

Sphaeropsidin A: A Pimarane Diterpene with Interesting Biological Activities and Promising Practical Applications

Marco Masi^[a] and Antonio Evidente^{*[a]}

Sphaeropsidin A (SphA) is a tetracyclic pimarane diterpene, first isolated as the main phytotoxin produced by *Diplodia cupressi* the causal agent of a severe canker disease of Italian cypress (*Cupressus sempervirens* L.). It was also produced, together with several analogues, by different pathogenic *Diplodia* species and other fungi and showed a broad array of biological activities suggesting its promising application in agriculture and medicine. The anticancer activity of SphA is very potent and cell

specific. Recent studies have revealed its unique mode of action. This minireview reports the structures of SphA and its family of natural analogues, their biosynthetic origins, their fungal sources, and biological activities. The preparation of various SphA derivatives is also described as well as the results of structure-activity relationship (SAR) studies and on their potential practical applications.

1. Introduction

Diterpenes are widely distributed among the plant and fungal kingdoms and were classified in different subgroups according to their carbon skeleton architecture resulting from the different arrangements of the geranylgeranyl pyrophosphate (GGPP), that is the precursor of all diterpenes.^[1,2] Among the diterpenes, the pimarane diterpenes are a very representative subgroup and have showed several interesting biological activities.^[3] Sphaeropsidin A (1, SphA, Figure 1; Table 1) is a tetracyclic pimarane diterpene first produced as the main phytotoxin by *Diplodia cupressi* (syn. of *Sphaeropsis sapinea* f. sp. *cupressi*), the causal agent of a severe canker disease of Italian cypress (*Cupressus sempervirens* L.) in the Mediterranean basin.^[4,5]

Sphaeropsidin A together to sphaeropsidin B (1 and 2, Figure 1 and Table 1) were previously isolated from fermentations of *Aspergillus chevalieri* in 1972 at the Lederle Laboratories in Pearl River, New York and named LL-S491 β and γ , respectively. Their structures were determined by a combination of chemical and IR, UV, and ¹H NMR methods.^[3,6]

The disease induced on cypress by *D. cupressi* differs from another form of canker induced by *Seiridium cardinale* that caused the death of millions of cypresses especially in central Italy. Other two *Seiridium* species, namely *S. cupressi* and *S. unicorne*, induced cankers on cypress, including also *Cupressus arizonica* and *Cupressus macrocarpa*, in the Mediterranean basin.^[7] The three *Seiridium* species produced a plethora of phytotoxic metabolites that differed from those produced by

D. cupressi.^[8] The diseases induced by both *S. cardinale* and *D. cupressi* are responsible of severe damage to forest and ornamental varieties and also heavy losses to nursery and lumber industries. Thus, extensive efforts have been made to control both diseases with ecofriendly methods based on the use of natural compounds. The production of SphA by other pathogenic fungi was intensively investigated and including other interesting and promising biological activities.

SphA has shown antimicrobial, insecticidal, herbicidal, and potent anticancer activity associated with a novel mode of action as detailed below. Thus, SphA is an important natural compound with promising practical application in agriculture and medicine.

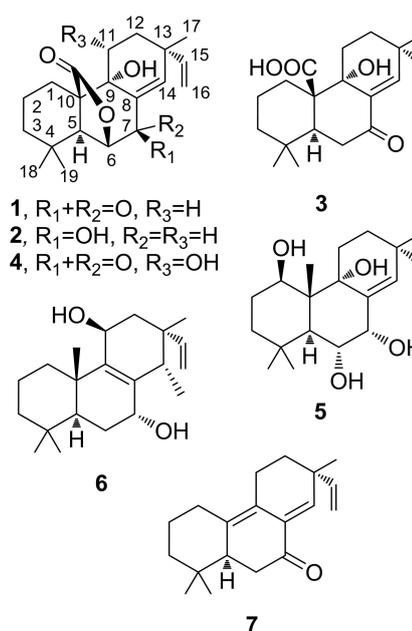


Figure 1. Sphaeropsidins A–G (1–7) produced by different pathogenic *Diplodia* species.

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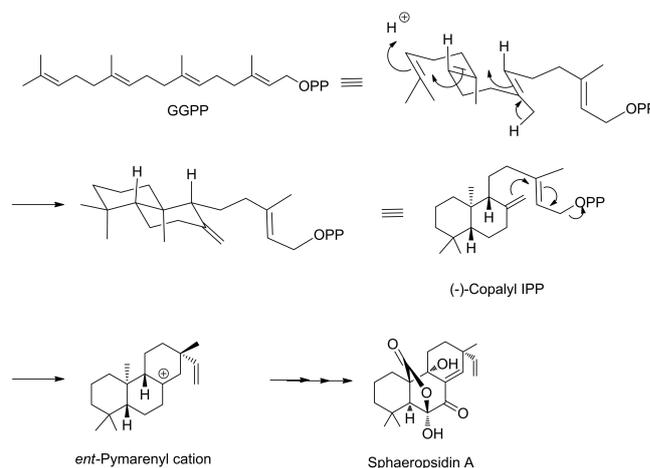
2. Structure, Biosynthesis and Biological Activities of SphA

Among the terpene family, several subgroups are classified based on their biosynthetic origin starting from the two 5-carbon isoprene units: dimethylallyl diphosphate (DMAPP) and isopentenyl diphosphate (IPP) which were generated from mevalonate (MVA) produced starting from acetylCoA. More recently, an alternative biosynthetic via for terpene was discovered and started from 1-deoxyxylulose-5-phosphate.^[3] C10 monoterpenes, C15 sesquiterpenes, C20 diterpenes, and C25 sesterterpenes are generated by the head-tail union of DMAPP and IPP by electrophile addition followed by nucleophile E₁ elimination of pro-*R* hydrogen. Triterpene (C30) and tetraterpenes (C40) were generated by tail-tail union of the intermediate C15 farnesyl diphosphate and the dimerization of C20 geranylgeranyl diphosphate, respectively. The biosynthetic mechanism, which starting from DMAPP and IPP firstly generated the geranylIPP (GPP) and this in turn farnesylIPP (FPP) and geranylgeranylIPP (GGPP), the precursor of all diterpenes, then to the (–)-copalyl and the *ent*-pimarenyl cation intermediates, is reported in Scheme 1.^[1] The *ent*-pimarenyl cation is the precursor of all pimarane diterpenes as SphA.

The structure of **1** was determined by spectroscopic methods and essentially 1D and 2D ¹H and ¹³C NMR and HR ESIMS techniques which also allowed to assign its relative configuration.^[9] Its absolute configuration was assigned comparing its specific optical rotation and ECD spectrum with those reported for the antibiotic labelled LL-S491 β isolated from *A. chevalieri*.^[3,6]

Furthermore, the structure and absolute configuration of **1** was confirmed by X-ray analysis, which was only recently carried out on suitable crystals obtained from its 6-*O*-*p*-bromobenzoate derivative.^[10]

SphA, during its first isolation from *D. cupressi*, caused browning and necrosis on young twigs of *C. sempervirens*, *C. macrocarpa* and *C. arizonica* and yellowing and necrosis on cutting of tomato (*Lycopersicon esculentum* L.) and avena (*Avena sativa*) when tested at 0.1 mg mL⁻¹. However, SphA showed some other different and interesting biological activities in



Scheme 1. Biosynthetic pathway from geranylgeranylpyrophosphate (GGPP) to sphaeropsidin A.

addition to the phytotoxicity (Table 1) that are very promising for the potential ecofriendly application of SphA in agriculture. In fact, SphA showed antimicrobial activity when tested in the range 10–20 μg mL⁻¹ against twelve fungi of agricultural interest and two *Seiridium* species. The main inhibitory activity was observed on *Fusicoccum amygdali* while a lesser extended activity was observed on *Colletotricum acutatum*, *Fusarium solani*, *Pyrenochaeta lycopersici* and *Verticillium dahliae*.^[9] The results obtained against *C. acutatum* was successively confirmed in a study in which **1** showed no activity against *Colletotrichum fragariae*.^[11] Pea powdery mildew caused by *Erysiphe pisi* is one of the major constraints for pea crops worldwide. SphA, strongly inhibited *E. pisi* germination and haustoria formation and reduced colony size. These results were further confirmed by spraying **1** on plant leaves for preventive or curative control. The strongly reduced development of *E. pisi* observed was comparable with those obtained by application of the well-known fungicide Nimrod Quattro® (ADAMA).^[12] Considering its antifungal activity SphA was also tested for antimould activity against *Aspergillus niger* and *Penicillium roqueforti* that contaminate packaged food and bakery products generating heavy



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Prof. Antonio Evidente is Full Professor of Organic Chemistry at Department of Chemical Sciences of University of Naples, Federico II, Italy. He has over 45 years of experience in the organic chemistry of natural substances. In particular, he carried out research on the biosynthesis, biochemistry, spectroscopy, and synthesis of bioactive metabolites produced by phytopathogenic fungi and bacteria, toxic and non-toxic fungi, and plants. He has been a leader of several national and international research projects and in 2014 he was awarded with “Medaglia Quilico”, assigned for research in the field of chemistry of bioactive metabolites from the Organic Chemistry Division of Italian Chemical Society.

Table 1. Sphaeropsidin A and its natural analogues.

Compound	Fungal source	Biological activity	Reference
Sphearopsidin A (1, Figure 1)	<i>D. cupressi</i>	Phytotoxic	[8]
		Antifungal	[11]
		Anticandida	[16]
		Antirust	[12]
		Antibacterial	[14]
		Antibiotic-Antibiofilm	[17]
		Insecticidal	
		Herbicidal	[18]
		Antimould	[15]
		Anticancer	[13]
		Antibiotic-Antiprotozoal	[20–24]
		Phytotoxic	[6]
		"	
	<i>D. mutila</i>	"	[25]
	<i>D. corticola</i>	"	[35]
	<i>D. quercivora</i>	Anticancer	[28]
	<i>D. africana</i>	Phytotoxic	[36]
	<i>Smardaea</i> sp.	Antifungal	[22]
	<i>D. olivarum</i>	Anticancer	[19]
"	Cytotoxic		
"	Anticancer	[37]	
<i>A. porosus</i>	No activity	[38]	
<i>Aspergillus</i> sp.		[39]	
<i>A. candidus</i>		[40]	
<i>B. laricina</i>			
Sphaeropsidin B (2, Figure 1)	<i>D. cupressi</i>	Phytotoxic	[25]
	<i>D. corticola</i>	"	[30]
Sphaeropsidin C (3, Figure 1)	<i>D. cupressi</i>	"	[25]
	<i>D. mutila</i>	"	[29]
	<i>D. corticola</i>	"	[30]
	<i>D. quercivora</i>	"	[28]
	<i>Smardaea</i> sp. AZ0432	No activity	[22]
	<i>D. africana</i>		
	<i>D. olivarum</i>	Phytotoxic	[36]
Sphaeropsidin D (4, Figure 1)	<i>D. cupressi</i>	No activity	[19]
	<i>Smardaea</i> sp.	Phytotoxic	[26]
Sphaeropsidin E (5, Figure 1)	<i>D. cupressi</i>	Cytotoxic	[36]
Sphaeropsidin F (6, Figure 1)	"	No activity	[26]
Sphaeropsidin G (7, Figure 1)	"	"	[27]
	<i>D. coticola</i>	Zootoxic	[35]
Smardaesidin A (8, Figure 2)	<i>D. olivarum</i>	"	[19]
	<i>Smardaea</i> sp.	No activity	[22]
Smardaesidin B (9, Figure 2)	"	"	"
Smardaesidin C (10, Figure 2)	"	"	"
Smardaesidin D (11, Figure 2)	"	"	"
Smardaesidin E (12, Figure 2)	"	"	"
Smardaesidin F (13, Figure 2)	"	"	"
Smardaesidin G (14, Figure 2)	"	"	"
Aspergilloid E (15, Figure 2)	<i>Aspergillus</i> sp.	Moderate cytotoxic activity	[38]
Botryosphin G (16, Figure 2)	<i>Botryospheria laricina</i>	Quinone reductase inducing activity	[40]
Botryosphin H (17, Figure 2)	"	"	"
Botryosphin I (18, Figure 2)	"	"	"
Chenopodolin (19, Figure 2)	<i>Phoma Chenopodiicola</i>	Herbicidal	[41]

economic losses.^[13] The antagonistic activity of SphA was also tested against bacteria responsible of severe rice diseases as *Xanthomonas oryzae* pv. *oryzae*, *Pseudomonas fuscovaginae*, and *Burkholderia glumae*. Compound **1** showed a strong and specific activity against *X. oryzae* pv. *oryzae*, while no activity was observed against the other two pathogens.^[14] Broomrapes (*Orobanchae* and *Pelipanche*) are parasitic plants, and cause heavy yield losses of important crops, e.g., legumes, sunflower, cereals, cabbage, tomato etc. Massive treatments with chemical herbicides are used for their control with consequent environmental pollution and severe risks for human and animal health. An alternative control method could be the use of natural based herbicides. SphA strongly inhibited the radicle growth of the broomrapes *Orobanchae crenata*, *Orobanchae cumana*, *Orobanchae minor*, and *Phelipanche ramosa*. These results highlighted the potential of SphA for the development of ecofriendly herbicides for the management of parasitic weeds.^[15]

SphA also showed a significant activity against some *Candida* species including *C. albicans*.^[15] SphA together with other fungal and plant metabolites was tested against many bacteria (Gram+ and Gram-) involved in human infection that have in recent decades rapidly increased their antibiotic resistance, reducing the effectiveness of therapies. Many of these bacteria can form biofilms and there are no drugs efficient to treat these formations. SphA resulted to be active at low concentration with MIC values ranging from 6.25 µg/mL to 12.5 µg/mL against all clinical strains tested. Furthermore, SphA decreased biofilm formation of methicillin-resistant strains of *Staphylococcus aureus* and of *Pseudomonas aeruginosa* at sub-inhibitory concentration. When tested in combination with *epi*-epoformin, a fungal cyclohexeneoxide produced by *Diplodia quercivora*, SphA had antimicrobial synergistic effects with a concomitant reduction of cytotoxicity against human immortalized keratinocytes.^[17] In another study SphA also showed insecticidal activity against *Aedes aegypti* L., which is the major vector of the arboviruses responsible for dengue fever, one of the most devastating human diseases. Among other fungal metabolite assayed, SphA exhibited mosquito biting deterrent and larvicidal activities.^[18] Recently, SphA exhibited strong phytotoxicity on leaves of *Phaseolus vulgaris* L., *Juglans regia* L. and *Quercus suber* L. at 1 mg mL⁻¹ when it was isolated from the cultures of the pathogenic fungus *Diplodia olivarum*. Furthermore, SphA completely inhibited mycelial growth of *Athelia rolfsii*, *Diplodia corticola*, *Phytophthora cambivora* and *P. lacustris* at 200 µg per plug and was active in the *Artemia salina* assay.^[19]

Among the biological activities shown by SphA the most interesting appeared to be the anticancer in vitro. In fact, **1**, was tested together with two natural analogues as sphaeropsidins B and C (**2** and **3**, Figure 1) and some its semisynthetic derivatives to evaluate their in vitro anticancer activity against the OE21 esophageal cancer the A549 non-small-cell lung cancer, the SKMEL-28 melanoma, the Hs683 oligodendroglioma and the U373 glioblastoma cell lines, and the mouse cancer cell line relates to the B16F10 melanoma. The positive control includes cisplatin, carboplatin, VP16 (etoposide) and temozolomide and

