Chemical Studies on the Secondary Metabolites of the Marine-Derived Fungi, and Their Biological Activities

해양균류 2차대사성분에 대한 화학적연구와 생물활성

A thesis submitted in partial fulfillment of the requirements
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Pukyong National University

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Chemical Studies on the Secondary Metabolites of the Marine-Derived Fungi, and Their Biological Activities

A Dissertation

By

Yong Li

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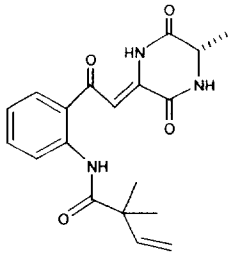
Abstract

During the investigation and development of the secondary metabolites from fungi for more than 70 years, so many interesting natural products were obtained from fungi. The marine-derived fungi, as being one important member of fungi family, have been regarded as a fresh source of secondary metabolites with significant therapeutic potential. The researches on the marine-derived fungi have been mostly involved in all fields nearly, not only in chemistry, biochemistry, pharmacology, and marine ecology, but also many diverse applying fields. Due to the unique ecological environment of the marine-derived fungi, it was approved that the marine-derived fungi may produce novel chemical structures and diverse biological activities on the basis of the previous reports, especially in the last 5 years. The advantage of the marine-derived fungi as compared to the marine macroorganisms are obvious in the following viewpoints: 1) Biotechnological fermentation are possible without ecological exploitation. 2) Some metabolites can be obtained after recultivation in large amounts, which are nearly impossible for the marine macroorganisms. 3) Microorganisms can be manipulated easier genetically.

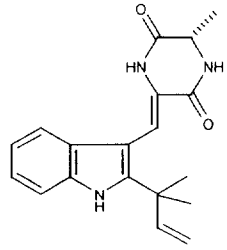
In our investigation for bioactive natural products, the marine-derived fungi were selected as our targets for chemical research. The secondary metabolites were obtained from combining the modern knowlwdge of microorganism and chemical techniques in culture, extraction, isolation, and purification. The stereochemistry of secondary metabolites and their biological activities have been investigated, and the results are as following:

1. A new diketopiperazine alkaloid, golmaenone (**1**) and related alkaloids, neoechinulin A (**2**) and *L*-alanyl-*L*-tryptophan anhydride (**3**), have been isolated from the culture broth of the marine-derived fungus *Aspergillus* sp. The structure and absolute stereochemistry of the compounds were assigned by spectroscopic methods and the advanced Marfey's method. Compounds **1** and **2** exhibited a significant radical scavenging activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH) with IC_{50} values of 20 and 24 μM, respectively, which were

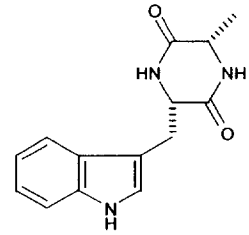
similar to the positive control, ascorbic acid (IC_{50}, 20 μM). They also showed an ultraviolet-A (UV-A) (320-390 nm) protecting activity with ED_{50} values of 90 and 170 μM, respectively, which were more active than oxybenzone (ED_{50}, 350 μM) currently being used as sunscreen.



golmaenone (**1**)

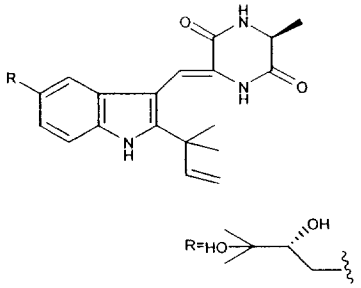


neoechinulin A (**2**)

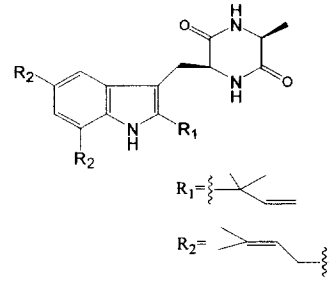


L-alanyl-*L*-tryptophan anhydride (**3**)

2. A more polar active fraction of *Aspergillus* sp. yielded a new metabolites, dihydroxyisoechinulin A (**4**) and related echinulin (**5**). The planar structures were elucidated on the basis of COSY, HMQC, HMBC, and NOESY correlations. The stereochemistry of **4** was carried out using the advanced Marfey's and Horeau's methods. Compound **4** exhibited a potent radical scavenging activity against DPPH (IC_{50}, 20 μM), and also showed an ultraviolet-A protecting activity with ED_{50} value of 130 μM, which was more active than oxybenzone (ED_{50}, 350 μM).

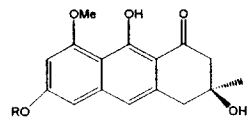


dihydroxyisoechinulin A (**4**)

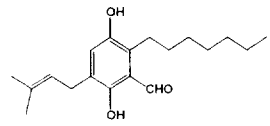


echinulin (**5**)

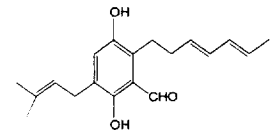
3. Bioassay-guided fractionation of the secondary metabolites from the marine-derived fungus *Microsporum* sp. led to the isolation of a new 6-*O*-α-*D*-asperflavin ribofuranoside (**6**), as well as the polyketides, flavoglaucin (**7**), isodihydroauroglaucin (**8**), citrinin (**9**), and neoechinulin B (**10**). The structures of **6-10** were elucidated on the basis of spectroscopic analyses, including 1D and 2D NMR comprehensive analyses. These compounds (**6-10**) showed the DPPH scavenging activity with IC_{50} values of 14.2, 11.3, 11.5, 48.5, and 26.0 μM, respectively, and **6** also exhibited *in vitro* mild antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and multidrug-resistant *S. aureus* (MDRSA) with MIC values of 50 μM, respectively. Furthermore, compound (**10**) exhibited an ultraviolet-A (UV-A) protecting activity with ED_{50} value of 165 μM.



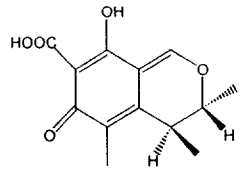
asperflavin ribofuranoside (**6**):
R=α-*D*-ribofuranoside
asperflavin (**6a**) : R=H



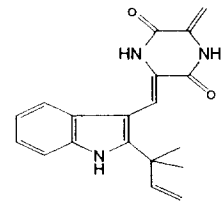
flavoglaucin (**7**)



isodihydroauroglaucin (**8**)

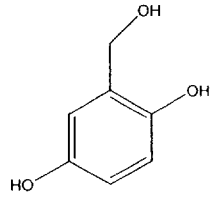


citrinin (**9**)

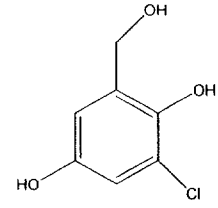


neoechinulin B (**10**)

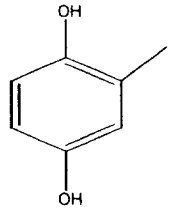
4. A series of active compounds were isolated from the culture broth of the marine-derived fungus of *Aspergillus*, which had been isolated from the surface of the marine red alga *Hypnea saidana*. On the basis of the comprehensive analyses of spectroscopic data, their structures were established as gentisyl alcohol (**11**), 3-chlorogentisyl alcohol (**12**), 2-methylhydroquinone (**13**), (+)-epoxydon (**14**), and (+)-epoxydon monoacetate (**15**), respectively. The biological activity of **11-15** showed *in vitro* mild antibacterial activity against MRSA and MDRSA with the MIC values (μg/mL) of 12.5, 50.0, 6.25, 12.5, 12.5, respectively.



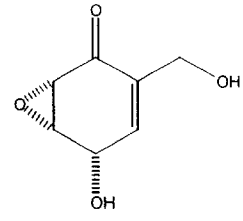
gentisyl alcohol (**11**)



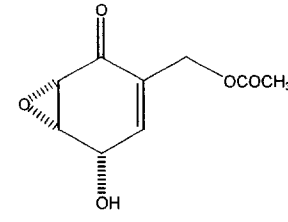
3-chlorogentisyl alcohol (**12**)



2-methylhydroquinone (**13**)

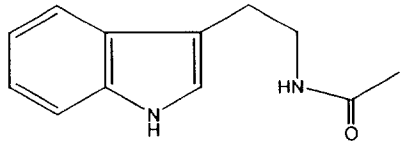


(+) epoxydon (**14**)



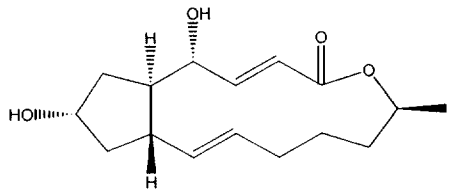
(+)-epoxydon monoacetate (**15**)

5. An indolyl alkaloid derivative, N_b-acetyltryptamine (**16**) was isolated from an unidentified marine-derived fungus, which had been separated from the red alga *Gracilaria verrucosa*. The structure of N_b-acetyltryptamine was firmly determined to be 2-(3-indolyl)ethylamine acetate (**16**) from the physicochemical analyses.



N_b-acetyltryptamine (**16**)

6. (+)-Brefeldin A (**17**) was obtained from an unidentified marine-derived fungus (Culture# MFA552). The unique structure of (+)-brefeldin A was established on the basis of comprehensive spectroscopic analyses. Due to its wide range of biological activities and well-functionalized macrolide structure, its biological mode of action has been disclosed by a number of important discoveries. Especially, (+)-brefeldin A is known as a disassembler of the Golgi apparatus because of blocking protein transport.



(+)-brefeldin A (**17**)

Acknowledgments

You may have noticed that this abstract and preface are almost entirely "we" and "our", not "I" and "my". That is because this is a team effort, especially, with my advisor, Dr. Byeng Wha Son. First of all, I am deeply indebted to him for his careful and outstanding academy guidance in all fields, not only excellent concepts, principles and methodology, but also valuable advice of converting our research manuscript to a real theses, and my heartfelt thanks for his constant financial supporting throughout these years of study period in Korea.

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Facilities for NMR and Mass spectral measurements were kindly provided by Korea Basic Science Institute; some activity assays were kindly completed by Korea Chemistry Bank, the same thanks for them so much.

I am everlastingly grateful for Board Chairman, Dr. Bung Kwon Park providing JS International Scholarship, and I would never forget those kind help from my friends in my lab –Jung Chul Kim, Sang-Mi Lee, Xi Feng Li and MD Atiqur Rahman. I am thankful to all Korean students in our graduate school for their profound friendship.

Finally, I would like to offer a deep appreciation to my loving wife and daughter, thanks a million for your understanding in my cause; thanks a million for your care in living; thanks a million for your so much time.

List of Abbreviations and Symbols

BHI.....Brain Heart Infusion

BHIA.....Brain Heart Infusion Agar

^{13}C NMR.....^{13}Carbon Nuclear Magnetic Resonance

CD.....Circular Dichroism

COSY.....Correlated Spectroscopy

1D NMR.....One Dimensional Nuclear Magnetic Resonance

2D NMR.....Two Dimensional Nuclear Magnetic Resonance

DEPT.....Distortionless Enhancement by Polarization Transfer

DMSO.....Dimethylsulfoxide

DPPH.....2,2-Diphenyl-picrylhydrazyl

ED_{50}.....Effective dose in 50% of the test samples

FABMS.....Fast Atom Bombardment Mass Spectroscopy

HMBC.....Heteronuclear Multiple Bond Correlation

HMQC.....Heteronuclear Multiple Quantum Coherence

^{1}H NMR.....Proton Nuclear Magnetic Resonance

HPLC.....High Performance Liquid Chromatography

HREIMS.....High Resolution Electron Impact Mass Spectrometry

IC_{50}.....Inhibition Concentration in 50% of the Test Samples

LREIMS.....Low Resolution Electron Impact Mass Spectrometry

MDRSA.....Multi-Drug Resistant *Staphylococcus aureus*

MFA.....Marine Fungus Strain Number A#

MIC.....Minimum Inhibitory Concentration

mp. .....Melting Point

MRSA.....Methicillin Resistant *Staphylococcus aureus*

MTPA.....α-Methoxy-α-Trifluoromethyl Phenylacetyl

MPLC.....Medium Pressure Liquid Chromatography

NOESY.....Nuclear Overhauser Effect Spectroscopy

OD.....Optical Density

ppm.....Parts Per Million

PTLC.....Preparative Thin Layer Chromatography

rHPLC.....Recycling High Performance Liquid Chromatography

TMS.....Tetramethylsilane

SA.....*Staphylococcus aureus*

SWS.....Soytone (0.1%), Soluble starch (1.0%), and Seawater (100%)

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Part I

Introduction

Fungi, as a potential source of bioactive natural products, are increasingly showing unique advantage. The investigation for new drugs from fungi started with the discovery of penicillin,[1,2] a potent antibiotic against Gram-positive bacteria, which was obtained from *Penicillium notatum.* During the investigation and development of fungi for more than 70 years, a further milestone for medicinal use of fungal metabolites was the discovery of the immunosuppressant cyclosporine which was from *Tolypocladium inflatum* and *Cylindrocarpon lucidum.*[3] It has been using as an antifungal and immunosuppressive metabolite which made cyclosporine useful for the treatment following organ transplantation.[4] Later the antifungal agent, griseofulvin isolated from *Penicillium* griseofulvum and the cholesterol biosynthesis inhibitor, lovastatin obtained *Aspergillus terreus* are two further evidences for supporting today's great interest in searching for new secondary metabolites from fungi.

Up to date more than 4000 fungal metabolites has been reported[5], and 5000-7000 taxonomic species have been studied with respect to their chemistry. It was estimated that the probable number of existing fungi to be 1.5 Mil. with only 71,000 being described so far.[6] It indicates that fungi are really an enormous source for natural products with unique chemical structures and interesting activities.

In addition, a diverse ecological niches may lead to various of secondary metabolites from the same fungus,[7] this enhances the capability of fungi to play an important role in yielding useful medicine as what we need.

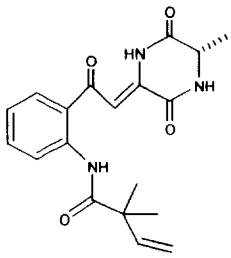
By the early 1960s, researchers began to view the oceans as a new and untouched source of potentially useful compounds-perhaps not surprising considering that more than 70% of the Earth's biosphere is ocean, since then, marine natural products chemists have demonstrated the chemical structures of over 10,000 new compounds.[8] Nearly all forms of life in the marine environment, e.g., algae, sponges, corals, tunicates, nudibranches, have been investigated for their natural products content,[9] However, the advantages of the investigation of microorganisms as compared to macroorganisms are obvious: biotechnological fermentation are possible without ecological exploitation, metabolites can be obtained after recultivation in large amounts which is nearly impossible for marine macroorganisms, and also microorganisms can be easier manipulated genetically. [10]

Most of the described fungi were isolated from not only algae, sponge, see weed, fishes, a coral, shell of crab, sea sediment, driftwood and mangrove wood, but also sea mud, sand and coastal marsh grass and so on. Marine fungi do not represent specific taxa, but are a group defined by their ecology and physiology. In generally, it can be classified into obligate and facultative marine fungi. Kohlmeyer et.al. (1979) defined obligate marine fungi as those "that grow and sporulate exclusively in a marine or estuarine (brackish water) habitat; facultative marine are fungi from freshwater or terrestrial areas also able to grow in the natural marine environment."[11] This means that the ability to germinate in the natural marine environment has to be assessed before defining a marine-derived fungal isolated as obligate or facultative marine. The isolation of a fungal strain from a marine sample does not prove that this fungus is active in the marine environment. Usually, it is possible to isolate a terrestrial fungus being a contaminant. Such a fungal isolate was dormant in the form of spore or hyphal fragments until the surrounding conditions in the laboratory became favorable for germination and growth. Most of fungi isolated from marine samples are not proven to be obligate or facultative marine. Thus, the more general expression **marine-derived fungi** are used always.

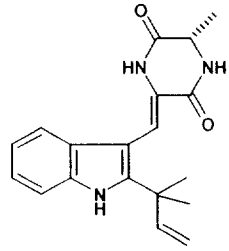
The increasing investigation of marine-derived fungi has been leading to numerous of secondary metabolites with various biological activities. Thus, we believe that more and more effective lead compounds or candidates for clinic drugs, which obtained from marine-derived fungus, are coming soon! [12-16]

In our investigation for bioactive natural product, the marine-derived fungi were selected as our targets for chemical research. The secondary metabolites were obtained from combining the modern knowledge for microorganism and chemical techniques in culture, extraction, isolation, and purification (Chart 1).

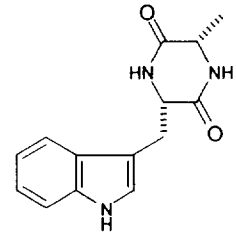
Their chemical structures were firmly elucidated on the basis of spectroscopic analyses, including 1D and 2D NMR comprehensive analyses, as well as physicochemical data (Chart 1), and all these secondary metabolites were screened by the radical scavenging activity (DPPH), antimicrobial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and multidrug-resistant *S. aureus* (MDRSA), and ultraviolet-A (UV-A) protecting activity.



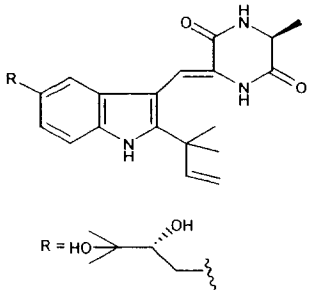
golmaenone (**1**)



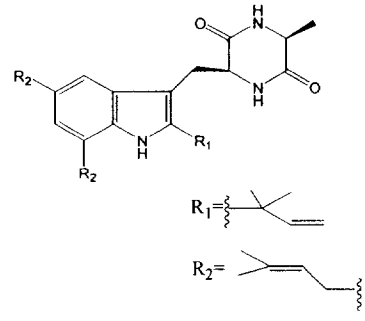
neoechinulin A (**2**)



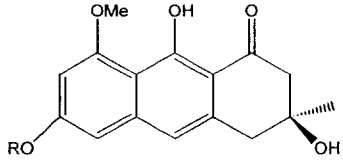
L-alanyl-*L*-tryptophan anhydride (**3**)



dihydroxyisoechinulin A (**4**)



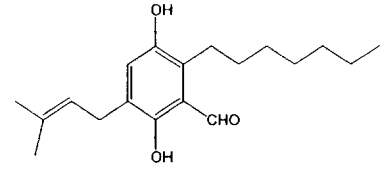
echinulin (**5**)



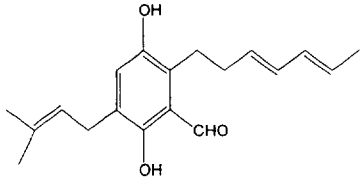
asperflavin ribofuranoside (**6**):

R=α-*D*-ribofuranoside

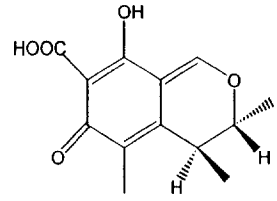
asperflavin (**6a**) : R=H



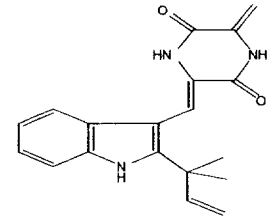
flavoglaucin (**7**)



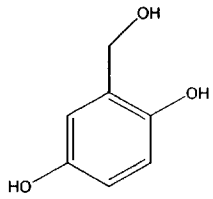
isodihydroauroglaucin (**8**)



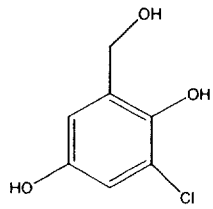
citrinin (**9**)



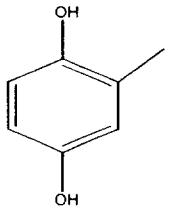
neoechinulin B (**10**)



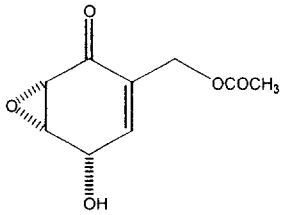
gentisyl alcohol (**11**)



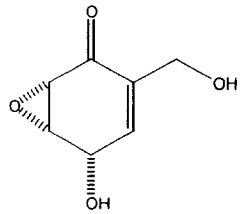
3-chlorogentisyl alcohol (**12**)



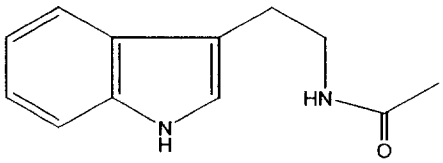
2-methylhydroquinone (**13**)



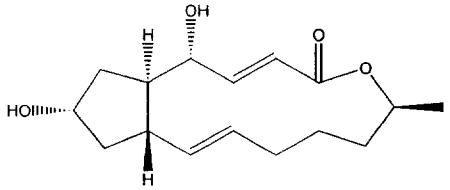
(+)-epoxydon (**14**)



(+)-epoxydon monoacetate (**15**)



N_b-acetyltryptamine (**16**)



(+)-brefeldin A (**17**)

Chart 1. Secondary metabolites from the marine-derived fungi.

Part II Experimental

1. Instruments and chemical reagents

1) Instruments

Melting points were determined on an Electothermal model IA9100 micro-melting point apparatus and uncorrected. Optical rotation was determined on a Pekerin Elmer model 341 polarimeter. IR spectrum was recorded on a Brucker FT-IR model IFS-88 spectrometer. ^{1}H NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were obtained on a JEOL JNM-ECP 400 NMR spectrometer, using TMS or solvent peaks as reference standard. MS spectra were obtained on a JEOL JMS-700 spectrometer. GC/MS spectrum was carried out on a Shimadzu GC/MS-QP-5050A spectrometer. UV/Visible spectra were measured on a Hitachi U-2001 UV/Vis spectrometer. CD spectra were taken on a JASCO J-715 spectropolarimeter.

2) Chemical reagents

NMR solvent: DMSO-d_6 (Cambridge Isotope Laboratories. Inc. deuterium degree 99.9%); $CDCl_3$ (Aldrich, deuterium degree 99.8%). Organic solvent: *n*-hexane, benzene, EtOAc, $CHCl_3$, CH_2Cl_2, MeOH, EtOH, acetone, *n*-BuOH (Duksan Pure Chemical, 99.5%). Coloring reagent: $Se(SO_4)_2$ (Sigma). Culture medium: soytone (Acuamedia), soluble starch (Duksan Pure Chemical), agar powder (Duksan Pure Chemical), *D*-mannitol (Sigma, 98%), yeast extract (Acuamedia), peptone (Acuamedia), *D*-(+)-glucose (Yakuri), glycerol (Sigma, 99%), penicillin G (Sigma). Assay reagent and medium: 1,1-dipnenyl-2-picrylhydrazyl (DPPH, Sigma, 90%), Ascorbic acid (Sigma, 99%), Oxacillin (Sigma, 99%), Brain Heart Infusion and Brain Heart Infusion Agar (Becton, Dickinson).

2. Isolation, culture, and extraction of the marine-derived fungus

1) *Aspergillus* sp. (MFA212)

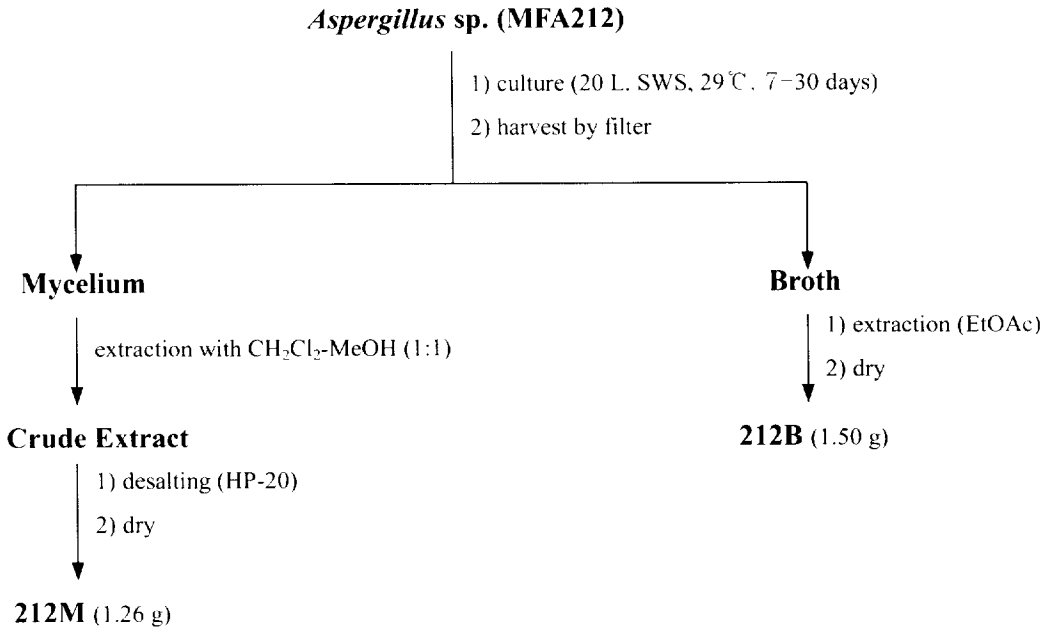
The fungal strain (stock # MFA 212) was isolated from the surface of the marine red alga *Lomentaria catenata* collected in the Golmae Village, Ulsan City, Korea in 2002 and identified as an *Aspergillus* sp. based on fatty acid methyl ester analysis (Korean Culture Center of Microorganisms, Seoul, Korea, similarity index 0.62) (Fig. 1). The fungal strain (MFA212) was stored in the 10% glycerol YPG medium at –75℃. The further culture for investigation was completed on YPG medium from 10 mL to large scale (1 L and 10 L). The fungus was cultured (20 L) for 30 days (static) at 29℃ in SWS medium.

The culture broth and mycelium were separated and the filtered broth was extracted with ethyl acetate to provide the broth extract (212B, 1.5 g). The mycelium was dried under vacuum, and then extracted with CH_2Cl_2-MeOH (1:1) to produce the mycelium extract (212M, 1.26 g).

MFA212 (YPG agar)

MFA212 (1 L)

Fig. 1. Morphology of the marine-derived fungus *Aspergillus* sp. (MFA212).

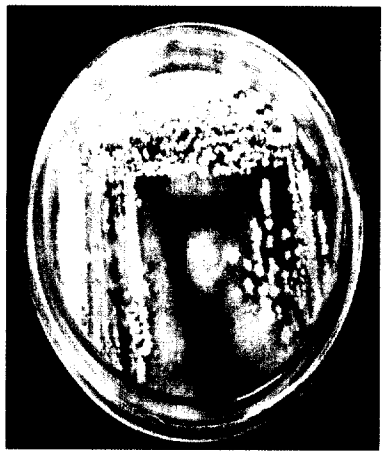


Scheme 1. Extraction of the marine-derived fungus *Aspergillus* sp. (MFA212).

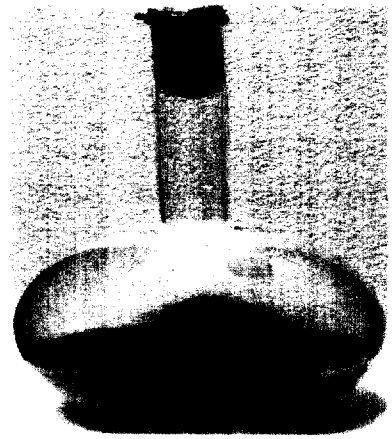
2) *M*icrosporum sp. (MFA212-1)

The fungal strain (MFA 212-1) was isolated from the surface of the marine red alga *Lomentaria catenata* collected in the Golmae Village, Ulsan City, Korea in 2002 and identified as a *Microsporum* sp. based on fatty acid methyl ester analysis (Korean Culture Center of Microorganisms, Seoul, Korea, similarity index 0.62) (Fig. 2). The fungal strain (MFA212-1) was stored in the 10% glycerol YPG medium at −75℃. The further culture for investigation was completed on YPG medium from 10 mL to large scale (1 L and 10 L). The fungus was cultured (20 L) for 30 days (static) at 29℃ in SWS medium.

The culture broth and mycelium were separated and the filtered broth was extracted with ethyl acetate to provide the broth extract (212-1B, 800 mg). The mycelium was dried under vacuum, and then extracted with CH_2Cl_2-MeOH (1:1) to produce the mycelium extract (212-1M, 600 mg).

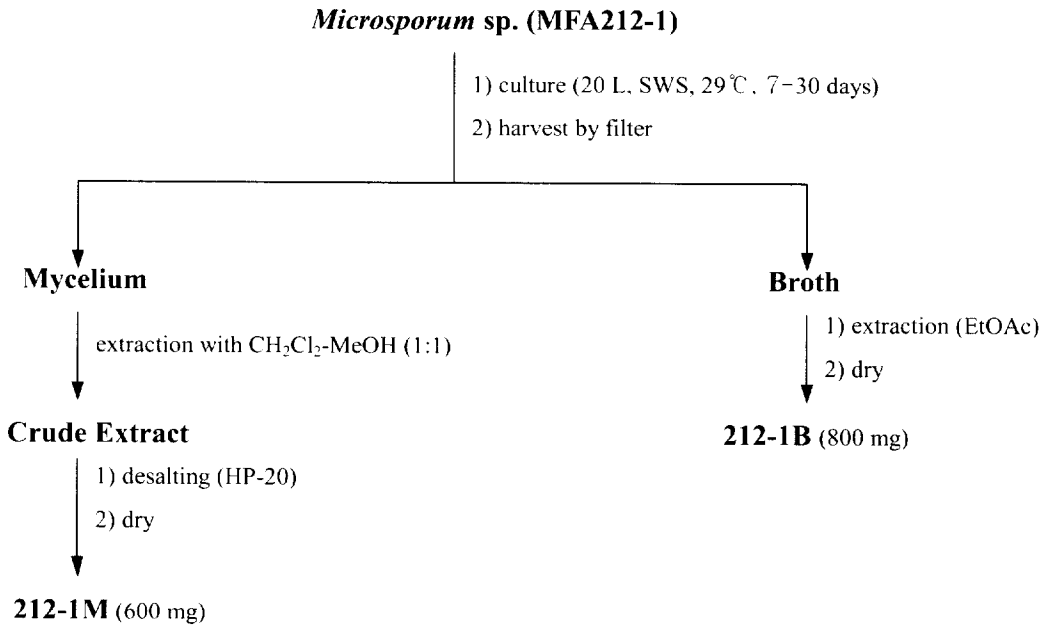


MFA212-1 (YPG agar)



MFA212-1 (1 L)

Fig. 2. Morphology of the marine-derived fungus *Microsporum* sp. (MFA212-1).



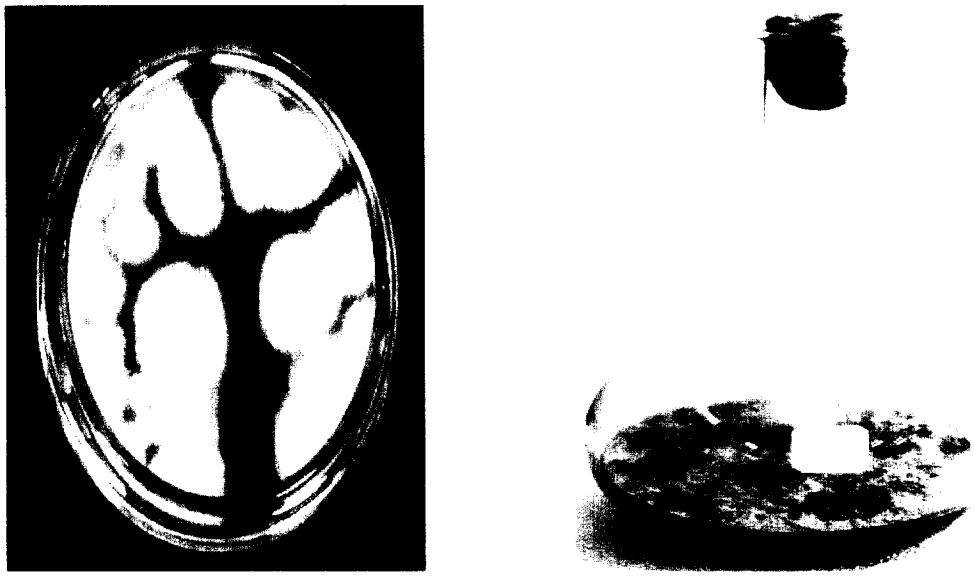
Scheme 2. Extraction of the marine-derived fungus *Microsporum* sp. (MFA212-1).

3) *Aspergillus* sp. (MFA292)

The fungal strain (MFA 292) was isolated from the surface of the marine red alga *Hypnea saidana* collected in the Golmae Village, Ulsan City, Korea in 1999 and identified as an *Aspergillus* sp. based on fatty acid methyl ester analysis (Korean Culture Center of Microorganisms, Seoul, Korea, similarity index 0.855) (Fig. 3). The strain was cultured in YPG agar medium for four times and then got the pure fungus (MFA292). The fungal strain (MFA292) was stored in the 10% glycerol YPG medium at −75℃.

