, CD_3OD): δ = 172.5 and 172.4 (s, 2 C, C-1′′′ and C-5′′′), 168.9 and 168.6 (s, 2 C, C-1′ and C-1′′), 142.1 and 141.8 (d, 2 C, C-3′ and C-3′′), 140.2 (s, Ar), 136.3 (s, Ar), 136.2 (s, Ar), 135.4 (s, Ar), 132.2 (s, Ar), 130.9 (d, Ar), 130.8 (d, Ar), 130.0 (d, Ar), 129.9 (d, Ar), 129.0 (d, Ar), 128.9 (d, Ar), 128.8 (d, Ar), 127.3 (d, Ar), 126.7 (d, Ar), 126.4 (d, Ar), 124.2 (d, Ar), 123.6 (d, Ar), 121.8 and 121.6 (d, 2 C, C-2′ and C-2′′), 72.3 (d, C-2), 71.6 (s, C-3′′), 47.7 (t, C-2′′′ or C-4′′′), 46.8 (t, C-1), 46.0 (d, C-1′′′′), 43.3 (t, C-4′′′ or C-2′′′), 36.9 (t, C-4), 32.5 (t, C-3), 28.2 (q, C-6′′′), 21.6 (q, C-2′′′′) ppm. MS (ESI+): *m*/*z* = 684.4 [M + Na]$^+$, 662.4 [M + H]$^+$.

Methyl (*S*)-4-[(*S*)-1-(1-Naphthyl)ethylcarbamoyl]-3-hydroxy-3-methylbutanoate (14): Amide **13** (0.015 g, 22.7 μmol) was dissolved in MeOH (2 mL) and a freshly prepared 10% solution of MeONa in MeOH (0.5 mL) was added. The mixture was stirred at 30 °C for 30 min and then quenched with 5% aqueous HCl. Volatiles were evaporated under reduced pressure, and the residue was chromatographed on a silica gel column (toluene/EtOAc, 7:3 to EtOAc/CH_3OH, 9:1) to afford dicinnamide **4** (7.2 mg, 87%), which was identical to the natural sample, and ester **14** (6.5 mg) as a waxy solid. $[\alpha]_D^{22}$ = –6.5 (*c* = 0.25, MeOH).[34] The ^{1}H and ^{13}C NMR spectra (CD_3OD) of compound **14** matched those reported in the literature.[23] MS (ESI+): *m*/*z* = 330.1 [M + H]$^+$, 352.2 [M + Na]$^+$, 680.8 [2M + Na]$^+$.

Studies on Human Tumour Cell Lines

Cell Culture and Treatments: Androgen-responsive LNCaP cells and androgen-insensitive prostate cancer cells DU-145 cells were obtained from American Type Culture Collection (ATCC, Rockville, MD, USA). LNCaP cells were grown in RPMI-1640 medium supplemented with 10% fetal calf serum, 100 U mL^{-1} penicillin, 100 μg mL^{-1} streptomycin and 1 mM glutamine. Androgen-nonresponsive DU-145 human prostate cancer cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% FCS, 100 U mL^{-1} penicillin, 100 μg mL^{-1} streptomycin, 1 mM glutamine and 1% nonessential amino acids. The cells were plated at a constant density to obtain identical experimental conditions in the different tests to thus achieve a high accuracy of the measurements. After 24 h incubation at 37 °C under a humidified 5% carbon dioxide environment to allow cell attachment, the cells were treated with different concentrations of maytenine (**3**) and pholiotic acid (**5**) and incubated for 72 h under the same conditions. Stock solutions of natural compounds were prepared in DMSO. Control cultures received DMSO alone.

MTT Bioassay: Cellular growth was determined by using the MTT assay on 96-well microplates as described previously.[12] The optical density of each sample well was measured with a microplate spec-

 © 2007 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

trophotometer reader (Digital and Analog Systems, Rome, Italy) at 550 nm.

Lactic Dehydrogenase (LDH) Release: LDH activity was measured spectrophotometrically in the culture medium and in the cellular lysates at 340 nm by analyzing NADH reduction during the pyruvate–lactate transformation, as reported previously.[12] The percentage of LDH released was calculated as a percentage of the total amount, considered as the sum of the enzymatic activity present in the cellular lysate and that in the culture medium. A Hitachi U-2000 spectrophotometer (Hitachi, Tokyo, Japan) was used.

DNA Analysis by COMET Assay: The presence of DNA fragmentation was examined by single-cell gel electrophoresis (COMET assay), as reported previously.[13] At the end of the electrophoretic run, the "minigels" were neutralized in 0.4 M Tris-HCl, pH 7.5, stained with 100 μL of ethidium bromide (2 $\mu g\,mL^{-1}$) for 10 min and scored by using a fluorescence microscope (Leica, Wetzlar, Germany) interfaced with a computer. Software (Leica-QWIN) was used for the analysis and quantification of DNA damage by measuring: (1) tail length (TL), intensity (TI) and area (TA); (2) head length (HL), intensity (HI) and area (HA). These parameters are employed by the software to determine the level of DNA damage as: (1) the percentage of the fragmented DNA (TDNA) and (2) tail moment (TMOM) expressed as the product of TD (distance between head and tail) and TDNA.

Supporting Information (see footnote on the first page of this article): High-resolution mass spectra (ESI) of compounds **3**–**5** and **12**.

Acknowledgments

This work was funded by the Dirección General de Investigación y Postgrado (DGIP), Universitad Técnica Federico SantaMaria (Proyect 13.05.21).