the MTT (3-[4,5-dimethylthiazol-2yl])-diphenyl tetrazolium bromide method was used.^[20] Among the thirteen compounds tested, SphA and its 6-O-acetyl and 15,16-dihydro derivatives displayed 50% growth-inhibition in the low micromolar range for all cell lines analyzed. Structure-activity relationships appeared to be comparable with the phytopathogenic and antimicrobial assays except for the vinyl group at C-13 that does not seem to be required for the cell viability.^[21] Similar results were reported by Wang et al. (2011)^[22] when SphA was isolated together with sphaeropsidins C-F (**3-7**, Figure 1), five new isopimarane diterpenes, named smardaesidins A-E, and two new 20-nor-isopimarane diterpenes, named smardaesidins F and G, from the endophytic fungal strain, *Smardaea* sp. AZ0432, occurring in the moss *Ceratodon purpureus*. In fact, when all the metabolites were tested for their potential anticancer activity using several cancer cell lines and cells derived from normal human primary fibroblasts, only SphA, its 6-O-acetyl derivative and sphaeropsidin D exhibited significant cytotoxic activity. SphA also showed a significant cell-type selectivity in the cytotoxicity assay and inhibited metastatic breast adenocarcinoma (MDA-MB-231) cells migration at sub-cytotoxic concentrations.^[22] SphA, display specific anticancer activity in vitro against melanoma and kidney cancer subpanels in the National Cancer Institute (NCI) 60-cell line screen, which are two tumors with intrinsic chemotherapy resistance and their prognosis remains poor. A mean LC₅₀ of ca. 10 µM was measured for SphA as well as a cellular sensitivity profile that did not match that of any other agent in the 765,000 compounds database. The calculated "Compare Correlation Coefficients" (the CCC index) came close (CCC[0.7]) to only one compound, NSC 205098 or 5-iodo-1-methyl-30-methylidenespiro[indole3,50-oxolane]-2,20-dione, which displayed GI₅₀ and LC₅₀ profiles similar to the those of sphaeropsidin A. Mechanistic studies in melanoma and other multidrug-resistant in vitro cancer models were carried out. The results demonstrated that SphA can overcome apoptosis as well as multidrug resistance and induces a marked and rapid cellular shrinkage related to the loss of intracellular Cl⁻ and the decreased HCO₃⁻ concentration in the culture supernatant. These changes in ion homeostasis and the absence of effects on the plasma membrane potential indicated that SphA induced impairment of regulatory volume increase (RVI). The effects of SphA on RVI, which are the type of cancer depending, could be related to Na-K-2Cl electroneutral cotransporter or Cl⁻/HCO₃⁻ anion exchanger(s) targeting. These results highlighted a new promising therapy to combat drug-resistant cancers based on adequate pharmacological SphA formulation.^[23] Thus, four melanoma cell lines were used in a set of experiments testing SphA together with two anticancer drugs as cisplatin or temozolomide to determine the optimal in vitro combinations. The combination of 4 µM SphA with 75 µM cisplatin for 72 h treatment synergistically improved its cytotoxic effects on melanoma cells. Similar results were also obtained using the combination of SphA with temozolomide.^[24]

3. Fungal Sources and Natural Analogues of SphA

SphA has been isolated from different pathogenic fungi of forest plants such as *Diplodia* spp.,^[7] but also from other pathogenic and not pathogenic fungi. It was isolated from *D. cupressi* together with its analogues sphaeropsidins B–F (2–6, Figure 1).^[25–27] SphA and compound 3 were also produced by *Diplodia mutila*, which was obtained from infected cypress (*Cupressus sempervirens* L.) trees from Morocco and from infected cypress (*C. sempervirens*) and oak (*Quercus cerri*, *Q. ilex* and *Q. robur*) trees from Southern and Northern Italy, respectively.^[25] Compounds 1 and 3 were successively isolated from *Diplodia quercivora*, a fungus responsible of canker disease of *Quercus canariensis* trees in Tunisia and *Quercus virginiana* in Florida.^[24,28] SphA was also produced together a plethora of different metabolites with phytotoxic, antifungal, and antibacterial activities from *Diplodia corticola*,^[13,15,29,30] the most widely distributed and aggressive pathogen of oak trees worldwide.^[31–34] Successively, from the cultures of the same fungus, a new *nor*-diterpene pimarane, named sphaeropsidin G (7, Figure 1), was isolated together with a new monosubstituted bifuranylone named diplobifuranylone and the well-known 4-hydroxycytalone and diorcinol.^[35] While diorcinol exhibited remarkable phytotoxicity on *Quercus afares*, *Quercus suber*, *Quercus ilex* and *Celtis australis*, sphaeropsidin G did not; however both exhibited zootoxicity on brine shrimp larvae (*Artemia salina* L.).^[35] SphA, was also isolated from the endophytic fungus *Smardaea* sp. AZ0432 (Pyronemataceae, Ascomycota) isolated from the fire moss (*Ceratodon purpureus*, Ditrichaceae) together with sphaeropsidins C–F (3–6), five new isopimarane diterpenes, named smardaesidins A–E (8–12, Figure 2) and two new 20-*nor*-isopimarane diterpenes, named smardaesidins F and G (13 and 14, Figure 2).^[22]