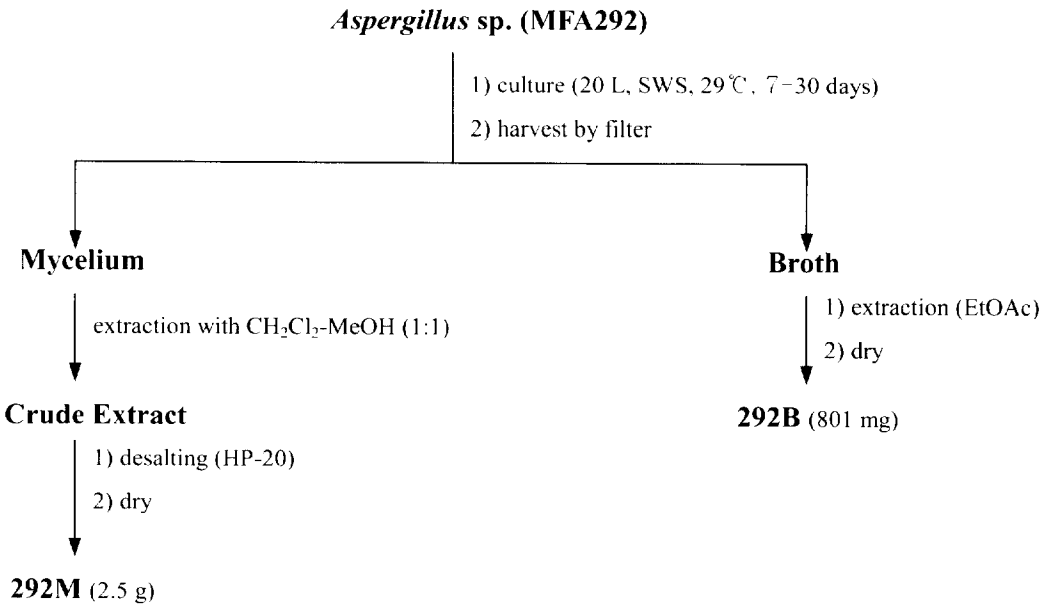
The further culture for investigation was completed on SWS medium from 10 mL to large scale (1 L and 10 L). The fungus was cultured (20 L) for 30 days (static) at 29℃ in SWS medium.

The culture broth and mycelium were separated and the filtered broth was extracted with ethyl acetate to provide the broth extract (292B, 801 mg). The mycelium was dried under vacuum, and then extracted with CH_2Cl_2-MeOH (1:1) to produce the mycelium extract (292M, 2.5 g).



MFA292 (YPG agar)　　MFA292 (1 L)

Fig. 3. Morphology of the marine-derived fungus *Aspergillus* sp. (MFA292).



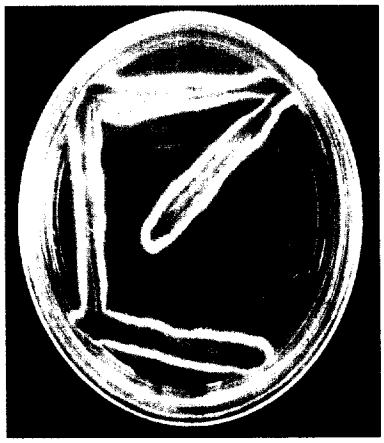
Scheme 3. Extraction of the marine-derived fungus *Aspergillus* sp. (MFA292).

4) MFA936 (unidentified strain)

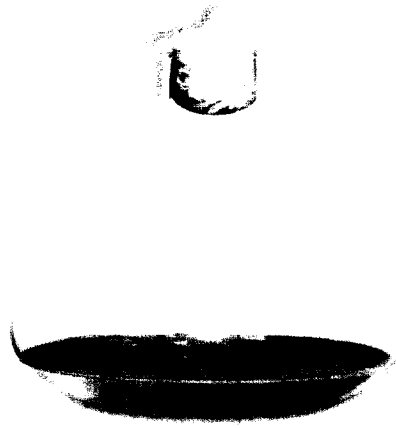
An unidentified marine-derived fungal strain (MFA936) was isolated from the surface of the marine red alga *Gracilaria verrucosa* collected in Hamdeok Beach, Cheju Island, Korea in 2001. The strain was cultured in YPG agar medium for four times and then got the pure fungus (MFA936) (Fig. 4). The fungal strain (MFA936) was stored in the 10% glycerol YPG medium at −75℃. The 10 mL and 1 L culture used the liquid culture medium—SWS medium at 29℃ for 7 days and 30 days (static), respectively. The mycelium and broth were separated by filter.

The further culture for investigation was completed on SWS medium from 10 mL to large scale (1 L and 10 L). The fungus was cultured (20 L) for 30 days (static) at 29℃ in SWS medium.

The culture broth and mycelium were separated and the filtered broth was extracted with ethyl acetate to provide the broth extract (936B, 1.9 g). The mycelium was dried under vacuum, and then extracted with CH_2Cl_2-MeOH (1:1) to produce the mycelium extract (936M, 870 mg).

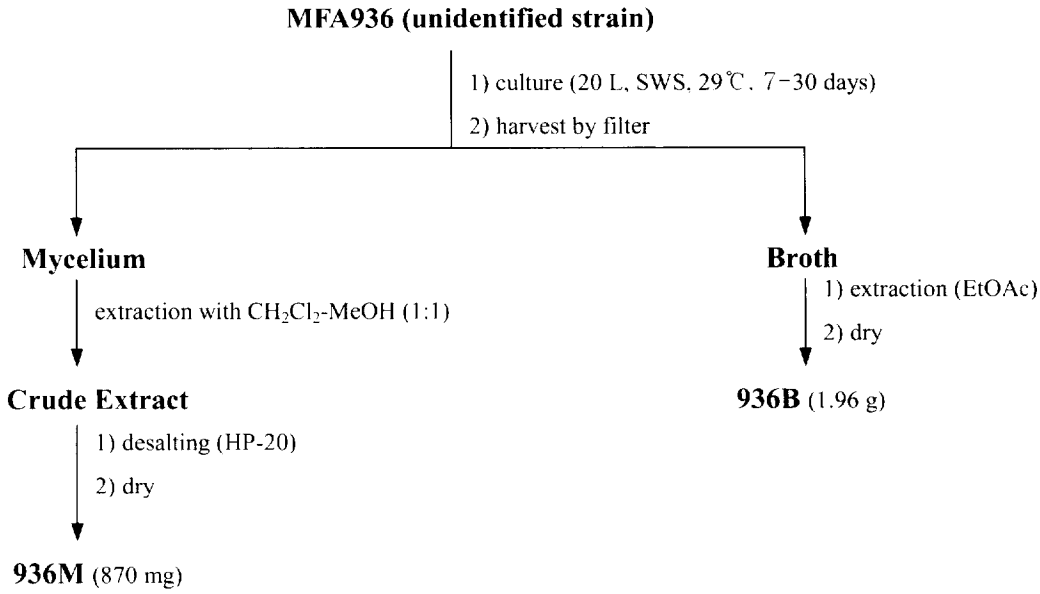


MFA936 (YPG agar)



MFA936 (1 L)

Fig. 4. Morphology of the marine-derived fungus MFA936 (unidentified strain).



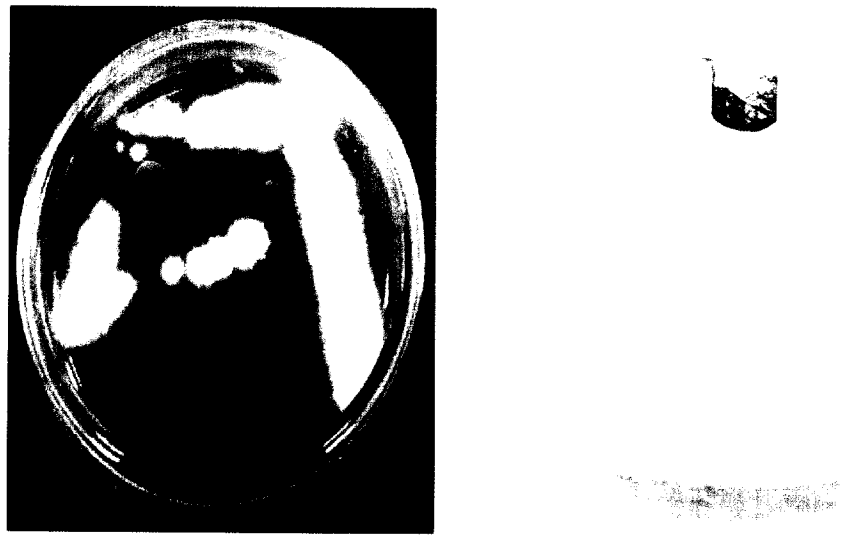
Scheme 4. Extraction of the marine-derived fungus MFA936 (unidentified strain).

5) MFA552 (unidentified strain)

An unidentified fungal strain (MFA552) was isolated from the surface of the marine brown alga *Padina arborescens* collected in Zhaseongpo, Geoje, Gyeongnam, Korea in 1998, The strain was cultured in YPG agar medium for four times and then got the pure fungus (MFA552) (Fig. 5). The fungal strain (MFA552) was stored in the 10% glycerol YPG medium at –75℃. The 10 mL and 1 L culture used the liquid culture medium—SWS medium at 29℃ for 7 days and 30 days (static), respectively. The mycelium and broth were separated by filter.

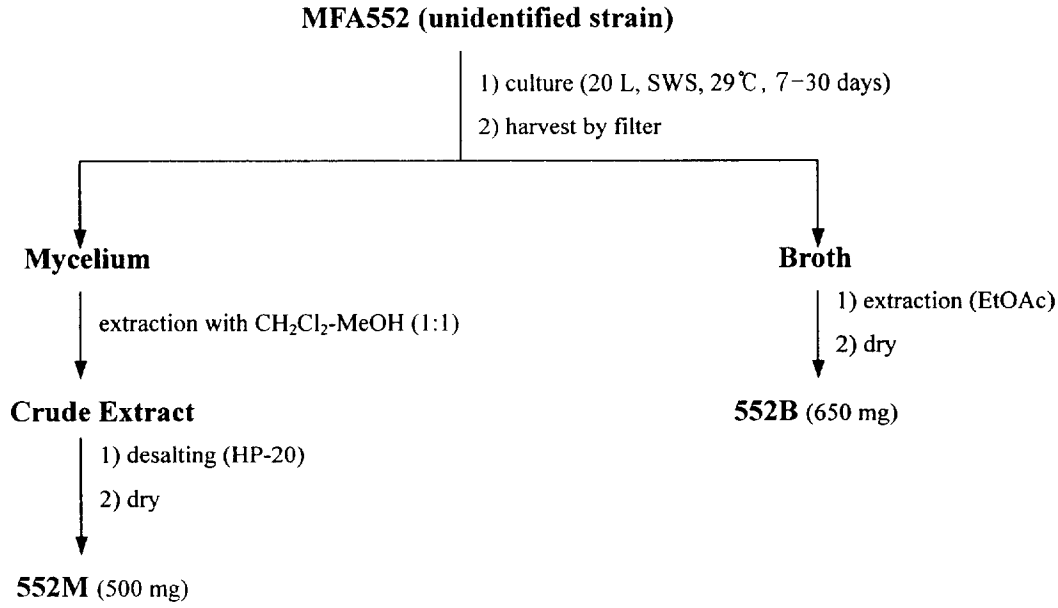
The further culture for investigation was completed on SWS medium from 10 mL to large scale (1 L and 10 L). The fungus was cultured (20 L) for 30 days (static) at 29℃ in SWS medium.

The culture broth and mycelium were separated and the broth was extracted with ethyl acetate to provide the broth extract (650 mg). The mycelium was dried under vacuum, and then extracted with CH_2Cl_2-MeOH (1:1) to produce the mycelium extract (500 mg).



MFA552 (YPG agar) **MFA552 (1 L)**

Fig. 5. Morphology of the marine-derived fungus MFA552 (unidentified strain).



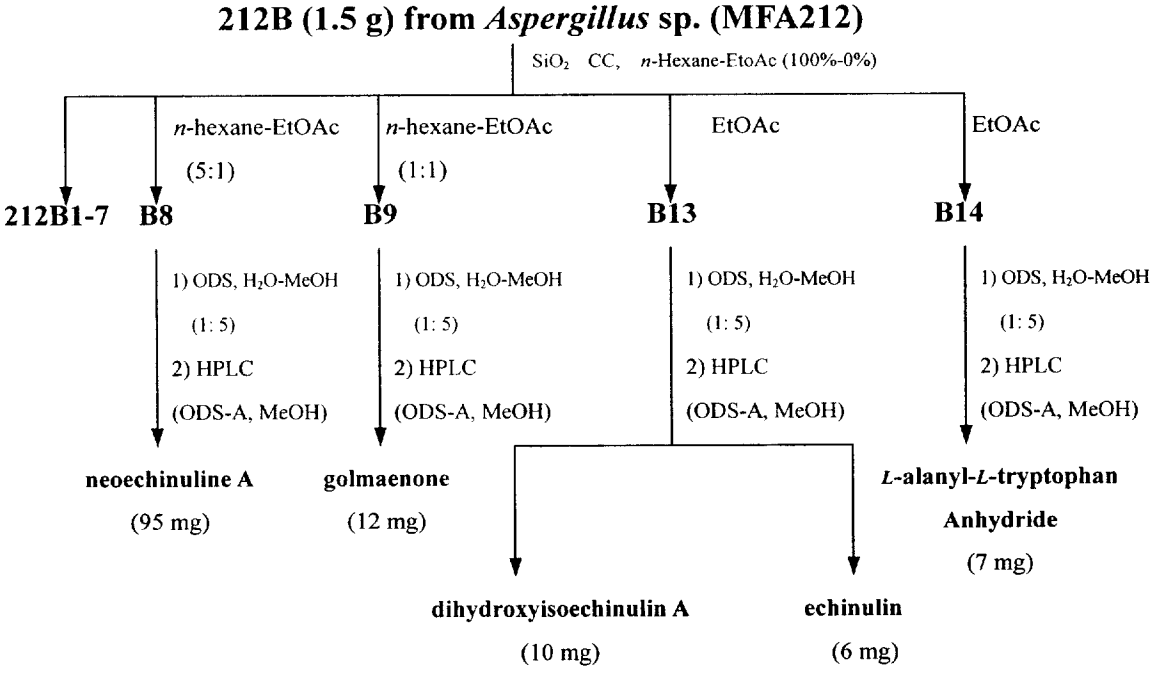
Scheme 5. Extraction of the marine-derived fungus MFA552 (unidentified strain).

3. Isolation, purification, and spectroscopic data of the secondary metabolites from the marine-derived fungi

1) *Aspergillus* sp. (MFA212)

(1) Isolation and purification of 212B

The resulting broth and mycelium were separated and the broth was extracted with ethyl acetate to provide the broth extract (1.5 g), which was fractionated by silica gel flash chromatography (*n*-hexane/EtOAc) to generate five fractions containing diketopiperazines. The further purification of the fractions by ODS column chromatography (H_2O in MeOH), followed by HPLC (YMC ODS-A, MeOH), yielded two new compounds golmaenone (12 mg) and dihydroxyisoechinulin A (10 mg), together with three known compounds, neoechinulin A (95 mg), *L*-alanyl-*L*-tryptophan anhydride (7 mg), and echinulin (6 mg).



Scheme 6. Isolation of the bioactive diketopaperazine derivatives from *Aspergillus* sp. (MFA212).

Golmaenone was obtained as a yellow solid which showed: mp 160-161℃ (from $CHCl_3$); $[\alpha]_D^{20}$ = + 7.1° (*c* = 0.4, $CHCl_3$); IR (KBr) ν_{max} 3433, 3242, 1697, 1629, 1578, 1510, 1446, 1378, 1310, 1218, 1164, 1021, 916, 752 cm^{-1}; UV λ_{max} ($CHCl_3$) (logε) 222 (1.8), 247 (2.0), 327 (1.9), 368 (1.7) nm; CD λ_{max} ($CHCl_3$) ($\Delta\varepsilon$): 230 (+0.5), 239 (-0.2), 253 (-0.4), 305 (+0.1), 346 (-0.1); ^1H NMR (400 MHz $CDCl_3$) δ 7.96 (1H, dd, *J* = 8.5, 1.5 Hz, H-3), 7.14 (1H, ddd, *J* = 8.2, 8.0, 1.0 Hz, H-4), 7.57 (1H, ddd, *J* = 8.6, 8.2, 1.5 Hz, H-5), 8.74 (1H, dd, *J* = 8.6, 1.0 Hz, H-6), 7.22 (1H, s, H-8), 11.44 (1H, s, H-11), 4.40 (1H, qd, *J* = 7.0, 1.8 Hz, H-12), 11.57 (1H, s, H-14), 8.01 (1H, brs, H-15), 6.12 (1H, dd, *J* = 17.5, 10.5 Hz, H-18), 5.37 (1H, d, *J* = 17.5 Hz, Ha-19), 5.31 (1H, d, *J* = 10.5 Hz, H_b-19), 1.43 (1H, s, H_3-20/21), 1.66 (3H, d, *J* = 7.0 Hz, H_3-22); ^{13}C NMR (100 MHz $CDCl_3$) δ 141.4 (s, C-1), 123.6 (s, C-2), 130.5 (d, C-3), 122.6 (d, C-4), 135.4 (d, C-5), 121.3 (d, C-6), 195.1 (s, C-7), 102.3 (d, C-8), 140.0 (s, C-9), 157.3 (s, C-10), 51.8 (d, C-12), 166.4 (s, C-13), 175.9 (s, C-16), 46.8 (s, C-17), 142.4 (d, C-18), 114.9 (t, C-19), 24.8 (q, C-20/21), 21.1 (q, C-22); LRFABMS *m/z* 378 $[M+Na]^+$, 356 $[M+H]^+$; HRFABMS *m/z* 378.1428 (calcd for $C_{19}H_{21}N_3O_4Na$, 378.1430).

Acid hydrolysis of golmaenone

Sample (0.5 mg) of golmaenone was subjected to acid hydrolysis with 6 N HCl (1 mL) at 110°C for 12h. The hydrolyzates were dried, resuspended in H_2O (100 μL), and derivatized with L-FDAA. The L-FDAA derivatives, from the hydrolyzates, were compared with similarly derivatized standard amino acids (L-alanine and D-alanine) by HPLC [YMC ODS-A (10 x 250 mm), flow rate 1 mL/min, UV detection at 340 nm] using an isocratic elution of MeCN-0.1% (v/v) aqueous TFA (1:1). The retention times of L-FDAA derivatives of standard amino acids, L-alanine and D-alanine, were 9.6 and 10.6 min, respectively, and the retention times of L-FDAA derivatives of hydrolyzates were 9.6 min, respectively.

19, 20- Dihydroxyisoechinulin A was isolated as a yellowish oil which showed: $[\alpha]_D^{20}$ = -47° (*c* = 0.4, $CHCl_3$); IR (neat) ν_{max} 3358, 3262, 3085, 1673, 1629, 1425, 1381, 1323, 1242, 1160, 1024, 1000, 902, 756 cm^{-1}; UV (MeOH) λ_{max} (logε) 209 (3.9), 226 (3.9), 289 (3.4), 340 (3.5) nm; CD λ_{max} (MeOH) ($\Delta\varepsilon$) 212 (-6.6) ,239 (+3.2), 266 (+1.7), 284 (+1.6), 341 (-1.1); ^1H NMR (400 MHz DMSO-d_6) δ 10.91 (1H, s, H-1), 7.02 (1H, brs, H-4), 6.98 (1H, dd, *J* = 8.2, 1.3 Hz, H-6), 7.29 (1H, d, *J* = 8.2 Hz, H-7), 6.87 (1H, s, H-8), 8.36 (1H, d, *J* = 1.9 Hz, H-11), 4.10 (1H, qd, *J* = 6.5, 1.9 Hz, H-12), 8.51 (1H, s, H-14), 6.06 (1H, dd, *J* = 17.0, 10.5 Hz, H-16), 5.01 (1H, d, *J* = 17.0 Hz, Ha-17), 5.03 (1H, d, *J* = 10.5

Hz,H_b-17), 2.36 (1H, dd, J = 13.5, 10.0 Hz, H-18), 2.93 (1H, d, J = 13.5 Hz, H_b-18), 3.27 (1H, m, H-19), 1.09 (3H, s, H_3-21), 1.06 (3H, s, H_3-22), 1.46 (3H, s, H_3-23), 1.45 (3H, s, H_3-24), 1.39 (3H, d, J = 6.5 Hz, H_3-25), 4.11 (1H, s, 19-OH), 4.16 (1H, s, 20-OH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 144.0 (s, C-2), 103.1 (s, C-3), 126.2 (s, C-3a), 119.2 (d, C-4), 132.3 (s, C-5), 123.0 (d, C-6), 111.1 (d, C-7), 133.9 (s, C-7a), 110.8 (d, C-8), 124.8 (s, C-9), 160.0 (s, C-10), 51.0 (d, C-12), 166.6 (s, C-13), 39.2 (s, C-15), 145.4 (d, C-16), 111.6 (t, C-17), 38.0 (t, C-18), 80.0 (d, C-19), 72.0 (s, C-20), 26.5 (q, C-21), 24.7 (q, C-22), 27.6 (q, C-23), 27.7 (q, C-24), 20.3 (q, C-25); LREIMS *m/z* 425 $[M]^+$; HREIMS *m/z* 425.2320 $[M]^+$ (calcd for $C_{24}H_{31}N_3O_4$, 425.2315).

Acid hydrolysis and Marfey analysis [37]of 19, 20-dihydroxyisoechinulin A

Samples (0.5 mg) of dihydroxyisoechunulin A were subjected to acid hydrolysis with 6 N HCl (1 mL) at 110 °C for 12 h. The hydrolyates were dried, resuspended in H_2O (100 μL), and derivatized with 1-fluoro-2, 4-dinitrophenyl-5-*L*-alaninamide. The derivatives were compared with similarly derivatized *L*- and *D*- alanine by HPLC [HiQ sil C18 W (4.6 x 250 mm), 5 μm, flow rate 1 mL/min, UV detection at 340 nm], using a linear gradient of MeCN in 0.1% (v/v) aqueous TFA (30~70% MeCN over 50 min). The retention times of the derivatives of *L*- and *D*-alanine were 14.4 and 17.1 min, respectively, and the retention time of the derivative from both hydrolyzates was 14.4 min.

Absolute stereochemistry at C-19 of 19, 20-dihydroxyisoechinulin A

±-α-Phenylbutyric anhydride (15 mg) was added to a solution of dihydroxyisoechinulin A (3.5 mg) and dimethylaminopyridine (1.0 mg) in pyridine (0.5 mL), and the mixture was stirred under N_2 atmosphere for 48 h at r.t. The reaction mixture was partitioned into an EtOAc-sat. aq. $NaHCO_3$ mixture. The organic phase was dried under vacuum and the residue was chromatographed on silica gel (EtOAc), followed by HPLC (Applo-C-18, MeOH-H_2O = 5:1) to afford the ester (1.0 mg). The aq. $NaHCO_3$ phase was acidified with aq. 2 $_N$ HCl and extracted with EtOAc. Work-up of the EtOAc extract in usual manner afforded the recovered acid, which was purified by HPLC (ODS-A, MeOH-H_2O=10:1) to furnish α-phenylbutric acid (5 mg) of $[\alpha]_D^{20}$ = +4.9° (c = 0.6, benzene). The following data were recorded for ester: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (1H, br.s, H-1), 7.00 (1H, br.s, H-4), 6.82 (1H, dd, J = 8.3, 1.5 Hz, H-6), 7.03 (1H, d, J = 8.3 Hz, H-7), 7.21 (1H, s, H-8), 5.88 (1H, br.s, H-11), 4.32 (1H, q, J = 7.0 Hz, H-12), 7.37 (1H, br.s, H-14), 6.08 (1H, dd, J = 17.5, 10.5 Hz, H-16), 5.21, 5.25 (each 1H, d, J = 17.5, 10.5 Hz, respectively, H_2-17), 2.78 (1H, dd, J = 14.5, 9.0 Hz, Ha-18), 3.06 (1H, dd, J =

14.5, 4.5 Hz, H_b-18), 5.09 (1H, dd, J = 9.0, 4.5 Hz, H-19), 1.19 (3H, s, H_3-21), 1.17 (3H, s, H_3-22), 1.54 (6H, s, H_3-23/24), 1.60 (3H, d, J = 7.0 Hz, H_3-25), 7.09 (2H, m, ph-α), 7.15 (3H, m, ph-α), 3.39 (1H, t, J = 7.6 Hz, H- α), 1.69 (2H, m, H_2-β), 0.78 (3H, t, J = 7.5 Hz, H_3-γ); LREIMS *m/z* 571 $[M]^+$ (rel. int. 24), 407 [M-(2-phenylbutyric acid)]$^+$ (22), 338 (19), 336 (18), 320 (8), 194 (12), 164 [2-phenylbutyric acid]$^+$ (22), 119 (35), 91 (100); HREIMS m/z 571.3092 $[M]^+$ (calcd for $C_{34}H_{41}N_3O_5$, 571.3046).

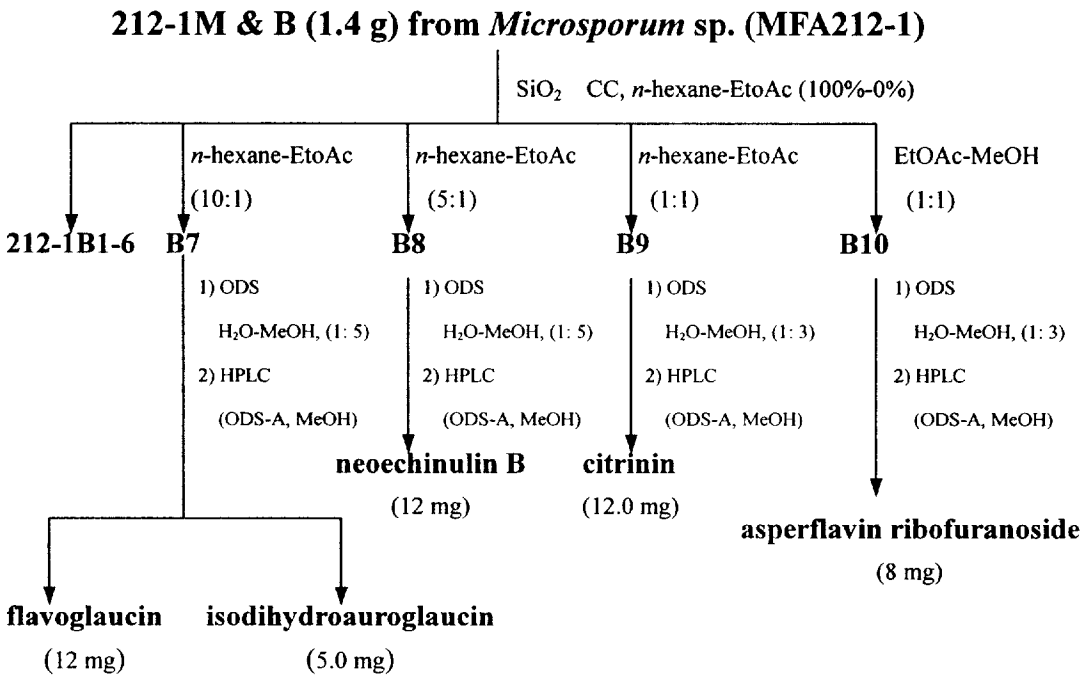
Neoechinulin A was isolated as a colorless solid; $[\alpha]_D^{20}$ = –13.3° (*c* = 0.3, AcOH); IR (KBr) ν_{max} 3449, 3351, 3190, 2971, 1687, 1634, 1442, 1424, 1309, 929, 739 cm^{-1}; UV (MeOH) λ_{max} (log ε) 209 (1.85), 230 (1.95), 285 (1.50), 291 (1.51), 325 (1.50) nm; CD (MeOH) (Δ ε) 230 (+0.5), 239 (-0.1), 253 (-0.4), 305 (+0.1), 346 (-0.1); ^{1}H NMR (400 MHz $CDCl_3$) δ 8.32 (1H, s, H-1), 7.27 (1H, d, J = 7.8 Hz, H-4), 7.18 (1H, dd, J = 7.8, 7.5 Hz, H-5), 7.16 (1H, dd, J = 7.5, 7.3 Hz, H-6), 7.36 (1H, d, J = 7.3 Hz, H-7), 7.21 (1H, s, H-8), 7.45 (1H, brs, H-11), 4.30 (1H, qd, J = 7.0, 1.7 Hz, H-12), 6.40 (1H, s, H-14), 6.07 (1H, dd, J = 17.5, 10.5 Hz, H-16), 5.23 (1H, d, J = 10.5 Hz, Ha-17), 5.19 (1H, d, J = 17.5 Hz, H_b-17), 1.53 (6H, s, H_3-18/19), 1.60 (3H, d, J = 7.0 Hz, H_3-20); ^{13}C NMR (100 MHz $CDCl_3$) δ 143.8 (C-2), 102.9 (C-3), 126.0 (C-3a), 118.9 (C-4), 121.0 (C-5), 122.3 (C-6), 111.2 (C-7), 134.3 (C-7a), 111.9 (C-8), 124.5 (C-9), 159.8 (C-10), 51.7 (C-12), 165.7 (C-13), 39.2 (C-15), 144.3 (C-16), 113.3 (C-17), 27.3 (C-18), 27.4 (C-19), 20.9 (C-20); LREIMS *m/z* 323 $[M]^+$.

L-Alanyl-L-tryptophan Anhydride: was isolated as a colorless needle, which showed: mp. 290~292°*C* (decomp), $[\alpha]_D^{20}$ = +33° (*c* = 0.06, AcOH), IR (KBr) ν_{max} 3406, 1673, 1668, 1456, 1323, 1285, 1098, 970, 922, 743 cm^{-1}; UV λ_{max} (MeOH) (logε), 228 (3.68), 273 (3.63), 279 (3.63), 289 (3.56) nm; CD λ_{max} (MeOH) nm (Δε): 213 (-22.4), 233 (+1.96), 251 (+0.69), 267 (+1.36), 332 (-0.77), 408 (+0.17); ^{1}H NMR (400 MHz DMSO-d_6) δ 10.9 (1H, s, H-1), 7.04 (1H, s, H-2), 7.56 (1H, d, J = 7.8 Hz, H-4), 6.93 (1H, dd, J = 7.8, 7.0 Hz, H-5), 7.02 (1H, dd, J = 8.0, 7.0 Hz, H-6), 7.30 (1H, d, J = 8.0 Hz, H-7), 3.02 (1H, dd, J = 14.4, 4.0 Hz, Ha-8), 3.24 (1H, dd, J = 14.4, 4.0 Hz, H_b-8), 4.10 (1H, m, H-9), 7.90 (1H, s, H-11), 3.57 (1H, qd, J = 7.0, 2.9 Hz, H-12), 8.02 (1H, s, H-14), 0.40 (3H, d, J = 7.0 Hz, H_3-15); ^{13}C NMR (100 MHz DMSO-d_6) δ 124.5 (d, C-2), 135.7 (s, C-3), 108.4 (s, C-3a), 120.7 (d, C-4), 118.3 (d, C-5), 118.9 (d, C-6), 111.0 (d, C-7), 127.7 (s, C-7a), 28.8 (t, C-8), 55.3 (d, C-9), 166.7 (s, C-10), 49.7 (d, C-12), 167.7 (s, C-13), 19.5 (d, C-15); LREIMS *m/z* 257 (26) $[M]^+$, 130 (100), 115 (6), 103 (24), 89 (3), 77 (37), 51 (6); HREIMS *m/z* 257.1195 (calcd for $C_{14}H_{15}N_3O_2$, 257.1164, Δ mmμ = +3.1).

Echinulin was isolated as a white needle, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (1H, s, H-1), 5.98 (1H, s, H-4), 5.66 (1H, s, H-6), 4.09 (1H, d, J = 6.8 Hz, H_a-8), 4.41(1H, d, J = 11.1 Hz, H_b-8), 5.41 (2H, dd, J = 7.2, 7.4 Hz, H-9), 6.80 (1H, s, H-10), 5.35 (q, J = 7.0 Hz, H-12), 1.54. (3H, d, J = 7.0 Hz, 12-CH_3), 7.13 (1H, s, H-13), 6.10 (1H, dd, J = 10.7, 6.6 Hz, H-16), 5.14 (1H, s, H_a-17), 5.16 (1H, d, J = 6.6 Hz, H_b-17), 1.50 (3H, s, H_3-18), 1.50 (3H, s, H_3-19), 3.38 (2H, d, J = 7.2 Hz, H_2-20), 3.18 (1H, dd, J = 13.2, 3.1 Hz, H-21), 1.80 (3H, s, H_3-23), 1.86 (3H,s, H_3-24); 3.53 (2H, d, J = 7.2 Hz, H_2-25), 3.65 (1H, dd, J = 14.8, 3.7 Hz, H-26), 1.74 (6H, s, H_3-28/29); ^{13}C NMR (100 MHz, $CDCl_3$) δ 141.3 (s, C-2), 104.0 (s, C-3), 124.4 (s, C-3a), 123.4 (d, C-4), 122.8 (s, C-5), 115.0 (d, C-6), 132.9 (s, C-7), 133.8 (s, C-7a), 54.5 (d, C-8), 34.5 (d, C-9), 167.6 (s, C-11), 50.8 (d, C-12), 19.8 (q, 12-CH_3), 168.3 (s, C-14), 38.9 (s, C-15), 145.7 (d, C-16), 112.3 (t, C-17), 27.8 (q, C-18), 27.9 (q, C-19), 25.8 (t, 20), 31.3 (d, C-21), 128.9 (s, C-22), 131.5 (q, C-23), 17.9 (q, C-24), 17.8 (q, C-25), 29.3 (d, C- 26), 122.8 (s, C-27),.132.2 (q, C- 28), 25.7 (q, C-29). LREIMS *m/z* 461 (8) $[M]^+$, 335 (27), 334 (100), 319 (2), 279 (2), 266 (1), 264 (6), 234 (6), 208 (6), 194 (11), 180 (4), 99 (29), 85 (2), 69 (14).

2) *Microsporum* sp. (MFA212-1)

The culture broth and mycelium were separated, and the resulted broth and mycelium were extracted with ethyl acetate and CH_2Cl_2-MeOH (1:1) to provide the broth extract (800 mg) and the mycelium extract (600 mg), respectively. Both extracts were combined, and the total extract (1.4 g) was fractionated by silica gel flash chromatography (*n*-hexane/EtOAc) to generate four fractions containing the active compounds, respectively. Final purification of each fraction by ODS column chromatography (H_2O in MeOH), followed by HPLC (YMC ODS-A, MeOH), yielded the asperflavin ribofuranoside (7.0 mg), flavoglaucin (19.0 mg), isodihydroauroglaucin (5.0 mg), neoechinulin B (12 mg), and citrinin (12.0 mg), respectively (Scheme 7).



