Article Type: Full Paper

Corresponding author mail id: gabyc@qo.fcen.uba.ar

Metabolites from the dark septate endophyte *Drechslera sp.* Evaluation by LC/MS and principal component analysis of culture extracts with histone deacetylase inhibitors

Gastón Siless[a,b], Gabriela L. Gallardo[a], M. Alejandra Rodriguez[c,d], Yuliet A. Rincón[a,b], Alicia M. Godeas[c,d], Gabriela M. Cabrera[a,b*]

[a]Departamento de Química Orgánica, [b]Facultad de Ciencias Exactas y Naturales, CONICET-Universidad de Buenos Aires, Unidad de Microanálisis y Métodos Físicos aplicados a la Química Orgánica (UMYMFOR). Ciudad Universitaria, Pabellón II, 3° piso, C1428EHA, Buenos Aires, Argentina. [c]Departamento de Biodiversidad y Biología Experimental, dFacultad de Ciencias Exactas y Naturales, CONICET- Universidad de Buenos Aires, INBA. Ciudad Universitaria, Pab. II, 4° piso, C1428EHA, Buenos Aires, Argentina.

Secondary metabolites from the cultures of the dark septate fungal endophyte (DSE) *Drechslera sp.*, isolated from the roots of rye grass (*Lollium sp.*) and cultured under different experimental conditions, are described here for the first time. The use of suberoylanilide hydroxamic acid (SAHA) and other histone deacetylase inhibitors as epigenetic modifiers in the culture medium was evaluated by LC/MS and LC/MS/MS. Several differences in the metabolite production were detected by means of supervised principal component analysis (PCA) of LC/MS data. The presence of the compounds in the culture medium or in the mycelium was compared.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/cbdv.201800133

The structure of many of the metabolites was confirmed by isolation from a larger scale culture. These metabolites were characterized as prenylhydroxybenzoic acids and chromans, two compounds, one of each class were previously undescribed, prenylquinoids, diketopiperazines and macrosphelides. Some of the compounds which were released to the medium showed good antifungal activity, suggesting that these compounds could protect *Lollium* from fungal phytopatogens.

The use of SAHA as an additive of the cultures also induced the release of hexosylphytosphyngosine to the culture medium. The biotransformation of the inhibitors was observed in addition to the production of antifungal metabolites, showing the ability of this endophytic strain to control xenobiotics.

Keywords: Dreschlera • asperpentyn • macrosphelides • lysosphingolipid • SAHA

Introduction

Dark septate endophytes (DSE) are a group of root-colonizing fungi that belong mostly to a few orders of the phylum Ascomycota. These widely distributed fungi are septate and generally have melanized hyphae that colonize intracellularly and intercellularly the roots, improving plant´s tolerance against harsh climatic conditions, as well as pathogen resistance. There has been an increasing interest in DSE fungi but many aspects of these species are not as well understood as with other type of endophytes. Although it has been established that DSE can improve plant growth, their ecological role is not fully understood, and their metabolome or the activity of their secondary metabolites in plant-host interaction is mostly unknown.[1,2]

The genus *Drechslera* is a vast complex of species commonly associated to algae[3,4] and plants as endophytes[5] or pathogens.[6] Many metabolites produced by *Drechslera spp* have shown toxicity to plants, animals and humans,[7] suggesting an ecological role for these compounds. Interestingly, the isolated metabolites from these genera belong to several metabolic routes, with terpenoids among the most common, but also with the finding of macrocyclic esters and alkaloids. The rare sesquiterpenes drechslerines[3] were produced by *D. dematoidea*, while eremophilanes[8] were produced by *D. gigantea*. The sesterterpene mycotoxins opiobolins were produced by several *Drechslera spp*[9,10] and a related *Cochliobolus sp.*[11,12] The macrocyclic ester pyrenophorin[13], was isolated from *Pyrenophora avenae*, a teleomorph of *Drechslera*, while a related pyrenophorol[6] was isolated from *D. avenae*. Other non-related metabolites as alkaloids,[14] spirocyclic γ-lactams[15] and anthraquinones[16] have also been reported, showing the great metabolic variability of this genus.

The production of most fungal secondary metabolites is known to be influenced by environmental factors and nutrients. Under standard laboratory culture conditions, only a subset of biosynthetic pathway genes are expressed and therefore only a fraction of potential secondary metabolites are commonly described. Aiming to increase the production of previously unreported secondary metabolites, many culture techniques had been developed in order to induce gene expression, in what has been called the OSMAC approach (one strain, many compounds). This strategy is opposed to the selection of a particular metabolite-producer strain among hundreds of others under standard conditions.[17] Medium composition, solid or liquid media, vessel configuration, shaking, aeration, irradiation and even water composition[18] are some common parameters that had been explored.

The repression of secondary metabolite gene clusters has been attributed to different epigenetic mechanisms, which affect the chromatin structure, restricting the access to the genetic material and thus result in reversible gene silencing. Post-traslational modifications of the chromatin structure, mainly histone acetylation and methylation, are factors involved in the silencing or expression of genes. Histone acetylation is controlled by the antagonic histone acetyltransferases (HATs) and deacetylases (HDACs). It had been noticed that inhibition of HDAC activity and subsequent hyperacetylation of histone residues leads to an open, and transcriptionally more active chromatin structure. The production of new metabolites was assessed by this approach.[19-21]

Rational or random variation of culture conditions could then lead to a better insight to the fungal metabolome, which is essential to understand chemical biology of plant-host interaction, pathogenicity processes and adaptation to harsh climatic conditions.

In the present work, the study of the secondary metabolome of a fungal DSE, *Drechslera sp.*, isolated from the roots of *Lollium sp.*, was conducted in order to understand the role of this strain as a plant colonizer. For this purpose and to gain knowledge of the metabolome, small scale cultures were performed using two different culture media and epigenetic modifiers, HDAC inhibitors (HDACIs), as additives. Suberoylanilidehydroxamic acid (SAHA), sodium valproate (VPA), and a SAHA analog, octanoylhydroxamic acid (OHA) were used as HDACIs (Figure 1). Liquid Chromatography coupled to Mass Spectrometry (LC/MS) was performed on all the culture media and the extracts of the mycelia. Principal component analysis (PCA) of LC/MS data was performed in order to reduce the dimensionality and a graphical comparison was performed between different culture replicates and runs.

Aiming to identify the secondary metabolites, large scale cultures were carried out, followed by the isolation and structural elucidation of the compounds by means of Nuclear Magnetic Resonance (NMR) and MS. Antifungal activity against common phytopathogens was also assessed by direct bioautography on TLC plates for some abundant metabolites in order to obtain some insights on their possible ecological role.

Figure 1. HDACIs used as epigenetic modifiers.

Results and Discussion

Screening of culture conditions

Since the three HDCAs inhibitors used in this work can inhibit the growth of the studied strain of *Dreschlera*, it was first necessary to determine the optimal concentration of HDCA and time for inoculation. Once these conditions were obtained, small scale cultures were performed using two different culture media: malt extract liquid culture (ME) and a minimal medium liquid culture (MM). The ME culture was used as a control culture and as the base medium for the addition of the different epigenetic modifiers. Three replicates of each culture condition were performed.

The cultures were filtered to separate medium and mycelium; their corresponding organic extracts were obtained and analyzed separately by LC/MS. LC conditions were selected in order to obtain a well resolved- well spread profile of signals in the chromatogram. ESI, in positive ion mode, was used as the standard ionization method. APCI, in both positive and negative ion mode, was also used in some cases for compound identification since it ionizes molecules as $[M+H]^+$ ions, which give better MS/MS spectra. A peak to peak inspection was made to discriminate common impurities and also to build and interpret the PCA model in later stages. LC/MS Base Peak Chromatograms (BPCs) of medium extracts are shown (Figure 2).

Figure 2. BPC chromatograms of medium extracts.

Several compounds originated by biotransformation of the employed HDACs were easily recognized in the LC/MS runs (*Table 1*) based on their MS/MS spectra (Table ST1, Supplementary Material).These compounds were identified as dihexosyl and hexosyl SAHA (**12** and **16**) in the cultures where SAHA was used as additive. In turn, dihexosyl and hexosyl OHA (**20** and **21**) and octanamide (**23**) were identified in the cultures where OHA was an additive and two different hydroxyvalproic acids (**13** and **15**) in those cases where VPA was used. The identifications were straightforward by analysis of the MS and MS/MS spectra of the protonated molecules or deprotonated molecules when using negative ion mode. For example, hexosyl-SAHA (**16**) with $[M+H]^+$ at m/z 427.2070 (consistent with a molecular formula $C_{20}H_{31}N_2O_8^+$), showed in the MS/MS spectrum a signal at m/z 265.1547, corresponding with the loss of an hexose (loss of 162u) from the protonated ion, in addition to the characteristic fragments of SAHA at m/z 232.1327 and 172.0979 (neutral losses of hydroxylamine and aniline respectively from SAHA). As these hexosyl conjugates were only present in the culture broths treated with SAHA and OHA, it is probable that the hydroxamic acid functionality is the glycosylation site. Octanamide (**23**) was identified by comparison with an authentic sample.