[1] M. Clericuzio, M. Piovano, M. C. Chamy, J. A. Garbarino, M. Milanesio, D. Viterbo, G. Vidari, P. Vita Finzi, *Croat. Chem. Acta* **2004**, *77*, 605–611.
[2] E. I. Mbadiwe, *Phytochemistry* **1973**, *12*, 2546–2546.
[3] A. Ehmann, *Phytochemistry* **1974**, *13*, 1979–1983.
[4] J. Martin-Tanguy, F. Cabanne, E. Perdrizet, C. Martin, *Phytochemistry* **1978**, *17*, 1927–1928.
[5] M. Ponchet, J. Martin-Tanguy, A. Marais, C. Martin, *Phytochemistry* **1982**, *21*, 2865–2869.
[6] V. U. Ahmad, A.-ur.-R. Amber, S. Arif, M. H. M. Chen, J. Clardy, *Phytochemistry* **1985**, *24*, 2709–2711.
[7] A. Takahashi, G. Kusano, T. Ohta, Y. Ohizumi, S. Nozoe, *Chem. Pharm. Bull.* **1989**, *37*, 3247–3250.
[8] F. Cabanne, J. Martin-Tanguy, C. Martin, *Physiol. Veg.* **1977**, *15*, 439–441.
[9] T. Niwa, U. Doi, T. Osawa, *J. Agric. Food Chem.* **2003**, *51*, 90–94.
[10] R. E. Altman Jr, I. L. Honiberg, *J. Pharm. Sci.* **1972**, *61*, 610–613.
[11] W. Steglich, B. Steffan, K. Stroech, M. Wolf, *Z. Naturforsch., Teil C* **1984**, *39*, 10–12.
[12] A. Russo, M. Piovano, M. Clericuzio, L. Lombardo, S. Tabasso, M. C. Chamy, G. Vidari, V. Cardile, P. Vita-Finzi, J. A. Garbarino, *Phytomedicine* **2007**, *14*, 185–191.
[13] G. Englert, K. Klinga, Raymond-Hamet, E. Schlittler, W. Vetter, *Helv. Chim. Acta* **1973**, *56*, 474–478.
[14] J. Martin-Tanguy, *Plant Growth Regul.* **2001**, *34*, 135–148.
[15] S.-I. Murahashi, T. Naota, N. Nakajima, *Chem. Lett.* **1987**, 879–882. For a general review on the bioactivities and the synthesis of spermidine alkaloids, see G. Karigiannis, D. Papaioannou, *Eur. J. Org. Chem.* **2000**, 1841–1863.
[16] R. K. Kullnig, C. L. Rosano, M. E. Coulter, C. Hurwitz, *J. Biol. Chem.* **1973**, *248*, 2487–2488.
[17] H. Staudinger, J. Meyer, *Helv. Chim. Acta* **1919**, *2*, 635–644.
[18] A. Goti, M. Cacciarini, F. Cardona, A. Brandi, *Tetrahedron Lett.* **1999**, *40*, 2853–2856.
[19] E. F. V. Scriven, K. Turnbull, *Chem. Rev.* **1988**, *88*, 298–360.
[20] J. Tobari, T. T. Tchen, *J. Biol. Chem.* **1971**, *246*, 1262–1265.
[21] A. Stoessl, R. Rohringer, D. J. Samborski, *Tetrahedron Lett.* **1969**, *10*, 2807–2809.
[22] T. Shiba, H. Akiyama, I. Umeda, S. Okada, T. Wakamiya, *Bull. Chem. Soc. Jpn.* **1982**, *55*, 899–902.
[23] L. Garlaschelli, G. Vidari, M. Virtuani, P. Vita-Finzi, G. Mellerio, *J. Nat. Prod.* **1995**, *58*, 992–1002.
[24] Z. J. Kaminski, *Synthesis* **1987**, 917–920.
[25] B. Frydman, A. Valasinas, *Expert Opin. Ther. Pat.* **1999**, *9*, 1055–1068.
[26] P. Srinath, S. A. McQuarrie, M. R. Suresh, *Nucl. Med. Biol.* **2002**, *29*, 497–503.
[27] A. V. Fraser, P. M. Woster, H. M. Wallace, *Biochem. J.* **2002**, *367*, 307–312.
[28] A. C. Wolff, D. K. Armstrong, J. H. Fetting, M. K. Carducci, C. D. Riley, J. F. Bender, R. A. Casero Jr, N. E. Davidson, *Clin. Cancer Res.* **2003**, *9*, 5922–5928.
[29] Y. Huang, A. Pledgie, R. A. Casero, N. E. Davidson, *Anti-Cancer Drugs* **2005**, *16*, 229–241.
[30] B. J. Feldman, D. Feldman, *Nat. Rev. Cancer* **2001**, *1*, 34–45.
[31] D. E. McCloskey, P. M. Woster, R. A. Casero Jr, N. E. Davidson, *Clin. Cancer Res.* **2000**, *6*, 17–23.
[32] R. G. Schipper, J. C. Romijn, V. M. Cuijpers, A. A. Verhofstad, *Biochem. Soc. Trans.* **2003**, *31*, 375–380.
[33] T. Godard, E. Deslandes, P. Lebailly, C. Vigreux, F. Sichel, J. M. Poul, P. Gauduchon, *Histochem. Cell Biol.* **1999**, *112*, 155–161.
[34] In ref.[23], the optical rotation of **14** was reported in dichloromethane as $[\alpha]_D^{22} = -17.7$ ($c = 0.3$, CH_2Cl_2); an authentic sample of **14** was available and a new measurement in methanol gave $[\alpha]_D^{22} = -7.3$ ($c = 0.5$, CH_3OH).

Received: June 19, 2007
Published Online: September 25, 2007

 © 2007 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim