Advances in Biological Chemistry, 2014, 4, 109-115
Published Online April 2014 in SciRes. http://www.scirp.org/journal/abc
http://dx.doi.org/10.4236/abc.2014.42015

Pharmacological Prospects of Oxygenated Abietane-Type Diterpenoids from *Taxodium distichum* Cones

Norihisa Kusumoto[1,2*], Nobuhiro Aburai[2], Tatsuya Ashitani[2,3], Koetsu Takahashi[2,3], Ken-ichi Kimura[2,4]

[1]Institute of Wood Technology, Akita Prefectural University, Noshiro, Japan
[2]The United Graduate School of Agricultural Sciences, Iwate University, Morioka, Japan
[3]Faculty of Agriculture, Yamagata University, Tsuruoka, Japan
[4]Graduate School of Agriculture, Iwate University, Morioka, Japan
Email: [*]kusumoto@iwt.akita-pu.ac.jp

Received 28 January 2014; revised 6 March 2014; accepted 15 March 2014

Abstract

Eight naturally occurring diterpenoids, including 6,7-dehydroroyleanone, taxodal, taxodione, salvinolone, 14-deoxycoleon U, 5,6-dehydrosugiol, sandaracopimaric acid, and xanthoperol were isolated from *Taxodium distichum* cones and their biological properties evaluated *in vitro* against six different biological screening targets. Taxodione showed potent activity against a number of different targets, and salvinolone and 14-deoxycoleon U showed remarkable inhibitory activities against prolyl oligopeptidase (POP) and 17α-hydroxylase/$C_{17,20}$-lyase (CYP17), respectively. These three compounds also showed strong cytotoxic activities against HL60 and K562 human leukemia cells. The structure-activity relationships of these compounds have also been considered. The findings in this study could lead to enhanced pharmacological prospects for the natural abietane-type diterpenoids consisting in conifer cones.

Keywords

Abietane Diterpenoid; Conifer Cone; Antibacterial Activity; 17α-Hydroxylase/$C_{17,20}$-Lyase; Prolyl Oligopeptidase; Cytotoxicity

1. Introduction

Natural occurring terpenoids such as mono-, sesqui- and diterpenoids are formed in large amounts as secondary

[*]Corresponding author.

How to cite this paper: Kusumoto, N., *et al.* (2014) Pharmacological Prospects of Oxygenated Abietane-Type Diterpenoids from *Taxodium distichum* Cones. *Advances in Biological Chemistry*, **4**, 109-115. http://dx.doi.org/10.4236/abc.2014.42015

metabolites in coniferous species. Mono- and diterpenoid mixtures are well-known to be the major components of resins produced by plants belonging to this particular species, and their synergistic self-defense functions, including their chemical and physical defensive roles, have been reported [1]. Furthermore, the chemical defenses of conifers are strongly reliant on specific components being present in the different parts of the tree (e. g., trunk, branch, root, needle, and cone). In our previous work, we focused on the hydrophobic terpenoid constituents in the cones of *Taxodium distichum*, and reported the isolations and identifications of twelve oxygenated diterpenoids, including ten abietane-type diterpenoids, as well as one novel norabietane-type diterpenoid and one isopimarane-type diterpenoid, together with an evaluation of the antitermitic and antifungal properties of these compounds against wood decomposers [2] [3]. Oxygenated abietane-type diterpenes are widely distributed in the plant kingdom and have been reported to show potent cytotoxic, antifungal, and antibacterial activities [4]-[7].

Based on our previous investigations of the natural terpenoids in conifer cones, it was envisaged that the diterpenoids isolated from *T. distichum* cones would not only show activities against wood decomposers but would also show activity against other organisms. Furthermore, the results of our previous studies effectively highlighted the interesting bioactivities of oxygenated abietane-type diterpenoids [8]-[11]. The aim of the current study, therefore, was to evaluate the pharmacological prospects of eight natural diterpenoids, including 6,7-dehydroroyleanone (**A**), taxodal (**B**), taxodione (**C**), salvinolone (**D**), 14-deoxycoleon U (**E**), 5,6-dehydrosugiol (**F**), sandaracopimaric acid (**G**) and xanthoperol (**H**), which were isolated from *T. distichum* cones against six different biological targets *in vitro*. These unique methods for the evaluation of natural products were previously developed by our group during the course of several screening programs [12]-[18]. We also considered the structure-activity relationships of these compounds.

2. Materials and Methods

2.1. Chemicals

The eight natural diterpenoids (**Figure 1**), including 6,7-dehydroroyleanone (**A**), taxodal (**B**), taxodione (**C**), salvinolone (**D**), 14-deoxycoleon U (**E**), 5,6-dehydrosugiol (**F**), sandaracopimaric acid (**G**) and xanthoperol (**H**), were isolated from the *n*-hexane extract of fallen *Taxodium distichum* cones. The isolation procedures and techniques used for the structural identification of these compounds have been described previously in the literature [2]. Liposidomycin C-(III) and propeptin were isolated from the fermentation broth of *Streptomyces* sp. SN-1061M [12] and *Microbispora sp*. SNA-115 [14], respectively. Pisiferdiol was isolated from *Chamaecyparis pisifera* [17]. Ketoconazole and camptothecin were purchased from Wako Pure Chemical Industries Ltd., Osaka, Japan. Sanguinarine chloride was purchased from Funakoshi Co. Ltd., Tokyo, Japan. The immunosuppressive drug FK506 was kindly provided by Fujisawa Pharmaceutical Co., Ltd (currently known as Astellas Pharma Inc., Tokyo, Japan).

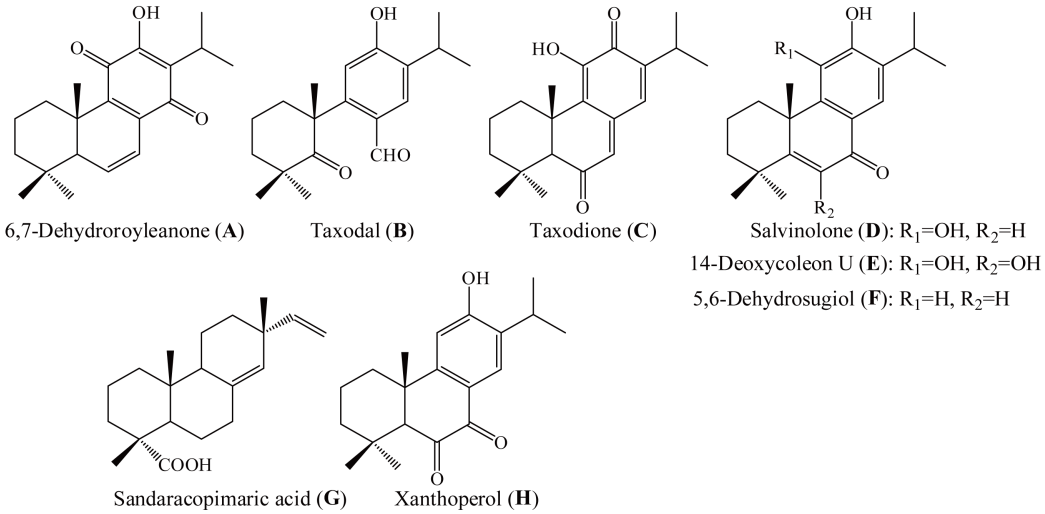

Figure 1. Eight diterpenoids isolated from the cones of *Taxodium distichum*.

2.2. Antimicrobial Activity against Acid-Fast Bacillus

A inoculating loop of *Mycobacterium phlei* (IFO3158) was pre-cultured in a tube for 4 days at 37˚C and then suspended in agar medium (pH 6.8) consisting of glycerin (30 g), meat extract (5 g), peptone (10 g), NaCl (3 g) and agar (1.5 g) per liter of distilled water (pH 6.7). Each of the compounds (**A**-**H**) was soaked into an 8 mm paper disc (40 μg/disc) before being placed on the prepared agar surface. Liposidomycin C-(III) (1.2 μg/disc) was used as a positive control for the antimicrobial screening [12]. The prepared dishes were incubated for 2 days at 37˚C and the diameters of inhibition and partial inhibition zones (mm) were measured.

2.3. Inhibition of 17α-Hydroxylase/$C_{17,20}$-Lyase (CYP17)

The compounds (**A**-**H**) were screened for their CYP17 inhibitory activity according to a previously reported procedure [13]. Each compound (**A**-**H**) was used at 20 μg/ml of the final concentration, and the inhibition (%) of the $C_{17,20}$-lyase reaction was calculated from the area of androstenedione using 17α-hydroxyprogesterone as a substrate. An antifungal clinical drug ketoconazole was used as a positive control at a concentration of 50 μM [13].

2.4. Inhibition of Prolyl Oligopeptidase (POP)

The POP [formerly known as prolyl endopeptidase (PEP): EC 3.4.21.26] inhibition assay was performed as previously reported [14]. Each compound (**A**-**H**) was used at a final concentration of 10 μg/ml and the IC_{50} values (μM) were measured when significant levels of POP inhibition (%) were observed. A new peptide inhibitor, propeptin (IC_{50} = 0.55 μM) was used as a positive control [14].

2.5. Inhibition of Ca^{2+}-Signal Transduction in the Mutant Yeast

The Ca^{2+}-signal transduction inhibitory activities of the compounds were tested according to a previously reported procedure [15]. Each compound (**A**-**H**) was soaked into an 8 mm paper disc (40 μg/disc) before being placed on the surface of YPD ager medium containing the mutant strain of *Saccharomyces cerevisiae* (*zds*1Δ *erg*3Δ *pdr*1Δ *pdr*3Δ) (YNS17) and 0.3 M $CaCl_2$. After 3 days of incubation at 28˚C, the diameters of the growth and inhibitory zones around the disc were measured. FK506 (0.02 μg/disc) was used as a positive control [15].

2.6. Inhibition or Activation of Protein Phosphatase (PP) 2C

Screening for PP2C inhibitors or activators was carried out according to a previously reported procedure [16] [17]. The phosphatase activity of PP2C was assayed using α-casein as a substrate on the malachite green detection method. BIOMOL GREEN™ (AK-111, Biomol Research Laboratories, Inc., Plymouth Meeting, PA, USA) was used for the PP2C reaction. Each compound (**A**-**H**) was used at a final concentration of 40 μg/ml and the IC_{50} values (μg/ml) or activation activities (%) were measured when significant levels of PP2C inhibition or activation were observed. Sanguinarine (IC_{50} = 2.50 μM) was used as an inhibition control [16] and pisiferdiol (130% at 100 μM) was used as an activation control [17].

2.7. Cytotoxicity against HL60 and K562 Cells

The antitumor activities of the compounds were evaluated against human promyelocytic leukemia HL60 cells (RCB-0041, 4×10^4 cells/ml) and human chronic myelogenous leukemia K562 cells (ATCC CCL-243, 5×10^4 cells/ml) using an MTT assay, according to a previously described procedure [18]. The cells were grown in 96-well plates and treated with the compounds at a variety of different concentrations at 37˚C under a humidified atmosphere containing 5% CO_2 for 4 days. The viable cell percentages were calculated from the ratio of the A_{570} values of the treated and control cells. Camptothecin (IC_{50} = 0.02 μM to HL60 cells and IC_{50} = 0.10 to K562 cells) was used as a positive control [18].

3. Results and Discussion

The results for the six different *in vitro* biological screening experiments that were conducted for each compound are shown in **Table 1**. In terms of the antimicrobial activities of the compounds against *Mycobacterium*

Table 1. Results of the *in vitro* screening experiments for the eight abietane-type diterpenes isolated from the cones of *Taxodium distichum*.

Sample	*M. phlei* (mm)[a]		CYP17 inhibition (%)[b]	POP inhibition (%)[c]	Ca^{2+}-signal (mm)[d]		PP2C inhibition (%)[e]	Cytotoxicity (μM)[f]	
	Inhibition inner diam.	Partial inhibition outer diam.			Growth diam.	Inhibition diam.		HL60 cells	K562 cells
A	-	-	18	13	-	-	−16	**4.46**	36.0
B	-	-	14	0	-	-	−9	45.0	60.6
C	**12.8**	21.3	16	2	**20.1**	**10.5**	**92** (IC_{50} = 56.4 μM)	**4.14**	**6.05**
D	10.5	-	19	**79** (IC_{50} = 26.0 μM)	**14.4**	**12.0**	−30	30.3	**6.05**
E	**13.6**	19.6	**53**	29	**15.2**	**14.7**	4	**3.94**	**5.15**
F	-	-	11	29	14.2	9.3	−11	39.9	18.5
G	-	-	2	25	-	-	−17	42.4	>64.9
H	-	-	0	8	-	-	−8	37.6	34.7
Control	14.6 - 14.8 [a]		63 [b]	IC_{50} = 0.55 μM[c]	17.8 [d]	-	IC_{50} = 2.50 μM[e]	0.02	0.10

The hyphen indicates no activity was observed. [a]Antibacterial activities on the growth of *Mycobacterium phlei* (IFO 3158) at a concentration of 40 μg/disc. The inner inhibitory diameters (mm) are shown with the outer diameter. Liposidomycin C-(III) was used as a positive control at the concentration of 1.2 μg/disc. [b]Inhibitions of CYP17 (17α-hydroxylase/$C_{17,20}$-lyase) at a final concentration of 20 μg/ml. Ketoconazole was used as a positive control at a concentration of 50 μM. [c]Inhibitions of prolyl oligopeptidase (POP: EC 3.4.21.26) at a final concentration of 10 μg/ml. Propeptin was used as a positive control. [d]Ca^{2+}-signal transduction inhibitory activities using a mutant strain of *Saccharomyces cerevisiae* (*zds*1Δ *erg*3Δ *pdr*1Δ *pdr*3Δ) (YNS17) at a final concentration of 40 μg/ml. FK506 was used as a positive control at a concentration of 0.02 μg/ml. [e]Inhibitions of protein phosphatase (PP) 2C at a final concentration of 40 μg/ml. Sanguinarine was used as an inhibition control and pisiferdiol (130% at 100 μM) was used as an activation control. [f]Cytotoxic values (IC_{50}) against the HL60 (RCB-0041, 4×10^4 cells/ml) and K562 (ATCC CCL-243, 5×10^4 cells/ml) human leukemic cell lines. Camptothecin was used as a positive control.

phlei (IFO 3158), only taxodione (**C**), salvinolone (**D**), and 14-deoxycoleon U (**E**) showed inhibition zones at 40 μg/ml. The anti-*Mycobacterium* activities of compounds **C** and **E** were the highest of the eight diterpenoids tested, and partial inhibition zones were also observed for these two compounds (**Figure 2(a)**).

14-Deoxycoleon U (**E**) was the only compound of the eight compounds tested to show inhibitory activity towards CYP17 (17*α*-hydroxylase/$C_{17,20}$-lyase), with 53% inhibition at a concentration of 20 μg/ml against $C_{17,20}$-lyase. The screening results for prolyl oligopeptidase (POP: EC 3.4.21.26) activity revealed that salvinolone (**D**) showed 79% inhibition at 10 μg/ml. The IC_{50} value of salvinolone (**D**) was determined to be 26.0 μM (8.15 μg/ml) using serial dilution. All of the other compounds tested showed weak levels of inhibition.

The Ca^{2+}-signal transduction inhibitory activities of the compounds were evaluated using the hypersensitive mutant yeast *Saccharomyces cerevisiae* (*zds*1Δ *erg*3Δ *pdr*1Δ *pdr*3Δ) (YNS17), and a notable growth restored zone (20.1 mm) was observed on taxodione (**C**) at 40 μg/ml together with an inhibitory zone (10.5 mm) (**Figure 2(b)**). 7-Keto formed abietatrienes, compounds **D** (14.4 mm), **E** (15.2 mm) and **F** (14.2 mm), also showed restored zones around the discs. Compounds **A**, **B**, **G** and **H** did not show any signs of activity.

Of the eight compounds tested, only taxodione (**C**) showed any activity as a protein phosphatase (PP) 2C inhibitor, with 92% inhibition at 40 μg/ml. The IC_{50} value of taxodione (**C**) was determined to be 56.4 μM (17.7 μg/ml) by serial dilution.

In terms of their cytotoxic activities against the human leukemia cell lines, 6,7-dehydroroyleanone (**A**) (IC_{50} = 4.46 μM), taxodione (**C**) (IC_{50} = 4.14 μM) and 14-deoxycoleon U (**E**) (IC_{50} = 3.94 μM) showed notable activities against HL60 cells; whereas taxodione (**C**) (IC_{50} = 6.05 μM), salvinolone (**D**) (IC_{50} = 6.05 μM) and 14-deoxycoleon U (**E**) (IC_{50} = 5.15 μM) showed notable activities against K562 cells. The only compounds to show specific cytotoxic activity against towards one cell line were 6,7-dehydroroyleanone (**A**) and salvinolone (**D**), which showed specificity towards the HL60 and K562 cell lines, respectively.

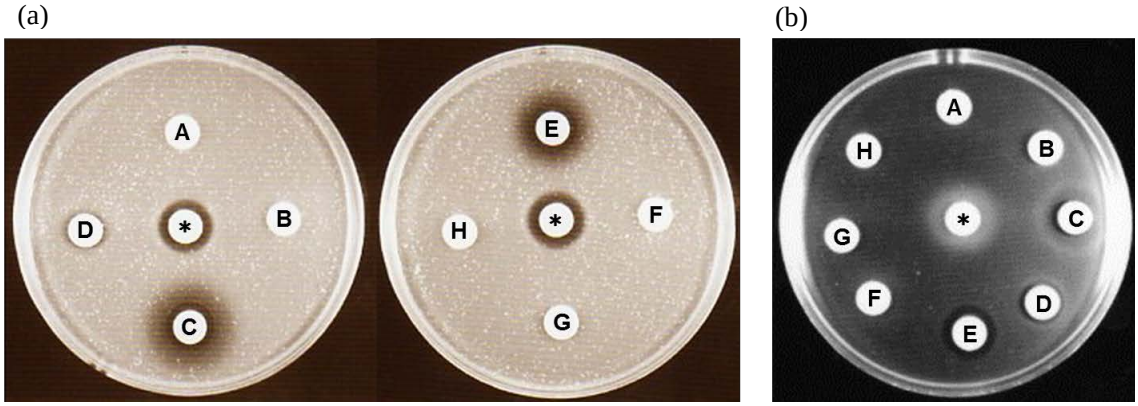

Figure 2. The growth effects of the abietane-type diterpenes (**A**-**H**) on *Mycobacterium phlei* (a) and *Saccharomyces cerevisiae* (b). Figure Labels: (a) Antibacterial activities on the growth of *M. phlei* (IFO 3158) at a concentration of 40 μg/disc. Liposidomycin C-(III) (asterisk) was used as a positive control at a concentration of 1.2 μg/disc. (b) Ca^{2+}-Signal transduction inhibitory activities using a mutant strain of *S. cerevisiae* (*zds*1Δ *erg*3Δ *pdr*1Δ *pdr*3Δ) at a concentration of 40 μg/disc. FK506 (asterisk) was used as a positive control at a concentration of 0.02 μg/disc.

From the results of the six different *in vitro* screening assays, quinone methide taxodione (**C**), 7-keto formed abietatrienes 14-deoxycoleon U (**E**), and salvinolone (**D**) showed the highest levels of activity in a number of different assays, and were the most remarkable of the eight diterpenoids tested. The biochemical activities of taxodione-type compounds containing two double bonds conjugated with a carbonyl group have previously been investigated [4] [5] [11], and the IC_{50} value for the cytotoxicity of compound **C** against HL60 cells (4.14 μM, 4 days) from the current study was similar to a result reported in a recent study [11] (4.8 μM, 3 days). In contrast, the structures of the notable POP inhibitor salvinolone (**D**) and the CYP17 inhibitor 14-deoxycoleon U (**E**) are based on a 5,6-dehydrosugiol framework. Interestingly, however, 5,6-dehydrosugiol (**F**) was much less active than these two compounds in all of the screening experiments conducted in the current study. The higher activity of salvinolone (**D**) compared with 5,6-dehydrosugiol (**F**) was attributed to the presence of the C-11 hydroxy group in salvinolone (**D**), which represents the only difference between the two compounds (**Figure 3**). Furthermore, a report concerning the structure-activity relationships of oxidized abietane diterpenes against methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE) has already been reported [5], where a catechol with a carbonyl function at C-7 together with an alcohol or ketone at C-6 of the 12-hydroxyabietatriene structure was required structures for potent antimicrobial activity. Although the results of this study were in agreement with these requirements, further consideration could be needed because xanthoperol (**H**), which is a 12-hydroxyabietatriene compound that is oxidized at C-6 and C-7, showed no activity against any of the targets tested in the current study. Thus, the hydroxyl group at C-11 could be a more important factor in determining the activities of these compounds than the presence of an alcohol or ketone at C-6 position.

4. Conclusions

Remarkable levels of biological activity have been observed in some of the eight naturally occurring diterpenoids isolated from *Taxodium distichum* cones. Taxodione (**C**) and 14-deoxycoleon U (**E**), in particular, were the most active of the eight compounds tested and showed good levels of *in vitro* activity against five of the targets investigated. The antibacterial activities of compounds **C** and **E** against *M. phlei* have not been reported previously elsewhere. However, further considerations will be necessary, because these compounds also appeared to exhibit antimicrobial activity against *M. tuberculosis* or *M. avium complex* (MAC) [12]. It is noteworthy that taxodione (**C**) inhibited Ca^{2+}-signal transduction. The prospects of natural terpenoids that showed inhibitory activity towards Ca^{2+}-signal transduction in the mutant strain of *S. cerevisiae* (*zds*1Δ *erg*3Δ *pdr*1Δ *pdr*3Δ) were discussed in our previous studies [19] [20]. Furthermore, taxodione (**C**) was the only compound to show potent inhibitory activity against PP2C with an IC_{50} value of 56.4 μM. Sanguinarine, which is a plant benzo (*c*) phenanthridine alkaloid, has been reported to show potent PP2C inhibition with an IC_{50} value of 2.50 μM [16]. Although taxodione (**C**) provided a lower level of PP2C inhibition than that of sanguinarine and the structurally

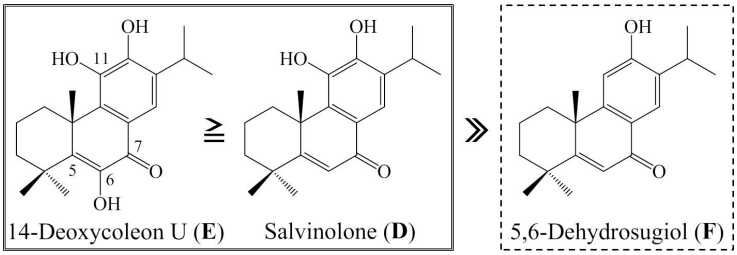

Figure 3. Comparison of pharmacological activities of the 11-hydroxylated (double line) and non-hydroxylated (broken line) 5,6-dehydrosugiol structure compounds investigated with the six different screening methods.

related compound chelerythrine (IC_{50} = 11.0 μM) [16], the PP2C inhibitory activity of this compound is particularly interesting because of its unique character.

From our previous studies, 6,7-dehydroroyleanone (**A**) showed remarkable termicidal activity and taxodione (**C**), 14-deoxycoleon U (**E**) and xanthoperol (**H**) showed potent antifeedant activity against the subterranean termite *Reticulitermes speratus* Kolbe [2]. Furthermore, taxodione (**C**), salvinolone (**D**) and 14-deoxycoleon U (**E**) showed remarkable levels of mycelial growth inhibition against wood-decaying fungi [3]. Most of the previously reported antitermitic and antifungal compounds showed notable pharmacological activities during the *in vitro* biological screening experiments conducted in this study. It is necessary to investigate the functional mechanisms of each active compound, because the novel findings of this study could enhance the pharmacological prospects of the natural abietane-type diterpenoids found in conifer cones.