Table 1. Chemical composition of the extracts of *Dreschlera sp.*

RT	N°	Compound	MF	$[M+H]^+$ m/z	Mass accuracy (ppm)	$[M+Na]^+$ m/z	Mass accuracy (ppm)
3.6	1	cyclo (Gly-Pro)	$C_7H_{10}N_2O_2$	155.0816	-0.5	177.0633	1.1
6.4	2	cochlione B	$C_{11}H_{14}O_5$	227.0920	-2.5	249.0729	1.9
7.3	3	5-methoxy-cochlione B	$C_{12}H_{16}O_5$	241.1061	4.0	263.0892	0.9
8.1	4	cyclo (Pro-Val)	$C_{10}H_{16}N_2O_2$	197.1283	1.0	219.1108	-1.7
8.9	5	cyclo (Leu/Ile-Hyp)	$C_{11}H_{18}N_2O_3$	227.1397	-2.2	249.1203	2.6
9.4	6	Asperpentyn	$C_{11}H_{12}O_3$	193.0867	-4.3	215.0692	-6.3
10.0	7	cyclo (Phe-Hyp)	$C_{14}H_{16}N_2O_3$	261.1233	2.9	283.1038	5.5
10.7	8	cyclo (Phe-Hyp)	$C_{14}H_{16}N_2O_3$	261.1234	0	283.1048	1.9
11.3	9	cyclo (Leu/Ile-Pro)	$C_{11}H_{18}N_2O_2$	211.1443	-1.0	233.1260	0.4
11.8	10	cyclo (L-Leu-L-Pro)	$C_{11}H_{18}N_2O_2$	211.1445	-2.0	233.1259	0.8
13.6	11	cyclo (L-Phe-L-Pro)	$C_{14}H_{16}N_2O_2$	245.1280	1.7	267.1094	3.7
13.9	12	di(hexosyl)-SAHA[a]	$C_{26}H_{40}N_2O_{13}$	589.2611	-1.4	611.2444	-3.5
13.9	13	3-hydroxyvalproic acid	$C_8H_{16}O_3$	161.1160	7.7	183.0987	2.4
14.0	14	Harveynone	$C_{11}H_{10}O_3$	191.0693	4.9	213.0512	4.7
14.4	15	4-hydroxyvalproic acid	$C_8H_{16}O_3$	161.1171	0.8	183.0985	3.7
14.9	16	hexosyl-SAHA	$C_{20}H_{30}N_2O_8$	427.2070	1.7	449.1897	-0.5
16.4	17	Siccayne[c]	$C_{11}H_{10}O_2$	175.0754	0.3		
16.5	18	4-hydroxy-3-(1'-hydroxy-3'- methyl-3'-buten)-benzoic acid	$C_{12}H_{14}O_4$	223.0965	-4.4	245.0780	-1.6
17.2	19	macrosphelide A or isomer	$C_{16}H_{22}O_8$	343.1402	-4.4	365.1223	-4.4
17.4	20	di(hexosyl)-OHA[b]	$C_{20}H_{37}NO_{12}$	484.2380	1.7	506.2222	-2.8
19.0	21	hexosyl-OHA	$C_{14}H_{27}NO_7$	322.1857	1.1	344.1699	-5.6
19.3	22	macrosphelide B or isomer	$C_{16}H_{20}O_8$	341.1226	1.6	363.1044	1.7
20.6	23	Octanamide[c]	$C_8H_{17}NO$	144.1390	-5.2		
20.7	24	4-hydroxy-3-(3'-methyl-3'- buten-1'-inyl)-benzoic acid	$C_{12}H_{10}O_3$	203.0709	-3.1	225.0531	-3.8
20.8	25	macrosphelide C	$C_{16}H_{22}O_7$	327.1448	-3.0	349.1275	-4.9
21.2	26	macrosphelide C isomer	$C_{16}H_{22}O_7$	327.1446	-2.4	349.1254	1.1
22.1	27	4-hydroxy-3-prenyl-benzoic acid	$C_{12}H_{14}O_3$	207.1018	-1.1	229.0835	1.5
23.4	28	anofinic acid	$C_{12}H_{12}O_3$	205.0864	-2.4	227.0688	-4.4
23.7	29	Benzophenone	$C_{13}H_{10}O$	183.0805	0.4	205.0620	1.8
24.2	30	14-deoxy-macrosphelide C	$C_{16}H_{22}O_6$	311.1488	0.2	333.1311	-0.9

26.4	31	hexosyl-phytosphingosine[c]	$C_{24}H_{49}NO_8$	480.3526	1.1		
27.6	32	hexosyl-sphingosine	$C_{24}H_{47}NO_7$	462.3424	0.2	484.3217	5.7

[a]SAHA: suberoyl-anilide hydroxamic acid, [b]OHA: octanoyl-hydroxamic acid, [c]$[M+Na]^+$ not observed

The distribution of the HDACIs and its metabolized products from mycelium and medium are listed in *Table 2*. It is evident that SAHA, OHA and VPA were mostly present in the medium as expected, but the distribution of the corresponding metabolized products was not the same for all the compounds. Hexosyl-OHA and octanamide were found exclusively in the medium, thus indicating that the biotransformation of OHA is an efficient detoxification process. SAHA and its derivatives were distributed between medium and mycelium with some tendency to be higher in the mycelium. This fact would be related with the known high affinity of SAHA for several membrane Zn^{2+} binding proteins. The hydroxy-valproic acids were found to be most abundant in the mycelium indicating they would interact with a biomolecule in the mycelium.

Table 2. Relative abundances of HDACIs and its derivatives in mycelium and medium extracts.

N°	RT	Compound	% mycelium	% medium
12	13.9	di(hexosyl)-SAHA	32	68
16	14.9	hexosyl-SAHA	55	45
A	15.2	SAHA	27	73
13	13.9	3-hydroxyvalproic acid	71	29
15	14.4	4-hydroxyvalproic acid	88	12
B	23.8	valproic acid	12	88
21	19.0	hexosyl-OHA	0	100
C	19.7	OHA	18	82
23	20.6	octanamide	0	100

Independently of whether these metabolized products were present in the extracts of medium or mycelium, their presence shows the plasticity of this fungal strain to metabolize xenobiotics by different routes.

PCA model and differences observed in different culture conditions

Principal component analysis allowed the reduction of the dimensionality of the raw data in order to detect the signals with higher variability between runs. Initially, unsupervised principal component analysis of the raw data of LCMS runs of medium extracts was performed, but although the replicates of each experiment clustered well on the scores chart, and the signals of structurally

related metabolites showed some degree of correlation, the first 5 PCs represented 75% of the total variance. For this reason, the 88 buckets corresponding to the signals of the most relevant metabolites on the chromatograms of the medium extracts were selected for the supervised PCA model and cluster analysis. In this improved model, the first 3 PCs accounted for 96% of the total variance. The scores biplot (Figure 3a) showed a clear difference between the control experiments, which clustered together with OHA and VPA, being more similar, and SAHA and MM signals, which clustered separately. Coherence between replicates of each culture run could be appreciated in the dendrogram chart (Figure 1S, Supplementary Material); all the SAHA LC/MS data grouped together at a low level, in the same way as for the MM data, while the OHA, VPA and control data were grouped together at a higher level.

As it is known, signals belonging to buckets of largest variability between runs have larger values on the different PCs of the loadings biplot, and buckets circumscribed to the same direction are correlated, that is, they vary in a coordinated fashion among runs. As shown in Figure 3b*,* the *s*ignals of the diketopiperazines (DKPs) **1**, **4**, **5**, **7**-**11** (Figure 4) are correlated; when one DKP signal was attenuated, which was observed when HDACIs were added to the cultures, all other signals of the DKPs were also attenuated, probably because these metabolites are biosynthetically related to each other. SAHA signals showed to be correlated with compounds **29** and **31**, which were later identified as hexosyl-phytosphingosine and benzophenone respectively.

These results indicated that all the experiments gave different profiles, and SAHA was the additive which produced the greatest effect in the culture, although it did not trigger the biosynthesis of other compounds than those observed on control experiments. The use of minimal medium produced significant changes in the metabolite profile: the production of DKPs decayed significantly, while at the same time the signals of other metabolites started to appear (Figure 2). The MS/MS mass spectra of the metabolites which were produced exclusively in the MM experiment showed many fragments and neutral losses in common, indicating that these compounds belonged to the same family.

Figure 3. a) Scores and b) loadings biplots of supervised PCA analysis.

Secondary metabolites produced by Dreschlera sp.

A detailed analysis was performed of the MS and MS/MS spectra in order to identify the compounds as tentative candidates or probable structures by a database search, or by *de novo*analysis. The chromatographic processing of large-scale cultures allowed the isolation and identification of the secondary metabolites which couldn´t be identified by means of MS and MS/MS and also the confirmation of tentative candidates. The chemical composition of the extracts of *Dreschlera sp.* is shown in *Table 1* and complementary data are presented in *Table ST1* (Suplementary material). The structures of the compounds are exhibited in Figure 4.

Figure 4. Compounds produced by *Drechslera sp.*

Diketopiperazines (DKPs) were the compounds with greater relative abundance in the LC/MS runs of medium and mycelium extracts. The identification of DKPs **1**, **4-5** and **7**-**11** was straightforward based on their MS and MS/MS spectra.[22] Two of them, cyclo(L-Leu-L-Pro) (**10**) and cyclo(L-Phe-L-Pro) (**11**), were also isolated and identified by their NMR and MS spectra. DKPs are common fungal metabolites with structural characteristics that enable them to bind with high affinity to a large variety of receptors, showing a broad range of biological activities.[23]

Compound **2** showed NMR and MS spectra identical with those of cochlione B, previously isolated from the endophyte *Cochliobolus sp.*,[24] a member of the *Pleosporaceae* family, the same as *Dreschlera.* Molecular modeling was employed to confirm the relative configuration of the asymmetric centers (Supplementary Material).

Compound **3** is described here for the first time. Its mass spectrum showed a signal at m/z 241.1061, indicating a molecular formula of $C_{12}H_{16}O_5$. The MS/MS spectrum of the precursor ion m/z 241 showed signals corresponding to the loss of C_4H_8 in the same way as in compound **2**. The ^{1}H and ^{13}C NMR spectra showed most of the signals in common with compound **2**, except for the presence of an additional methoxy group at δ 3.42 in compound **3**, suggesting that this compound is closely related to **2** with an additional methoxy group. The HMBC experiment allowed the unambiguous localization of this methoxy group at C-5, as the signal of CH_3O-protons at 3.42 ppm correlated with C-5 at 73.7 ppm. Thus, the structure of **3** was confirmed as 5-methoxy-cochlione B.

Related deoxygenated chromanones were previously reported from a common grapevine pathogen, *Eutypa lata*[25] and also some chlorinated derivatives were isolated from a mangrove-derived fungus *Pestalotiopsis sp.*[26] There are no reports about any biological activity for these related compounds.

Compound **6**, the most abundant metabolite of the ME big scale-extract, showed ^{1}H, ^{13}C, 2D NMR, HR-ESI-MS spectra and optical rotation coincident with those of (-)-asperpentyn. This highly functionalized prenylquinoid was previously isolated from several strains obtained from very different environments like the soil fungus *Aspergillus duricaulis*[27,28] and the marine fungus *Curvularia inaequalis*[29]. Both genera belong also to the *Pleosporaceae* family. The absolute configuration of **6** was confirmed by comparison with data of the synthesized (+) and (-) – asperpentyns.[30,31]

Other biosynthetically related compounds were also isolated and identify like harveynone (**14**),[32]siccayne (**17**),[33, 34] the 3-prenyl-4-hydroxybenzoic acids **18**, **24**, **27** and the chromene **28**. Compound **18** is described here for the first time. Its mass spectrum was consistent with a molecular formula $C_{12}H_{12}O_3$; the ^{1}H and ^{13}C and 2D NMR spectra were consistent with a trisubstituted benzene

ring, with a prenyl chain with a terminal double bond, a carboxylic acid and a hydroxylatedmethine as substituents. Further analysis of the HMBC and NOESY spectra allowed the location of each substituent in the benzene ring, leading to the structure shown in Figure 4.

The MS and MS/MS spectra and the retention time of compound **29** matched with those of a standard of commercial benzophenone.

Compounds **31** and **32** were identified as a hexosyl-phytosphingosine and hexosyl-sphingosine respectively by means of their MS and MS/MS spectra.[35] Collisional induced decomposition (CID) of the corresponding $[M+H]^+$ ions m/z 480.3526 and 462.3424respectively yielded product ions with the loss of a hexose (162 u) in addition to those typical of sphingosines. (Glyco)sphingolipids are an important class of outer membrane compounds involved in structure, recognition and signal transduction, found in essentially all animals, plants, and fungi, as well as in some prokaryotic organisms.[36]*Lyso*-glycosphingolipids have been implicated in a variety of regulatory roles in cell signaling, and the galactosyl-sphingosine psychosine, was linked to the pathogenesis of several diseases like Krabbe disease.[37, 38]Psychosine was already known to be cytotoxic, inhibiting protein kinase C and disturbing cytokinesis.[39]In particular, sphingolipids have also been reported to be involved in the growth and virulence in *Fusarium graminearum*.[40]

The loadings plot of the unsupervised PCA analysis, Figure 5, showed several aligned (correlated) signals on MM extracts, not present in ME extracts. Closer inspection of LCMS and MS/MS runs yielded fragmentation patterns for compounds belonging to the same family. Losses of $C_4H_6O_2$ and $C_6H_8O_2$ in their MS/MS spectra were characteristic for these compounds. For this reason, a larger scale culture on MM was also performed. From the extract of this culture, two compounds, **25** and **30**, were isolated and identify by NMR and MS analysis as macrosphelide C and 14-deoxy-macrosphelide C, 16-membered macrolides having two subunits of sorbic acid (C_6) and one subunit of 3-hydroxybutyric acid (C_4). Careful interpretation of the MS/MS spectra allowed the recognition of a characteristic pattern which in turn led to the characterization of the minor compounds of the same family directly from the LCMS runs without need for isolation. Compounds **19** and **22** are macrosphelides A, B or diastereoisomer of these, while **26** is a diastereoisomer of macrosphelide C. 14-deoxy-macrosphelide C (**30**) has been previously obtained as an intermediate in the synthesis of macrosphelides[41] and is reported here as a natural product for the first time. Macrosphelides A and B were first isolated from the soil fungi *Microsphaeropsis sp*.[42] Their ability to inhibit the cell-cell adhesion of HL-60 cells to human umbilical vein endothelial cells, among many other intriguing bioactivities, have driven to extensive biological studies and their total synthesis.[43, 44]

Macrosphelides **25** and **30**, compound **27** and anofinic acid (**28**) showed antifungal activity against the fungal pathogen of soybean *Fusarium tucumaniae,* responsible for the sudden death syndrome, with inhibition halos of 20, 12, 20 and 20 (± 1) mm respectively, comparable with those of the positive controls carbendazim and benomyl. Compounds **27** and **28** also showed antifungal activity

against the fungal pathogen of soybean *Macrophomina phaseolina*, with inhibition halos of 8 and 12 (± 1) mm respectively, also comparable with those of the positive controls. These results suggest a possible ecological role for these compounds in the defense of the plant against pathogens.

Other metabolites previously described from other *Dreschlera* strains have not been detected in this work: sesterpenes like ophiobolines from *Dreschlera gigantea*[10] or sesquiterpenes like drechslerines.[3,4]

Figure 5. Scores and loadings plot of unsupervised PCA analysis of LC-MS dataset. Arrow on loadings plot points were data from MM experiments which correlate each other. Red: ME, Blue: MM, circle: no HDACI, ♦: SAHA, x: OHA, +: VPA.

Metabolite distribution

It is generally accepted that the extra-cellular metabolites present in a culture filtrate may be associated with the combative relationship of the organism with its environment, whilst the metabolites present in the mycelium extract may have a biological related to the protection of the organism.[45] For this reason, the distribution of the metabolites between medium and mycelium is relevant to have a clue about their possible biological role.

The relative abundances (RA) of the identified metabolites produced by *Dreschlera sp.* in both media and mycelia in all the experiments are shown in *Table 3***.** DKPs showed a tendency to have higher relative abundances in the medium extracts than mycelium extracts, higher in ME than ME with additives, and always presented the minimum RA values in MM experiments. These facts may be related to phytotoxicity, which has been previously associated with DPKs. [45,46]

Asperpentyn(**6**) was present in all the experiments with minimal changes in RA, becoming then a good biomarker for the strain. Prenylquinoids(PQs) **14** and **17**, prenylhydroxybenzoic acids(PHBAs) **18**, **24**, **27** and **28** and chromanes **2**, **3** exhibited higher abundances in the medium than in the mycelium extracts. In general, these compounds were more abundant in the control experiments than in presence of the HDACs inhibitors, with the highest differences for cochlione B (**2**) and anofinic acid (**28**). Harveynone (**14**) was at least 15 times more abundant in MM experiments and cochlione B (**2**) was especially abundant in ME with VPA.

It is particularly remarkable that the most antifungic compound, anofinic acid (**28**), was mostly released to the medium, allowing it to exert efficiently its biological role.

Medium extracts of culture broths treated with SAHA showed a prominent peak corresponding to hexosyl-phytosphingosine (**31**) at 26.4min (Figure 2) which was almost absent in the medium extracts of control experiments (Table 3). This fact suggests that the HDAC inhibitor SAHA triggers the overproduction of **31** and its release to the medium. In the opposite sense, hexosyl-sphyngosine (**32**) was observed mainly in the mycelium extracts. These compounds are known to be signal transducers, cytotoxic and are related to pathogenesis.[47] Their release to the medium as a response to the presence of the inh.ibitor may indicate that compound **31** may play a role as an activation factor. Benzophenone (**29**) was also produced with higher abundances in the medium and mycelium extracts of the SAHA experiments.

Macrosphelides were produced almost exclusively in MM, and were highly abundant in both medium and mycelium extracts. Considering its antifungal activity, these compounds also could protect *Lollium sp.* from common phytopathogens.