Scheme 7. Isolation of flavoglaucin, isodihydroauroglaucin, neoechinilin B, asperflavin ribofuranoside, and citrinin from the marine-derived fungus *Microsporum* sp. (MFA212-1).

Asperflavin ribofuranoside was isolated as a yellow oil; $[\alpha]_D^{20}$ = +23.8^O (*c* = 0.2, MeOH); IR (neat) ν_{max} 3429, 2921, 2853, 2360, 2341, 1632, 1605, 1432, 1383, 1330, 1289, 1245, 1201, 1167, 1113, 1045, 1017, 827, 667 cm^{-1}; UV (MeOH) λ_{max} (logε) 225 (0.91), 269 (1.22), 312 (0.44), 388 (0.62) nm; ^{1}H NMR (400 MHz, DMSO-d_6) δ2.64 (1H, d, *J* = 17.0 Hz, H-2), 2.86 (1H, d, *J* = 17.0 Hz, H-2), 1.27 (3H, s, 3-CH_3), 2.91 (1H, d, *J* = 16.3 Hz, H-4), 3.02 (1H, d, *J* = 16.3 Hz, H-4), 6.84 (1H, d, *J* = 2.0 Hz, H-5), 6.58 (1H, d, *J* = 2.0 Hz, H-7), 3.87 (3H, s, 8-OCH_3), 6.91 (1H, s, H-10), 4.85 (1H, s, 3-OH), 14.87 (1H, s, 9-OH), 5.75 (1H, d, *J* = 4.5 Hz, H-1'), 4.11 (1H, m, H-2'), 3.95 (1H, m, H-3'), 4.01 (1H, m, H-4'), 3.49 (2H, m, H_2-5'), 4.75 (1H, d, *J* = 8.8 Hz, 2'-OH), 4.93 (1H, d, *J* = 5.3 Hz, 3'-OH), 4.82 (1H, t, *J* = 5.6 Hz, 5'-OH); ^{13}C NMR (100 MHz, DMSO-d_6) δ203.7 (s, C-1), 51.5 (t, C-2), 69.4 (s, C-3), 28.8 (q, 3-CH_3), 42.7 (t, C-4), 138.1 (s, C-4a), 102.2 (d, C-5), 159.4 (s, C-6), 98.7 (d, C-7), 160.6 (s, C-8), 55.8 (q, 8-OCH_3), 109.6 (s, C-8a), 164.5 (s, C-9), 109.5 (s, 9a), 116.5 (d, C-10), 141.0 (s, C-10a), 99.9 (d, C-1'), 71.5 (d, C-2'), 69.2 (d, C-3'), 86.5 (d, C-4'), 61.5 (t, C-5'); LRFABMS *m/z* 421 $[M+H]^+$; HRFABMS *m/z* 421.4265 [calcd for $C_{21}H_{25}O_9$ $(M)^+$, 421.4257].

Acid hydrolysis of asperflavin ribofuranoside, and sugar analysis

A solution of asperflavin ribofuranoside (10 mg) in 9% dry HCl-MeOH (0.5 mL) was stirred at r.t. for 3h (N_2 atmosphere). The reaction mixture was neutralized with Ag_2CO_3 and filtered. The residue, obtained by removal of the solvent, was purified by Silica gel (CH_2Cl_2-MeOH=10:1), and then with HPLC (ODS-A, MeOH) to furnish an aglycone (6 mg), which was identical to asperflavin[46], and methyl ribofuranoside fraction (2 mg). The sugar fraction was dissolved in pyridine (0.5 mL) and treated with bis(trimethylsily)trifluoroacetamide (BSTFA) (0.5 mL) at r.t. for 30 min. Solvent was removed by a nitrogen stream and the residue dissolved in hexane was used for GC-MS analysis [DB-1MS column, 60 m X 0.32 mm]; N_2 as a carrier gas at 0.7 mL/min; the program rate: 80-260 °*C* at 5 °*C*/min], showing peak at t_R 19.54 min, which corresponded to those of TMS/Me derivatives of ribose (*m/z* 380 $[M]^+$).

Asperflavin was isolated as a green amorphous; $[\alpha]_D^{20}$ = + 8.8 ^{O}(*c* = 0.3, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ2.85, 2.89 (2H, each, d, *J* = 17.0 Hz, H-2), 1.26 (3H, s, 3-CH_3), 2.98, 3.00 (2H, each s, H_2-4), 6.54 (1H, d, *J* = 2.1Hz, H-5), 6.42 (1H, d, *J* = 2.1 Hz, H-7), 3.84 (3H, s, 8-OCH_3), 6.79 (1H, s, H-10), 4.82 (1H, s, 3-OH), 10.27 (1H, s, 6-OH), 14.97 (1H, s, 9-OH). LREIMS *m/z* 288 $[M]^+$(20), 284 (2.9), 270 (31), 255 (5), 241 (8), 230 (9), 212 (12).

Determination of the stereochemistry of the ribofuranose

A solution of asperflavin ribofuranoside (14.7 mg) in 9% aq. HCl (10 mL) was stirred at 80 °*C* for 1h. The reaction mixture was treated as described above, and the residue thus was partitioned into H_2O and CH_2Cl_2 to give the organic phase and aqueous phase. Acetylation of the aqueous portion (10 mg), obtained after removal of the solvent under reduced pressure, in the usual manner (acetic anhydride-pyridine) furnished the ribose tetraacetate (11.6 mg). The optical rotation recorded for ribopyranose tetraacetate isolated in this study was $[\alpha]_D^{20}$ = –55.2° (*c* = 0.5, MeOH), which showed of *D*-ribose in asperflavin ribofuranoside (lit. -55.4° in *D*-ribose; +55.2° in *L*-ribose) [58].

Ribopyranosyl tetraacetate $[\alpha]_D^{20}$ = –55.2° (*c* = 0.5, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ6.01 (1H, d, *J* = 4.8 Hz, H-1), 5.02 (1H, dd, *J* = 4.8, 3.7 Hz, H-2), 5.47 (1H, dd, *J* = 3.7, 3.2 Hz, H-3), 5.14 (1H, ddd, *J* = 4.3, 3.2, 3.2 Hz, H-4), 3.90 (1H, dd, *J* = 12.3, 4.3 Hz, H_a-5), 4.01 (1H, dd, *J* = 12.3, 3.2 Hz, H_b-5), 2.12, 2.09, 2.08, and 2.07 (each 3H, s, Ac); ^{13}C NMR (100 MHz, $CDCl_3$) δ90.8 (d, C-1), 67.2 (d, C-2), 66.1 (d, C-3), 66.2 (d, C-4), 62.6 (t, C-5), 169.8, 169.7, 169.4, and 168.7 (each s, Ac), 20.6, 20.6, 20.7, and 20.8 (each q, Ac).

Flavoglaucin was isolated as a yellow needle (from CH_2Cl_2);; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.89 (1H, s, H-5), 10.23 (1H, s, H-7), 2.87 (2H, br.t, *J* = 7.0 Hz, H-8), 2.32 (dt, *J* = 7.0, 5.6 Hz, H-9), 1.30 (8H, brm, H_2-10, 11, 12 and 13), 0.91 (3H, t, *J* = 7.0 Hz, H-14), 3.32 (2H, br.d, *J* = 7.0 Hz, H-15), 5.28 (1H, br.t, *J* = 7.0 Hz, H-16), 1.69 (3H, s, H_3-18), 1.75 (3H, s, H_3-19), 11.92 (1H, s, 1-OH), 4.44 (1H, br.s, 4-OH). LREIMS *m/z* 304 $[M]^+$.

Isodihydroauroglaucin was isolated as a yellow needle (from CH_2Cl_2); ^{1}H NMR (400 MH_Z, $CDCl_3$) δ 6.90 (1H, s, H-5), 10.02 (1H, s, H-7), 2.98 (2H, t, *J* = 7.7 Hz, H_2-8), 2.33 (2H, dt, *J* = 7.7, 6.9 Hz, H_2-9), 5.60 (2H, dt, *J* = 12.6, 6.9 Hz, H-10/11), 6.02 (2H, dd, *J* = 12.6, 10.2 Hz, H-12/13), 1.69 (3H, s, H_3-14), 3.29 (2H, d, *J* = 7.4 Hz, H_2-15), 5.28 (1H, dt, *J* = 7.4, 1.0 Hz, H-16), 1.73 (3H, d, *J* = 7.9 Hz, H_3-18), 1.75 (3H, d, *J* = 7.9 Hz, H_3-19), 11.93 (1H, s, 1-OH), 4.52 (1H, br.m, 4-OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.8 (s, C-1), 127.3 (s, C-2), 128.9 (s, C-3), 145.0 (s, C-4), 121.0 (d, C-5), 117.2 (s, C-6), 195.3 (d, C-7), 24.0 (t, C-8), 34.2 (t, C-9), 131.9 (d, C-10), 129.3 (d, C-11), 131.0 (d, C-12), 128.3 (d, C-13), 17.7 (q, C-14), 27.0 (t, C-15), 125.8 (d, C-16), 133.8 (s, C-17), 25.8 (q, C-18),

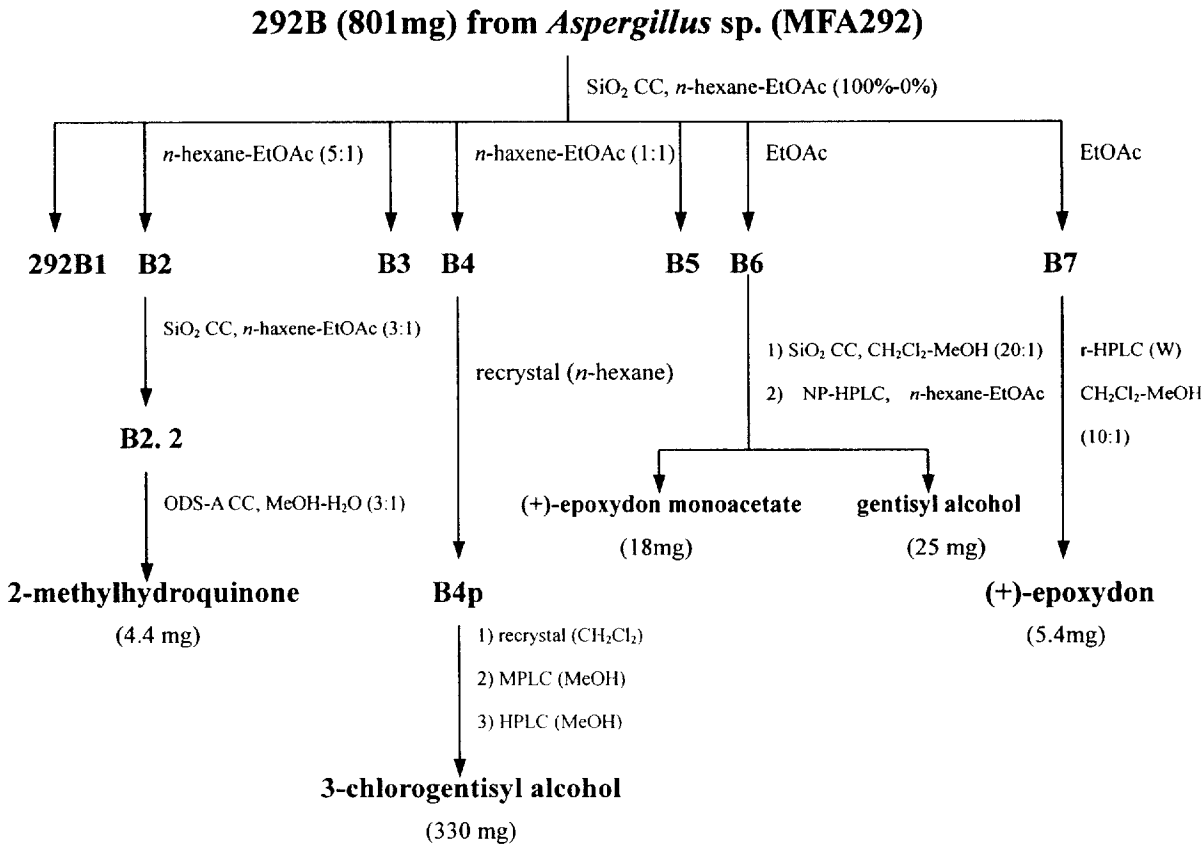
18.0 (q, C-19); LREIMS *m/z* 300 $[M]^+$ (16), 267 (7), 239 (6), 219 (15), 211 (11), 189 (5), 163 (100), 161 (6), 121 (8), 95 (13), 81 (38), 77 (28), 53 (27).

Citrinin was isolated as a yellow needle (from MeOH); ^1H NMR (400 MHz, $CDCl_3$) δ 8.23 (1H,s, H-1), 4.78 (1H, d, J = 6.7 Hz, H-4), 2.98 (1H, d, J = 7.2 Hz, H-4), 15.11 (1H, s, 8-OH), 1.22 (3H, d, J = 7.2 Hz, H_3-11), 1.34 (3H, d, J = 6.7 Hz, H_3-12), 2.02 (3H, s, H_3-13), 15.86 (1H, s, 14- OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.7 (C-1), 81.6 (C-3), 34.6 (C-4), 123.1 (C-5), 183.8 (C-6), 100.3 (C-7), 174.5 (C-8), 139.0 (C-9), 107.4 (C-10), 18.2 (C-11), 9.4 (C-12), 18.5 (C-13), 177.2 (C-14). LREIMS *m/z* 250 $[M]^+$ (51), 232 (22), 217 (54), 206 (100), 191 (41), 177 (18), 163 (27), 135 (14), 105 (17), 91 (62), 77 (72), 65 (45), 51 (32).

Neoechinulin B was isolated as yellow crystals (from MeOH); ^1H NMR (400 MHz, $CDCl_3$) δ 7.66 (1H, s, H-1), 7.32 (1H, d, J = 7.6 Hz, H-4), 7.22 (1H, dd, J = 7.6, 7.3 Hz, H-5), 7.18 (1H, dd, J = 7.3, 6.9 Hz, H-6), 7.37 (1H, d, J = 6.9 Hz, H-7), 7.28 (1H, s, H-8), 8.35 (1H, s, H-11), 8.63 (1H, s, H-14), 6.07 (1H, dd, J = 17.2, 10.6 Hz, H-16), 5.23 (1H, dd, J = 17.2, 1.0 Hz, H_a-17), 5.26 (1H, dd, J = 10.6, 1.0 Hz, H_b-17), 1.54 (6H, s, H_3-18/19), 4.95 (1H, d, J = 1.0 Hz, Ha-20), 5.61 (1H, d, J = 1.0 Hz, H_b-20); ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.1 (C-2), 102.9 (C-3), 125.8 (C-3a), 118.9 (C-4), 121.3 (C-5), 122.5 (C-6), 111.3 (C-7), 134.2 (C-7a), 113.1 (C-8), 124.4 (C-9), 155.8 (C-10), 60.4 (C-12), 157.5 (C-13), 39.2 (C-15), 144.2 (C-16), 113.5 (C-17), 27.3 (C-18), 27.4 (C-19), 102.1 (C-20); LREIMS *m/z* 321 $[M]^+$ (100), 306 (8), 278 (18), 252 (91), 196 (23), 182 (79), 168 (25), 167 (46), 97 (23), 69 (8).

3) *Aspergillus* sp. (MFA292)

The culture broth and mycelium were separated, and the resulted broth and mycelium were extracted with ethyl acetate and CH_2Cl_2-MeOH (1:1) to provide the broth extract (801 mg) and the mycelium extract (2.5 g), respectively. The broth extract (801 mg) was fractionated by silica gel flash chromatography (*n*-hexane/EtOAc) to generate seven fractions containing the active compounds, respectively. Final purification of each fraction by ODS column chromatography (H_2O in MeOH), followed by HPLC (YMC ODS-A, MeOH), yielded the gentisyl alcohol, 3-clorogentisyl alcohol, 2-methylhydroquinone, (+)-epoxydon, (+)-epoxydon monoacetate and (Scheme 8, 9).



Scheme 8. Isolation of gentisyl, 3-chlorogentisyl alcohol, 2-methylhydroquinone, (+)-epoxdon, and (+)-epoxdon monoacetate from the marine-derived fungus *Aspergillus* sp. (MFA292).

Gentisyl alcohol was isolated as a brown solid. mp 82-85 °C; IR (KBr) ν_{max} 3415, 1630, 1454, 1251, 1203, 1163, 1109, 1006, 877 cm^{-1}; UV (MeOH) λ_{max} (logε) 210 (3.60), 225 (3.60), 295 (3.50) nm; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.53 (1H, s, 2-OH), 6.54 (1H, d, J = 8.5 Hz, H-3), 6.42 (1H, dd, J = 8.5, 3.0 Hz, H-4), 8.54 (1H, s, 5-OH), 6.73 (1H, d, J = 3.0 Hz, H-6), 4.40 (2H, d, J = 5.6 Hz, H_2-7), 4.89 (1H, t, J = 5.6 Hz, 7-OH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 129.3 (s, C-1), 146.4 (s, C-2), 115.1 (d, C-3), 113.2 (d, C-4), 149.7 (s, C-5), 114.0 (d, C-6), 58.3 (t, C-7); LREIMS *m/z* 140 $[M]^+$ (52), 122 $[M-H_2O]^+$ (100), 94 (62), 65 (42), 44 (46).

3-Chlorogentisyl alcohol was isolated as a red solid; mp 127-129 °C (MeOH); IR (KBr) ν_{max} 3435, 1627, 1599, 1478, 1451, 1306, 1167, 1112, 1026 cm^{-1}; UV (MeOH) λ_{max} (logε) 220 (3.70), 297 (3.60) nm; ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.66 (1H, d, J = 5.6 Hz, H-4), 6.72 (1H, d, J = 5.6 Hz, H-6), 4.60 (2H, s, H_2-7), 9.10 (1H, s, 2-OH), 8.40 (1H, s, 5-OH), 5.20 (1H, br.s, 7-OH); ^{13}C NMR (100 MHz, DMSO-d_6), δ 120.6 (s, C-1), 141.8 (s, C-2), 150.6 (s, C-3), 113.5 (d, C-4), 132.7 (s, C-5), 113.0 (d, C-6), 58.9 (t, C-7); LREIMS *m/z* 176 $[M]^+$ (9), 174 (26), 156 (100), 128 (53), 110 (9), 87 (3), 65 (18), 53 (24).

2-Methylhydroquinone was isolated as a colorless needle; ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.45 (1H, d, J = 2.8 Hz, H-3), 6.36 (1H, dd, J = 8.5, 2.8 Hz, H-5), 6.53 (1H, d, J = 8.5 Hz, H-6), 2.02 (1H, s, H-7), 8.44 (1H, s, 1-OH), 8.48 (1H, s, 4-OH); ^{13}C NMR (100 MHz, DMSO-d_6), δ 147.6 (s, C-1), 124.3 (s, C-2), 112.6 (d, C-3), 149.5 (s, C-4), 115.0 (d, C-5), 117.1 (d, C-6), 16.1 (q, C-7).

(+)-Epoxydon was isolated as an unstable colorless oil; $[\alpha]_D^{20}$ = + 71.6° (*c* = 0.3, MeOH); IR (neat) ν_{max} 3356, 1680, 1400, 1236, 1027, 903, 867 cm^{-1}; UV (MeOH) λ_{max} (logε) 203 (3.72), 237 (3.68) nm; CD (MeOH) ($\Delta\varepsilon$) 338 (+0.95), 245 (-1.76) nm; ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.39 (1H, dddd, J =2.2, 2.2, 2.0, 2.0 Hz, H-3), 4.70 (1H, ddddd, J = 6.2, 3.1, 3.1, 2.5, 2.5 Hz, 4-OH), 5.78 (1H, d, J = 6.4 Hz, H-4), 3.40 (1H, d, J = 4.2 Hz, H-5), 3.76 (1H, ddd, J = 4.2, 2.7, 2.5 Hz, H-6), 3.96 (1H, dddd, J = 15.3, 5.5, 2.0, 1.8 Hz, H-7), 4.07 (1H, dddd, J = 15.3, 5.5, 2.2, 2.0 Hz, H-7), 5.00 (1H, t, J = 5.5 Hz, 7-OH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 193.9 (s, C-1), 133.8 (s, C-2), 141.4 (s, C-3), 63.7 (d, C-4), 52.9 (d, C-5), 54.0 (d, C-6), 57.3 (t, C-7); LREIMS *m/z* 156 $[M]^+$ (100), 138 $[M - H_2O]^+$ (7), 122 $[M - H_2O - O]^+$ (2), 110 (3), 86 (3).

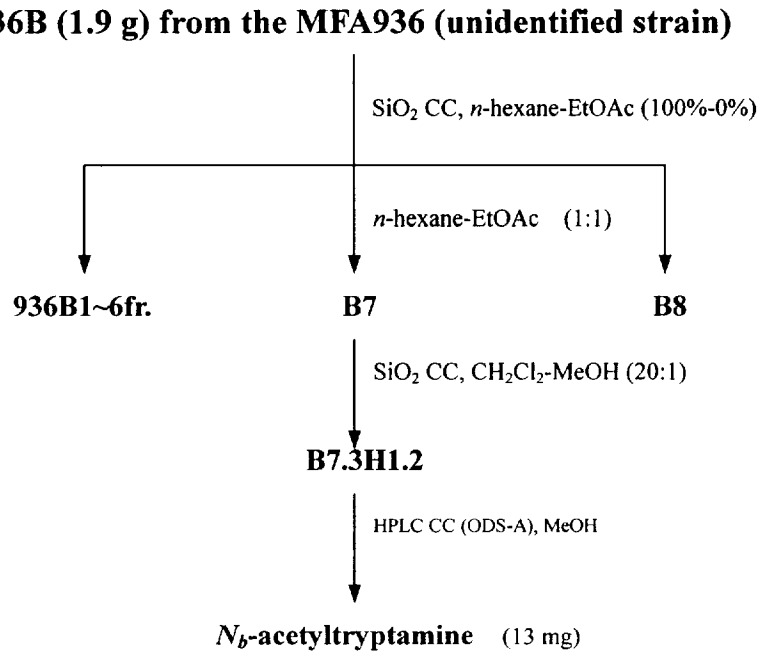
(+)-Epoxydon monoacetate was isolated as an unstable colorless oil; $[\alpha]_D^{20}$ = +66.0° (*c* = 0.56, MeOH), IR (neat) ν_{max} 3392, 1683, 1374, 1240, 1032, 902, 866 cm^{-1}; UV (MeOH) λ_{max} (logε) 213 (3.63), 233 (3.70) nm; ^1H NMR (400 MHz DMSO-d_6) δ 6.51 (1H, dddd, *J* = 1.3, 1.3, 1.0, 1.0 Hz, H-2), 4.72 (1H, m, 4-OH), 3.46 (1H, d, *J* = 4.2 Hz, H-5), 3.78 (1H, ddd, *J* = 2.6, 1.3, 1.3 Hz, H-6), 4.56 (1H, ddd, *J* = 12.9, 1.3, 1.0 Hz, H-7), 4.63 (1H, ddd, *J* = 13.1, 1.6, 1.3 Hz, H-7); 5.90 (1H, d, *J* = 6.7 Hz, H-4); 2.02 (3H, s, 7-OAc); ^{13}C NMR (100 MHz DMSO-d_6), δ 193.0 (s, C-1), 128.7 (s, C-2), 145.8 (d, C-3), 63.6 (d, C-4), 52.7 (d, C-5), 54.1 (d, C-6), 60.0 (t, C-7), 169.8 (s, 7-OAc), 20.5 (q, 7-OAc); HREIMS *m/z* 198.0532 [calcd for $C_9H_{10}O_5$ $(M)^+$, 198.0528].

Acetylation of (+)-epoxydon monoacetate

Acetylation of (+)-epoxydon monoacetate (11.4 mg) in the usual manner (acetic anhydride-pyridine) yielded the diacetate (6.6 mg), which showed spectral data virtually to that of (+)-diacetate reported, previously.[17]

4) MFA936 (unidentified strain)

The mycelium and broth were separated by filtration. The filtered broth was extracted with EtOAc. The crude extract (1.9 g) was subjected to silica gel flash column chromatography, eluting with *n*-hexane/EtOAc (from 100% to 0%) to obtained 8 fractions. Further purification of fraction 7 (400 mg) by silica gel column chromatography using CH_2Cl_2/MeOH (20:1), followed by HPLC (YMC ODS-A, 10 x 250 mm) (MeOH), yielded an N_b-acetyltryptamine (13 mg) and oxaciline (6.0 mg) (Scheme.9).



Scheme 9. Isolation of N_b-acetyltryptamine from the marine-derived fungus MFA936 (unidentified strain).

N_b-Acetyltryptamine was iosolated as a yellow oil; IR (neat) ν_{max} 3400 (NH), 1638 (amide), 1384, 744 cm^{-1}; UV (MeOH) λ_{max} (logε) 222 (4.0), 283 (2.3) nm; ^1H NMR (400 MHz $CDCl_3$) δ 8.14 (1H, br.s, H-1), 7.04 (1H, d, J = 2.2 Hz, H-2), 7.60 (1H, d, J = 8.0 Hz, H-4), 7.13 (1H, dd, J = 8.0, 8.0 Hz, H-5), 7.21 (1H, dd, J = 8.0, 8.0 Hz, H-6), 7.38 (1H, d, J = 8.0 Hz, H-7), 2.98 (2H, t, J = 6.5 Hz, H-10), 3.60 (2H, dt, J = 6.5, 6.0 Hz, H-11), 5.53 (1H, br.s, H-12), 1.92 (3H, s, H-14); ^{13}C NMR (100 MHz, $CDCl_3$) δ 122.0 (d, C-2), 113.1 (s, C-3), 118.7 (d, C-4), 119.5 (d, C-5), 122.2 (d, C-6), 111.2 (d, C-7), 136.4 (s, C-8), 127.3 (s, C-9), 25.3 (t, C-10), 39.8 (t, C-11), 170.0 (s, C-13), 23.4 (q, C-14); LREIMS *m/z* 202 $[M]^+$ (rel. int., 7), 154 (47), 143 (62), 130 (58), 86 (18), 70 (100); HREIMS *m/z* 202.1060 (calcd for $C_{12}H_{14}N_2O$, 202.1106).

5) MFA552 (unidentified strain)

The mycelium and broth were separated by filtration. The filtered broth was extracted with EtOAc. The broth extract (126 mg) was subjected to silica gel flash column chromatography, eluting with *n*-hexane/EtOAc (from 100% to 0%), to obtain the fraction 6 containing (+)-brefeldin A. Further purification of fraction 6 (35 mg) by r-HPLC (ODS-A, MeOH), followed by HPLC (YMC ODS-A, 10 x 250 mm) (MeOH) yielded (+)-brefeldin A (15 mg).

(+)-brefeldin A was isolated as a white needle (EtOAc); ^{1}H NMR (400 MHz DMSO-d_6) δ 5.72 (1H, m, H-2), 7.32 (1H, ddd, J = 12.6, 2.9, 2.1 Hz, H-3), 4.48 (1H, d, J = 3.2 Hz, H-4), 1.75 (1H, m, H-5), 1.65 (2H, m, H-6), 4.04 (1H, m, H-7), 1.80 (2H, m, H-8), 2.31 (1H, ddd, J = 8.8, 8.6, 8.3 Hz, H-9), 5.20 (1H, dt, J = 9.6, 5.3 Hz, H-10), 5.68 (1H, m, H-11), 1.96 (2H, m, H-12), 1.29 (1H, m, H-13), 0.75 (1H, m, H-13), 1.49 (1H, m, H-14), 1.60 (1H, m, H-14), 4.70 (1H, dd, J = 6.4, 4.8 Hz, H-15), 1.18 (3H, d, J = 6.4 Hz, H-16), 5.10 (1H, d, J = 5.6 Hz, 4-OH), 2.29 (1H, dd, J = 4.8, 4.3 Hz, 7-OH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 115.6 (s, C-1), 116.2 (d, C-2), 154.3 (d, C-3), 74.3 (d, C-4), 51.7 (d, C-5), 31.4 (t, C-6), 70.5 (d, C-7), 40.9 (t, C-8), 43.3 (d, C-9), 138.1 (d, C-10), 129.2 (d, C-11), 33.4 (t, C-12), 26.4 (t, C-13), 43.0 (t, C-14), 70.8 (d, C-15), 20.7 (q, C-16); LREIMS *m/z* 280 (3), 257 (0.2), 244 (7), 207 (32), 165 (13), 157 (33), 137 (21), 122 (32), 119 (83), 93 (64), 79 (93).

4. Bioactive assay

1) Free radical scavenging activity [DPPH (1,1-diphenyl-2-picrylhydrazyl)] [19-26]

Samples to be tested were dissolved in MeOH (three gradient concentrations by 10 times) and the solution (160 μL) was dispensed into a 96-well microtiter tray. 40 μL of the DPPH solution in MeOH (1.5×10^{-4} M) was added to each well. The mixture was shaken and left to stand for 30 min, and the absorbance of the resulting solution was measured at 520 nm with microplate reader (Packard Co., Spectra Count ^{TM}).

The scavenging activity on DPPH radical was expressed as IC_{50}, which is the concentration of the tested compound required to give a 50% decrease of the absorbance from that of blank solution [consisting of MeOH (160 μL) and DPPH solution (40 μL)]. *L*-Ascorbic acid (Vitamin C, 1 mg/1 mL MeOH, A-7506, Sigma) was used as positive control with the IC_{50} value of 3.5 μg/mL (20 μM). The estimation of the results was expressed by % inhibition for acetone sample and IC_{50} (μg/mL and μM) for fractions and the pure secondary metabolites, respectively.

%Inhibition = {[Control-(OD-Color control)]/Control} X 100

2) Antimicrobial activity against methicillin-resisitant *Staphylococcus aureus* (MRSA) and multidrug-resistant *S. aureus* (MDRSA)

a) **Bacteria and positive control:** Staphylococcus aureus ATCC29213 (SA), Staphylococcus aureus CCARM3167 (methicillin-resistance) (MRSA), Staphylococcus aureus CCARM3089 (multidrug-resistance) (MDRSA); Oxacillin (positive control, Sigma Co.).

b) **Culture of bacteria** : cultured on the Brain Heart Infusion (BHI) agar at 37 ^{o}C in incubator for 17 h.

c) **Preparation of sample:** to take 1 mL acetone extraction solution into vial to remove acetone under vacuum completely; take 1 mg mycelium extract (or broth extract) to make sample solution dissolved in 20% DMSO aq.; oxacillin (8 μg/mL) as the positive control.

d) **Method:** samples to be tested were dissolved in 20% DMSO (diluted into five different concentrations at 50% difference) and the solution (1 mL) was dispensed into four test tubes with 1 mL sterilized Mueller-Hinton medium, and then seed pathogens (SA, MRSA, and MDRSA) respectively, and together with positive control and blank control (sample solution and BHI medium). After culture in incubator at 37 ^{o}C for 20 h, to check if the bacteria grow or not and turbidity compared with the positive control as well as blank.

e) **Estimation: t**he antimicrobial activity against SA, MRSA, and MDRSA was expressed as MIC (μg/mL or μM). The positive control (oxacillin) shows the inhibition against SA at MIC 8 μg/mL only, and negative for both MRSA and MDRSA.

f) **Preparation of BHIA Medium:** Suspend 52 g of the powder in 1 L of purified water and mix thoroughly, heat with frequent agitation and boil for 1 min. to completely dissolve the powder.

g) **Preparation of Mueller-Hinton Medium:** Suspend 21 g of the powder in 1 L of purified water and warm gently to dissolve. Autoclave at 121 ^{o}C for 15 min.

3) Ultraviolet-A (UV-A) protecting assay [30, 31]

Samples to be tested were dissolved in MeOH (three gradient concentrations by 10 times) and the solution (200 μL) was dispensed into a 96-well microtiter tray, and the absorbance of the resulting solution was measured at 340 nm (UV-A, 320-390 nm) with microplate reader (Packard Co., Spectra Count ^{TM}).

The protecting activity on UV-A protecting assay was expressed as ED_{50}, which is the concentration of the tested compound required to give a 50% decrease of the absorbance from that of blank solution [consisting of MeOH (200 μL)]. Oxybenzone (0.1 mg/mL) was used as positive control with the ED_{50} value of 80 μg/mL (350 μM). The estimation of the results was expressed by % inhibition for acetone sample and ED_{50} (μg/mL and μM) for fractions and the pure secondary metabolites, respectively.

%Abs = [(Abs of sample)/(Abs of standard)] X 100

Part III Results and Discussions

1. Structural elucidation of the secondary metabolites

1) *Aspergillus* sp. (MFA212)

(1) Golmaenone

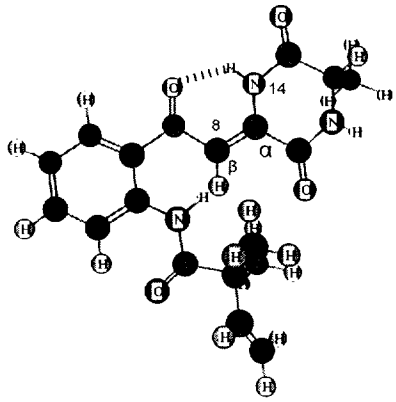
Golmaenone was isolated as a yellow solid which was thought to have a molecular composition of $C_{19}H_{21}N_3O_4$ from the HRFABMS and ^{13}C NMR data (Fig. 6, 10). Since golmaenone showed eleven unsaturations in HRFABMS, it implied that golmaenone contained four carbonyls, five double bonds, and two rings. The IR spectrum of golmaenone showed absorptions for free amide (3433, 1698 cm^{-1}) and hydrogen-bonded amide (3246, 1629cm^{-1}) functionalities (Fig.8). The UV spectrum of golmaenone showed the presence of conjugated amide [222 nm (logε 1.8), 327 (1.9), 368 (1.7)] chromophores (Fig.7).