Acknowledgements

This work was supported by a Grant-in-Aid from the Japan Society for the Promotion of Science Fellows (Grant no. 225181).

References

[1] Gershenzon, J. and Dudareva, N. (2007) The Function of Terpene Natural Products in the Natural World. *Nature Chemical Biology*, **3**, 408-414. http://dx.doi.org/10.1038/nchembio.2007.5

[2] Kusumoto, N., Ashitani, T., Hayasaka, Y., Murayama, T., Ogiyama, K. and Takahashi, K. (2009) Antitermitic Activities of Abietane-Type Diterpenes from *Taxodium distichum* Cones. *Journal of Chemical Ecology*, **35**, 635-642. http://dx.doi.org/10.1007/s10886-009-9646-0

[3] Kusumoto, N., Ashitani, T., Murayama, T., Ogiyama, K. and Takahashi, K. (2010) Antifungal Abietane-Type Diterpenes from the Cones of *Taxodium distichum* Rich. *Journal of Chemical Ecology*, **36**, 1381-1386. http://dx.doi.org/10.1007/s10886-010-9875-2

[4] Kupchan, S.M., Karim, A. and Marcks, C. (1969) Tumor Inhibitors. XLVIII. Taxodione and Taxodone, Two Novel Diterpenoid Quinone Methide Tumor Inhibitors from *Taxodium distichum*. *Journal of Organic Chemistry*, **34**, 3912-3919. http://dx.doi.org/10.1021/jo01264a036

[5] Yang, Z., Kitano, Y., Chiba, K., Shibata, N., Kurokawa, H., Doi, Y., Arakawa, Y. and Tada, M. (2001) Synthesis of Variously Oxidized Abietane Diterpenes and Their Antibacterial Activities against MRSA and VRE. *Bioorganic & Medicinal Chemistry*, **9**, 347-356. http://dx.doi.org/10.1016/S0968-0896(00)00253-4

[6] Kofujita, H., Ota, M., Takahashi, K., Kawai, Y. and Hayashi, Y. (2002) A Diterpene Quinone from the Bark of Cryptomeria Japonica. *Phytochemistry*, **61**, 895-898. http://dx.doi.org/10.1016/S0031-9422(02)00352-7

[7] Marques, C.G., Rijo, P., Simões, M.F., Duarte, M.A. and Rodriguez, B. (2006) Abietanes from *Plectranthus grandidentatus* and *P. hereroensis* against Methicillin- and Vancomycin-Resistant Bacteria. *Phytomedicine*, **13**, 267-271. http://dx.doi.org/10.1016/j.phymed.2005.06.002

[8] Moujir, L. and Gutierrez-Navarro, A.M. (1996) Bioactive Diterpenoids Isolated from *Salvia mellifera*. *Phytotherapy Research*, **10**, 172-174. http://dx.doi.org/10.1002/(SICI)1099-1573(199603)10:2<172::AID-PTR797>3.0.CO;2-Q

[9] Fronza, M., Lamy, E., Günther, S., Heinzmann, B., Laufer, S. and Merfort, I. (2012) Abietane Diterpenes Induce Cytotoxic Effects in Human Pancreatic Cancer Cell Line MIA PaCa-2 through Different Modes of Action. *Phytochemistry*, **78**, 107-119. http://dx.doi.org/10.1016/j.phytochem.2012.02.015

[10] Wang, W.-X., Xiong, J., Tang, Y., Zhu, J.-J., Li, M., Zhao, Y., Yang, G.-X., Xia, G. and Hu, J.-F. (2013) Rearranged

Abietane Diterpenoids from the Roots of *Clerodendrum trichotomum* and Their Cytotoxicities against Human Tumor Cells. *Phytochemistry*, **89**, 89-95. http://dx.doi.org/10.1016/j.phytochem.2013.01.008