SphA was also isolated from liquid culture of *Diplodia africana*, a fungal pathogen responsible for branch dieback of Phoenician juniper in Italy, together with two phytotoxic dihydrofuropyran-2-ones, named afritoxinones A and B, the well-known oxysporone, *epi*-sphaeropsidone, *R*-(–)-mellein, (3*R*,4*R*)-4-hydroxymellein, and (3*R*,4*S*)-4-hydroxymellein were also isolated.^[36] Recently, sphaeropsidins A, C, and G, and diplopimarane (2–5) were isolated together with a new cleistanthane *nor*-diterpenoid, named olicleistanone, from the culture filtrates of *Diplodia olivarum*, an emerging pathogen involved in the etiology of branch canker and dieback of several plant species typical of the Mediterranean maquis in Sardinia, Italy.^[19] SphA was also isolated from the endophytic fungus *Aspergillus porosus* during a screening carried out to isolate cytotoxic compounds. The same fungus was shown to produce four new polyketides named porosuphenols A–D.^[37] SphA was produced by *Aspergillus* sp. (strain no. YXf3), an endophytic fungus isolated from *Ginkgo biloba*, together with four new diterpenoids, named aspergiloids E–H, a new flavonol, named chlorflavinon A and eight known compounds. Aspergilloid E (15, Figure 2) is closely related to SphA. Compounds 1 and 15 showed moderate cytotoxic activity with IC₅₀ values ranging

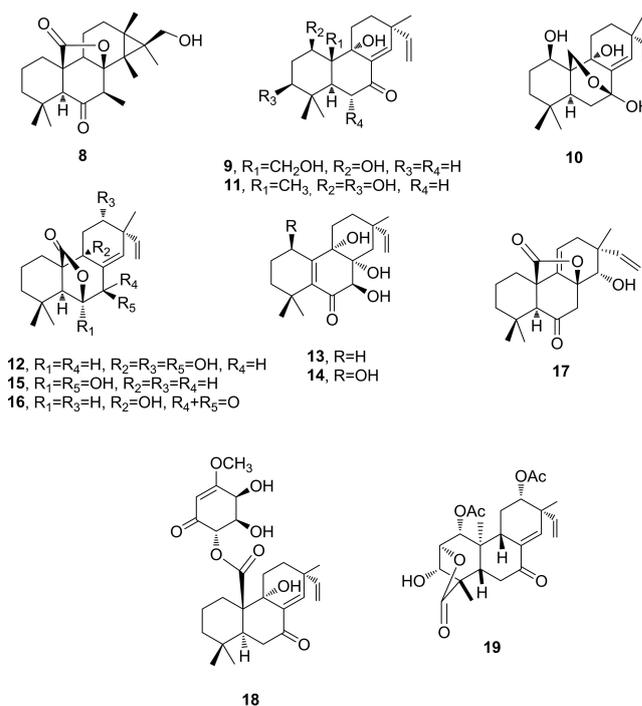


Figure 2. Sphaeropsidins natural analogues (8–19).