Table 3. Relative abundances of the identified compounds in the different experiments.

			Mycelium extract					Medium extract				
compound class[a]	**N°**	**Compound**	**ME**	**SAHA**	**OHA**	**VPA**	**MM**	**ME**	**SAHA**	**OHA**	**VPA**	**MM**
DKPs	**1**	cyclo (Gly-Pro)	30.7	2.0	1.6	2.9	1.0	6.2	2.8	6.3	7.4	1.0
	4	cyclo (Pro-Val)	7.7	5.0	1.8	8.3	1.0	26.6	2.7	22.0	15.6	1.0
	5	cyclo (Leu/Ile-Hyp)	7.4	5.1	2.1	7.9	1.0	23.1	5.4	1.0	20.8	2.5
	7	cyclo (Phe-Hyp)	8.3	6.6	2.0	4.1	1.0	18.2	2.7	12.2	15.7	1.0
	8	cyclo (Phe-Hyp)	10.1	6.6	2.4	4.9	1.0	9.0	1.8	3.9	7.7	1.0
	9	cyclo (Leu/Ile-Pro)	4.1	2.3	1.0	5.0	0.0	131.2	14.2	107.9	80.4	1.0
	10	cyclo (L-Leu-L-Pro)	4.8	2.9	1.2	4.0	1.0	16.1	1.6	15.2	8.0	1.0
	11	cyclo (L-Phe-L-Pro)	14.8	8.1	3.1	17.3	1.0	23.1	2.4	21.0	16.3	1.0
PQs	**6**	asperpentyn	3.6	1.2	1.0	2.6	2.5	2.3	1.0	1.3	1.9	2.1
	14	harveynone	1.5	1.2	1.0	2.1	5.2	2.6	1.0	4.5	18.7	79.6
	17	siccayne	1.0	0.0	0.0	0.0	0.0	11.7	1.5	1.8	1.0	4.2
PHBAs	**18**		5.0	1.0	0.0	3.7	1.2	10.5	1.0	6.1	4.4	1.4
	24		5.2	1.0	4.6	1.0	3.0	11.5	3.0	19.8	2.1	1.0
	27		2.9	2.6	5.0	1.2	1.0	6.2	1.0	4.9	1.0	1.0
Chromanes	**2**	cochlione B	8.1	1.0	5.0	8.1	4.6	64.9	1.0	1.7	80.4	15.8
	3	5-methoxy-cochlione B	0.0	0.0	0.0	0.0	1.0	1.6	0.0	1.0	4.3	1.2
	28	anofinic acid	9.3	1.0	6.9	3.2	3.3	107.4	3.7	1.0	8.5	17.1
	19	macrosphelide	1.8	5.8	1.2	1.0	391.0	0.0	1.0	0.0	0.0	249.6

		A/isomer										
	22	macrosphelide B/isomer	2.9	1.0	0.0	1.5	68.4	31.9	8.6	3.4	1.0	499.3
MSP	**25**	macrosphelide C	3.8	1.0	1.3	15.6	72.1	18.0	4.5	1.0	3.4	452.6
	26	macrosphelide C isomer	1.0	0.0	0.0	0.0	45.2	3.5	3.5	1.8	1	577.4
	30	14-deoxy-MSP C	1.0	1.2	1.0	0.0	101.0	1.8	1.0	4.0	0.0	92.7
	29	benzophenone	1.2	17.6	1.0	0.0	12.0	0.0	73.9	1.3	1.0	1.4
SphL	**31**	(hexosyl)-phytosphingosine	1.0	2.1	1.0	0.0	1.7	1.2	65.4	1.2	0.0	1.0
	32	(hexosyl)-sphingosine	2.9	3.2	1.0	1.2	3.2	0.0	1.0	0.0	0.0	0.0

[a] DKPs: diketopiperazines, PQs:prenylquinoids, PHBAs: prenylhydroxybenzoic acids, MSP: Macrospherelides, SphL: sphingolipids, ME: control

Conclusions

From the cultures of a strain of *Drechslera sp*., a dark septate endophyte (DSE) isolated from the rye grass *Lollium sp.*, twenty five metabolites were identified. Two of them, the chromanone **3** and the prenylhydroxybenzoic acid **18**, were isolated and identified for the first time. Other metabolites belonging to different compound classes, the chromanes **2**,**3**, the prenylquinoids derivatives **6**, **14**,**17**, the prenylhydroxybenzoic acids **18**, **24**,**27**, and the macrosphelides **19**,**22**, **25**, **26** and **30** were first described for *Drechslera*, being **30** first described also as a natural product. Metabolites **18**, **25**, **27**, **28** and **30** displayed good antifungal activity against the phytopathogen *F. tucumaniae*, and a role for these compounds as chemical defenses in the host plant is plausible.

It was established that the addition of different HDACIs as epigenetic modifiers to the culture broths produced changes in the concentration of the metabolites. Particularly, the use of SAHA produced the greatest changes, with a noticeable increment in the production of benzophenone (**29**) in the medium and mycelium extracts, and the release of lyso-glycosphingolipids to the medium, which are known cellular response inducers.

Additionally, this *Drechslera* strain presented a high ability to metabolize xenobiotics, a capability that may be useful for the fungal protection from the environment.

.

Experimental Section

General

Optical rotations were recorded on a Perkin-Elmer 343 polarimeter. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance II spectrometer operating at 500.13 and 125.77 MHz, respectively. Two-dimensional NMR spectra COSY, HSQC-DEPT and HMBC were performed using standard Bruker software. The residual non-deuterated solvent signal was used for calibration. LC/MS grade methanol and water were purchased from Carlo Erba (Milan, Italy). DMSO, sodium valproate and hydroxylamine hydrochloride were purchased from Sigma Aldrich. SAHA and OHA were synthesized by known procedures[48]. Octanamide was synthesized from octanoic acid through the octanoyl-chloride treated with aqueous conc. ammonium hydroxide.

Fungal material

DSE strain Drechslera sp. was isolated from the roots of rye grass (Lollium sp.) as previously reported[49] and was deposited in the BAFC culture Collection (FCEN-UBA, CONICET) under the Accession Number BAFC 3419. This strain was previously characterized molecularly and the ITS sequence was deposited in the GenBank with accession number FJ868975.

Fermentation of small scale cultures with HDACIs

DSE strain was cultured and maintained in malt extract agar (MEA), composed of 30g malt extract, 5g peptone and 15g agar per liter. A 5 mm diameter agar disk of cultured fungus was cut with a sterile cork bore and used to inoculate 125 ml Erlenmeyer flasks containing 50 ml of malt extract medium (ME). Fermentation was carried out at 25°C for 48hs with orbital shaking. After this time, 3mL of culture broth were used to inoculate 125 mL Erlemeyer flasks containing 50mL malt extract medium (3 x control, 3 x SAHA, 3 x OHA, 3 x VPA) or minimal medium (3 x MM). Fermentation was carried out at 25°C for 72hs with orbital shaking, when inhibitors were added in 100 µL DMSO to a final concentration of 500 µM. Control cultures (3 x control and 3 x MM) without the addition of inhibitors were spiked with 100µL DMSO. Finally, fermentation was carried out for further 12 days under static conditions at 25°C. All the experiments were made at least by triplicate.

Fermentation of large scale cultures

Solid medium culture was cut into 1x1cm plugs and used for inoculation of three 250mL Erlenmeyer flasks containing 75mL of malt extract medium composed of 30g malt extract and 5g peptone per liter. Fermentation was carried out for 7 days at 25°C, and the fermentation broth was inoculated to five 4L Erlenmeyer flasks containing 1L each of the same liquid medium. Finally, fermentation was carried out under static conditions at 25°C for 7 days.

In the same way, a large-scale culture was performed on minimal medium, composed by sacharose (10g) and a solution of salts (100mL) per liter. The solution of salts contained $MgSO_4.7H_2O$ (7.31g), KNO_3 (0.8g), KCl (0.65g), KH_2PO_4 (48 mg), $Ca(NO_3)_2.4H_2O$ (2.88g), NaFeEDTA (0.08g), KI (7.5mg),

$MnCl_2.4H_2O$ (60mg), $ZnSO_4.7H_2O$ (26.5mg), H_3BO_3 (15mg), $CuSO_4.5H_2O$ (1.3mg), $Na_2MoO_4.2H_2O$ (0.2mg), glycine (30mg), thiamine chlorhydrate (1mg), pyridoxine chlorhydrate (1mg) and nicotinic acid (5mg) per liter.

Extract preparation

The culture broths (50mL) were filtered. The mycelia were sonicated in EtOH for 30 min, then filtered and extracted with EtOAc (3x50mL); the media of the culture broths were extracted with 3x50mL of EtOAc. In both cases, mycelia and media, the organic solvents were removed at reduced pressure; the residue was dissolved in 2mL of methanol and filtered through a 0.22µm pore size nylon membrane.