[11] Burmistrova, O., Simões, M.F., Rijo, P., Quintana, J., Bermejo, J. and Estévez, F. (2013) Antiproliferative Activity of Abietane Diterpenoids against Human Tumor Cells. *Journal of Natural Products*, **76**, 1413-1423. http://dx.doi.org/10.1021/np400172k

[12] Kimura, K., Ikeda, Y., Kagami, S. and Yoshihama, M. (1998) Selective Inhibition of the Bacterial Peptidoglycan Biosynthesis by the New Types of Liposidomycins. *The Journal of Antibiotics*, **51**, 1099-1104. http://dx.doi.org/10.7164/antibiotics.51.1099

[13] Kimura, K., Itakura, Y., Goto, R., Tojima, M., Egawa, N. and Yoshihama, M. (2007) Inhibition of 17α-Hydroxylase/$C_{17,20}$-Lyase (CYP17) from Rat Testis by Green Tea Catechins and Black Tea Theaflavins. *Bioscience, Biotechnology, and Biochemistry*, **71**, 2325-2328. http://dx.doi.org/10.1271/bbb.70258

[14] Kimura, K., Kanou, F., Takahashi, H., Esumi, Y., Uramoto, M. and Yoshihama, M. (1997) Propeptin, a New Inhibitor of Prolyl Endopeptidase Produced by *Microbispora* I. Fermentation, Isolation and Biological Properties. *The Journal of Antibiotics*, **50**, 373-378. http://dx.doi.org/10.7164/antibiotics.50.373

[15] Ogasawara, Y., Yoshida, J., Shiono, Y., Miyakawa, T. and Kimura, K. (2008) New Eremophilane Sesquiterpenoid Compounds, Eremoxylarins A and B Directly Inhibit Calcineurin in a Manner Independent of Immunophilin. *The Journal of Antibiotics*, **61**, 496-502. http://dx.doi.org/10.1038/ja.2008.66

[16] Aburai, N., Yoshida, M., Ohnishi, M. and Kimura, K. (2010) Sanguinarine as a Potent and Specific Inhibitor of Protein Phosphatase 2C *in Vitro* and Induces Apoptosis *via* Phosphorylation of p38 in HL60 Cells. *Bioscience, Biotechnology, and Biochemistry*, **74**, 548-552. http://dx.doi.org/10.1271/bbb.90735

[17] Aburai, N., Yoshida, M., Ohnishi, M. and Kimura, K. (2010) Pisiferdiol and Pisiferic Acid Isolated from *Chamaecyparis pisifera* Activate Protein Phosphatase 2C *in Vitro* and Induce Caspase-3/7-Dependent Apoptosis via Dephosphorylation of Bad in HL60 Cells. *Phytomedicine*, **17**, 782-788. http://dx.doi.org/10.1016/j.phymed.2009.12.015

[18] Shiono, Y., Motoki, S., Koseki, T., Murayama, T., Tojima, M. and Kimura, K. (2009) Isopimarane Diterpene Glycosides, Apoptosis Inducers, Obtained from Fruiting Bodies of the Ascomycete *Xylaria polymorpha*. *Phytochemistry*, **70**, 935-939. http://dx.doi.org/10.1016/j.phytochem.2009.03.023

[19] Kimura, K., Minamikawa, Y., Ogasawara, Y., Yoshida, J., Saitoh , K., Shinden, H., Ye, Y.-Q., Takahashi, S., Miyakawa, T. and Koshino, H. (2012) Kujigamberol, a New Dinorlabdane Diterpenoid from 85 Million Years Old Kuji Amber Using a Biotechnological Assay. *Fitoterapia*, **83**, 907-912. http://dx.doi.org/10.1016/j.fitote.2012.03.024

[20] Aburai, N., Yoshida, J., Kobayashi, M., Mizunuma, M., Ohnishi, M. and Kimura, K. (2013) Pisiferdiol Restores the Growth of a Mutant Yeast Suffering from Hyperactivated Ca^{2+} Signaling through Calcineurin Inhibition. *FEMS Yeast Research*, **13**, 16-22. http://dx.doi.org/10.1111/1567-1364.12003