from 6.74 to 46.64 μM when tested against KB, SGC-7901, SW1116, and A549 cell lines.^[38] SphA was also isolated from *Aspergillus candidus* strains in a study to find alternative sources to produce the toxin.^[39] Recently, compound 1, was also isolated from the fungus *Botryosphaeria laricina* associated with the moss *Rhodobryum umgiganteum* together with three new isopimarane-type diterpenoids, named botryosphaerins G–I (16–18, Figure 2), a new muurolane-type sesquiterpenoid and two new triketides. Botryosphaerins G and H showed quinone reductase inducing activity.^[40] Chenopodolin (19, Figure 2), another phytotoxic unrearranged *ent*-pimaradiene diterpene, close to 1, was isolated from *Phoma chenopodolica*, a fungus proposed as mycoherbicide for the biological control of *Chenopodium album*, a ubiquitous weed of arable crops such as sugarbeet and maize. When tested at a concentration of 2 mg mL⁻¹, 19 caused necrotic lesions on *Mercurialis annua*, *Cirsium arvense*, and *Setaria viride*.^[41]

4. Semisynthetic Derivatives of SphA: SAR Studies

Fourteen derivatives (20–34, Figure 3) were prepared starting from sphaeropsidins A–C to carry out SAR investigation in three different studies testing the phytotoxic and antifungal,^[42] antibacterial^[14] and anticancer^[21] activities.

Eight derivatives were prepared by chemical modification of sphaeropsidins A–C (1–3) and assayed, compared to the parent compounds, to evaluate their phytotoxicity on host (three

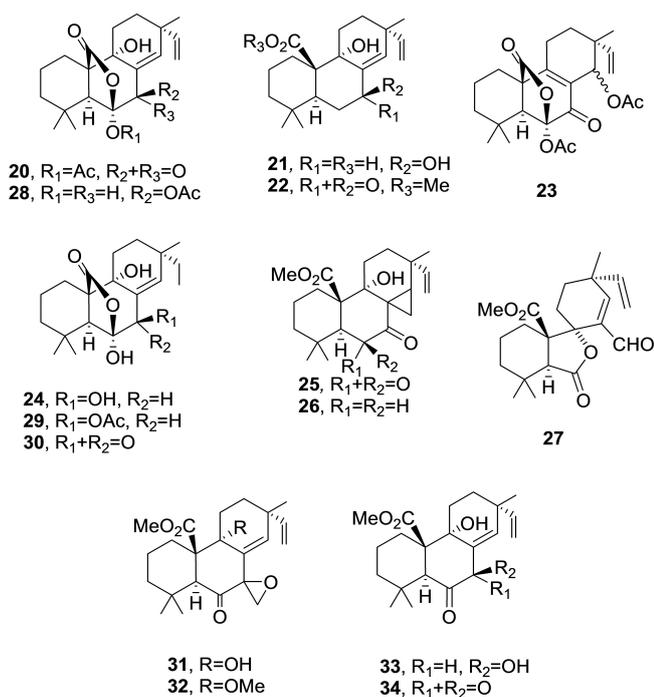


Figure 3. Hemisynthetic derivatives of sphaeropsidins A–C (20–34).

Cupressus species) and two non-host plants and antifungal activity against eight phytopathogenic fungi (including the three *Seridium* species). By common acetylation and catalytic hydrogenation SphA (1) was converted, respectively, into the corresponding 6-*O*-acetyl derivative and 21–22-dihydro derivative (20 and 30, Figure 3), while by reaction with Fritz and Shenk reagent (1959)^[43] in the derivative 23 (Figure 3), which showed in addition to the acetylation of the C-6 hydroxy group, the acetoxylation of C-24 with consequent shift of the double bond from C(8)–C(14) to C(8)–C(9) and C-9 dehydroxylation. Furthermore, by reaction with an ethereal solution of diazomethane 1 was converted in derivative 25 (Figure 3). Compound 25 showed the opening of the lactone ring with consequent methylation of the carboxy group at C-10, the reconstitution of the carbonyl at C-6 and the cyclopropanation of the double bond between C(8)–C(14). Sphaeropsidin B (2) by NaO₄ oxidation was converted into the derivative 27 (Figure 3) which showed the complete rearrangement of the carbon skeleton of the pimarane diterpene. In fact, as consequence of the oxidation of the diol system at C-6 and C-7 was generated a carboxyl group at C-6 which by lactonization with the hydroxyl group at C-9 generate the dihydrofuranone ring, while the C-7 was converted into the corresponding formyl group. Finally, sphaeropsidin C (3) by stereospecific NaBH₄ reduction and esterification with ethereal solution of diazomethane gave the derivatives 21, 22 and 26 (Figure 3). Derivatives 21 and 22 showed the reduction of the ketone group at C-7 and the methyl esterification of the carboxyl group at C-10, respectively. Derivative 26 differed from 3 for the methylation of the carboxylic group and the cyclopropanation of the double bond between C(8)–C(14). As above cited each compound was tested