In the ^1H NMR spectrum ((Fig.9) , three protons were exchanged by D_2O, suggesting that it has three amide protons [δ 6.61 (1H, s, H-11), 11.57 (1H, s, H-14), 11.44 (1H, s, H-15)]. Detailed analyses of the ^1H and ^{13}C NMR spectra of golmeanone (Fig.10), including the results from distortionless enhancement by polarization transfer (DEPT), ^1H-detected heteronuclear multiplequantum correlation (HMQC) experiments, revealed signals ascribable to a methyl substituted diketopiperazine [δ 6.61 (1H, br.s, H-11), 4.40 (1H, qd, J = 7.0, 1.8 Hz, H-12), 11.57 (1H, s, H-14), 1.66 (3H, d, J = 7.0 Hz, H_3-22), 140.0 (C-9), 157.3 (C-10), 51.8 (C-12), 166.4 (C-13), 21.1 (C-22)], 1,2-disubstituted benzene [δ 7.96 (1H, dd, J = 8.0, 1.5 Hz, H-3), 7.14 (1H, ddd, J = 8.2, 8.0, 1.0 Hz, H-4), 7.57 (1H, ddd, J = 8.6, 8.2, 1.5 Hz, H-5), 8.74 (1H, dd, J = 8.6,1.0 Hz, H-6); 141.4 (C-1), 123.6 (C-2), 130.5 (C-3) ,122.6 (C-4), 135.4 (C-5), 121.3 (C-6)], 2,2-dimethyl-3-butenamide [δ 11.44 (1H, s, H-15), 6.12 (1H, dd, J = 17.5, 10.5 Hz, H-18), 5.31, 5.37 (each 1H, d, J = 10.5 and 17.5 Hz, respectively, H_2-19), 1.43 (6H, s, CH_3-20/21)], and 1,3,3-trisubstituted propenone [δ 7.22 (1H, s, H-8), 195.1 (C-7), 102.3 (C-8), 140.0 (C-9)] (Table1).

The connection of the functional groups, which led to the planar structure, was achieved on the basis of HMQC and HMBC correlations. Key HMBC correlations between H-15 and C-2, C-6 and C-16; between H-3 and C-7; between H-8 and C-7 and

C-10; between H_3-20/21 and C-16 and C-18; and between H-14 and C-8, C-10 and C-12, clearly established the planar structure (Chart 2).

The geometry of C-8/C-9 double bond was determined to be (*Z*) configuration on the basis of the chemical shifts of H-8 [δ 7.22 (1H, s)] and H-14 [δ 11.57 (1H, s)], which were shifted to the low field by the deshielding effect of the carbonyl group on β-vinyl proton and by the hydrogen bonding with 7-carbonyl group, respectively (Scheme 10).

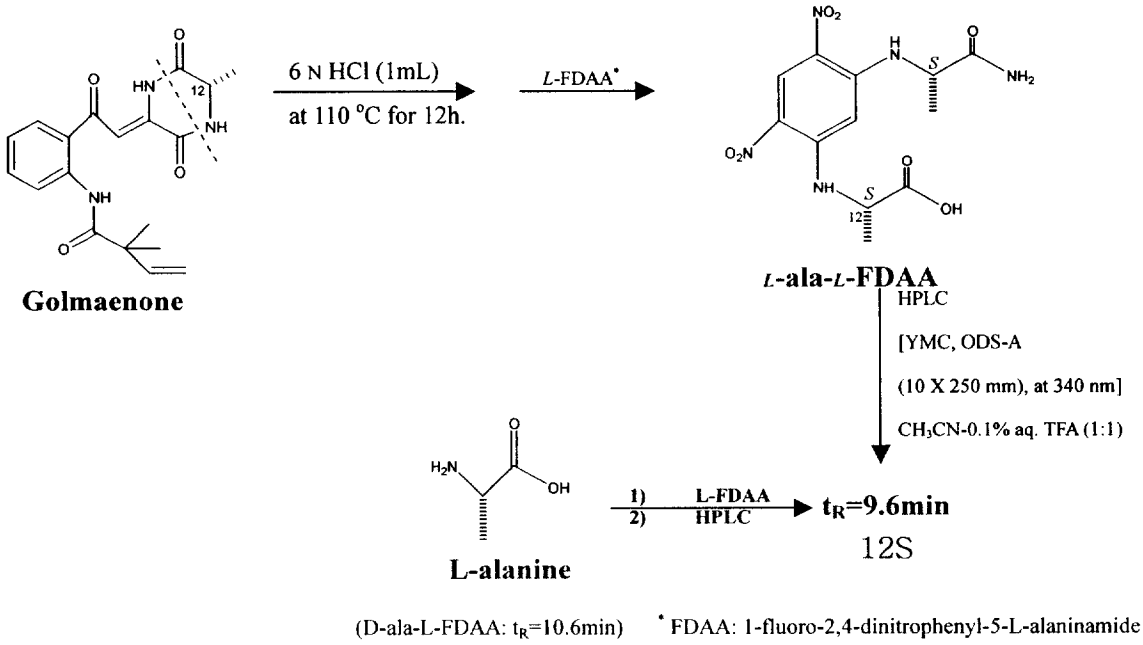


Scheme 10. 3-dimentional structure of golmaenone

The stereochemistry of the alanine residue was determined by the advanced Marfey's method. [35] For this analysis two enantiomeric alanine isomers were derivatized with 1-fluoro-2,4-dinitrophenyl-5-*L*-alaninamide (*L*-FDAA), and analyzed by reversed-phase HPLC. The retention times of the corresponding enantiomers (2*S* and 2*R*) were observed with 9.6 and 10.6 min, respectively. Analogous derivatization of the acid hydrolyzate of golmaenone followed by HPLC analysis and comparison with the standard derivatives enable us to deduce 12*S* configuration (Scheme 10).

On the basis of foregoing evidences, the absolute stereostructure of golmaenone was elucidated, as depicted in Chart 2.

Golmaenone exhibited a significant radical scavenging activity against DPPH with IC_{50} value of 20.2 μM, which is similar to positive control, ascorbic acid (IC_{50}, 20 μM), and also showed an UV-A protecting activity with ED_{50} value of 90 μM, which is more active than oxybenzone (ED_{50}, 350 μM) currently used as sunscreen.



Scheme 11. Stereochemistry at C-12 in golmeanone.

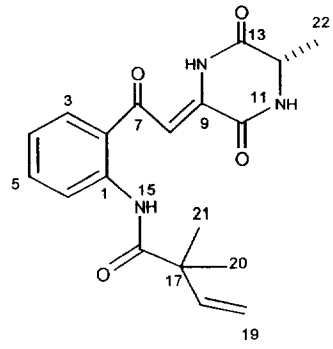


Chart 2. Chemical structure of golmaenone.

Table 1. Tabulated NMR data for golmaenone

Carbon no.	δ_H (mult, *J*)	δ_C (mult)	HMBC (H to C)
1		141.4 (s)	
2		123.6 (s)	
3	7.96 (dd, 8.5, 1.5)	130.5 (d)	1, 4, 5, 7
4	7.14 (ddd, 8.2, 8.0, 1.0)	122.6 (d)	2, 3, 5, 6
5	7.57 (ddd, 8.6, 8.2, 1.5)	135.4 (d)	1, 3, 4
6	8.74 (dd, 8.6, 1.0)	121.3 (d)	1, 2, 4
7		195.1 (s)	
8	7.22 (s)	102.3 (d)	7, 10
9		140.0 (s)	
10		157.3 (s)	
11	11.44 (s)		9, 13
12	4.40 (qd, 7.0, 1.8)	51.8 (d)	10, 13, 22
13		166.4 (s)	
14	11.57 (s)		10, 12
15	8.01 (s)		2, 6, 16
16		175.9 (s)	
17		46.8 (s)	
18	6.12 (dd, 17.5, 10.5)	142.4 (d)	16, 17, 20/21
19	5.37 (d, 17.5)	114.9 (t)	17, 18
	5.31 (d, 10.5)		
20/21	1.43 (s)	24.8 (q)	16, 17, 18
22	1.66 (d, 7.0)	21.1 (q)	12, 13

* Recorded in $CDCl_3$ at 400 MHz (^1H) and 100 MHz (^{13}C).

(2) Dihydroxyisoechinulin A

19,20-dihydroxyisoechinulin A was isolated as colorless oil with a molecular composition of $C_{24}H_{31}N_3O_4$ from the HREIMS (Fig.12) and ^{13}C NMR data (Fig.16). T he eleven unsaturated degrees by HREIMS implied that it contained two carbonyl groups, six double bonds, and three rings.

The IR spectrum of 19, 20-dihydroxyisoechinulin A showed broad absorptions for multiple hydroxyl and amine (3358, 3262 cm^{-1}) and amide (1673, 1629 cm^{-1}) functionality (Fig.14). The UV spectrum of 19, 20-dihydroxyisoechinulin showed the presence of conjugated amide [209 nm (log ε 3.9), 226 (3.9)] and conjugated indole [289 nm (3.4), 340 (3.5)] chromophores (Fig.13).

In the ^1H NMR spectrum (Fig.15), five protons were exchanged by D_2O, suggesting that it has one aromatic amine proton [δ 10.91 (H-1)], two amide protons [δ 8.36 (H-11), 8.51 (H-14)], and two hydroxyl protons [δ 4.11 (19-OH), 4.16 (20-OH)].

The ^1H and ^{13}C NMR spectra of dihydroxyisoechinulin A showed signals ascribable to methyl substituted diketopiperazine [δ 8.36 (H-11), 4.10 (H-12), 8.51 (H-14), 1.39 (H_3-25), 124.8 (C-9), 160.0 (C-10), 51.0 (C-12), 166.6 (C-13), 20.3 (C-25)], a trisubstituted indole [δ 10.91 (H-1), 7.02 (H-4), 6.98 (H-6), 7.29 (H-7), 144.0 (C-2), 103.1 (C-3), 126.2 (C-3a), 119.2 (C-4), 132.3 (C-5), 123.0 (C-6), 111.1 (C-7), 133.9 (C-7a)], an isopentenyl [δ 6.06 (H-16), 5.01, 5.03 (H_2-17), 1.45, 1.46 (CH_3-23/24), 39.2 (C-15), 145.4 (C-16), 111.6 (C-17), 27.6 (C-23), 27.7 (C-24)], a dihyfroxyisopentanyl [δ 2.36 (Ha-18), 2.93 (H_b-18), 3.27 (H-19), 1.09, 1.06 (H_3-21/22), 4.11 (19-OH), 4.16 (20-OH), 38.0 (C-18), 80.0 (C-19), 72.0 (C-20), 26.5 (C-21), 24.7 (C-22)], and a trisubstituted double bond [δ 6.87 (H-8), 110.8 (C-8), 124.8 (C-9)] (Table 2).

The connection of the functional groups in dihydroxyisoechinulin A, which led to the planar structure, was achieved on the basis of COSY, HMQC, HMBC, and NOESY correlations. Key HMBC correlations between H-4 and C-18; between H-6 and C-18; between H-8 and C-2, C-3a, and C-10; between H-16 and C-2; between H-18 and C-4, C-6 and C-19; and between H-19 and C-5, clearly established planar structure of dihydroisoechinulin A (Chart 3).

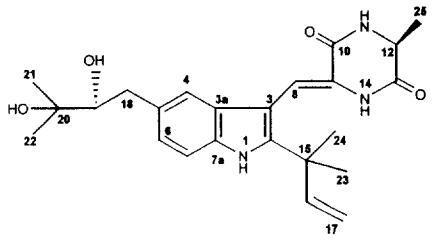


Chart 3. Chemical structure of dihydroxyisoechinulin A.

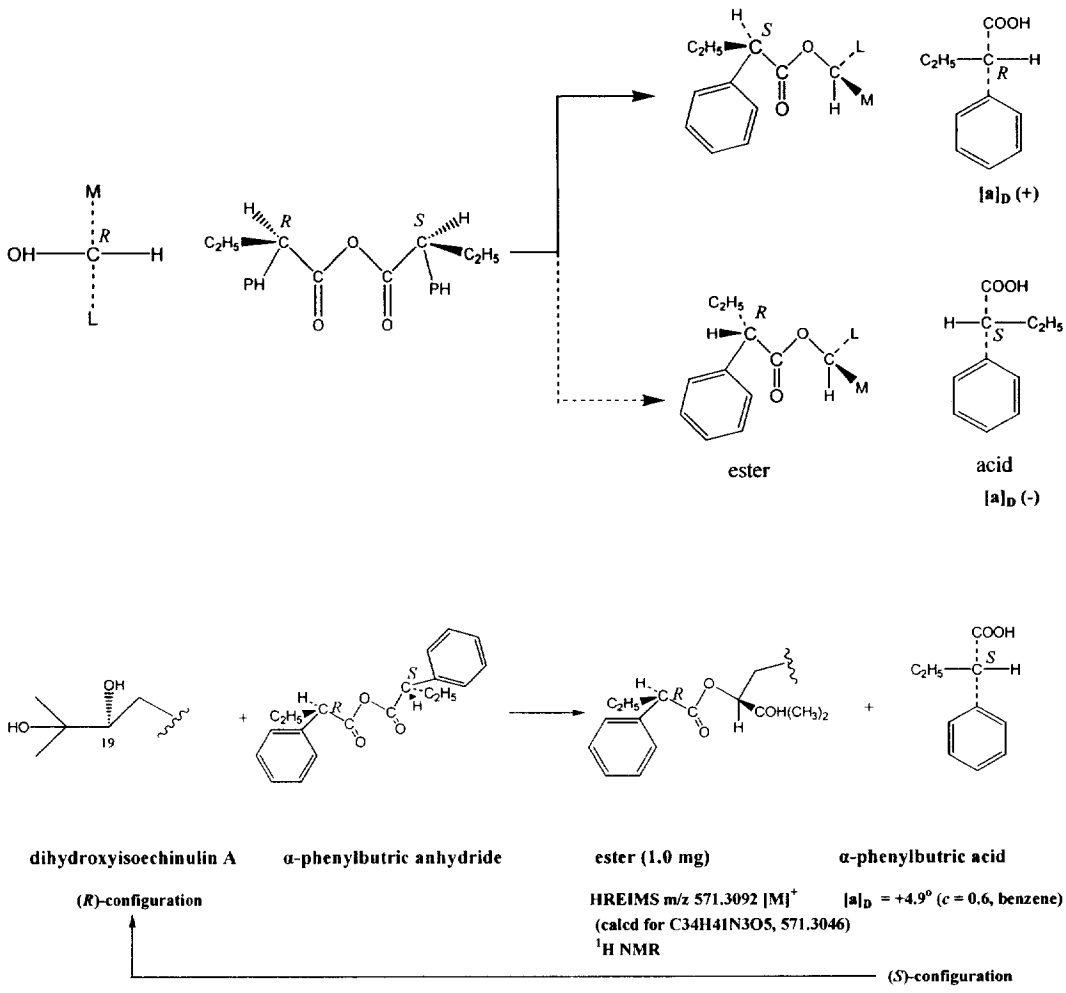
The 1.9 Hz coupling constant for H-11 and H-12 indicates a pseudoequatorial orientation at C-12. [30] The configuration at C-12 was established using Marfey's method[37] (Scheme 12).

For this analysis alanine enantiomers were derivatized with 1-fluoro-2, 4-dinitrophenyl-5-*L*-alaninamide and analyzed by reversed-phase HPLC. The retention times of the corresponding enantiomers (2*S* and 2*R*) were observed at 14.4 and 17.1 min, respectively. Analogous derivatives of the acid hydrolyzate of it followed by HPLC analysis and comparison with the standard derivatives enable us to deduce the (*S*) configuration at C-12 (Scheme 12).

Two factors enabled us to identify the geometry of C-8/C-9 double bond as (*Z*) configuration. The first is the NOE correlations between H-8 and H3-23 and H3-24, and the second is the chemical shift of H-8 (δ 6.87), which is shifted to low field by the deshielding effect of the carbonyl group on *β*-vinyl proton.[33]

The absolute configuration at C-19 in it was determined to be (*R*) by the application of Horeau's method to it , where the recovered *α*-phenylbutyric acid showed $[\alpha]_D^{20}$ = +4.9° (*c* = 0.6, benzene).[33, 36]

Dihydroisoechinulin A exhibited a significant radical scavenging activity against DPPH with IC_{50} values of 20.2 μM, which is similar to positive control, ascorbic acid (IC_{50}, 20 μM); and also showed an UV-A protecting activity with ED_{50} values of 130 μM, which is more active than oxybenzone (ED_{50}, 350 μM) currently used as sunscreen.



Scheme 12. Absolute configuration at C-18 in dihydroxyisoechinulin A by Horeau's method.

Table 2. Tabulated NMR data for dihydroxyisoechinulin A [a]

Carbon no.	δ_H (mult, *J*)	δ_C (mult)	HMBC (H to C)
1	10.91[b] (s)		2, 3, 3a, 7a
2		144.0(s)	
3		103.1 (s)	
3a		126.2 (s)	
4	7.02 (br.s)	119.2 (d)	3, 6, 7a, 18
5		132.3 (s)	
6	6.98 (dd, 8.2,1.3)	123.0 (d)	4, 7a, 18
7	7.29 (d, 8.2)	111.1 (d)	3a, 5
7a		133.9 (s)	
8	6.87 (s)	110.8 (d)	2, 3a, 10
9		124.8 (s)	
10		160.0 (s)	
11	8.36[b] (d, 1.9)		9, 13
12	4.10 (qd, 6.5, 1.9)	51.0 (d)	10, 13, 25
13		166.6 (s)	
14	8.51[b] (s)		10, 12, 13
15		39.2 (s)	
16	6.06 (dd, 17.0, 10.5)	145.4 (d)	2, 15, 23, 24
17	5.01 (d, 17.0)	111.6 (t)	15, 16
	5.03 (d, 10.5)		
18	2.36 (dd, 13.5, 10.0)	38.0 (t)	4, 5, 6, 19
	2.93 (d, 13.5)		
19	3.27 (m)	80.0 (d)	5, 20, 21, 22
20		72.0 (s)	
21	1.09 (s)	26.5 (q)	19, 20, 22
22	1.06 (s)	24.7 (q)	19, 20, 21
23	1.46 (s)	27.6 (q)	2, 15, 16, 24
24	1.45 (s)	27.7 (q)	2, 15, 16, 23
25	1.39 (d, 6.5)	20.3 (q)	12, 13
19-OH	4.11[b] (s)		18, 19, 20
20-OH	4.16[b] (s)		20, 21, 22

[a] Recorded in $CDCl_3$ at 400 MHz (^1H) and 100MHz (^{13}C). [b] Exchangeable.

(3) Neoechinulin A

Neoechinulin A was isolated as a colorless oil with a molecular composition of $C_{19}H_{21}N_3O_2$ from the LREIMS (Fig.17) and ^{13}C NMR data (Fig.20). The eleven unsaturated degrees by molecular formula implied that it contained two carbonyl groups, six double bonds, and three rings. The IR spectrum of noechinulin A showed broad absorptions for multiple amine (3449, cm^{-1}) and amide (1687, 1634 cm^{-1}) functionality (Fig.18). The UV spectrum of noechinulin A showed the presence of conjugated amide [209 nm (log ε 1.85), 230 (1.95)] and conjugated indole [291 nm (1.51), 325 (1.50)] chromophores.

In the ^1H NMR spectrum (Fig.19), three protons were exchanged by D_2O, suggesting that it has one aromatic amine proton [δ 8.32 (H-1)] and two amide protons [δ 7.45 (H-11), 6.40 (H-14)].

The ^1H and ^{13}C NMR spectra of neoechinulin A showed signals ascribable to methyl substituted diketopiperazine [δ 7.45 (H-11), 4.30 (H-12), 6.40 (H-14), 1.60 (H_3-20), 124.5 (C-9), 159.8 (C-10), 51.7 (C-12), 165.7 (C-13), 20.3 (C-20)], a bisubstituted indole [δ 8.32 (H-1), 7.27 (H-4), 7.18 (H-5), 7.16 (H-6), 7.36 (H-7), 143.8 (C-2), 102.9 (C-3), 126.0 (C-3a), 118.9 (C-4), 121.0 (C-5), 122.3 (C-6), 111.2 (C-7), 134.3 (C-7a)], an isopentenyl [δ 6.07 (H-16), 5.23, 5.19 (H_2-17), 1.53 (H_3-18/19), 39.2 (C-15), 144.3 (C-16), 113.3 (C-17), 27.3 (C-18), 27.4 (C-19)], and a trisubstituted double bond [δ 7.21 (H-8), 111.9 (C-8), 124.5 (C-9)] (Table 3).

The connection of the functional groups in neoechinulin A, which led to the planar structure, was achieved on the basis of COSY, HMQC, HMBC, and NOESY correlations. Key HMBC correlations between H-8 and C-2, C-3a, and C-10 and between H-16 and C-2 clearly established planar structure of neoechinulin A. The 1.7 Hz coupling constant for H11-H12 indicates a pseudoequatorial orientation at C-12. According to the data of MS, 1D NMR (^1H &^{13}C), 2D NMR (HMQC & HMBC) and CD spectral data (Fig. 21), we can clearly elucidate the structure as neoechinulin A (Table 3).

The stereochemistry of neoechinulin A has been elucidated from detailed comparison of the data for neoechinulin A with those reported in the literature
(Chart 4).[34]

Neoechinulin A exhibited a significant radical scavenging activity against DPPH with IC_{50} value of 24 μM, which is similar to positive control, L-ascorbic acid (IC_{50}, 20 μM), and also showed an UV-A protecting activity with ED_{50} value of 170 μM, which is more active than oxybenzone (ED_{50}, 350 μM) currently used as sunscreen.[32, 34]

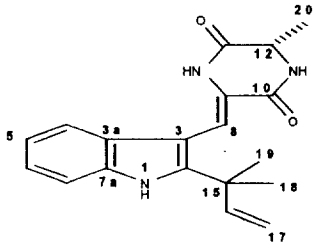


Chart 4. Chemical structure of neoechinulin A.

Table 3. Tabulated NMR data for neoechinulin A [a]

Carbon no.	δ_H (mult, *J*)	δ_C	HMBC (H to C)
1	8.32 (s)		2, 3, 3a, 7a, 16
2		143.8 (s)	
3		102.9 (s)	
3a		126.0 (s)	
4	7.27 (d, 7.8)	118.9 (d)	3, 3a, 6
5	7.18 (dd, 7.8, 7.5)	121.0 (d)	4, 13
6	7.16 (dd, 7.5, 7.3)	122.3 (d)	7, 7a
7	7.36 (d, 7.3)	111.2 (d)	5, 6, 7a
7a		134.3 (s)	
8	7.21 (s)	111.9 (d)	2, 3, 7a, 10, 16
9		124.5 (s)	
10		159.8 (s)	
11	7.45 (brs)		10, 12, 13
12	4.30 (qd, 7.0, 1.7)	51.7 (d)	10, 13, 20
13		165.7 (s)	
14	6.40 (s)		9, 10, 12, 13
15		39.2 (s)	
16	6.07 (dd, 17.5, 10.5)	144.3 (d)	2, 15, 18, 19
17	5.23 (d, 10.5)	113.3 (t)	15, 16, 18, 19
	5.19 (d, 17.5)		
18	1.53 (s)	27.3 (q)	2, 7a, 15, 16, 17, 18, 19
19	1.53 (s)	27.4 (q)	2, 7a, 15, 16, 17, 18, 19
20	1.60 (d)	20.9 (q)	12, 13

[a] Recorded in $CDCl_3$ at 400 MHz (^1H) and 100MHz (^{13}C). [b] Exchangeable.

(4) *L*-Alanyl-*L*-tryptophan anhydride

L-Alanyl-*L*-tryptophan anhydride was isolated as a colorless needle with a molecular composition of $C_{14}H_{15}N_3O_2$ from the HREIMS (Fig. 22) and ^{13}C NMR dat a (Fig.26). The nine unsaturated degrees by molecular formula implied that it containe d two carbonyl groups, four double bonds, and three rings. The IR spectrum of *L*-alanyl-*L*-tryptophan anhydride showed absorptions for a secondary amine group of an indole ring system (3406 cm^{-1}) and carbonyl absorption of amide groups (1673 and 1668 cm^{-1}) (Fig. 24). The UV spectrum of *L*-alanyl-*L*-tryptophan anhydride showed the presence of indole ring system [228 nm (log ε 3.68), 273 (3.63), 279 (3.63), and 289 (3.56) nm] (Fig. 23).

In the ^1H NMR spectrum (Fig.25), three protons were exchanged by D_2O, suggesting that it has one aromatic amine proton [δ 10.90 (H-1)], two amide protons [δ 7.90 (H-11), 8.02 (H-14)]. The ^1H and ^{13}C NMR spectra of *L*-alanyl-*L*-tryptophan anhydride showed signals ascribable to a methyl substituted diketopiperazine [δ 7.90 (H-11), 3.57 (H-12), 8.02 (H-14), 0.40 (H_3-15), 55.3 (C-9), 166.7 (C-10), 49.7 (C-12), 167.7 (C-13), 19.5 (C-15)], a substituted indole [δ 10.9 (H-1), 7.56 (H-4), 6.93 (H-5), 7.02 (H-6), 7.30 (H-7), 124.5 (C-2), 135.7 (C-3), 108.4 (C-3a), 120.7 (C-4), 118.3 (C-5), 118.9 (C-6), 111.0 (C-7), 127.7 (C-7a)], and methylene single bond [δ 3.02, 3.24 (H-8), 28.8 (C-8)] (Table 4).

Based on the above evidence, *L*-alanyl-*L*-tryptophan anhydride showed spectral data virtually identical to those reported in the literature[37]. The stereochemistry of *L*-alanyl-*L*-tryptophan anhydride has been determined previously (Chart 5).

L-Alanyl-*L*-tryptophan anhydride exhibited a significant radical scavenging activity against DPPH with IC_{50} value of 20.7 μM, which is similar to positive control, *L*-ascorbic acid (IC_{50}, 20 μM).

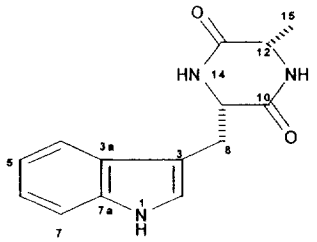


Chart 5. Chemical structure of *L*-alanyl-*L*-tryptophan anhydride.

Table 4. Tabulated NMR data for *L*-alanyl-*L*-tryptophan anhydride [a]

Carbon no.	δ_H (mult, *J*)	δ_C	Carbon no.	δ_H (mult, *J*)	δ_C
1	10.9 (s)		8	3.02 (dd, 14.4, 4.0)	28.8 (t)
				3.24 (dd, 14.4, 4.0)	
2	7.04 (s)	124.5 (d)	9	4.10 (m)	55.3 (d)
3		135.7 (s)	10		166.7 (s)
3a		108.4 (s)	11	7.90 (s)	
4	7.56 (d, 7.8)	120.7 (d)	12	3.57 (qd, 7.0, 2.9)	49.7 (d)
5	6.93 (dd, 7.8, 7.0)	118.3 (d)	13		167.7 (s)
6	7.02 (dd, 8.0, 7.0)	118.9 (d)	14	8.02 (s)	
7	7.30 (d, 8.0)	111.0 (d)	15	0.40 (d, 7.0)	19.5 (d)
7a		127.7 (s)			

[a] Recorded in DMSO at 400 MHz (^{1}H) and 100 MHz (^{13}C).

(5) Echinulin [44]

Echinulin was isolated as a white needle with a molecular composition of $C_{29}H_{39}N_3O_2$ by LREIMS (Fig. 28) and ^{13}C NMR data (Fig.30). The twelve Unsaturated degrees by molecular formula implied that it contained two carbonyl gro ups, seven double bonds, and three rings.

The ^{1}H and ^{13}C NMR spectra (Fig. 30) of echinulin showed signals ascribable to methyl substituted diketopiperazine [δ 6.80 (H-10), 5.35 (H-12), 7.13 (H-13), 1.54 (H_3-12), 34.5 (C-9), 167.6 (C-11), 50.8 (C-12), 168.3 (C-14), 19.5 (CH_3-12)], a tatrasubstituted indole [δ 8.04 (H-1), 5.98 (H-4), 5.66 (H-6), 141.3 (C-2), 104.0 (C-3), 124.4 (C-3a), 123.4 (C-4), 122.8 (C-5), 115.0 (C-6), 132.9 (C-7), 133.8 (C-7a)], a single bond [δ 4.09, 4.41 (H-8), 54.5 (C-8), 5.41 (H-9), 34.5 (C-9)] (Table 5).

Echinulin showed spectral data virtually identical to those reported in the literature. Based on the above evidence, the stereochemistry of echinulin has been determined (Chart 6).

Echinulin exhibited a significant radical scavenging activity against DPPH with IC_{50} value of 35.7 μM, which is similar to positive control, *L*-ascorbic acid (IC_{50}, 20 μM), and showed immunosuppressive activity reported previously. [33, 41]

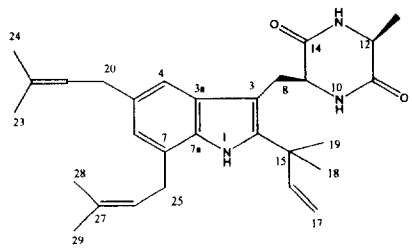


Chart 6. Chemical structure of echinulin.

Table 5. Tabulated NMR data for echinulin

Carbon no.	δ_H (mult, *J*)	δ_C	Carbon no.	δ_H (mult, *J*)	δ_C
1	8.04 (s)		14		168.3
2		141.3	15		38.9
3		104.0	16	6.10 (dd, 10.7, 6.6)	145.7
3a		124.4	17	5.14 (s)	112.3
				5.16 (d, 6.6)	
4	5.98 (s)	123.4	18	1.50 (s)	27.8
5		122.8	19	1.50 (s)	27.9
6	5.66 (s)	115.0	20	3.38 (d, 7.2)	25.8
7		132.9	21	3.18 (dd, 13.2, 3.1)	31.3
7a		133.8	22		128.9
8	4.09 (d, 6.8)	54.5	23	1.80 (s)	131.5
	4.41 (d, 11.1)				
9	5.41 (dd, 7.2, 7.4)	34.5	24	1.86 (s)	17.9
10	6.80 (s)		25	3.53 (d, 7.2)	17.8
11		167.6	26	3.65 (dd, 14.8, 3.7)	29.3
12	5.35 (q, 7.0)	50.8	27		122.8
12- CH3	1.54 (d, 7.0)	19.8	28	1.74 (s)	132.2
13	7.13 (s)		29	1.74 (s)	25.7

[a] Recorded in $CDCl_3$ at 400 MHz (^1H) and 100 MHz (^{13}C).

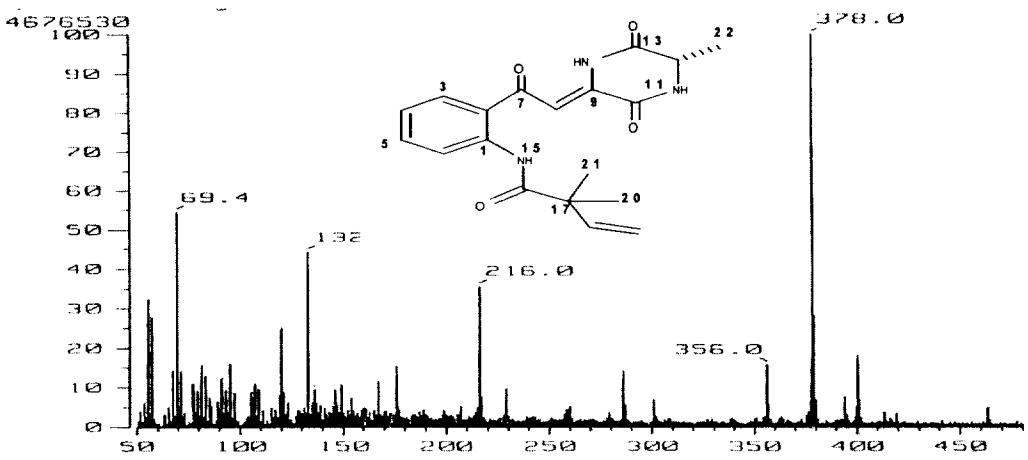


Fig. 6. LRFABMS spectrum of golmaenone.

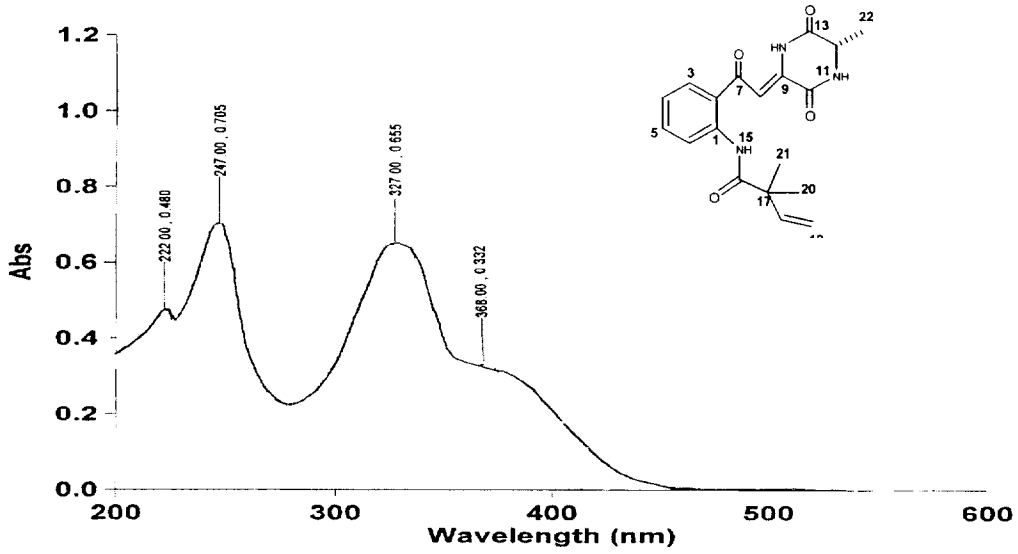


Fig. 7. UV spectrum of golmaenone.