Liquid Chromatography

LCMS analyses of each extract were performed on a RRLC Agilent 1200 using a Luna C_{18} column (3 µm, 2.0 × 100 mm; Phenomenex, Torrance, CA, USA). The mobile phase consisted of water containing 0.1% formic acid (A) and methanol (B). The flow rate was 0.3 mL/min and the column temperature was set at 30 °C. Linear gradient elution was performed as follows: 10-75 % B (0-25 min), 75-100 % B (25-26 min), 100 % B (26-46 min). Detection was performed with a DAD detector from 190 to 950 nm coupled to a mass spectrometer. All the runs were repeated at least three times.

Mass spectrometry

Mass spectrometric analyses were performed using a Bruker MicrOTOF-Q II mass spectrometer (BrukerDaltonics, Billerica, MA, USA), equipped with electrospray and APCI ion sources. The instrument was operated at a capillary voltage of 4.5 kV with an end plate offset of 500 V, a dry temperature of 200 °C using N_2 as dry gas at 6.0 l/min and a nebulizer pressure of 3.0 bar. Multi-point mass calibration was carried out using a sodium formate solution from m/z 50 to 1200 in positive ion mode. For APCI the corona was set at 4000 nA and the vaporizer temperature at 250°C and an Agilent tuning mix APCI/APPI was employed as calibrant. Data acquisition and processing were carried out using the software Bruker Compass Data Analysis version 4.0 supplied with the instrument.

Data analysis and Principal Component Analysis

Extraction and bucketing of the raw LC/MS data and unsupervised PCA model was performed using Profile Analysis 2.0 (BrukerDaltonics), obtaining a [31x9300] matrix data of [analyses x (tR; m/z)], which was converted to .csv data for further analysis. Supervised PCA, clustering and dendrogram were applied to simplified data matrix of most relevant data using MATLAB v.7.6 (R2008a, MathWorks Inc., Natick, MA).

Antifungal assay

Direct bioautography on TLC was employed as the method for detecting the antifungal activity[50]. A concentration level of 20 µg/spot of each assayed compound was used. Carbendazim and benomyl, which were used as positive control, showed inhibition zones of 22±2 and 20±1 mm respectively.

Extraction and isolation of metabolites

The 5x1L ME culture broths were filtered and the combined filtrates were partitioned with EtOAc. The extracts were subjected to dry column flash chromatography using silicagel, with mixtures of diclorometane: EtOAc as eluants, yielding fractions I to V. Fractions II and III resulted positive in the antifungal assay. Fraction II was subjected to column chromatography using silicagel using diclorometane: EtOAc as eluants, yielding 45 mg of **6** and a fraction, which was further purified by preparative silicagel TLC (dichloromethane: EtOAc (7:3)), yielding 3 mg of **2**. Fraction III was purified by column chromatography using silicagel and mixtures of dichloromethane: EtOAc yielding 1 mg of **3**. Fraction IV was subjected to preparative HPLC on a Phenomenex Synergi 4µm fusion-RP-80A (150 x 21.1mm) column, using methanol:water (40:60) under isocratic conditions (UV 215nm), obtaining compound **24** and the diketopiperazines **10** and **11**.

The 5x1L MM culture broths were filtered and the combined filtrates were partitioned with AcOEt. The extract was subjected to dry column flash chromatography using reverse phase silicagel (C-18) and mixtures of water: methanol as eluants, yielding fraction I to IV. Fraction I (180mg) contained asperpentyn as its main component. Fraction II was subjected to column chromatography using silicagel and mixtures of hexane: EtOAc as eluants, yielding asperpentyn (**6**, 16 mg), harveynone (**14,** 4.8 mg) and **18** (2.2 mg). Fraction III was subjected to preparative HPLC (YMC column C18, 5 µm, 22.5x2.5cm, eluant MeOH-H_2O (6:4), 5 mL/min; detection: UV 254 nm, RI), yielding compound **27** (5.5mg), anofinic acid (**28,** 4.7 mg), macrosphelide C (**25,** 1.6 mg) and 14-deoxy-macrosphelide C (**30,** 0.9 mg).

5-methoxy-cochlione B. 5-Methoxy-6-hydroxy-2,2-dimethyl-5,6,7,8-tetrahydro-7,8-epoxychroman-4-one **(3)**

Amorphous yellow solid; ^{1}H NMR ($CDCl_3$, 500MHz): δ 4.51 (brt, *J* = 2.1 Hz, 1H, H-6), 4.44 (brt, *J* = 2.1 Hz, 1H, H-5), 3.75 (dt, $J_{7\text{-}8}$=3.7, $J_{6\text{-}7}$= $^4J_{5\text{-}7}$ = 2.5 Hz, 1H, H-7), 3.46 (brd, *J* = 3.7 Hz, 1H, H-8), 3.42 (s, 3H, OCH_3), 2.68 (d, *J* = 16.5 Hz, 1H, H-3a), 2.51 (d, *J* = 16.5 Hz, 1H, H-3b), 1.53 (s, 3H, H-10), 1.43 (s, 3H, H-9); ^{13}C NMR ($CDCl_3$, 125MHz): δ 190.7 (C-4), 167.4 (C-8a), 107.5 (C-4a), 83.4 (C-2), 73.7 (C-5), 65.6 (C-6), 58.3 (OCH_3), 57.0 (C-7), 48.7 (C-8), 47.2 (C-3), 26.9 (C-10), 25.4 (C-9). EIMS (70 eV) m/z (relative abundance): 240 $[M]^{+\cdot}$ (5), 225 $[M- CH_3]^+$ (1), 182 (54), 83 (58).

(8R)-4-Hydroxy-3-(8-hydroxy-10-methyl-10-buten)-benzoic acid **(18)**

Amorphous yellow solid; $[\alpha]_D^{25}$ (MeOH, c=0.22): +16.5°; ^{1}H NMR ($CDCl_3$, 500MHz, δ in ppm): 8.88 (s, 1H, OH), 7.93 (dd, *J*=8.5Hz, 2.1Hz, 1H, H-6), 7.76 (d, *J*=2.1, 1H, H-2), 6.93 (d, *J*=8.5Hz, 1H, H-5), 5.05 (brs, 1H, H-11a), 5.03 (dd, *J*=10.8Hz, 3.4Hz, 1H, H-8), 4.93 (brs, 1H, H-11b), 2.60 (dd, *J*=13.8Hz, 10.8Hz, 1H, H-9a), 2.49 (dd, *J*=13.8Hz, 3.3Hz, 1H, H-9b), 1.86 (s, 3H, H-12). ^{13}C NMR (CD_3OD, 125MHz, δ in ppm): 169.6 (C-7), 161.0 (C-1), 141.3 (C-10), 131.6 (C-6), 129.8 (C-2), 125.9 (C-3) , 120.5 (C-1), 117.6 (C-5), 115.6 (C-11), 72.6 (C-8), 46.6 (C-9), 22.1 (C-12). Configuration of C-8 based on closely related structures.[51-53]

Supplementary Material

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/MS-number.

Acknowledgements

The authors thank Universidad de Buenos Aires (W670 and 100352), CONICET (PIP 100516) and ANPCyT (PICT 2008-1394) for partial financial support.

Author Contribution Statement

G. Siless made the fungal cultures in different conditions, analyzed LCMS runs and performed PCA analyses. G. Siless and G. Gallardo isolated and identified the compounds. G. Cabrera is the director of the project, and also performed and analyzed LCMS runs. Y. Rincón performed the bioassays. M. A. Rodriguez and A. Godeas isolated, characterized and classified the fungal strain.

References

[1] K. K. Newsham, 'A Meta-Analysis of Plant Responses to Dark Septate Root Endophytes', *New Phytol.* **2011**, *190*, 783-793.

[2] S. N. Kivlin, S. M. Emery, J. A. Rudgers, 'Fungal Symbionts Alter Plant Responses to Global Change', *Am. J. Bot.* **2013**, *100*, 1445–1457.

[3] C. Osterhage, G. M. König, U. Höller, A. D Wright, 'Rare Sesquiterpenes from the Algicolous Fungus *Drechslera dematioidea*', *J. Nat. Prod.* **2002**, *65*, 306-313.

[4] A. Abdel-Lateff, T. Okino, W. M. Alarif, S. S. Al-Lihaibi, 'Sesquiterpenes from the Marine Algicolous Fungus *Drechslera sp.*', *J. Saudi Chem. Soc.* **2013**, *17*, 161-165.

[5] M. M. A. A. El-Gendy, N. M Hassanein, H. A. E.-H. Ibrahim, D. H. A. El-Baky, 'Heavy Metals Biosorption from Aqueous Solution by Endophytic *Drechslera hawaiiensis* of Morusalba L. Derived from Heavy Metals Habitats', *Mycobiol.* **2017**, *45*, 73-83.

[6] M. Kastanias, M. Chrysayi-Tokousbalides, 'Herbicidal Potential of Pyrenophorol Isolated from a *Drechslera avenae* Pathotype', *Pest.Manag. Sci.* **2000**, *56*, 227-232.

[7] E. Read, J. Edwards, M. Deseo, G. Rawlin, S. Rochfor, 'Current Understanding of Acute Bovine Liver Disease in Australia', *Toxins* **2017**, *9*, 8.

[8] F. Sugawara, Y. F. Hallock, G.D. Bunkers, D.S. Kenfield, G. Strobel, S. Yoshida, 'Phytoactive Eremophilanes Produced by the Weed Pathogen *Drechslera gigantea*', *Biosci. Biotech. Biochem.* **1993**, *57*, 236-239.

[9] A. Evidente, A. Andolfi, A. Cimmino, M. Vurro, M. Fracchiolla, R. Charudattan, A. Motta, 'Ophiobolin E and 8-epi-ophiobolin J Produced by *Drechslera gigantea*, a Potential Mycoherbicide of Weedy Grasses', *Phytochemistry* **2006**, *67*, 2281–2287.

[10] F. Sugawara, G. Strobel, R. N. Strange, J. N. Siedow, G. D. Van Duyne, J. Clardy, 'Phytotoxins from the Pathogenic Fungi *Drechslera maydis* and *Drechslera sorghicola*', *Proc. Natl. Acad. Sci. USA.* **1987**, *84*, 3081-3085.

[11] G. F. Zhang, Z. K. Guo, W. Wang, J. T. Cui, R. X. Tan, H. M. Ge, 'Neuraminidase Inhibitory Terpenes from Endophytic *Cochliobolus sp.', J. Asian Nat. Prod. Res.* **2011**, *13*, 761-764.

[12] S. Nozoe, M. Morisaki, K. Tsuda, Y. Iitaka, N. Takahashi, S. Tamura, K. Ishibashi, M. Shirasaka, 'The Structure of Ophiobolin, a C_{25} Terpenoid having a Novel Skeleton', *J. Am. Chem. Soc.* **1965**, *87*, 4968–4970.

[13] S. Nozoe, K. Hirai, K. Tsuda,K. Ishibashi, M. Shirasaka, J. F. Grove, 'The Structure of Pyrenophorin', *Tetrahedron Lett.* **1965**, *51*, 4675-4677.

[14] A. Evidente, A. Andolfi, M. Vurro, M. Fracchiolla, M. C. Zonno, A. Motta, 'Drazepinone, a Trisubstituted Tetrahydronaphthofuroazepinone with Herbicidal Activity Produced by *Drechslera siccans', Phytochemistry* **2005**, *66*, 715-721.

[15] F. Sugawara, N. Takahashi, G. A. Strobel, S. A. Strobel, H. S. M. Lu, J. Clardy, 'Triticones A and B, Novel Phytotoxins from the Plant Pathogenic Fungus *Drechslera tritici-repentis*', *J. Am. Chem. Soc.* **1988**, *110*, 4086-4087.

[16] A. S. Awaad, H. M. Al-Zaylaee, S. I. Alqasoumi, M. E. Zain, E. M. Aloyan, A. M. Alafeefy, E. S. Awad, R. M. El-Meligy, 'Anti-Leishmanial Activities of Extracts and Isolated Compounds from *Drechslera rostrata* and *Eurotium tonpholium*', *Phytother Res.* **2014**, *28*, 774-780.

[17] H. B. Bode, B. Bethe, R. Höfs, A. Zeeck, 'Big Effects from Small Changes: Possible Ways to Explore Nature's Chemical Diversity', *ChemBioChem* **2002**, *3*, 619- 627.

[18] P. A. Paranagama, E. M. Kithsiri Wijeratne, A. A. Leslie Gunatilaka, 'Uncovering Biosynthetic Potential of Plant-Associated Fungi: Effect of Culture Conditions on Metabolite Production by *Paraphaeosphaeria quadriseptata* and *Chaetomium chiwersii*', *J. Nat. Prod.* **2007**, *70*, 1939-1945.

[19] C. Zutz, A. Gacek, M. Sulyok, M. Wagner, J. Strauss, K. Rychli, 'Small Chemical Chromatin Effectors Alter Secondary Metabolite Production in *Aspergillus clavatus*', *Toxins* **2013**, *5*, 1723-1741.

[20] J. C. Henrikson, A. R. Hoover, P. M. Joyner, R. H. Cichewicz, 'A Chemical Epigenetics Approach for Engineering the *in situ* Biosynthesis of a Cryptic Natural Product from *Aspergillus niger*', *Org. Biomol. Chem.* **2009**, *7*, 435–438.

[21] T. Asai, T. Yamamoto, Y. Oshima, 'Histone Deacetylase Inhibitor Induced the Production of Three Novel Prenylated Tryptophan Analogs in the Entomopathogenic Fungus, *Torrubiellaluteo rostrata*', *Tetrahedron Lett.* **2011**, *52*, 7042–7045.

[22] N. A. J. C. Furtado, R. Vessecchi, J. C. Tomaz, S. E. Galembeck, J. K. Bastos, N. P. Lopes, A. E. M. Crotti, 'Fragmentation of Diketopiperazines from *Aspergillus fumigatus* by Electrospray Ionization Tandem Mass Spectrometry (ESI-MS/MS)', *J. Mass Spectrom.* **2007**, *42*, 1279–1286.

[23] A. D. Borthwick, '2,5-Diketopiperazines: Synthesis, Reactions, Medicinal Chemistry, and Bioactive Natural Products', *Chem. Rev.* **2012**, *112*, 3641–3716.

[24] Q.-Z. Wang, H.-M. Ge, J. Zhang, J.-H. Wu, Y.-C. Song, Y.-F. Zhang, R.-X. Tan, 'Cochliones A–D, Four New Tetrahydrochromanone Derivatives from Endophytic *Cochliobolus sp.*', *J. Asian Nat. Prod. Res.* **2010**, *12*, 485-491.

[25] J.-M Renaud, G. Tsoupras, H. Stoeckli-Evans, R. Tabacchi, 'A Novel Allenicepoxycyclohexane and Related Compounds from *Eutypa lata* (Pers: F.) TUL.', *Helv. Chim. Acta* **1989**, *72*, 1262-1267.

[26] S. Klaiklay, V. Rukachaisirikul, K. Tadpetch, Y. Sukpondma, S. Phongpaichit, J. Buatong, J. Sakayaroj, 'Chlorinated Chromone and Diphenyl Ether Derivatives from the Mangrove-derived Fungus *Pestalotiopsis sp.* PSU-MA69', *Tetrahedron* **2012**, *68*, 2299-2305.

[27] A. Muhlenfeld, H. Achenbach, 'Asperpentyn, a Novel Acetylenic Cyclohexene Epoxide from *Aspergillus duricaulis*', *Phytochemistry* **1988**, *27*, 3853-3855.

[28] V. Rukachaisirikul, N. Rungsaiwattana, S. Klaiklay, S. Phongpaichit, 'γ-Butyrolactone, Cytochalasin, Cyclic Carbonate, Eutypinic Acid, and Phenalenone Derivatives from the Soil Fungus *Aspergillus sp*. PSURSPG185', *J. Nat. Prod.* **2014**, *77*, 2375−2382.

[29] O. F. Smetanina, A. N. Yurchenko, A. I. Kalinovskii, Y. V. Khudyakova, N. N. Kirichuk, M. V. S. Pivkin, Sh. Afiyatullov, V. V. Mikhailov, '(–)-Asperpentyn from the Facultative Marine Fungus *Curvularia inaequalis*', *Chem. Nat. Compd.* **2014**, *50*, 1120-1121.

[30] J. Li, S. Park, R. L. Miller, D. Lee, 'Tandem Enyne Metathesis-Metallotropic [1,3]-Shift for a Concise Total Syntheses of (+)-Asperpentyn, (-)-Harveynone, and (-)-Tricholomenyn A', *Org. Lett.* **2009**, *11*, 571-574.

[31] G. Mehta, S. Roy, S. C. Pan, 'Enantioselective Syntheses of Bioactive Epoxyquinone Natural Products (+)-Harveynone and (-)-Asperpentyn', *Tetrahedron Lett.* **2012**, *53*, 4093–4095.

[32] T. Nagata, Y. Ando, A. Hirota, 'Phytotoxins from Tea Gray Blight Fungi, *Pestalotiopsis longiseta* and *Pestalotiopsis theae*', *Biosci. Biotech. Biochem.* **1992**, *56*, 810-811.

[33] M. Pinault, Y. Frangin, J. P. Genet, H. Zamarlik, 'Total Synthesis of Siccayne', *Synthesis* **1990**, *10*, 935-937.

[34] J. Kupka, T. Anke, W. Steglich, L. Zechlin, 'Antibiotics from Basidiomycetes. XI. The Biological Activity of Siccayne, Isolated from the Marine Fungus *Halocyphinavillosa* J. & E. Kohlmeyer', *J. Antibiot.* **1981**, *34*, 298-304.

[35] S. Frank, H. Geyer, R. Geyer, 'Microscale Analysis of Glycosphingolipids from *Schistosomamansoni cercariae*', *J. Carbohyd. Chem.* **2011**, *30*, 233–248.

[36] A. H. Merrill Jr., K. Sandhoff, in 'Biochemistry of Lipids, Lipoproteins and Membranes', Ed. D. E. Vance, J. E. Vance, Elsevier Science B.V., Amsterdam, 2002, Vol. 36, p. 373.