for phytotoxic and antifungal activity. The results obtained showed that the integrity of the tricyclic pimarane system, the preservation of the double bond C(8)–C(14), the tertiary hydroxyl group at C-9, the vinyl group at C-13, and the carboxylic group at C-10 as well as the integrity of the A-ring are important structural features to impart non-selective phytotoxic and antimycotic activity.^[14,42]

Sphaeropsidins A–C (1–3) and nine semisynthetic derivatives above cited (20–27 and 30, Figure 3) and the acetyl sphaeropsidin B (28, Figure 3), prepared by common acetylation of 2, and the 7-*O*-acetyltetrahydro SphA (29, Figure 3) prepared by catalytic reduction of SphA, conducted for a more long time, were assayed *in vitro* for their anticancer activity using colorimetric MTT against five human (A549 (NSCLC) OE21 (esophageal) Hs683 (glioma) U373 (glioma) SKMEL28 (melanoma) and one mouse B16F10 (melanoma) cancer cells using as positive control the anticancer agent cisplatin, carboplatin, etoposide and temozolomide. The latter are drugs largely employed to treat a large variety of human cancers P16 (etoposide). SphA (1) appeared to be the most active compound among the thirteen analyzed and at the same level of cisplatin and etoposide, while more active than carboplatin and temozolomide.^[21] The results of SAR studies regarding the anticancer activity are essentially in agreement with those obtained in the previously studies,^[42] and with those obtained testing sphaeropsidins A–C and fourteen derivatives including the above cited (20–30) for antibacterial activity. To test this activity against Gram-negative pathogens, *Xanthomonas oryzae* pv. *oryzae*, *Pseudomonas fuscovaginae* and *Burkholderia glumae*, four new derivatives were prepared by reaction of SphA (1) with an ethereal solution of diazomethane for longer time obtaining the derivatives 31 and 32 (Figure 3) and by reaction of 2 with the same reagent but in normal conditions affording derivatives 33 and 34 (Figure 3).^[14] However, the vinyl group at C-13 also seemed to play a role in the antibacterial activity of SphA while the hydroxyl group at C-9 is a structural feature important to impart the biological activity tested.^[14,21,42]

5. Conclusions

Sphaeropsidin A (SphA) is a very promising natural product among the pimarane diterpenes. The noteworthy activity shown by SphA, essentially, as antibacterial, and anticancer agent allowed to hypothesize its possible application, with appropriate formulations in agriculture as biopesticide and as drug against malignant tumors in cancer therapy. However, although SphA is produced in relatively large amounts by different fungi, mostly belonging to different *Diplodia* species, to continue these studies there is a need to produce the metabolite in much larger quantities. This goal could be achieved by improving fermentation process and genetic modification of the best producing strains and developing an environmentally friendly extraction process as the total enantioselective synthesis appear to be very difficult to realize. In fact, the total synthesis of SphA is not yet reported but some

approaches to realize the synthesis of other pimarane diterpenes have been reviewed.^[44,45]

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Conflict of Interest

The authors declare no conflict of interest.

Keywords: biological activity · natural analogues and semisynthetic derivatives · Pimarane diterpenes · potential practical application · sphaeropsidin A

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