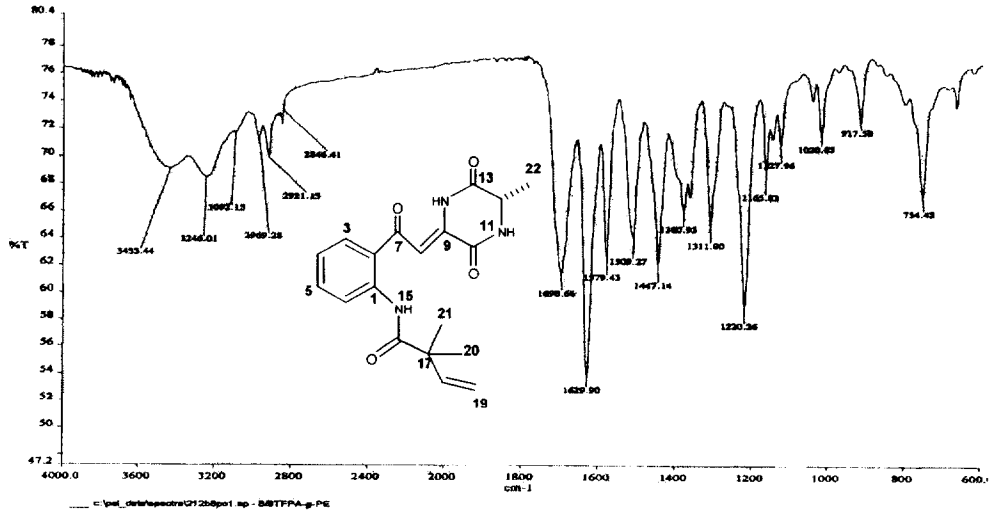


Fig. 8. IR spectrum of golmaenone.

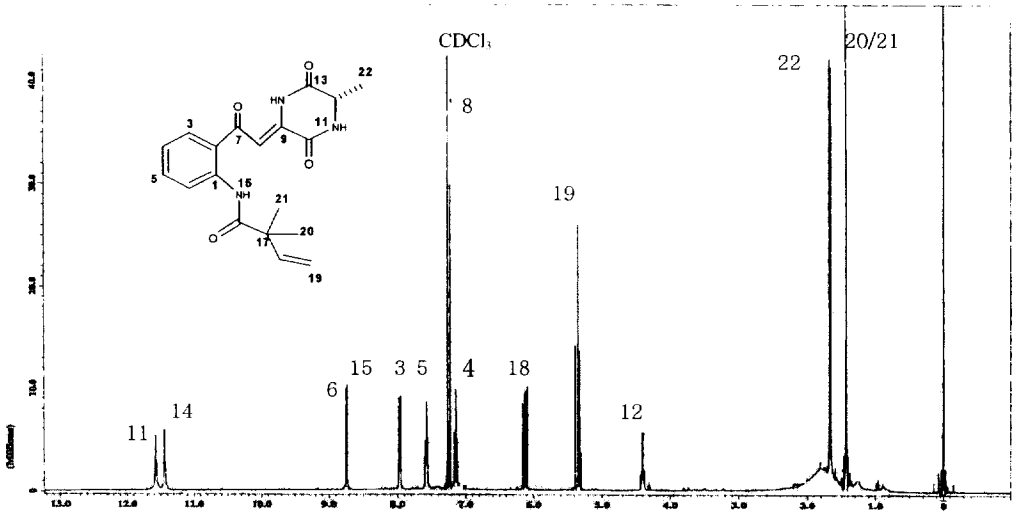


Fig. 9. ^{1}H NMR ($CDCl_3$) spectrum of golmaenone.

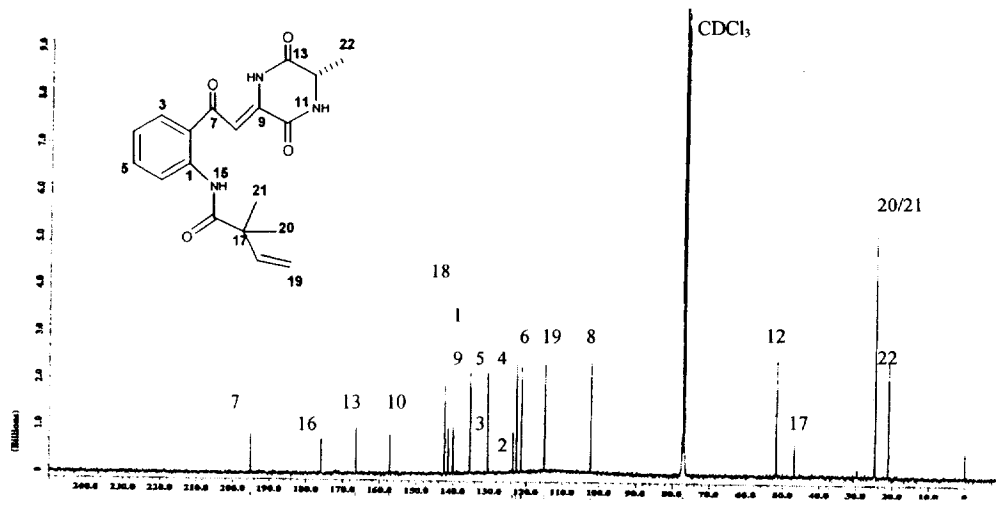


Fig. 10. ^{13}C NMR ($CDCl_3$) spectrum of golmaenone.

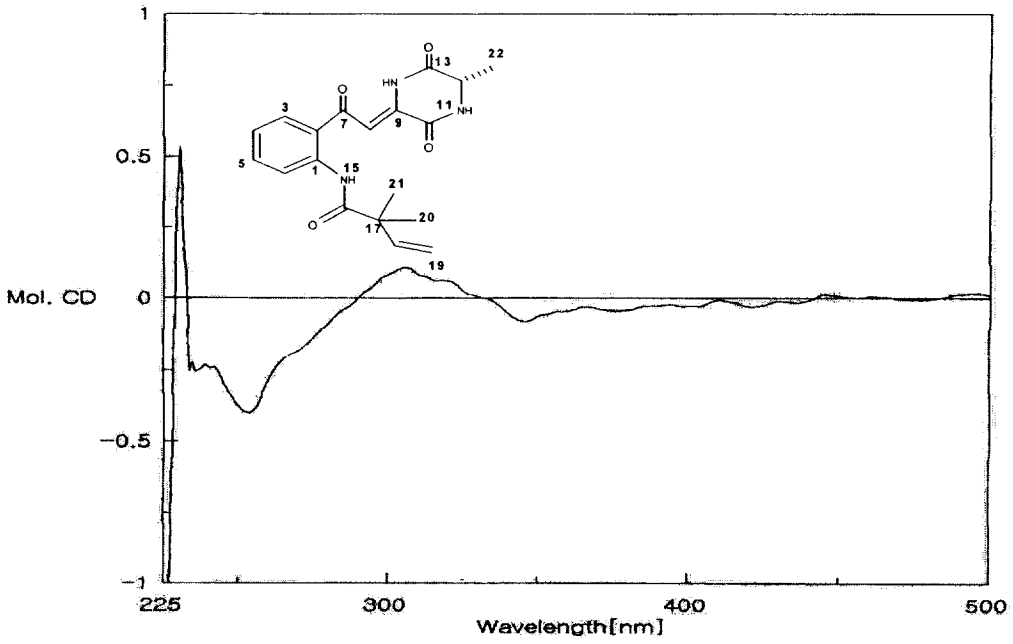


Fig. 11. CD spectrum of golmanone.

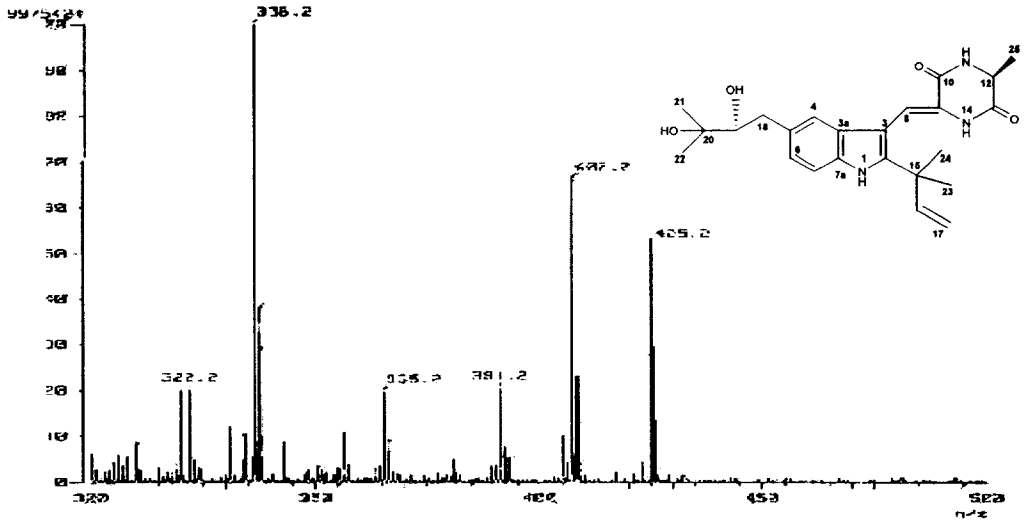


Fig. 12. LREIMS spectrum of dihydroxyisoechinulin A.

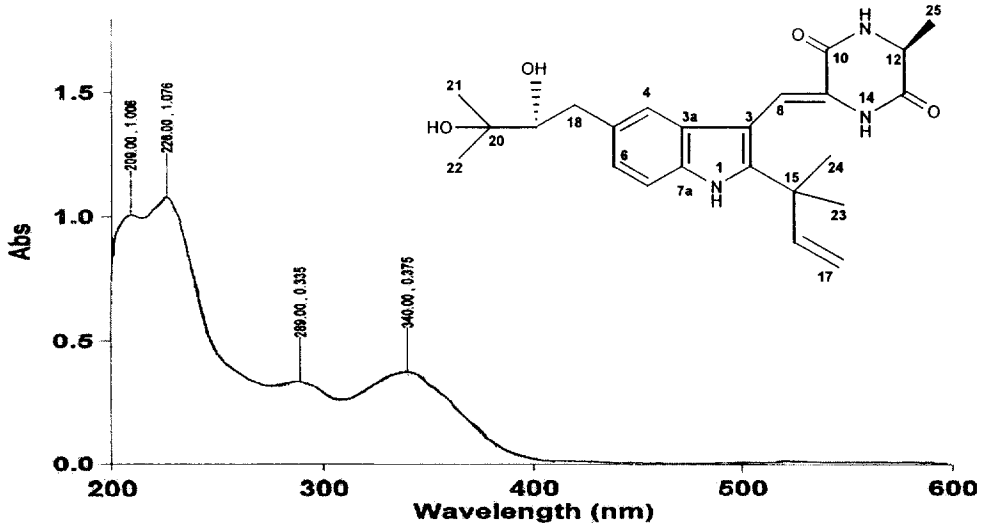


Fig. 13. UV of dihydroxyisoechinulin A.

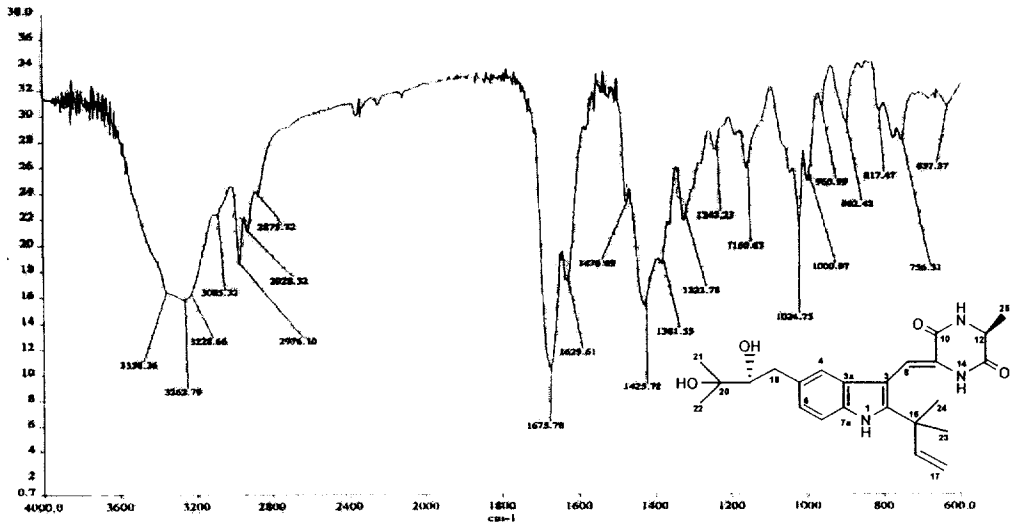


Fig. 14. IR spectrum of dihydroxyisoechinulin A.

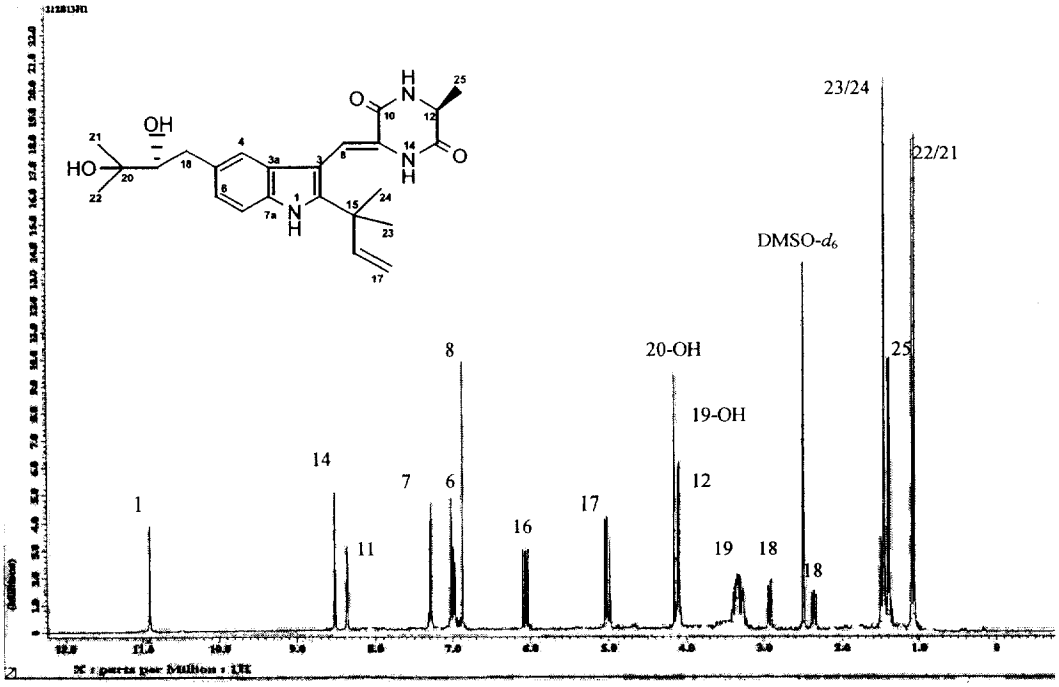


Fig. 15. ^{1}H NMR (DMSO-d_6) spectrum of dihydroxyisoechinulin A.

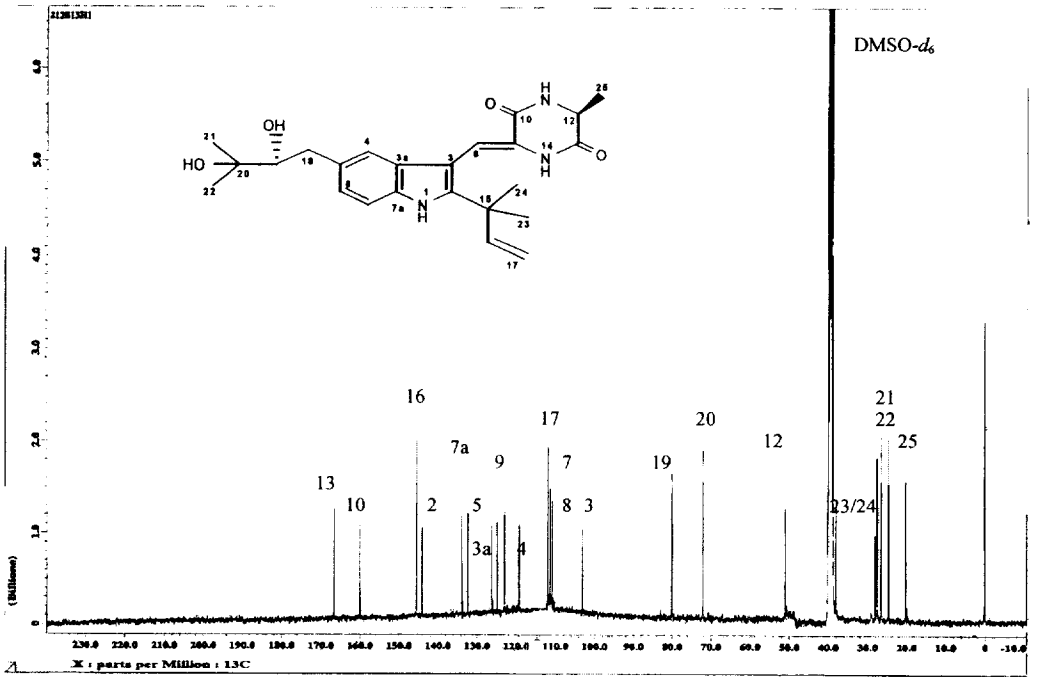


Fig. 16. ^{13}C NMR (DMSO-d_6) spectrum of dihydroxyisoechinulin A.

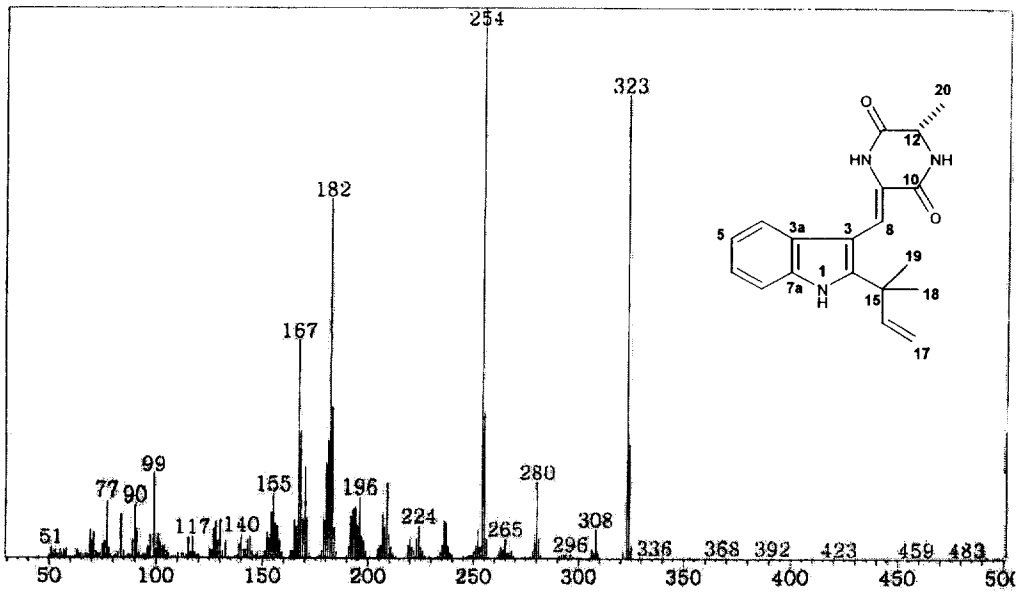


Fig. 17. LREIMS spectrum of neoechinulin A.

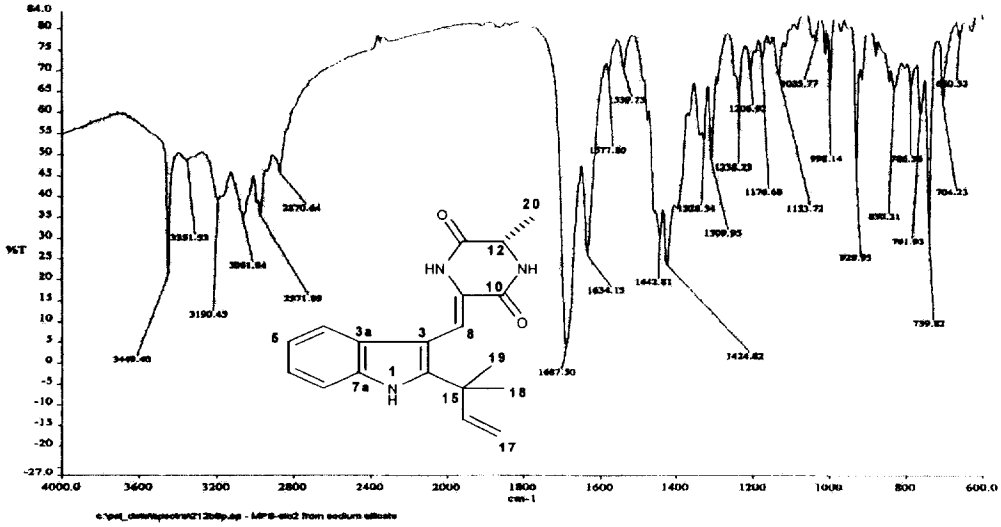


Fig. 18. IR spectrum of neoechinulin A.

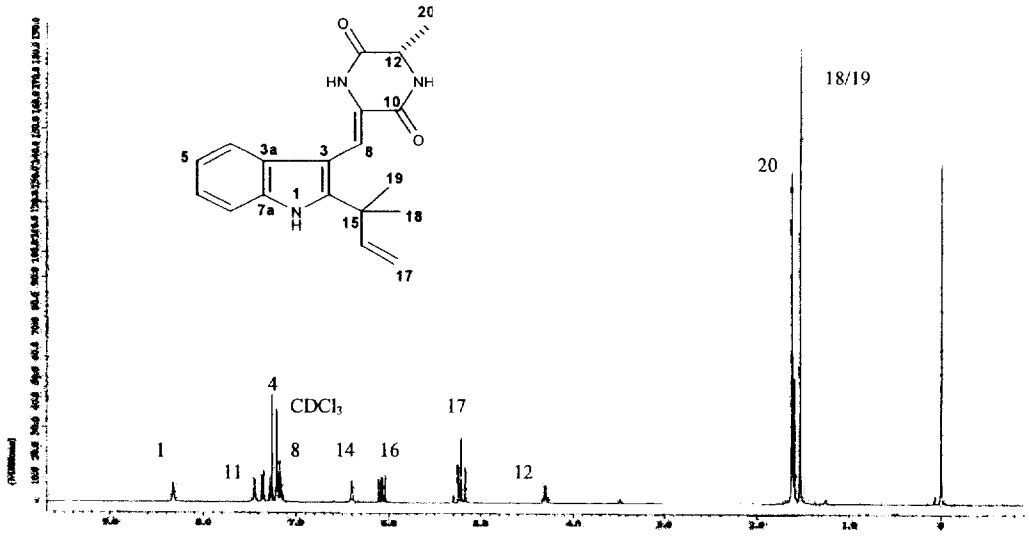


Fig. 19. ^{1}H NMR ($CDCl_3$) spectrum of neoechinulin A.

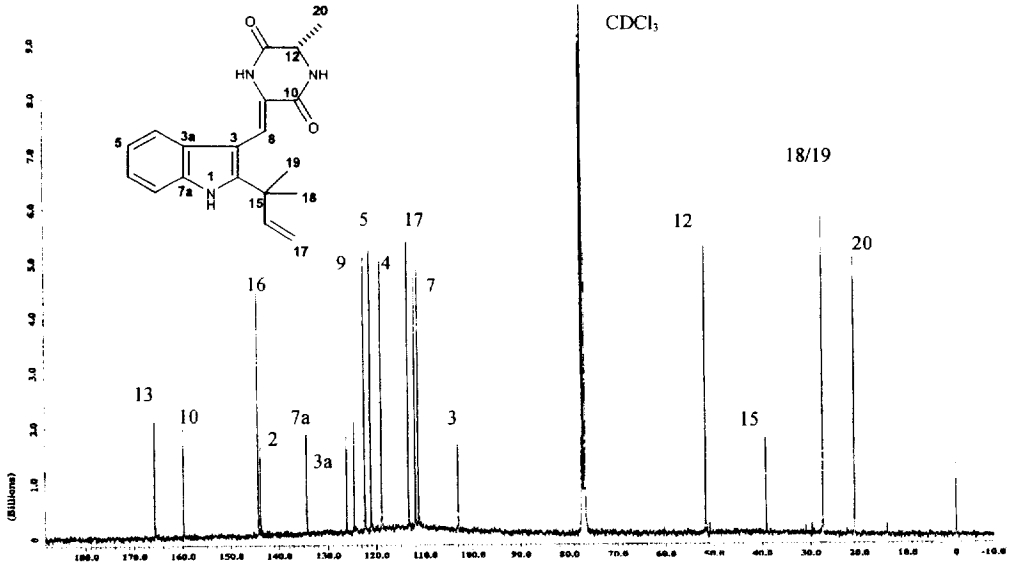


Fig. 20. ^{13}C NMR ($CDCl_3$) spectrum of neoechinulin A.

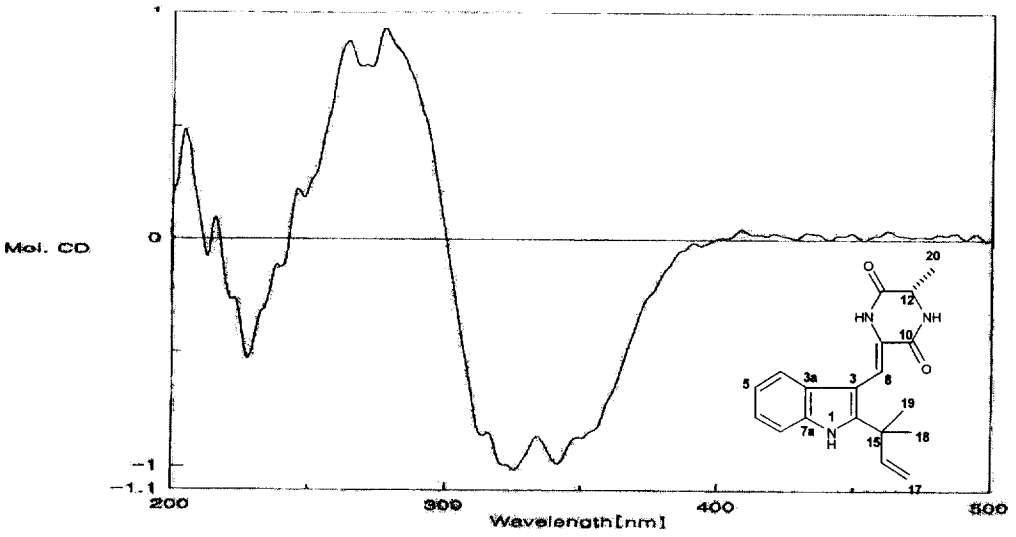


Fig. 21. CD spectrum of neoechinulin A.

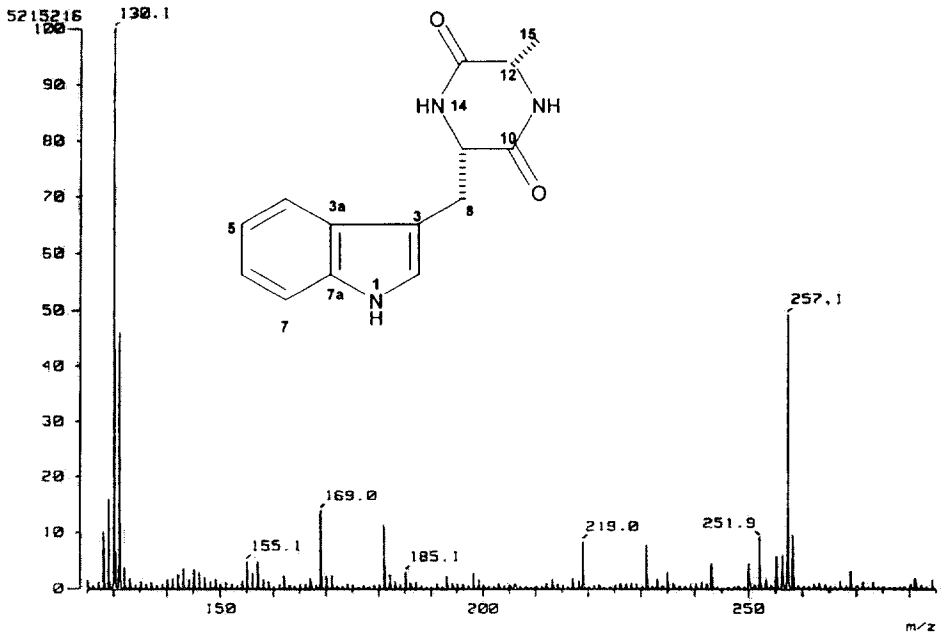


Fig. 22. HREIMS spectrum of *L*-alanyl-*L*-tryptophan anhydride.

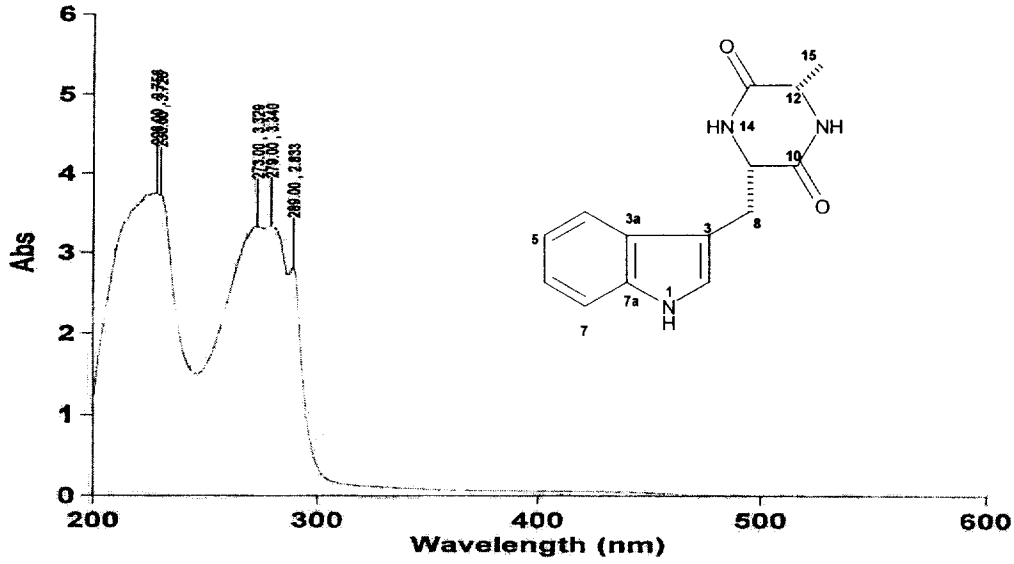


Fig. 23. UV spectrum of *L*-alanyl-*L*-tryptophan anhydride.

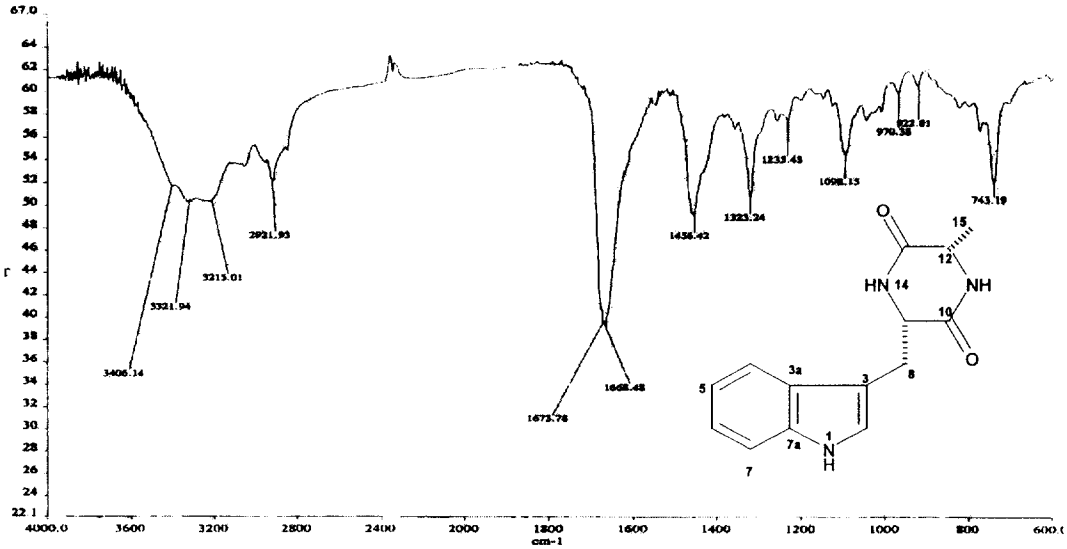


Fig. 24. IR spectrum of *L*-alanyl-*L*-tryptophan anhydride.