[37] L. M. Obeid, Y. Okamoto, C. Mao, 'Yeast Sphingolipids: Metabolism and Biology', *Biochim. Biophys. Acta* **2002**, *1585*, 163-171.

[38] L. C. Castelvetri, M. I. Givogri, A. Hebert, B. Smith, Y. Song, A. Kaminska, A. Lopez-Rosas, G. Morfini, G. Pigino, M. Sands, S. T. Brady, E. R. Bongarzone, 'The Sphingolipid Psychosine Inhibits Fast Axonal Transport in Krabbe Disease by Activation of GSK3β and Deregulation of Molecular Motors', *J. Neurosci*. **2013**, *33*, 10048–10056.

[39] C. Colacios, F. Sabourdy, N. Andrieu-Abadie, B. Ségui, T. Levade, 'Bioactive Sphingolipids in Cancer Biology and Therapy', Springer International Publishing, Switzerland, 2015.

[40] V. Ramamoorthy, E. B. Cahoon, M. Thokala, J. Kaur, J. Liand, D. M. Shah, 'Sphingolipid C-9 Methyltransferases are Important for Growth and Virulence but not for Sensitivity to Antifungal Plant Defensins in *Fusarium graminearum*', *Eukaryotic Cell* **2009**, *8*, 217-229.

[41] K. Ishihara, T. Kawaguchi, Y. Matsuya, H. Sakurai, I. Saiki, H. Nemoto, 'Synthesis and Biological Evaluation of Macrosphelide Cores', *Eur. J. Org. Chem.* **2004**, 3973-3978.

[42] S. Takamatsu, Y.-P. Kim, M. Hayashi, H. Hiraoka, M. Natori, K. Komiyama, S. Omura, 'Macrosphelide, a Novel Inhibitor of Cell-Cell Adhesion Molecule. II. Physicochemical Properties and Structural Elucidation', *J. Antibiot.* **1995**, *49*, 95-98.

[43] C. Hac̈ker, B. Plietker, 'General Stereodivergent Enantioselective Total Synthetic Approach toward Macrosphelides A−G and M', *J. Org. Chem.* **2015**, *80*, 8055−8064.

[44] S.-M. Paek, 'Development of Advanced Macrosphelides: Potent Anticancer Agents', *Molecules* **2015**, *20*, 4430-4449.

[45] J. R. Hanson, 'The chemistry of fungi', RSC Publishing, Cambridge, 2008.

[46] S. H. Park, A. Stierle, G. Strobel, 'Metabolism of Maculosin, a Host-Specific Phytotoxin Produced by *Alternaria alternata* on Spotted Knapweed (*Centaurea maculosa*)', *Phytochemistry* **1993**, *35*, 101-106.

[47] D. M. Heringdorf, K. H. Jakobs, 'Lysophospholipid Receptors: Signalling, Pharmacology and Regulation by Lysophospholipid Metabolism', *Biochim Biophys Acta.* **2007**, *1768*, 923-40.

[48] L. K. Gediya, P. Chopra, P. Purushottamachar, N. Maheshwari, V. C. O. Njar, 'A New Simple and High-Yield Synthesis of Suberoylanilide Hydroxamic Acid and its Inhibitory Effect Alone or in Combination with Retinoids on Proliferation of Human Prostate Cancer Cells', *J. Med. Chem.* **2005**, *48*, 5047-5051.

[49] V. A. Silvani, S. Fracchia, L. Fernández, M. Pérgola, A. Godeas, 'A Simple Method to Obtain Endophytic Microorganisms from Field-Collected Roots', *Soil Biol. Biochem.* **2008**, *40*, 1259–1263.

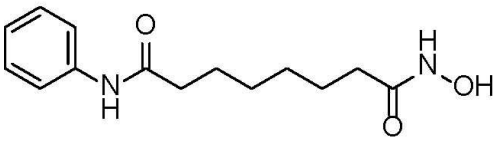
[50] F. Homans, A. Fuchs, 'Direct Bioautography on Thin-Layer Chromatography as a Method for Detecting Fungitoxic Substances', *J. Chromatogr*. **1970**, *51*, 325-328.

[51] J. H. Brewster, 'A Useful Model of Optical Activity. I. Open Chain Compounds', *J. Am. Chem. Soc*. **1959**, *81*, 5475–5483.

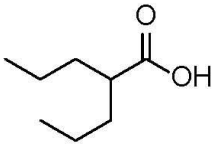
[52] T. Wang, X.-Q. Hao, J.-J. Huang, J.-L. Niu, J.-F. Gong, M.-P. Song, 'Chiral Bis(imidazolinyl)phenyl NCN Pincer Rhodium(III) Catalysts for Enantioselective Allylation of Aldehydes and Carbonyl–Ene Reaction of Trifluoropyruvates', *J. Org. Chem.* **2013**, *78*, 8712–8721.

[53] S. Zhao, X. Zhang, Y. Zhang, H. Yang, Y. Huang, K. Zhang, T. Du, 'A $Ce(OTf)_3$/PyBox Catalyzed Enantioselective Hosomi–Sakurai Reaction of Aldehydes with Allyltrimethylsilane', *New J. Chem*. **2015**, *39*, 7734-7737.