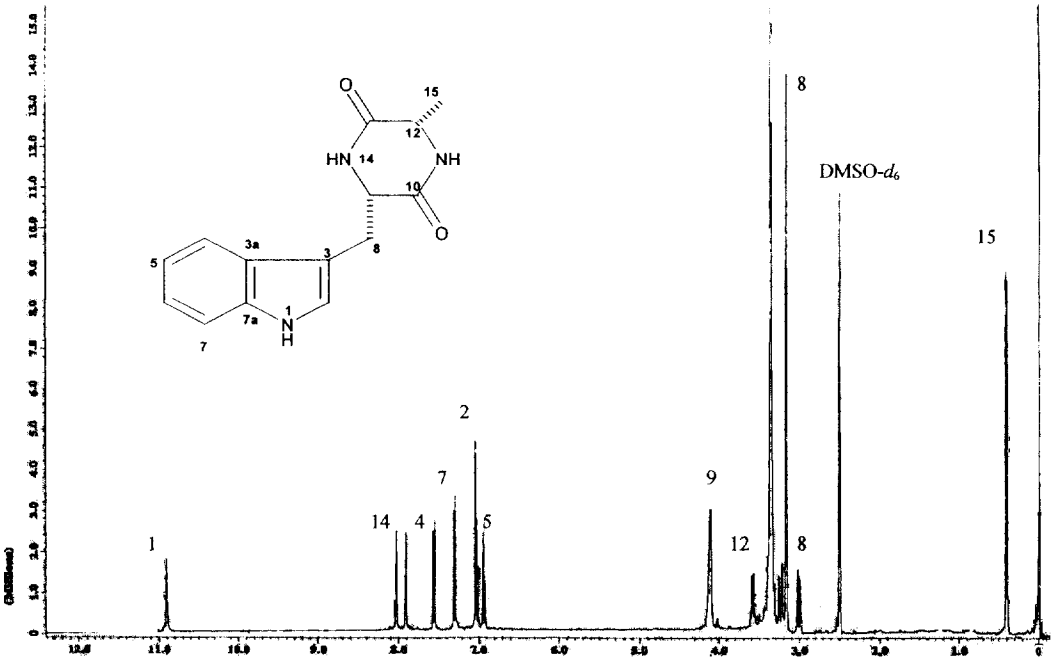


Fig. 25. ^{1}H NMR (DMSO-d_6) spectrum of *L*-alanyl-*L*-tryptophan anhydride.

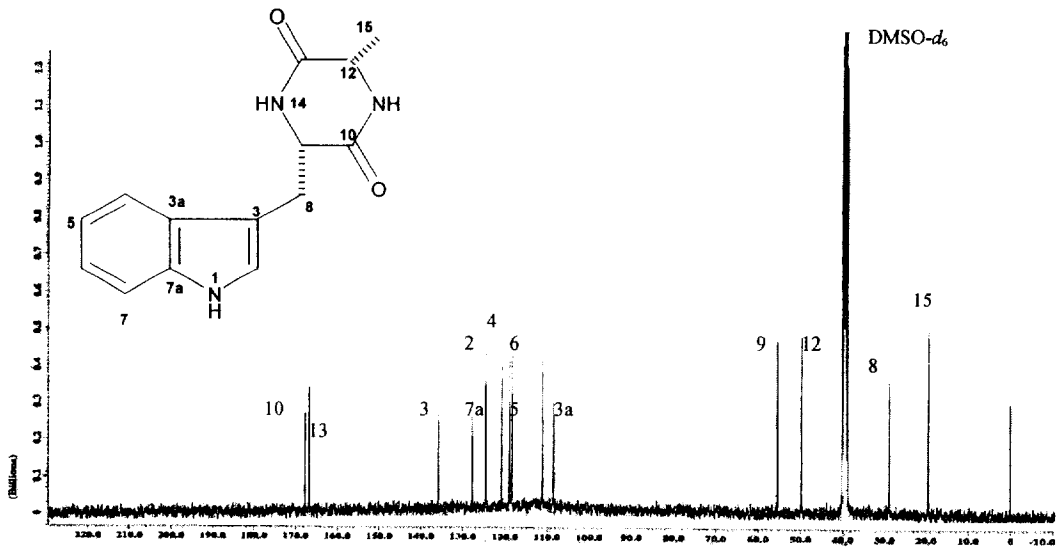


Fig. 26. ^{13}C NMR (DMSO-d_6) spectrum of *L*-alanyl-*L*-tryptophan anhydride.

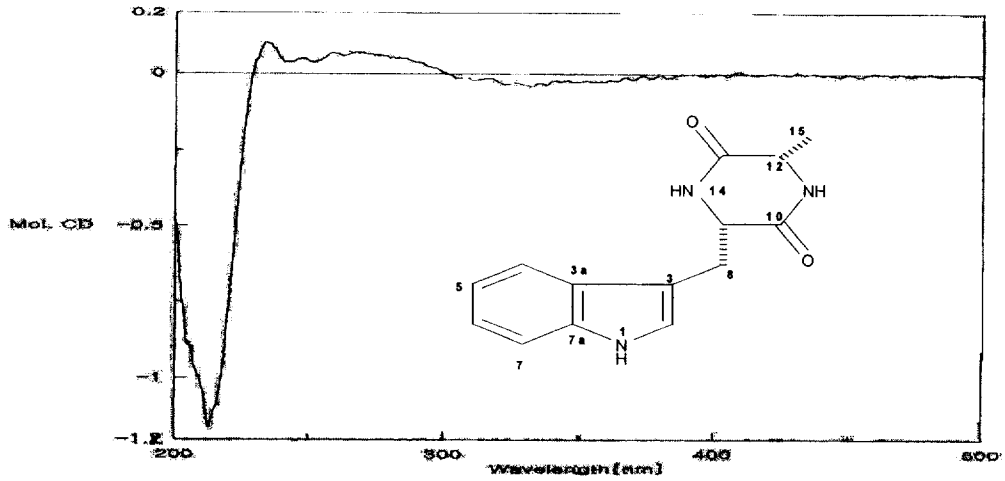


Fig. 27. CD spectrum of *L*-alanyl-*L*-tryptophan anhydride.

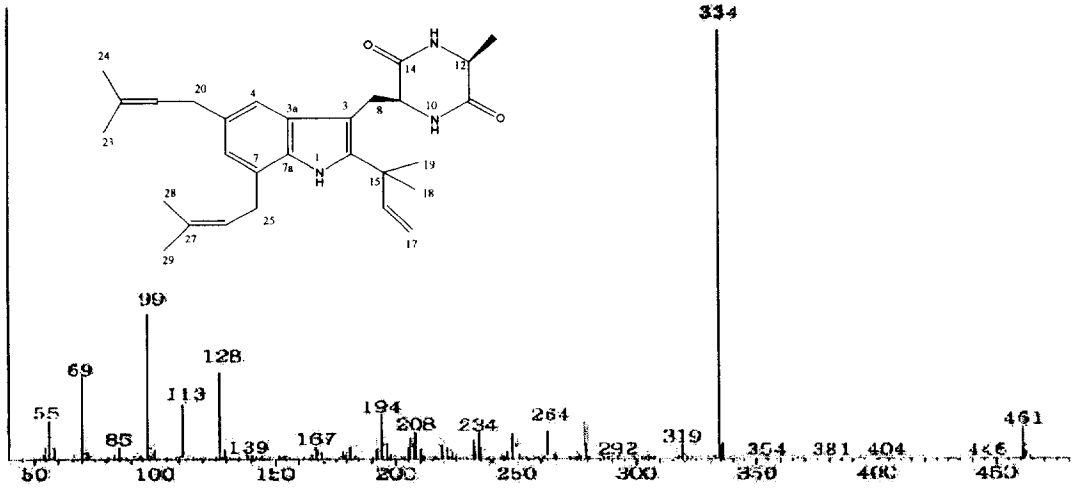


Fig. 28. LREIMS spectrum of echinulin.

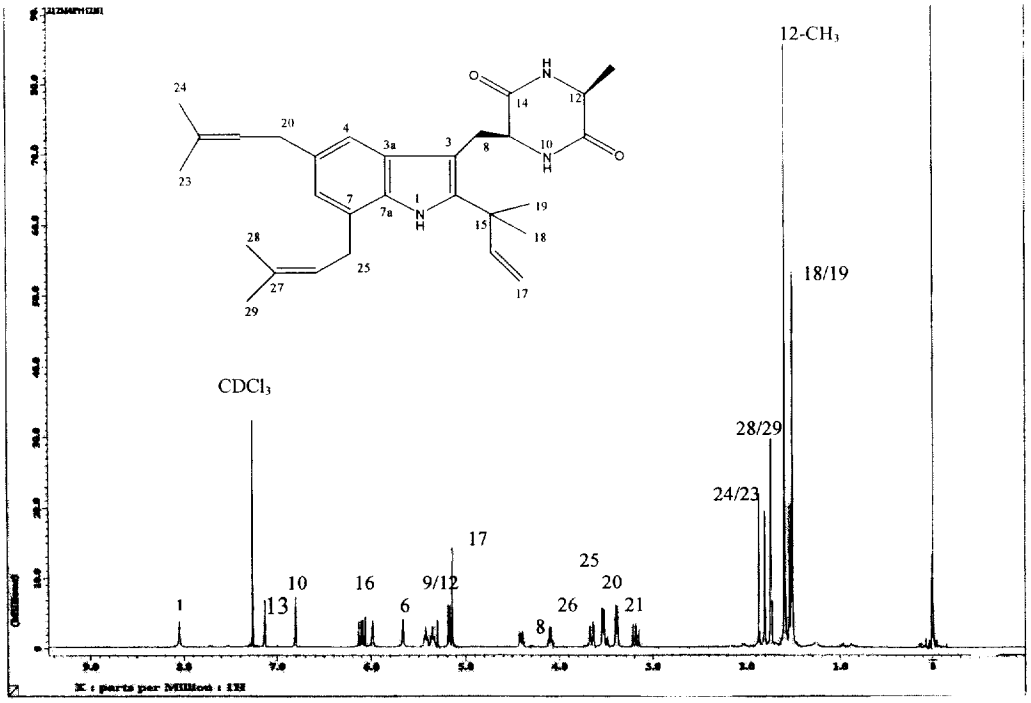


Fig. 29. ^{1}H NMR ($CDCl_3$) spectrum of echinulin.

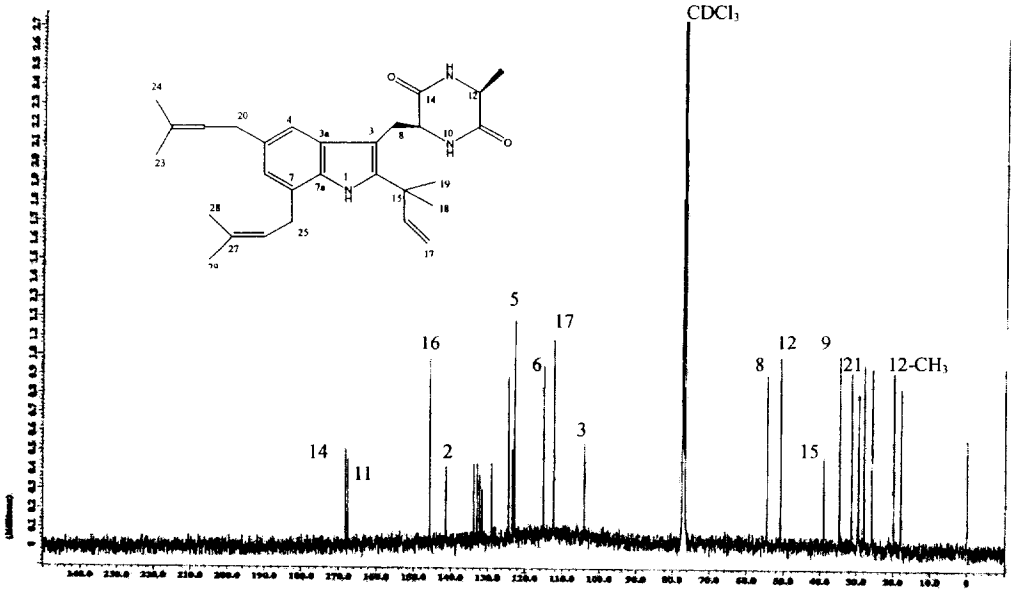


Fig. 30. ^{13}C NMR ($CDCl_3$) spectrum of echinulin.

2) *Microsporum* sp. (MFA212-1)

(1) Asperflavin ribofuranoside

Asperflavin ribofuranoside, $[\alpha]_D^{20}$ = +23.8^O (c = 0.2, MeOH), was obtained as a ye llow oil, which was deduced to have the molecular formula $C_{21}H_{25}O_9$ from HRFABMS (Fig.31) and ^{13}C NMR data (Fig.35). The IR spectrum of asperflavin ribofuranoside suggested the presence of hydroxyl (3429 cm^{-1}), conjugated enone (1632 cm^{-1}), aryl (1605 cm^{-1}), and glycosidic (1045 cm^{-1}) groups (Fig. 33).

Detailed analyses of the ^1H- (Fig.34) and ^{13}C-NMR data of asperflavin ribofuranoside revealed the presence of eight sp^2 and one sp^3 quaternary carbons, three sp^2, four sp^3 oxymethines, one oxymethylene, two methylenes, one oxymethyl, and one methyl (Table 6). Extensive analysis of 2D NMR spectra, correlated spectroscopy (COSY), ^1H-detected heteronuclear multiple-quantum coherence (HMQC), heteronuclear multiple-bond correlation (HMBC), and nuclear overhauser enhanced and exchange spectroscopy (NOESY), suggested that asperflavin ribofuranoside was a glycoside which consisted of a dihydroanthracene as aglycone and one sugar unit (Table 6).

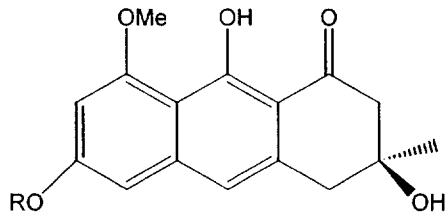
Acid hydrolysis of asperflavin ribofuranoside furnished asperflavin[44] (Fig. 36-38), $[\alpha]_D^{20}$ = +8.8^o (c = 0.3, MeOH), and methyl sugar, which was identified as ribose on the basis of GC-MS analysis of the trimethysilyl derivative. Key HMBC correlation from H-1' to C-6 in asperflavin ribofuranoside was critical in establishing the position of ribose attached at C-6.

The stereochemistry of the anomeric position of the sugar moiety was assigned as α-configuration on the basis of chemical shifts and coupling constant at C-1 [δ 5.75 (d, J = 4.5 Hz), 99.9 (d)] and the comparison of ^{13}C-NMR data of ribofuranose moiety with the reported value[41] (Fig. 40, 41).

The absolute configuration of the ribose unit was determined as *D* from the optical rotation of the tetraacetate derived from ribose. The specific rotation of ribose tetraacetate, $[\alpha]_D^{20}$ = –55.2^o (c = 0.5, MeOH), which was obtained from acid hydrolysis of asperflavin ribofuranoside with 9% aq. HCl, indicated the presence of *D*-ribose in asperflavin ribofuranoside.

From all of these results, the structure of asperflavin ribofuranoside was deduced as (+)-6-*O*-(α-*D*-ribofuranosyl)-3,4-dihydro-3,6,9-trihydroxy-8-methoxy-3-methylanthracen-1 (2*H*)-one.[48-56]

Asperflavin robofuranoside showed the DPPH scavenging activity with IC_{50} value of 14.2 μM, which was more potent than the positive control ascorbic acid (IC_{50} 20 μM), and also exhibited *in vitro* mild antibacterial activity against methicillin resistant *Staphylococcus aureus* (MRSA) and multidrug-resistant *S. aureus* (MDRSA) with MIC value of 50 μM, respectively.



R=α-*D*-ribofuranoside

Chart 7. Chemical structure of asperflavin ribofuranoside.

Table 6. Tabulated NMR data for asperflavin ribofuranoside[a]

Carbon no.	δ_H (mult, *J*).	δ_C (mult)	HMBC
1		203.7 (s)	
2	2.64 (d, 17.0) 2.86 (d, 17.0)	51.5 (t)	1, 3, 4, 9a, 3-CH_3
3		69.4 (s)	
3-CH_3	1.27 (s)	28.8 (q)	2, 3, 4,
4	2.91 (d, 16.3) 3.00 (d, 16.3)	42.7 (t)	2, 3, 4a, 9a, 10,
4a		138.1 (s)	
5	6.84 (d, 2.0)	102.2 (d)	6, 7, 8a, 10
6		159.4 (s)	
7	6.58 (d, 2.0)	98.7 (d)	5, 6, 8, 8a
8		160.6 (s)	
8- OCH_3	3.87 (s)	55.8 (q)	8
8a		109.6 (s)	
9		164.5 (s)	
9a		109.5 (s)	
10	6.91 (s)	116.5 (d)	4, 5, 8a, 9a, 10a
10a		141.0 (s)	
3-OH	4.85[b] (s)		2, 3, 3-CH_3,
9-OH	14.87[b] (s)		8a, 9, 9a
1'	5.75 (d, 4.5)	99.9 (d)	6, 3', 4'
2'	4.11 (m)	71.5 (d)	1', 3'
3'	3.95 (m)	69.2 (d)	1', 2', 5'
4'	4.01 (m)	86.5 (d)	
5'	3.49 (m)	61.5 (t)	3'
2'-OH	4.75[b] (d, 8.8)		1', 2', 3'
3'-OH	4.93[b] (d, 5.3)		2', 3', 4'
5'-OH	4.82[b] (d, 5.6)		4', 5'

[a] Recorded in DMSO at 400 MHz (^1H) and 100 MHz (^{13}C).

[b] Exchangeable

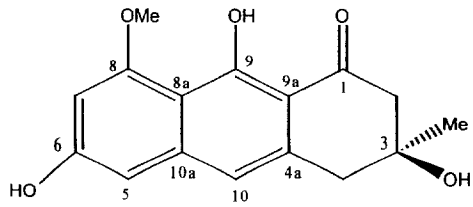


Chart 6. Chemical structure of asperflavin.

Table 7. Tabulated NMR data for asperflavin[a]

Carbon no.	δ_H (mult, J)
1	
2	2.85, 2.89 (each, d, 17.0)
3	
3-CH_3	1.26 (s)
4	2.98, 3.00 (each, s)
4a	
5	6.54 (d, 2.1)
6	
7	6.42 (d, 2.1)
8	
8-OCH_3	3.84 (s)
8a	
9	
9a	
10	6.79 (s)
10a	
3-OH	4.82 (s)
6-OH	10.27 (s)
9-OH	14.97 (s)

[a] Recorded in DMSO-d_6 at 400 MHz (^{1}H).

Table 8. Tabulated NMR data for D-ribopyranosyl tetraacetate[a]

Carbon no.	δ_H (mult, J).	δc (mult)
1	6.01 (d, 4.8)	90.8 (d)
2	5.02 (dd, 4.8, 3.7)	67.2 (d)
3	5.47 (dd, 3.7, 3.2)	66.1 (d)
4	5.14 (ddd, 4.3, 3.2, 3.2)	66.2 (d)
5	3.90 (dt, 12.3, 4.3) 4.01 (dt, 12.3, 3.2)	62.6 (t)
Ac		169.8 (s)
		169.7 (s)
		169.4 (s)
		168.7 (s)
	2.07 (s)	20.6 (q)
	2.08 (s)	20.6 (q)
	2.09 (s)	20.7 (q)
	2.12 (s)	20.8 (q)

[a] Recorded in $CDCl_3$ at 400 MHz (^{1}H) and 100 MHz (^{13}C).

(2) Flavoglaucin

Flavoglaucin was isolated as yellow needles with a molecular composition of $C_{19}H_{28}O_3$ from the LREIMS (Fig.41) and ^1H NMR data (Fig.42). The six unsaturated degrees by molecular formula implied that it contained one carbonyl group, four dou ble bonds, and one ring.

In the ^1H NMR spectrum (Fig.42), two protons were exchanged by D_2O, suggesting that it has two phenolic hydroxyl protons [δ 11.92 (1H, s, 1-OH), 4.44 (1H, brs, 4-OH)] .

The ^1H NMR spectrum showed twenty eight protons, indicated that flavoglaucin contained three methyl protons [δ_H 0.91 (3H, t, J = 7.0 Hz, H_3-14), 1.69 (3H, s, H_3-18), 1.75 (3H, s, H_3-19)], one carbonyl proton [δ_H 10.23 (1H, s, H-7)], one aromatic proton [δ_H 6.89 (1H, s, H-5)], one olefinic proton [δ_H 5.28 (1H, br.t, H-16)], seven methylene protons [δ_H 2.87 (2H, br.t, J = 7.0 Hz, H_2-8), 2.32 (2H, dt, J = 7.0, 5.6 Hz, H_2-9), 1.30 (2H, br.m, H_2-10), 1.30 (2H, br.m, H_2-11), 1.30 (2H, br.m, H_2-12/13), 3.32 (2H, br.d, J = 7.0 Hz, H_2-15)] (Table 9).

The stereochemistry of flavoglaucin has been elucidated from detailed comparison of the data for flavoglaucin with those reported in the literature[57] (Chart. 9).

Flavoglaucin showed the DPPH scavenging activity with IC_{50} value of 11.3 μM, which is more than the positive control ascorbic acid (IC_{50} 20 μM); and also exhibited antioxidation, cynergism with tocoplerol, and toxicity.[58]

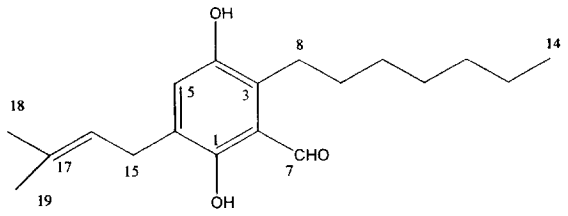


Chart 9. Chemical structure of flavoglaucin.

Table 9. Tabulated NMR data for flavoglaucin[a]

Carbon no.	δ_H (mult, *J*)	Carbon no.	δ_H (mult, *J*)
1		12	1.30 (br.m)
2		13	1.30 (br.m)
3		14	0.91 (t, 7.0)
4		15	3.32 (br.d, 7.0)
5	6.89 (s)	16	5.28 (br.t, 7.0)
6		17	
7	10.23 (s)	18	1.69 (s)
8	2.87 (br.t, 7.0)	19	1.75 (s)
9	2.32 (dt, 7.0, 5.6)	1-OH	11.92 (s)
10	1.30 (br.m)	4-OH	4.44 (br.s)
11	1.30 (br.m)		

[a]Recorded in $CDCl_3$ at 400 MHz (^1H).

(3) Isodihydroauroglaucin

Isodihydroauroglaucin was isolated as a yellow needle with a molecular composition of $C_{19}H_{24}O_3$ from the LREIMS (Fig. 43) and ^{13}C NMR data (Fig. 45). T he eight unsaturated degrees by molecular formula implied that it contained one carbonyl group, six double bonds, and one ring.

In the ^1H NMR spectrum, two protons were exchanged by D_2O, suggesting that it has two phenolic hydroxyl protons [δ 11.93 (1H, s, 1-OH), 4.44 (1H, br.m, 4-OH)].

The ^1H and ^{13}C NMR spectra of isodihydroauroglaucin showed signals ascribable to three methyl groups [δ_H 1.69 (3H, s, H_3-14), 1.73 (3H, d, J = 7.9 Hz, H_3-18), 1.75 (3H, d, J = 7.9 Hz, H_3-19); δ_C 17.7 (C-14), 25.8 (C-18), 18.0 (C-19)], one carbonyl group [δ_H 10.02 (1H, s, H-7), δ_C 195.3 (C-7)], three allylic double bonds [δ_H 5.60 (2H, dt, J = 12.6, 6.9 Hz, H-10/11), 6.02 (1H, dd, J = 12.6, 10.2 Hz, H-12/13), 5.28 (1H, dt, J = 7.4, 1.0 Hz, H-16); δ_C 131.9 (C-10), 129.3 (C-11), 131.0 (C-12), 128.3 (C-13), 125.8 (C-16), 133.8 (C-17)], one aromatic signal [6.90 (1H, s, H-5)] (Table 10).

The stereochemistry of isodihydroauroglaucin has been elucidated from detailed comparison of the data for isodihydroauroglaucin with those reported in the literature[57] (Chart. 10).

Isodihydroauroglaucin showed the DPPH scavenging activity with IC_{50} values of 11.5 μM, which is more than the positive control ascorbic acid (IC_{50} 20 μM); and also exhibited antioxidation, cynergism with tocoplerol, and toxicity.[58]

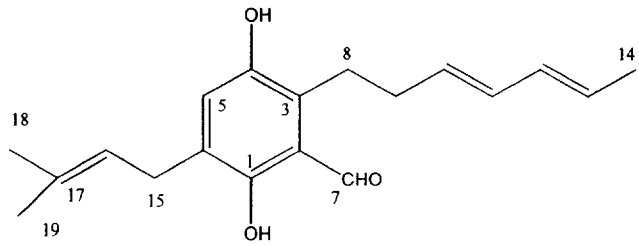


Chart 10. Chemical structure of isodihydroauroglaucin.

Table 10. Tabulated NMR data for isodihydroauroglaucin [a]

Carbon no.	δ_H (mult, *J*)	δ_C (mult)	Carbon no.	δ_H (mult, *J*)	δc (mult)
1		155.8 (s)	12	6.02 (dd, 12.6, 10.2)	131.0 (d)
2		127.3 (s)	13	6.02 (dd, 12.6, 10.2)	128.3 (d)
3		128.9 (s)	14	1.69 (s)	17.7 (q)
4		145.0 (s)	15	3.29 (d, 7.4)	27.0 (t)
5	6.90 (s)	121.0 (d)	16	5.28 (dt, 7.4, 1.0)	125.8 (d)
6		117.2 (s)	17		133.8 (s)
7	10.02 (s)	195.3 (d)	18	1.73 (d, 7.9)	25.8 (q)
8	2.98 (t, 7.7)	24.0 (t)	19	1.75 (d, 7.9)	18.0 (q)
9	2.33 (dt, 7.7, 6.9)	34.2 (t)	1-OH	11.93 (s)	
10	5.60 (dt, 12.6, 6.9)	131.9 (d)	4-OH	4.52 (br.m)	
11	5.60 (dt, 12.6, 6.9)	129.3 (d)			

Recorded in $CDCl_3$ at 400 MHz (^1H) and 100 MHz (^{13}C)

(4) Citrinin

Citrinin was isolated as a yellowish solid with a molecular composition of $C_{13}H_{14}O_5$ from the LREIMS (Fig. 46) and ^{13}C NMR data (Fig. 48). The seven unsaturated degrees by molecular formula implied that it contained one carbonyl group, one ketone group, three double bonds, and two rings.

In the ^1H NMR spectrum (Fig. 47), two protons were exchanged by D_2O, suggesting that it has two hydroxyl protons [δ 15.11 (1H, s, 8-OH), 15.86 (1H, s, 14-OH)].

The ^1H and ^{13}C NMR spectra of citrinin showed signals ascribable to three methyl groups [δ 1.22 (3H, d, J = 7.2 Hz, H_3-11), 1.34 (3H, d, J = 6.7 Hz, H_3-12), 2.02 (3H, s, H_3-14)], two carbonyl groups [δ 183.8 (C-6), 177.2 (C-14)], an three double bonds [δ 162.7 (C-1), 123.1 (C-5), 100.3 (C_3-7), 174.5 (C-8), 139.0 (C-9), 107.4 (C-10)] (Table 11).

The stereochemistry of citrinin has been elucidated from detailed comparison of the data for citrinin with those reported in the literature[42](Chart. 11).

Citrinin showed the DPPH scavenging activity with IC_{50} value of 48.5 μM, and also exhibited the high insecticidal activity and low toxicity.[47]

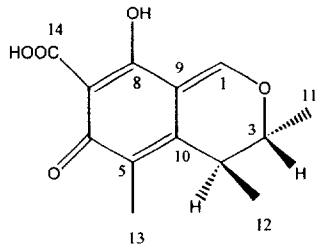


Chart 11. Chemical structure of citrinin.

Table 11. Tabulated NMR data for citrinin[a]

Carbon no.	δ_H (mult, J)	δ_C (mult)	Carbon no.	δ_H (mult, J)	δc (mult)
1	8.23 (s)	162.7	8-OH	15.11 (s)	
2			9		139.0
3	4.78 (d, 6.7)	81.6	10		107.4
4	2.98 (d, 7.2)	34.6	11	1.22 (d, 7.2)	18.2
5		123.1	12	1.34 (d, 6.7)	9.4
6		183.8	13	2.02 (s)	18.5
7		100.3	14		177.2
8		174.5	14-OH	15.86 (s)	

[a]Recorded in $CDCl_3$ at 400 MHz (^1H) and 100 MHz (^{13}C).

(5) Neoechinulin B

Noechinulin B was isolated as yellow crystals with a molecular composition of $C_{19}H_{19}N_3O_2$ from the LREIMS (Fig. 49) and ^{13}C NMR data (Fig. 51). The twelve unsaturated degrees by molecular formula implied that it contained two carbonyl groups, six double bonds, and three rings (Chart 12).

In the ^1H NMR spectrum (Fig. 50), three protons were exchanged by D_2O, suggesting that it has one aromatic amine proton [δ 7.66 (H-1)], two amide protons [δ 8.35 (H-11), 8.63 (H-14)].

The ^1H and ^{13}C NMR spectra of noechinulin B showed signals ascribable to methene substituted diketopiperazine [δ 8.35 (1H, s, H-11), 8.63 (1H, s, H-14), 5.61,

4.95 (2H, d, J = 1.0 Hz, H_2-20), 124.4 (C-9), 155.8 (C-10), 60.4 (C-12), 157.5 (C-13), 102.1 (C-20)], a bisubstituted indole [δ 7.66 (H-1), 7.32 (H-4), 7.22 (H-5), 7.18 (H-6), 7.37 (H-7), 144.1 (C-2), 102.9 (C-3), 125.8 (C-3a), 118.9 (C-4), 121.3 (C-5), 122.5 (C-6), 111.3 (C-7), 134.2 (C-7a)], an isopentenyl [δ 6.07 (H-16), 5.23, 5.26 (H_2-17), 1.54, 1.54 (CH_3-18/19), 39.2 (C-15),144.2 (C-16), 113.5 (C-17), 27.3 (C-18), 27.4 (C-19)], and a trisubstituted double bond [δ 7.28 (H-8), 113.1 (C-8), 124.4 (C-9)] (Table 12).

The stereochemistry of neoechinulin B has been elucidated from detailed comparison of the data for neoechinulin B with those reported in the literature[42] (Chart. 12).

Neoechinulin B exhibited a significant radical scavenging activity against DPPH with IC_{50} values of 26.0 μM, which is similar to positive control, ascorbic acid (IC_{50}, 20 μM), and also exhibited an ultraviolet-A (UV-A) protecting activity with ED_{50} value of 165 μM.

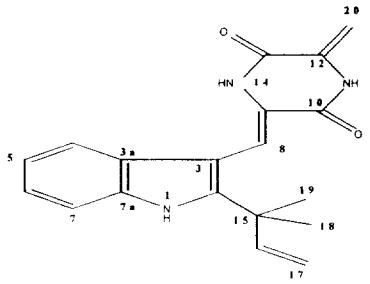


Chart 12. Chemical structure of neoechinulin B.

Table 12. Tabulated NMR data for neoechinulin B[a]

Carbon no.	δ_H (mult, J)	δ_C (mult)	Carbon no.	δ_H (mult, J)	δc (mult)
1	7.66 (s)		10		155.8
2		144.1	11	8.35 (s)	
3		102.9	12		60.4
3a		125.8	13		157.5
4	7.32 (d, 7.6)	118.9	14	8.63 (s)	
5	7.22 (dd, 7.6, 7.3)	121.3	15		39.2
6	7.18 (dd, 7.3, 6.9)	122.5	16	6.07 (d, 17.2, 10.6)	144.2
7	7.37 (d, 6.9)	111.3	17	5.23 (d, 17.2, 1.0) 5.26 (d, 10.6, 1.0)	113.5
7a		134.2	18	1.54 (s)	27.3
8	7.28 (s)	113.1	19	1.54 (s)	27.4
9		124.4	20	4.95 (d, 1.0) 5.61 (d, 1.0)	102.1

[a] Recorded in $CDCl_3$ at 400 MHz (^1H) and 100 MHz (^{13}C).

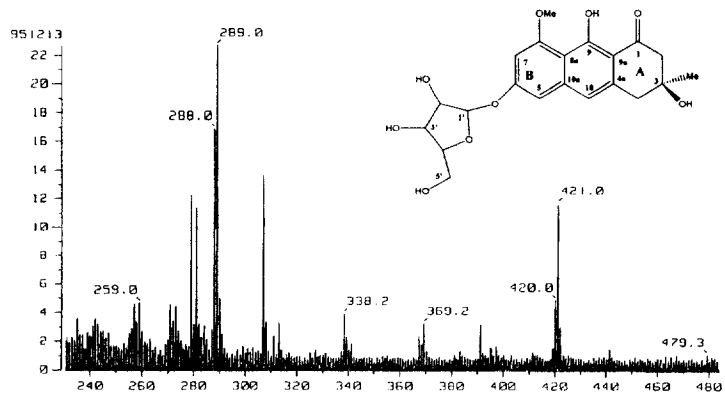


Fig. 31. LREIMS spectrum of asperflavin ribofuranoside.

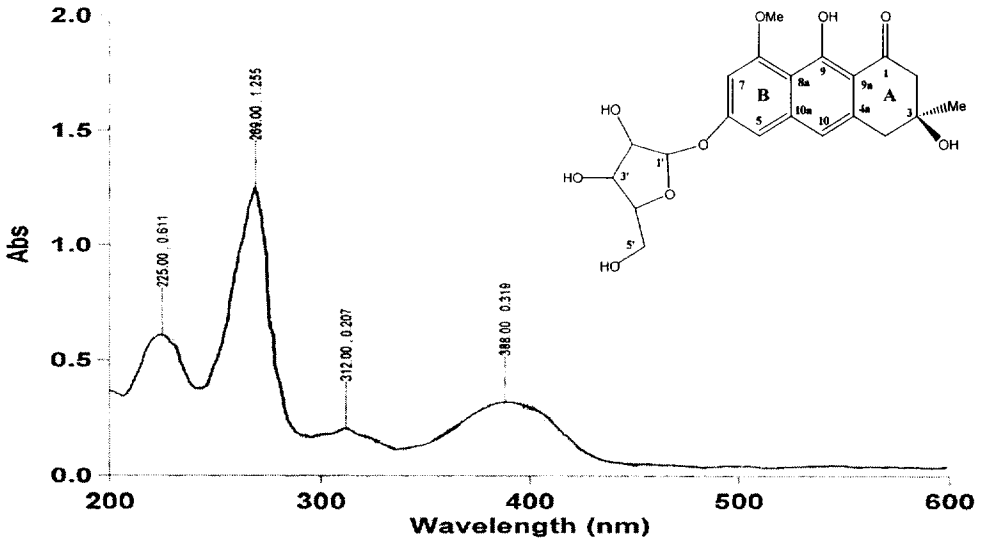


Fig. 32. UV spectrum of asperflavin ribofuranoside.

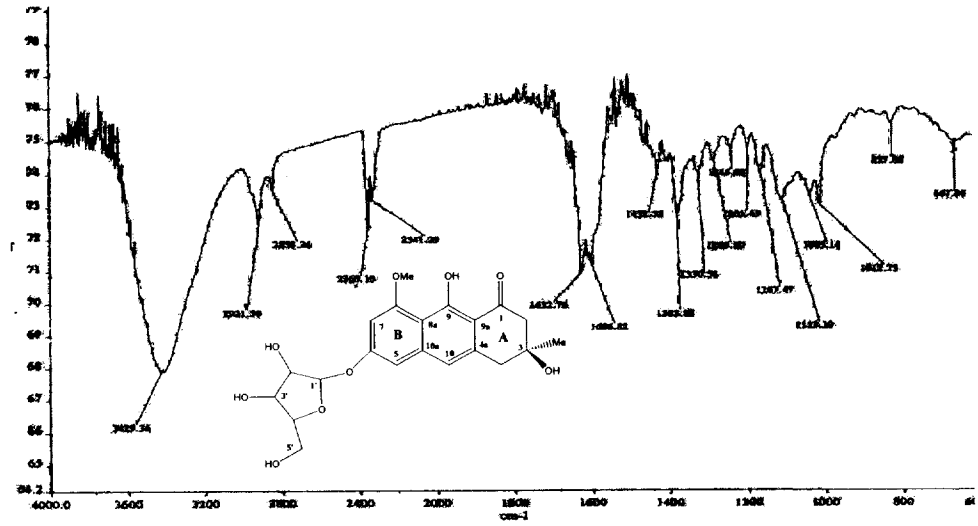


Fig. 33. IR spectrum of asperflavin ribofuranoside.

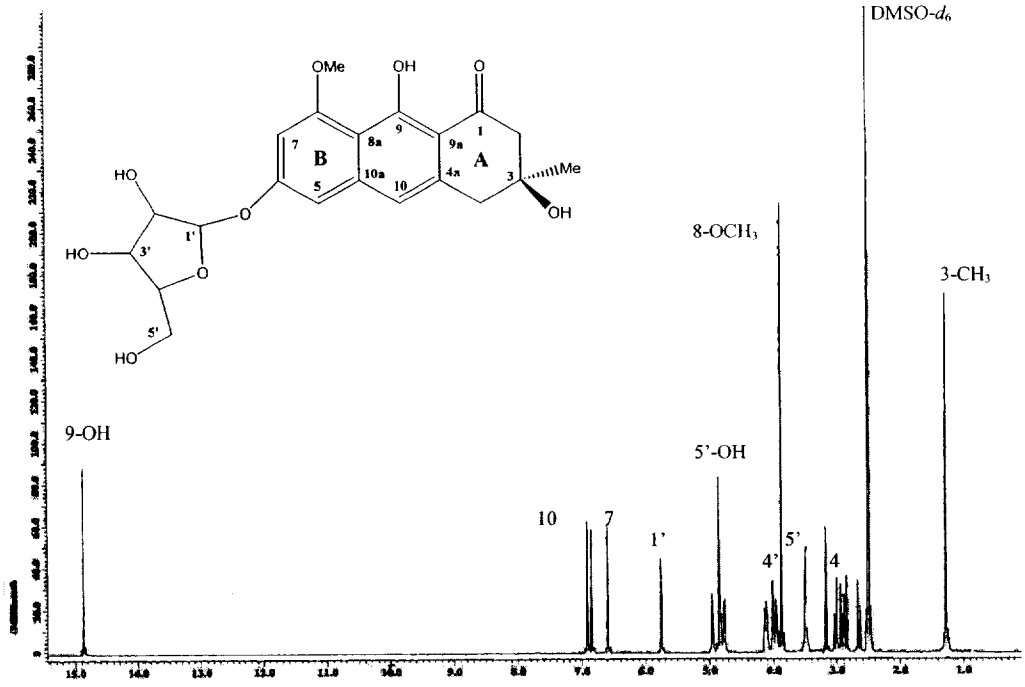


Fig. 34. ^{1}H NMR (DMSO-d_6) spectrum of asperflavin ribofuranoside.

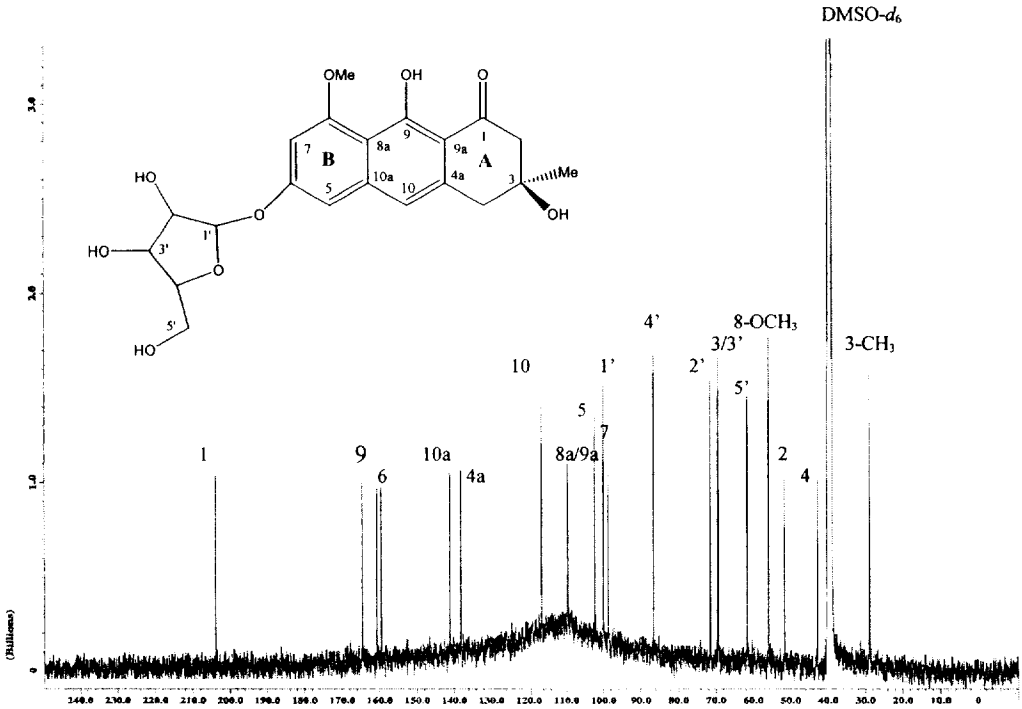


Fig. 35. ^{13}C NMR (DMSO-d_6) spectrum of asperflavin ribofuranoside.

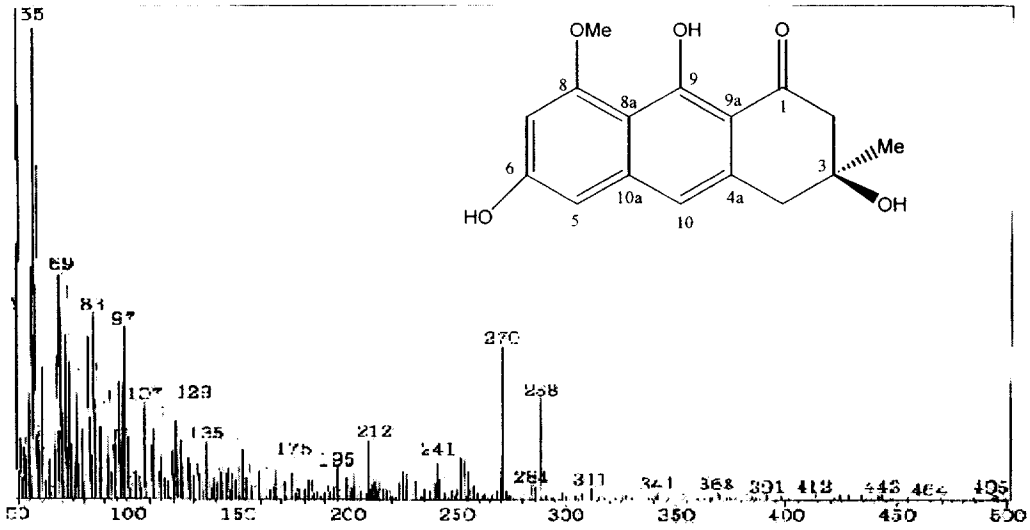


Fig. 36. LREIMS spectrum of Asperflavin.

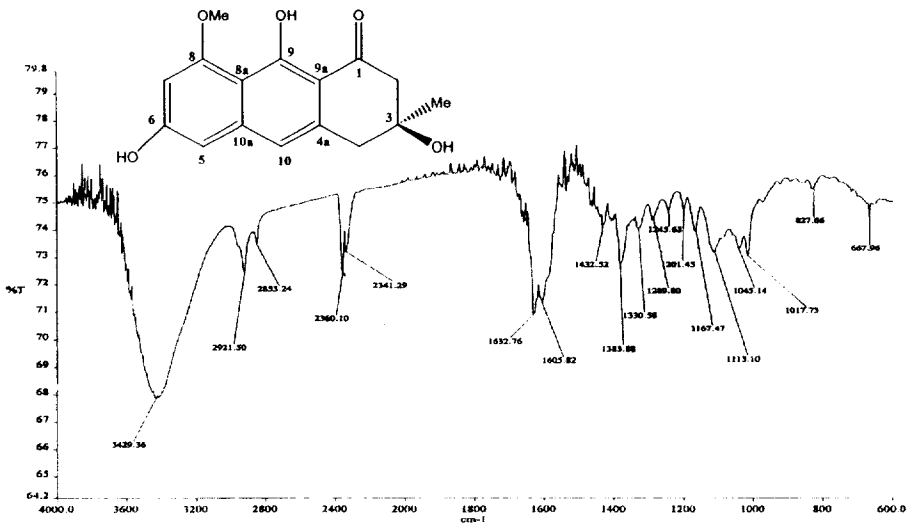


Fig. 37. IR spectrum of Asperflavin.

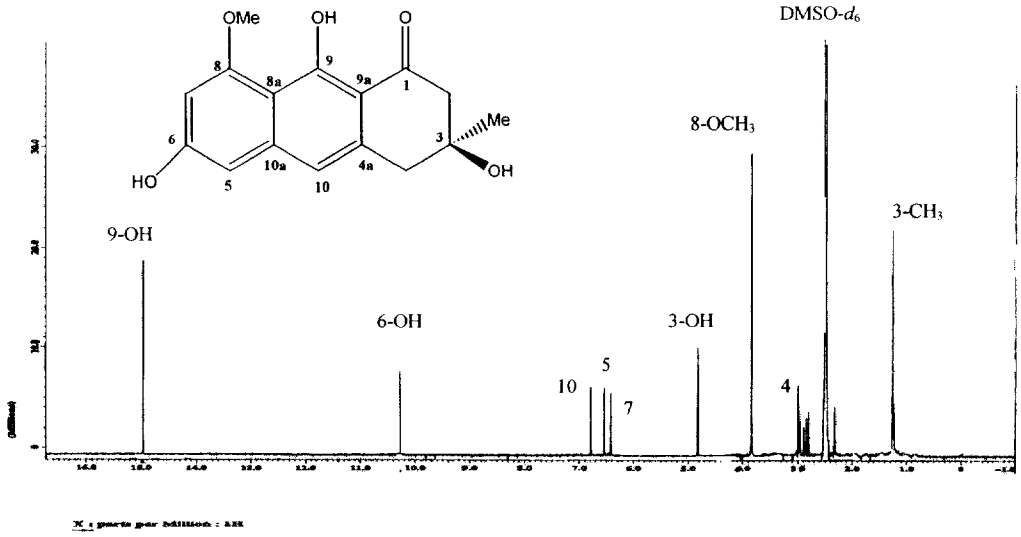


Fig. 38. ^{1}H NMR (DMSO-d_6) spectrum of Asperflavin.

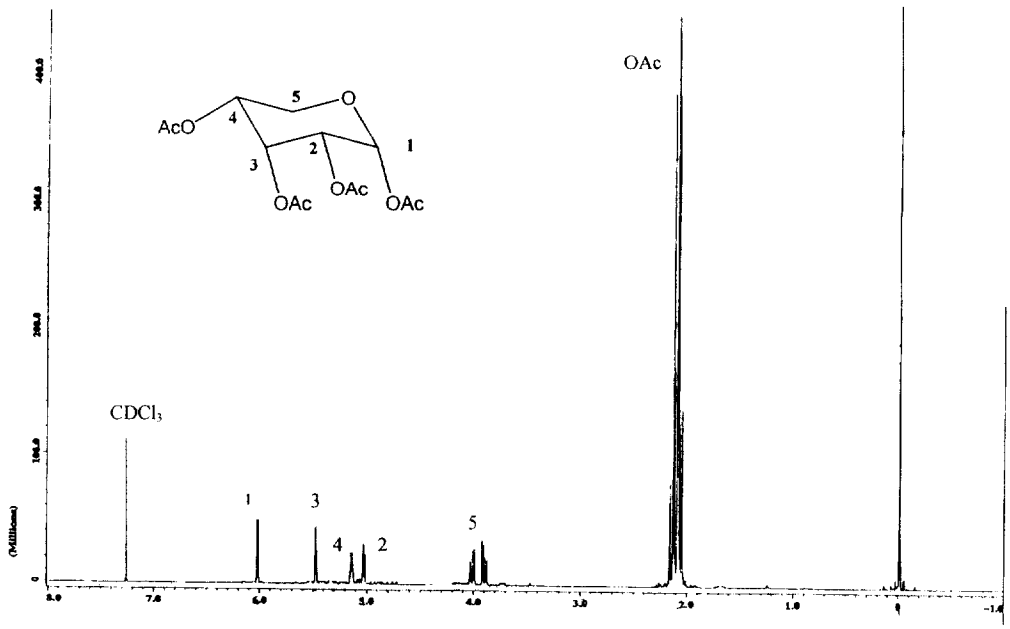


Fig. 39. ^{1}H NMR ($CDCl_3$) spectrum of D-ribopyranose tetraacetate[a].

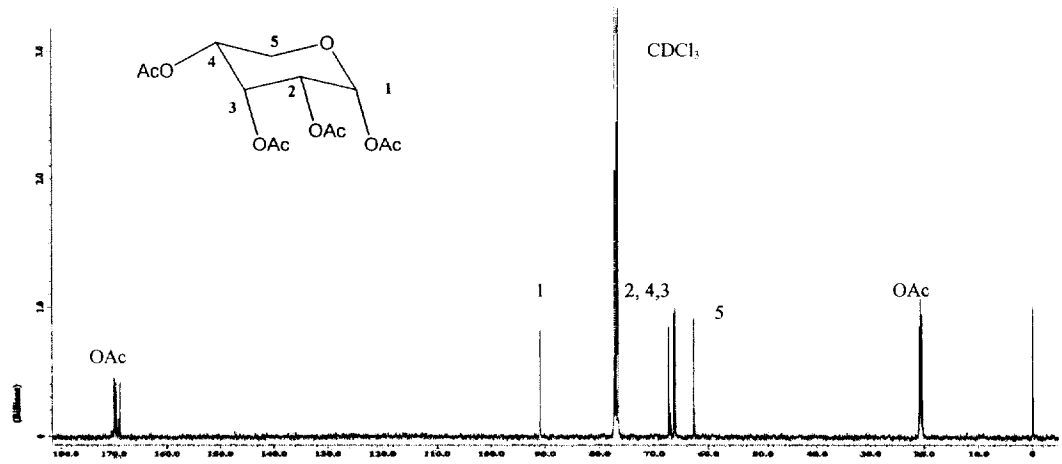


Fig. 40. ^{13}C NMR ($CDCl_3$) spectrum of D-ribopyranose tetraacetate[a].

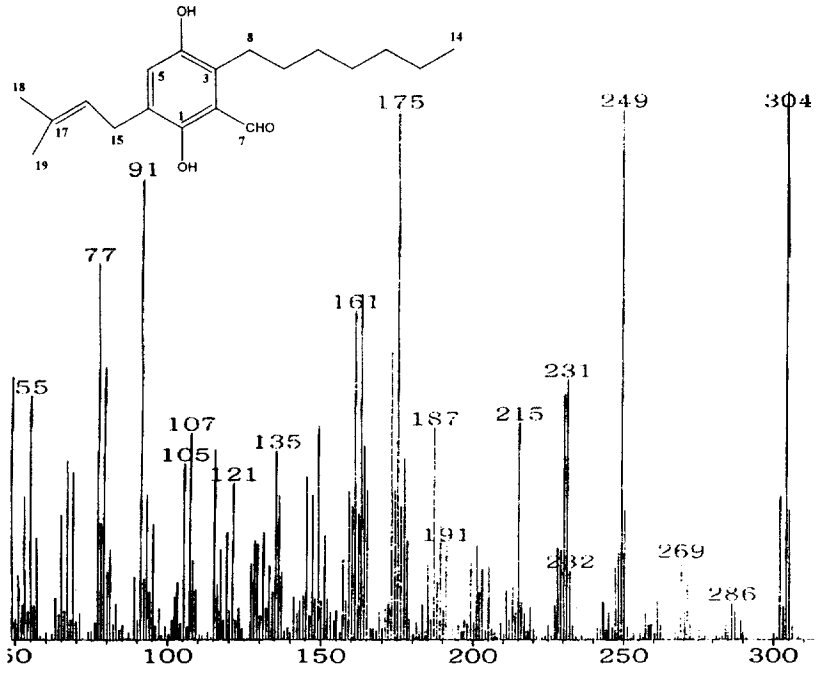


Fig. 41. LREIMS spectrum of flavoglaucin.

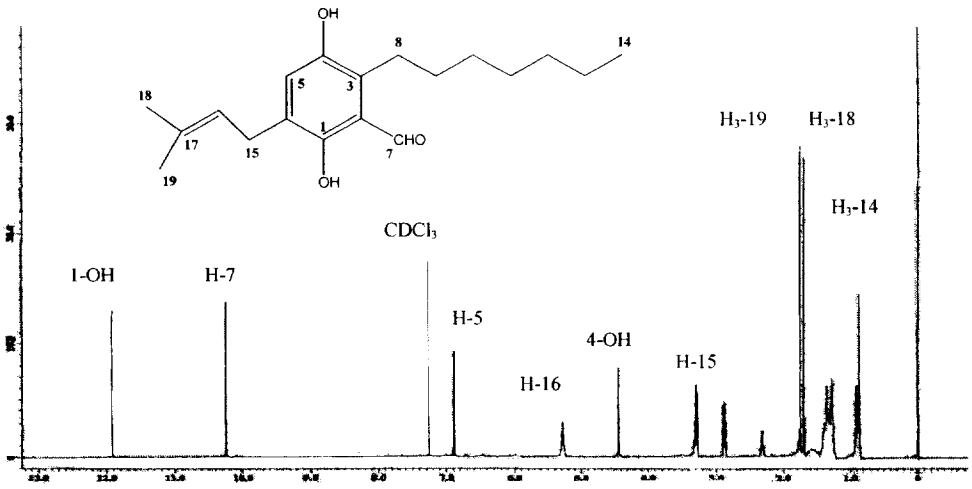


Fig. 42. ^{1}H NMR ($CDCl_3$) spectrum of flavoglaucin.

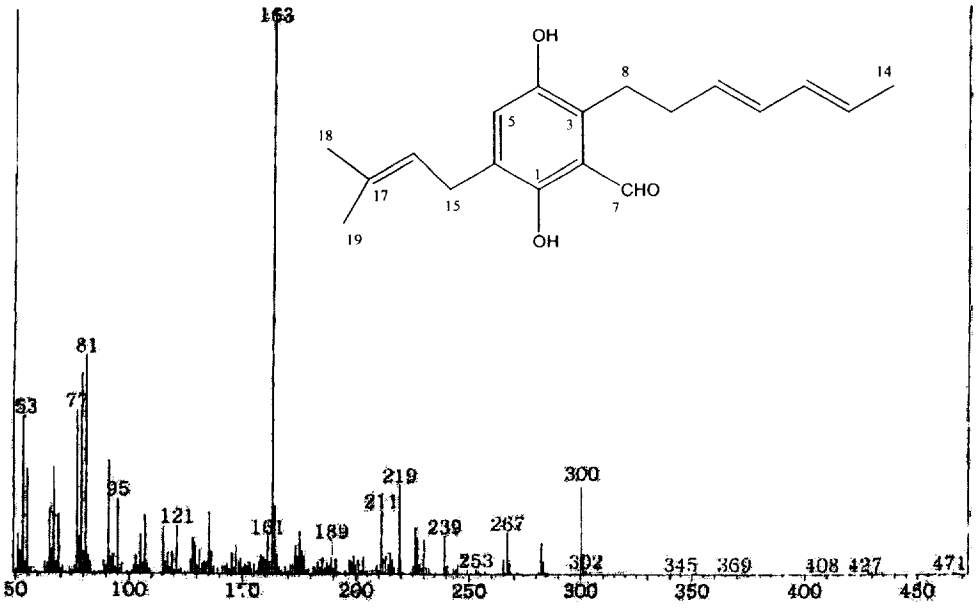


Fig. 43. LREIMS spectrum of isodihydroauroglaucin.

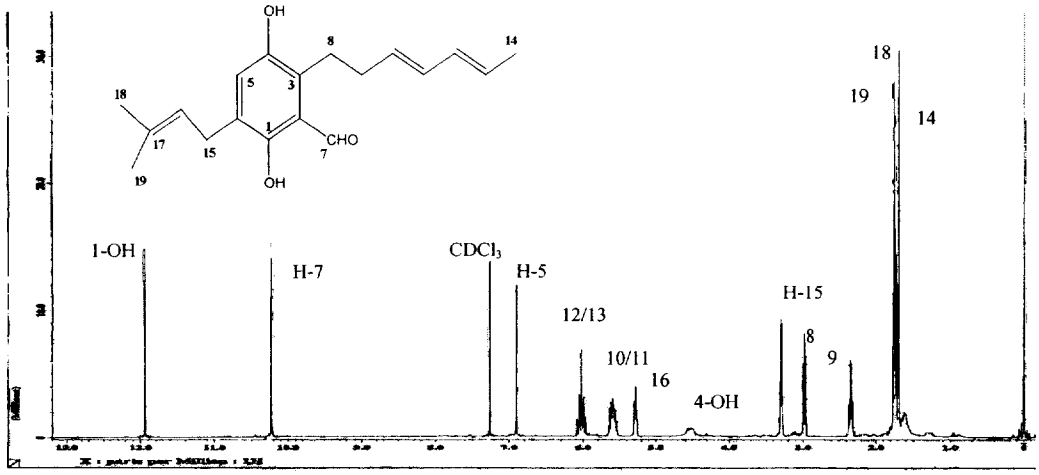


Fig. 44. ^{1}H NMR ($CDCl_3$) spectrum of isodihyroauroglaucin.

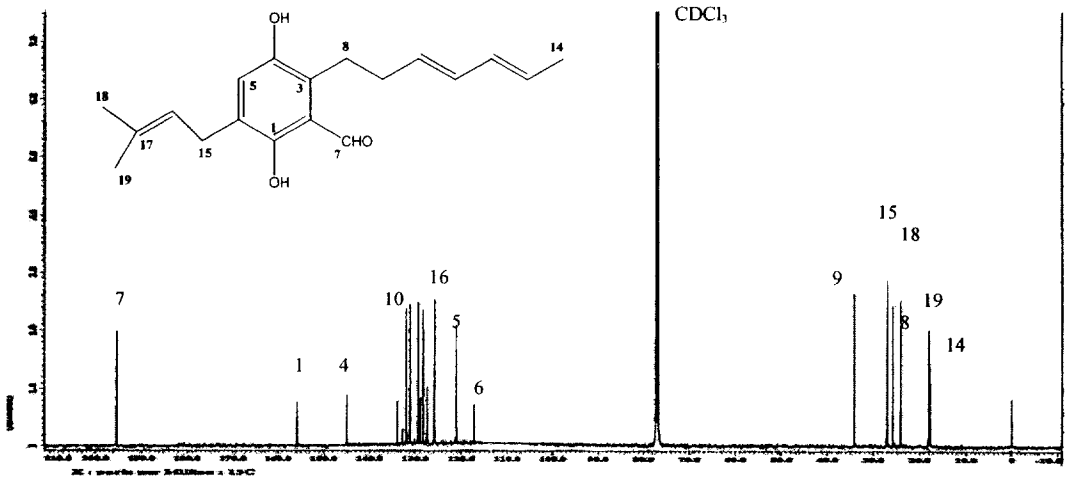


Fig. 45. ^{13}C NMR ($CDCl_3$) spectrum of isodihydroauroglaucin.

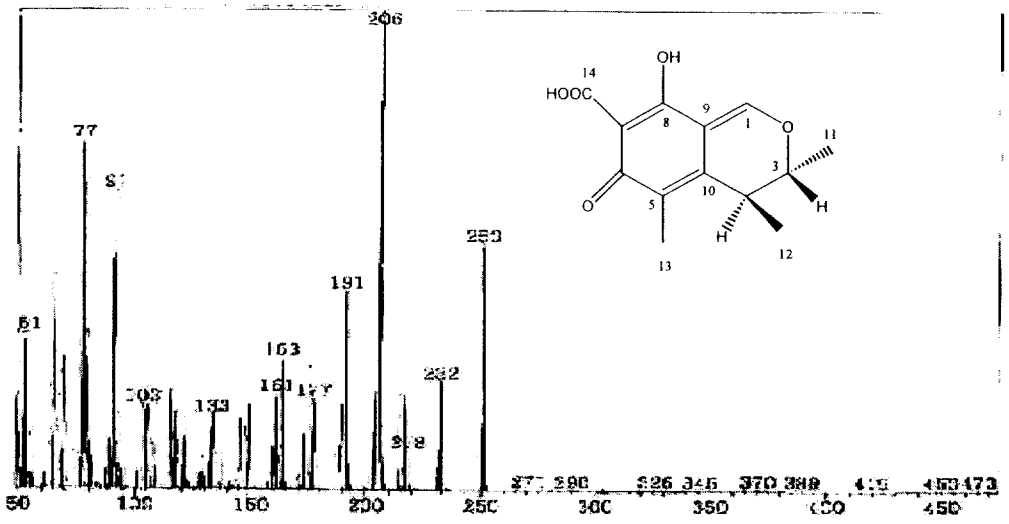


Fig. 46. LREIMS spectrum of citrinin.

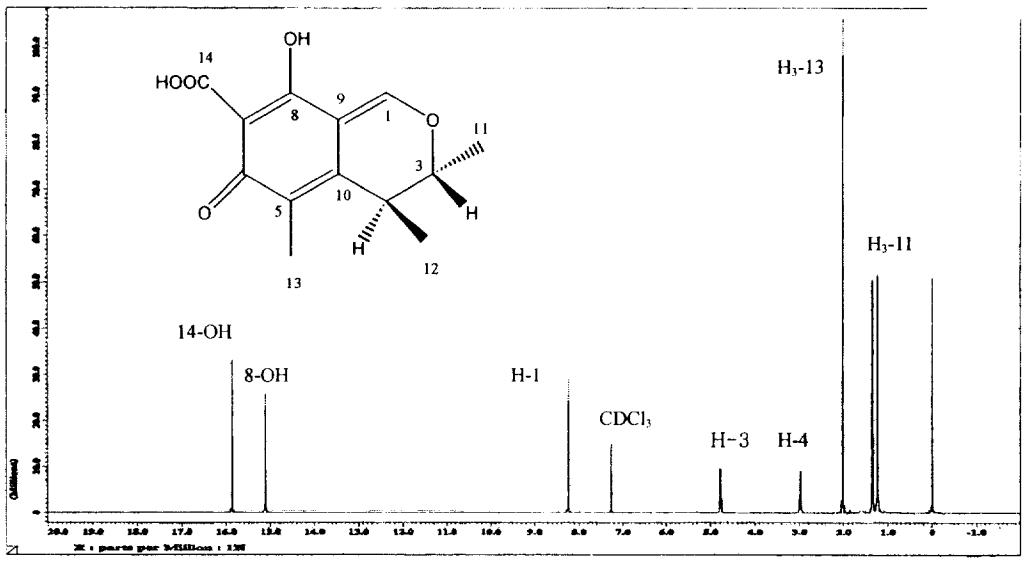


Fig. 47. ^{1}H NMR ($CDCl_3$) spectrum of citrinin.

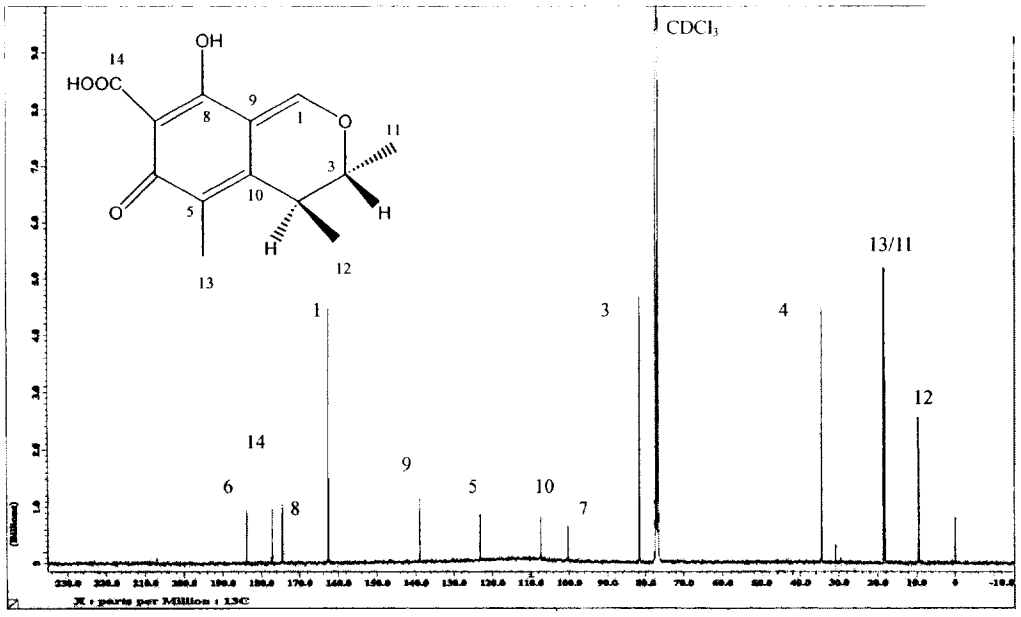


Fig. 48. ^{13}C NMR ($CDCl_3$) spectrum of citrinin.

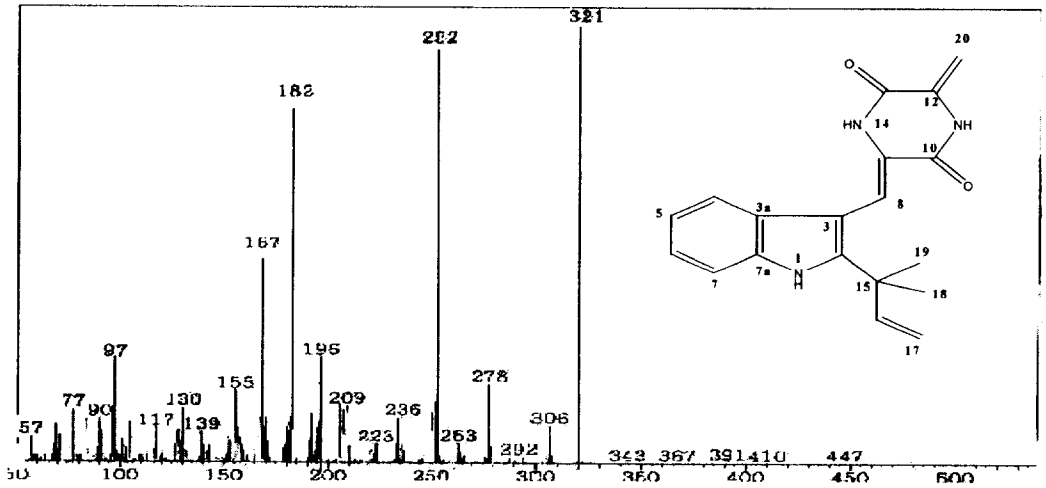


Fig. 49. LREIMS spectrum of neoechinulin B.

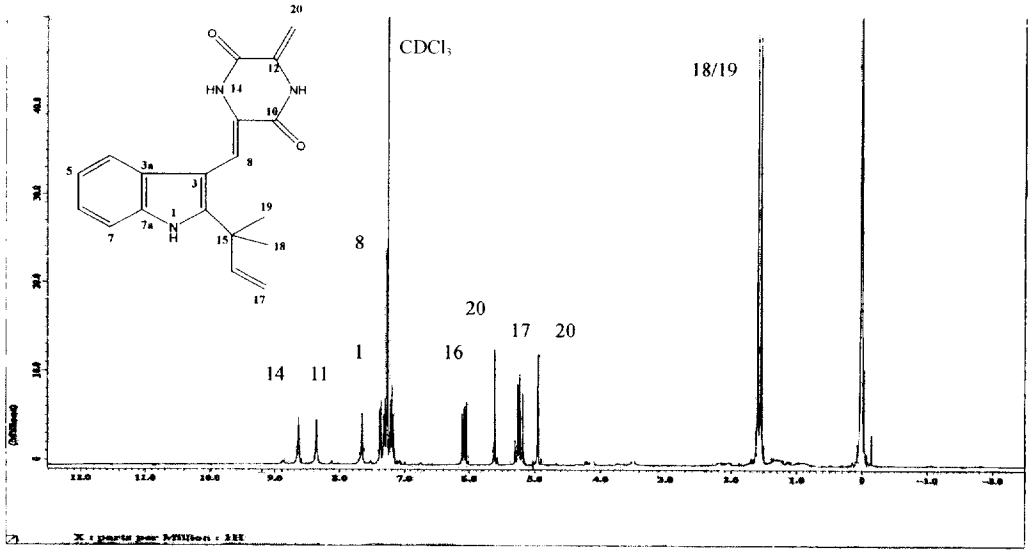


Fig. 50. ^{1}H NMR ($CDCl_3$) spectrum of neoechinulin B.