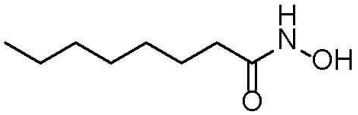
HDAC inhibitors



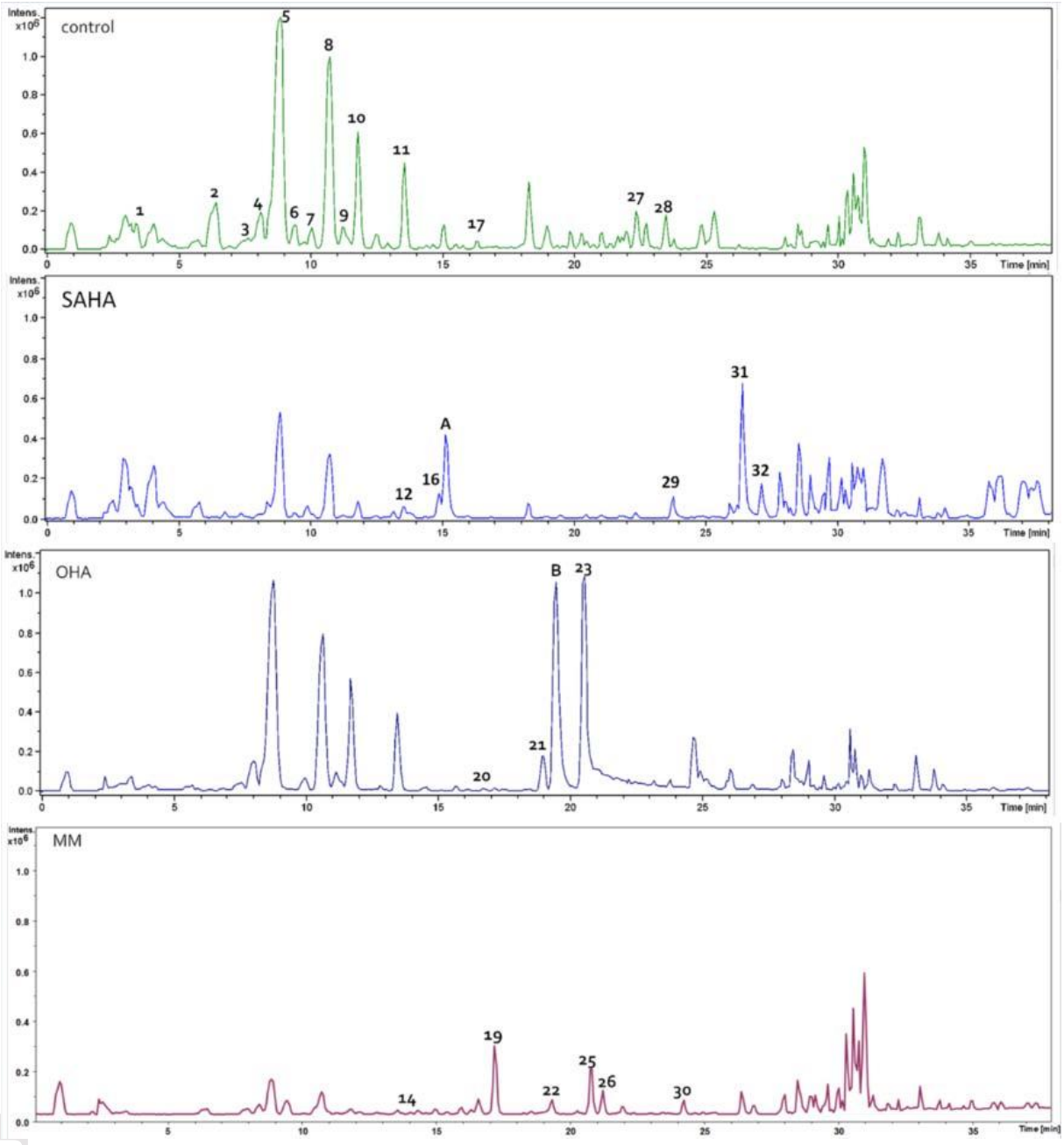
A
SAHA

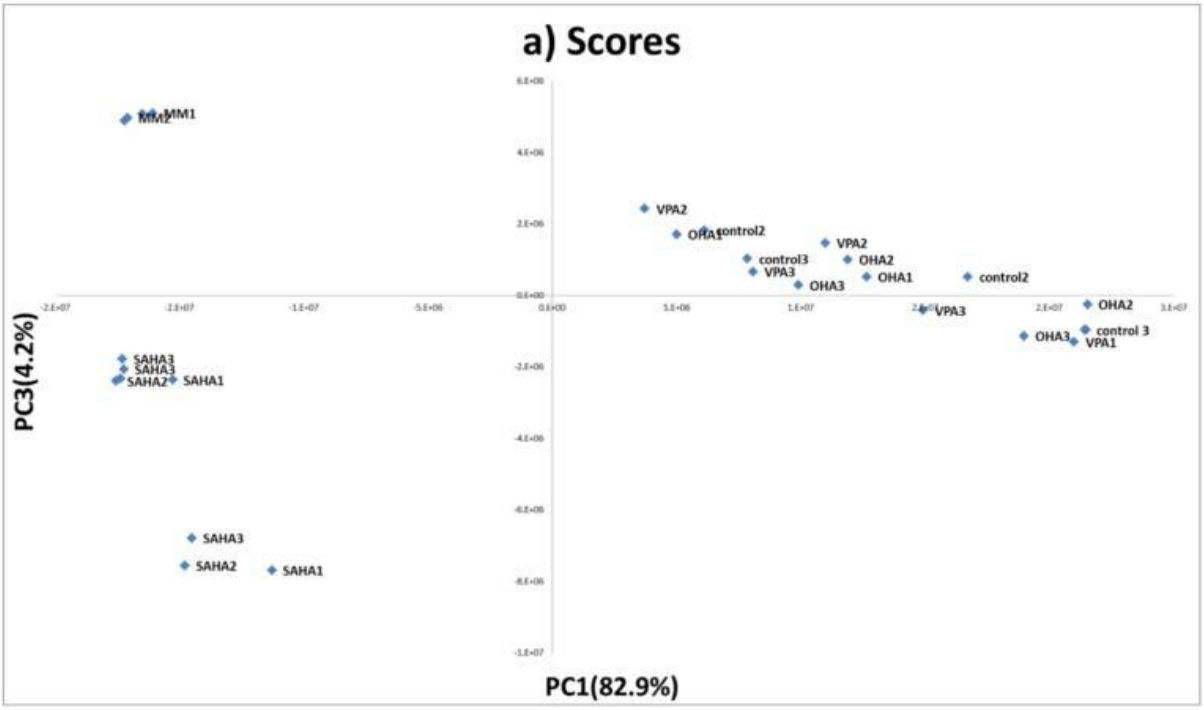


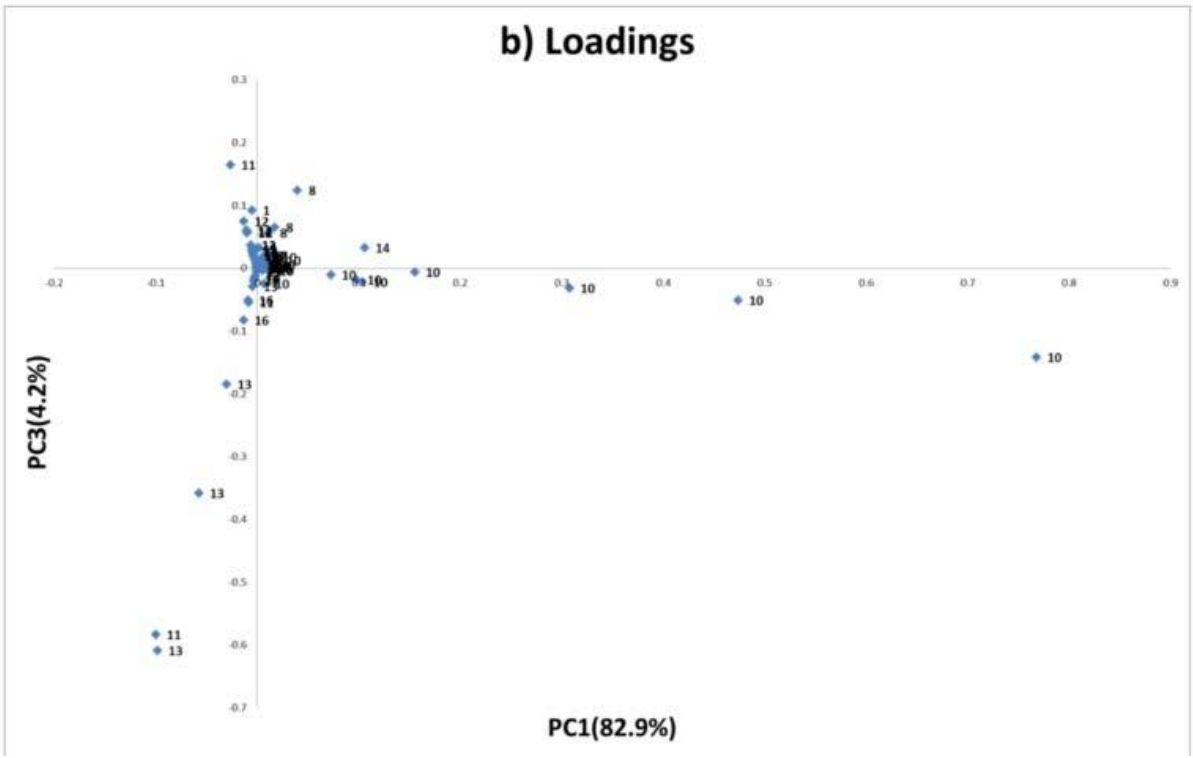
B
VPA

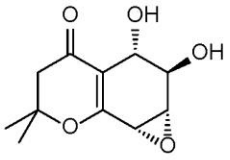


C
OHA

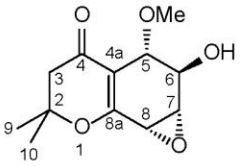




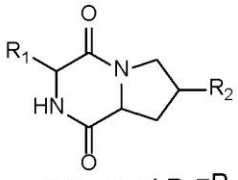




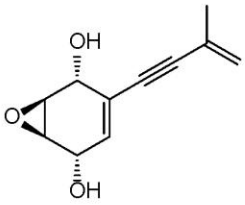
cochlione B
2



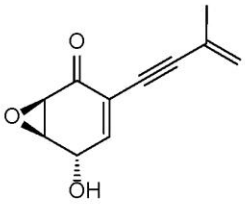
3



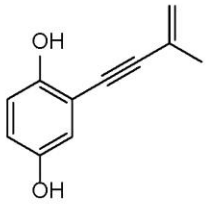
1: **cyclo(Gli-Pro)**: R_1=R_2=H
4: **cyclo(Val-Pro)**: R_1=iPr, R_2=H
5: **cyclo(Leu/Ile-Hyp)**: R_1=*i*Bu/*s*Bu, R_2=OH
7/8: **cyclo(Phe-Hyp)**: R_1=CH_2Ph, R_2=OH
9/10: **cyclo(Leu/Ile-Pro)**: R_1=*i*Bu/*s*Bu, R_2=H
11: **cyclo(Phe-Pro)**: R_1=CH_2Ph, R_2=H



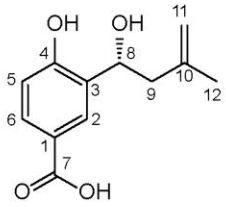
(-)-asperpentyn
6



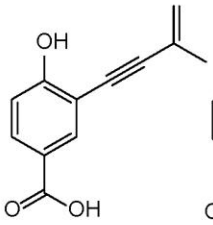
(+)-harveynone
14



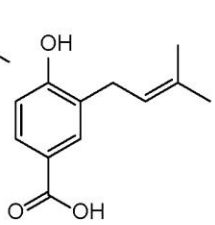
siccayne
17



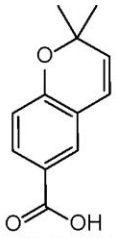
18



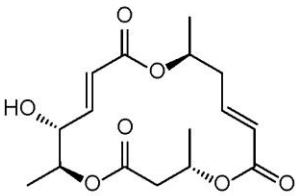
24



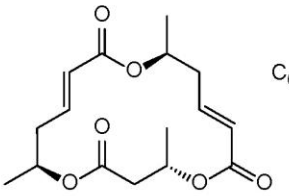
27



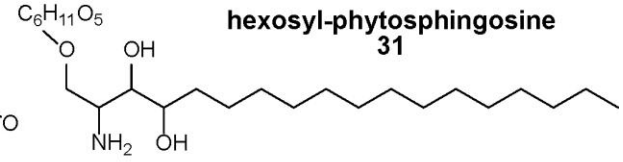
anofinic acid
28



macrosphelide C
25



14-deoxy-macrosphelide C
30



hexosyl-phytosphingosine
31