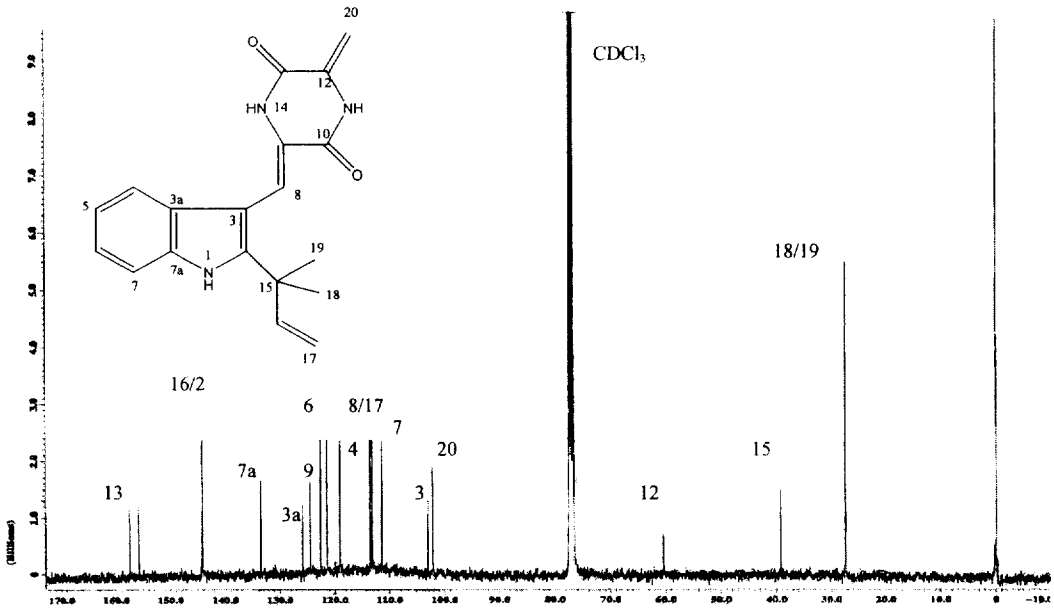


Fig. 51. ^{13}C NMR ($CDCl_3$) spectrum of neoechinulin B.

3) *Aspergillus* sp. (MFA292)

(1) gentisyl alcohol

Gentisyl alcohol was isolated as a brown solid, the LREIMS showed ion fragments at *m/z* 140 $[M]^+$ (52), 122 (100), 94 (62), 65 (42), 44 (46) (Fig. 52). The molecular formula was determined as $C_7H_8O_3$ by LREIMS and ^{13}C NMR spectroscopic data (Fig. 54). The four unsaturated degrees by molecular formula implied that it contained one benzene ring.

In the ^1H NMR spectrum (Fig. 53), three protons were exchanged by D_2O, suggesting that it has three hydroxyl protons [δ 8.53 (2-OH), 8.54 (5-OH), 4.89 (7-OH)].

The ^1H and ^{13}C NMR spectra of gentisyl alcohol showed signals ascribable to one hydroxymethyl [δ_H 4.39 (2H, d, J = 5.6 Hz, H_2-7), δ_C 58.3 (C-7)] and 1, 2, 5-trisubstituted benzene [δ_C 129.3 (C-1), 146.4 (C-2), 149.7 (C-5)], and three aromaic methine groups [δ_H 6.53 (1H, d, J = 8.5 Hz, H-3), δ_C 115.1 (C-3); δ_H 6.42 (1H, dd, J = 8.5, 3.0 Hz, H-4), δ_C 113.2 (C-4); δ_H 6.73 (1H, d, J = 3.0 Hz, H-6), δ_C 114.0 (C-6)] (Table 13).

The stereochemistry of gentisyl alcohol has been elucidated from detailed comparison of the data for gentisyl alcohol with those reported in the literature[17, 62] (Chart. 13).

Gentisyl alcohol showed the DPPH scavenging activity with IC_{50} value of 10.0 μM, which is more than the positive control L-ascorbic acid (IC_{50} 20 μM); and also exhibited *in vitro* mild antibacterial activity against methicillin resistant *Staphylococcus aureus* (MRSA) and multidrug-resistant *S. aureus* (MDRSA) with MIC value of 12.5 μg/mL, respectively.[17-19, 60]

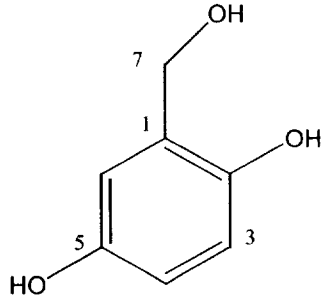


Chart 13. Chemical structure of gentisyl alcohol.

Table 13. Tabulated NMR data for gentisyl alcohol[a]

Carbon no.	δ_H (mult, *J*)	δ_C (mult)	Carbon no.	δ_H (mult, *J*)	δ_C (mult)
1		129.3 (s)	6	6.73 (d, 3.0)	114.0 (d)
2		146.4 (s)	7	4.40 (d, 5.6)	58.3 (t)
3	6.54 (d, 8.5)	115.1 (d)	2-OH	8.53 (s)	
4	6.42 (dd, 8.5, 3.0)	113.2 (d)	5-OH	8.54 (s)	
5		149.7 (s)	7-OH	4.89 (t, 5.6)	

[a] Recorded in DMSO-d_6 at 400 MHz (^{1}H) and 100 MHz (^{13}C).

(2) 3-chlorogentisyl alcohol

3-Chlorogentisyl alcohol was isolated as a red solid. Some characteristic fragmental ion peaks were observed from its LREIMS (Fig 55): *m/z*, 176 (9), 174 (26), 156 (100), 128 (53), 110 (9), 87 (3), 65 (18) and 53 (24). The abundance ratio of two ion peaks at 176 (9) and 174 (26) were about 1:3, which indicating the molecular weight is 174 $[M]^+$ (^{35}Cl) and 176 $[M]^+$ (^{37}Cl), and these characters are identical to that 3-chlorogentisyl alcohol published previously.

On account of NMR (^{1}H &^{13}C) data (Fig.56-57), we can suggest the molecular formula $C_7H_7ClO_3$ with four unsaturated degrees. The UV spectrum of 3-chlorogentisyl alcohol showed two strong absorptions at 217 (3.70) and 297 (3.60) nm, which was deduced to multi-substituted benzene derivative. The IR spectrum of 3-chlorogentisyl alcohol supported the presence of hydroxyl (3434 cm^{-1}). In ^{1}H NMR spectrum, three proton signals were observed: two aromatic protons at δ_H 6.66 (d, J = 5.6 Hz, H-4) and 6.72 (d, J = 5.6 Hz, H-6), and one hydroxymethyl [δ_H 4.60 (2H, s, H_2-7), and 1, 2, 3, 5-tetrasubstituted benzene [δ_c 120.6 (C-1), 141.8 (C-2), 150.6 (C-3) and 132.7 (C-5), however, the signals from two phenolic hydroxyl groups and one aliphatic hydroxyl group didn't appear. The ^{13}C & DEPT NMR spectra of 3-chlorogentisyl alcohol revealed one methylene carbon at δ_c 58.9 (C-7), and aromatic carbons at δ_c 113.5 (C-4) and 113.0 (C-6) (Table 14).

On the basis of the foregoing evidence of 3-chlorogentisyl alcohol, the structural elucidation was carried out, all spectral data were identified with those data published previously (Chart 13).[17]

3-Chlorogentisyl alcohol exhibited exhibited *in vitro* mild antibacterial activity against methicillin resistant *Staphylococcus aureus* (MRSA) and multidrug-resistant *S. aureus* (MDRSA) with MIC value of 50.0 μg/mL, and also showed a significant radical

scavenging activity against DPPH with IC_{50} value of 3.5 μM, also more than positive control L-ascorbic acid (20 μM), [17] as well as revealed the anticancer (HT 29) activity at the value of 42 % (cell viability, % of con., 50 μM).

Table 14. Tabulated NMR data for 3-chlorogentisyl alcohol[a]

Carbon no.	δ_H (mult, *J*)	δ_C (mult)	Carbon no.	δ_H (mult, *J*)	δ_C (mult)
1		120.6 (s)	6	6.72 (d, 5.6)	113.0 (d)
2		141.8 (s)	7	4.60 (s)	58.9 (t)
3		150.6 (s)	2-OH	9.10 (s)	
4	6.66 (d, 5.6)	113.5 (d)	5-OH	8.40 (s)	
5		132.7 (s)	7-OH	5.20 (br.s)	

[a] Recorded in DMSO-d_6 at 400 MHz (^{1}H) and 100 MHz (^{13}C)

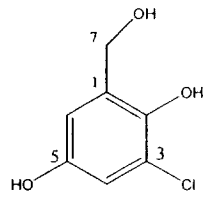


Chart 14. Chemical structure of 3-chlorogentisyl alcohol.

(3) 2-methylhydroquinone

2-Methylhydroquinone was isolated as a colorless needle, which was thought to have a molecular composition of $C_7H_8O_2$ from ^{1}H (Fig.58) and ^{13}C NMR data (Fig.59). 2-Methylhydroquinone showed four unsaturations on account of the molecular formula.

In the ^{1}H NMR spectrum, two protons were exchanged by D_2O, suggesting that 2-methylhydroquinone has two phenolic protons [δ 8.44 (1H, s, 1-OH), 8.48 (1H, s, 4-OH)]. Detailed analyses of the ^{1}H and ^{13}C NMR spectra of 2-methylhydroquinone, including DEPT spectral data, revealed signals ascribable to a methyl substituted hydroquinone [δ 2.02 (3H, s, H_3-7), and 6.45 (1H, d, J = 2.8 Hz, H-3), 6.36 (1H, dd, J = 8.5 , 2.8 Hz, H-5), and 6.53 (1H, d, J = 8.5 Hz, H-6); 147.6 (C-1), 124.3 (C-2), 112.6 (C-3), 149.5 (C-4), 115.0 (C-5), 117.1 (C-6), 16.1 (C-7)] (Table15).

On the basis of the foregoing evidence of 2-methylhydroquinone, the structural elucidation was assigned. All spectral data were identified with those data published previously (Chart 14). [59] 2-Methylhydroquinone exhibited *in vitro* mild antibacterial activity against methicillin resistant *Staphylococcus aureus* (MRSA) and

multidrug-resistant *S. aureus* (MDRSA) with MIC value of 6.25 μg/mL, which was more than the positive control oxacillin (8.0 μg/mL) and also showed a significant radical scavenging activity against DPPH with IC_{50} value of 16.9 μM, also more than positive control L-ascorbic acid (20 μM). [18, 19, 60]

Table 15. Tabulated NMR data for 2-methylhydroquinone [a]

Carbon no.	δ_H (mult, *J*)	δ_C (mult)	Carbon no.	δ_H (mult, *J*)	δ_C (mult)
1		147.6 (s)	6	6.53 (d, 8.5)	117.1 (d)
2		124.3 (s)	7	2.02 (s)	16.1 (q)
3	6.45 (d, 2.8)	112.6 (d)	1-OH	8.44 (s)	
4		149.5 (s)	4-OH	8.48 (s)	
5	6.36 (dd, 8.5, 2.8)	115.0 (d)			

[a] Recorded in DMSO-d_6 at 400 MHz (^{1}H) and 100 MHz (^{13}C).

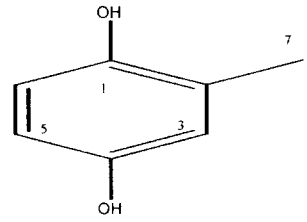


Chart 15. Chemical structure of 2-methylhydroquinone.

(4) (+)-epoxydon

(+)-Epoxydon was isolated as an unstable colorless oil. The molecular formula was supposed to be $C_7H_8O_4$ from its LREIMS (Fig.60) and ^{13}C NMR data (Fig.62). (+)-Epoxydon showed four unsaturated degrees from molecular formula analysis, which implied that (+)-epoxydon contained two rings. In the ^{1}H NMR spectrum (Fig. 61), two proton signals [δ_H 5.78 (1H, d, J = 6.4 Hz, 4-OH), 5.00 (1H, t, J = 5.5 Hz, 7-OH)] were exchanged by D_2O, suggesting that (+)-epoxydon had two hydroxyl groups. The ^{1}H and ^{13}C NMR spectra showed signals attributable to one ketone [δ_C 193.9 (C-1)], one trisubstituted double bond [δ_H 6.39 (1H, dddd, J = 2.2, 2.2, 2.0, 2.0 Hz, H-3), δ_C 133.8 (C-2); 141.4 (C-3)], one oxygenated methylene [δ_H 4.07, 3.96 (1H, H_2-7, each), δc 57.3 (C-7)], and three oxygenated methines [δ 5.78 (H-4), 63.7 (C-4); 3.40 (H-5), 52.9 (H-5); and 3.76 (H-6), 54.0 (C-6)] bearing oxygen. The upfield signals [δ 52.7 (C-5), 54.1(C-6)] in the ^{13}C NMR spectrum were assigned to an epoxy group (Table 16).

(+)-Epoxydon showed spectral data virtually identical to those reported in the literature. [18] Based on the above evidence, the stereochemistry of (+)-epoxydon has been determined (Chart 16).

(+)-Epoxydon exhibited *in vitro* mild antibacterial activity against methicillin resistant *Staphylococcus aureus* (MRSA) and multidrug-resistant *S. aureus* (MDRSA) with MIC value of 12.5 μg/mL, and also showed a significant radical scavenging activity against DPPH with IC_{50} value of 17.9 μM. [18, 19, 61]

(5) (+)-epoxydon monoacetate

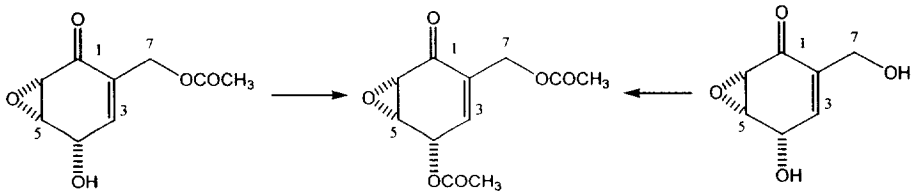
(+)-Epoxydon monoacetate was isolated as a very unstable colorless oil, which was deduced to have the molecular formula $C_9H_{10}O_5$ from its LREIMS and ^{13}C NMR data (Fig. 63, 67). The IR spectrum of (+)-epoxydon monoacetate suggested the presence of phenolic hydroxyl (3392 cm^{-1}), enone (1683, 1032 cm^{-1}) and epoxy (1240, 902, 866 cm^{-1}) groups (Fig. 65). The UV spectrum showed the presence of a β, β-disubstituted enone chromophore [213 nm (logε 3.63), 233 nm (3.70)] (Fig.64). It showed five unsaturated degrees from HREIMS analysis, it was implied that it contains two rings. In the ^1H NMR spectrum (Fig. 66), one proton signal [δ_H 4.72 (m, 4-OH)] was exchanged by D_2O, suggesting that it had one hydroxyl group. The ^1H and ^{13}C NMR spectra (Fig. 67) of (+)-epoxydon monoacetate showed signals attributable to one ketone (δ_C 193.0, C-1), one trisubstituted double bond [(δ_H 6.51, H-3), (δ_C 145.8, C-3), (δ_C 128.7, C-2)], one oxygenated methylene [(δ_H 4.56, 4.63, H_2-7), (δc 60.0, C-7)], and three oxygenated methines [(δ 5.90, H-4), (δ 63.6, C-6)] bearing oxygen. The upfield oxygenated ^{13}C NMR signals (δ 52.7, C-5; δ 54.1, C-6) were assigned to an epoxy group (Table 16).

Interpretation of 2D NMR (COSY, HMQC, HMBC) confirmed the presence of functional groups noted above and led to structure (+)-epoxydon monoacetate (Chart 15). The physicochemistry data of (+)-epoxydon monoacetate were not consistent with the data for the 4-epimer of (+)-epoxydon, which was previously reported as a synthetic intermediate in the synthesis of (-)-phyllostine. So, we elucidated the stereostructure of (+)- epoxydon monoacetate.

The spectral composition with the reported previously,[19] confirmed the presence of functional groups noted above and led to structure epoxydon monoacetate.

The relative stereochemistry of it was determined by the comparision of $[\alpha]_D^{20} = +70^\circ$ of its acetylation product with that of (+)-epoxydon diacetate, $[\alpha]_D^{20} = +67^\circ$. On the basis of the above evidence, the structure of (+)-epoxydon monoacetate was determined to be (4*S*,

5*S*, 6*S*)-5, 6-epoxy-4-hydroxy-2-acetoxy methylcyclohex-2-en-1-one (Scheme 13).



(+)-epoxydon monoacetate, $[\alpha]_D^{20} = +70°$ **(+)-epoxydon diacetate** $[\alpha]_D^{20} = +67°$

(+)-epoxydon, $[\alpha]_D^{20} = +72°$

Scheme 13. Stereochemistry of (+)-epoxydon, (+)-epoxydon monoacetate, and (+)-epoxydon diacetate.

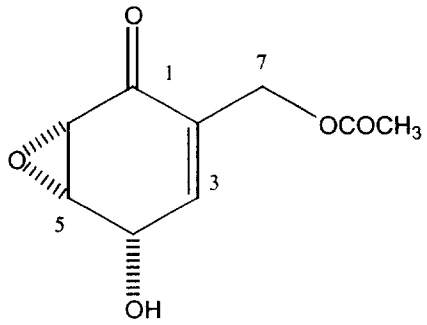
Based on the above evidence, (+)-epoxydon monoacetate showed spectral data virtually identical to those reported in the literature (Chart 15). The stereochemistry of (+)-epoxydon monoacetate has been determined previously. [19]

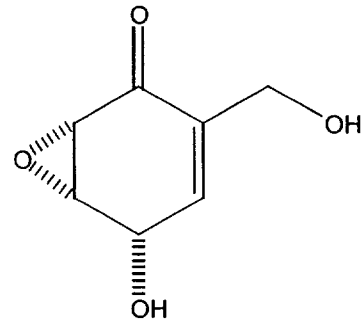
The polyoxygenated cyclohexenone derivatives known as gabosines have been previously reported from natural sources. Since they are biologically interesting for their phytotoxic, antibiotic, antitumor, and antigermination activities, thet have been used as targets or intermediates for the synthesis of biologically active compounds.[61]

Table 16. Tabulated NMR data for (+)-epoxydon and (+)-epoxydon monoacetate[a]

Carbon	(+)-epoxydon monoacetate		(+)-epoxydon	
No.	δ_H (mult, *J*)	δ_C	δ_H (mult, *J*)	δ_C
1		193.0 (s)		193.9 (s)
2		128.7 (s)		133.8 (s)
3	6.51(dddd, 1.3, 1.3, 1.0, 1.0)	145.8 (d)	6.39 (dddd, 2.2, 2.2, 2.0, 2.0)	141.4 (d)
4	5.90 (d, 6.7)	63.6 (d)	5.78 (d, 6.4)	63.7 (d)
5	3.46 (d, 4.2)	52.7 (d)	3.40 (d, 4.2)	52.9 (d)
6	3.78 (ddd, 2.6, 1.3, 1.3)	54.1 (d)	3.76 (ddd, 4.2, 2.7, 2.5)	54.0 (d)
7	4.56(ddd, 12.9, 1.3, 1.0)	60.0 (t)	3.96 (dddd, 15.3, 5.5, 2.0, 1.8)	57.3 (t)
	4.63 (ddd, 13.1, 1.6, 1.3)		4.07 (dddd, 15.3, 5.5, 2.2, 2.0)	
4-OH	4.72 (m)		4.70 (ddddd, 6.2, 3.1, 3.1, 2.5, 2.5)	
7-OH			5.00 (t, 5.5)	
7-OAc	2.02 (s)	169.8/20.5 (s/q)		

* Recorded in DMSO-d_6 at 400 MHz (^{1}H) & 100 MHz (^{13}C).





(+)-epoxydon monoacetate **(+)-epoxydon**

Chart 16. Chemical structures of (+)-epoxydon and (+)-epoxydon monoacetate.

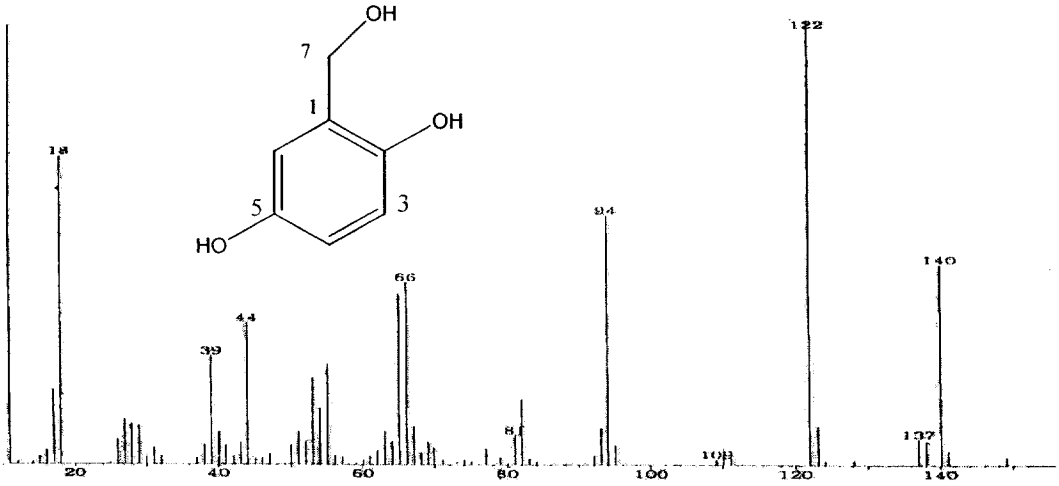


Fig. 52. LREIMS spectrum of gentisyl alcohol.

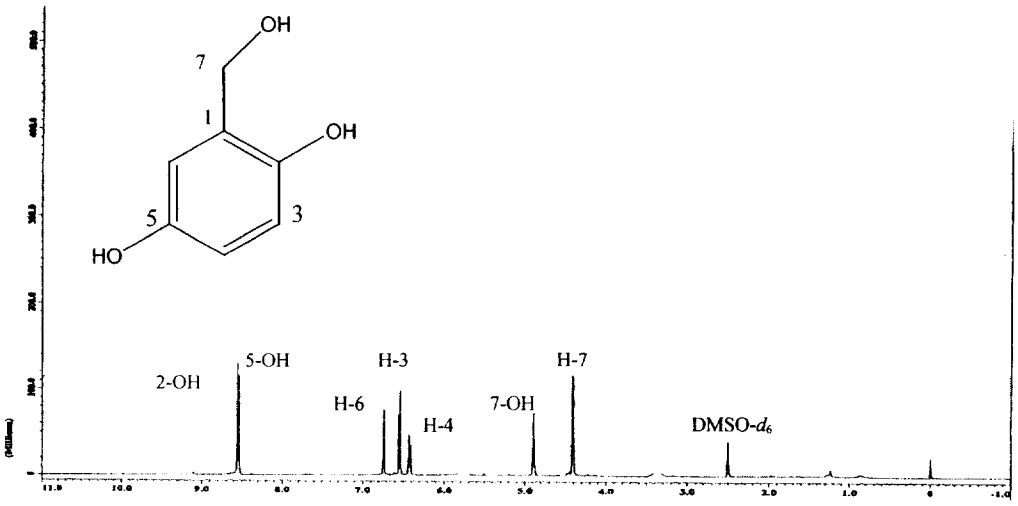


Fig. 53. ^{1}H NMR (DMSO-d_6) spectrum of gentisyl alcohol.

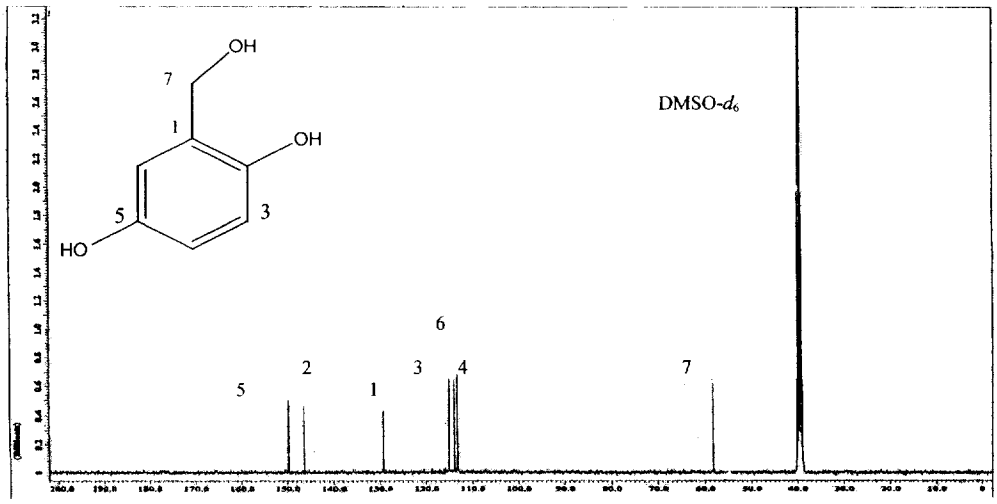


Fig. 54. ^{13}C NMR (DMSO-d_6) spectrum of gentisyl alcohol.

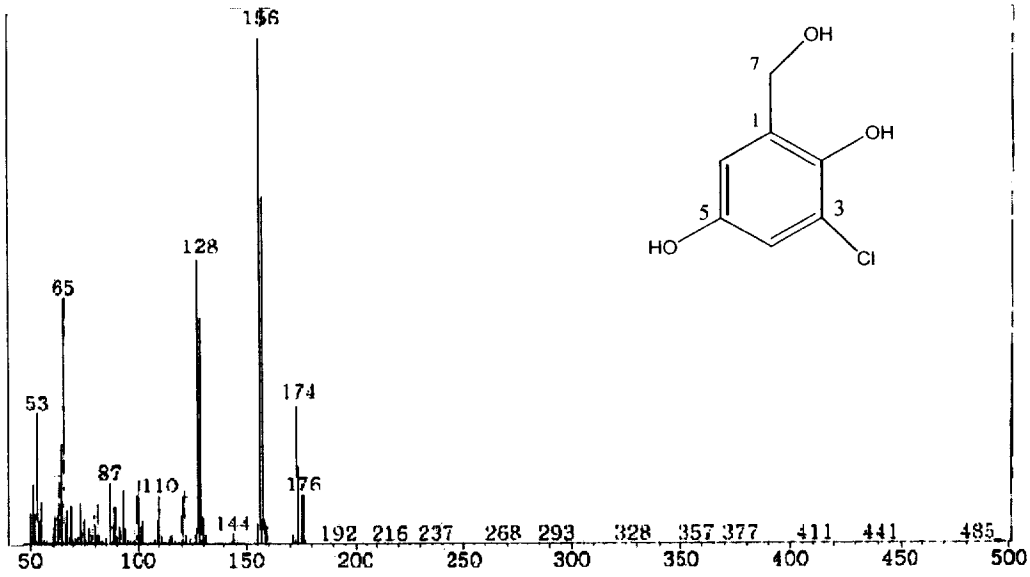


Fig. 55. LREIMS spectrum of 3-chlorogentisyl aldohol.

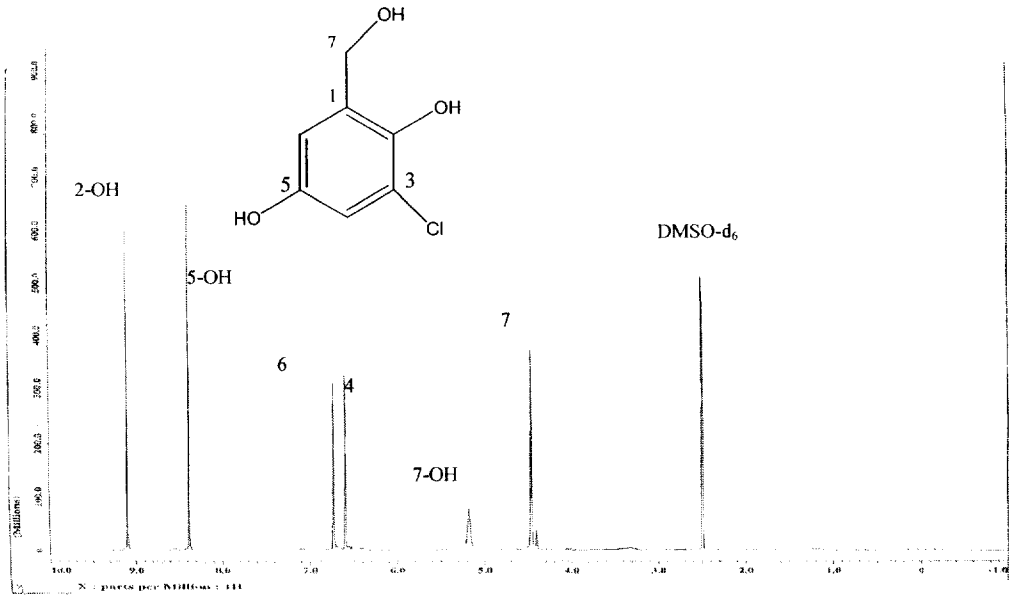


Fig. 56. ^{1}H NMR (DMSO-d_6) spectrum of 3-chlorogentisyl aldohol.

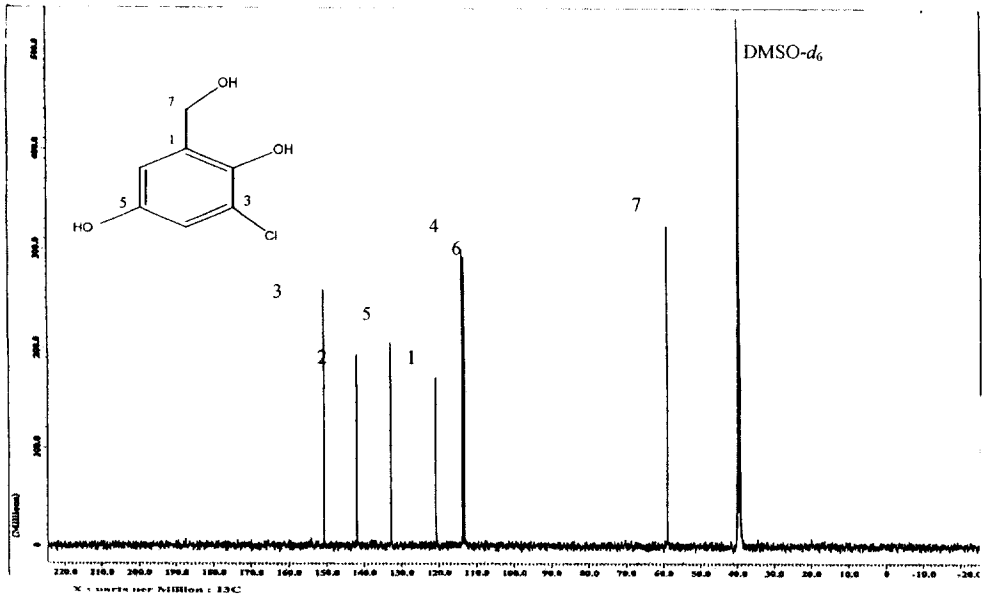


Fig. 57. ^{13}C NMR (DMSO-d_6) spectrum of 3-chlorogentisyl aldohol.

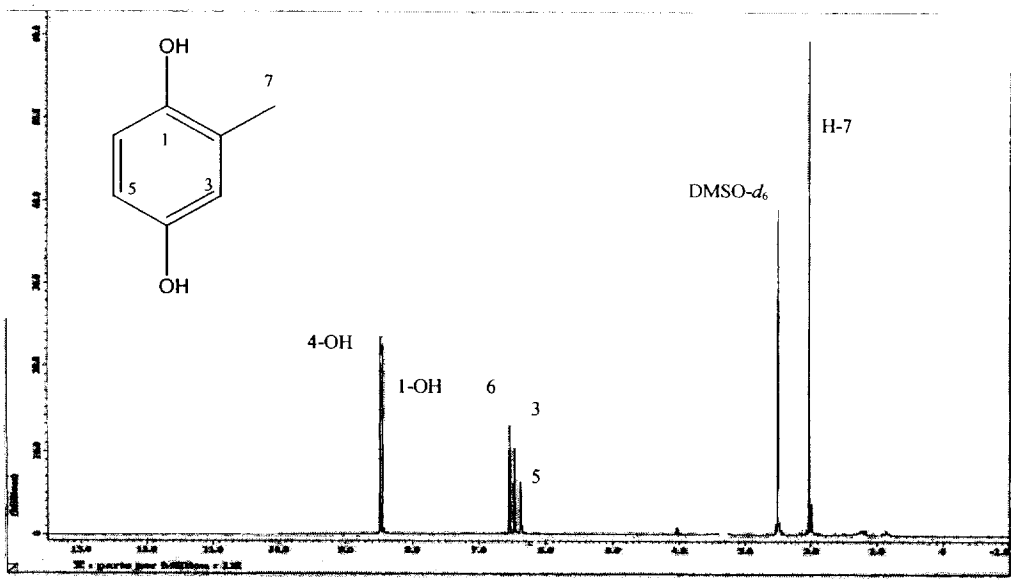


Fig. 58. ^{1}H NMR (DMSO-d_6) spectrum of 2-methylhydroquinone.

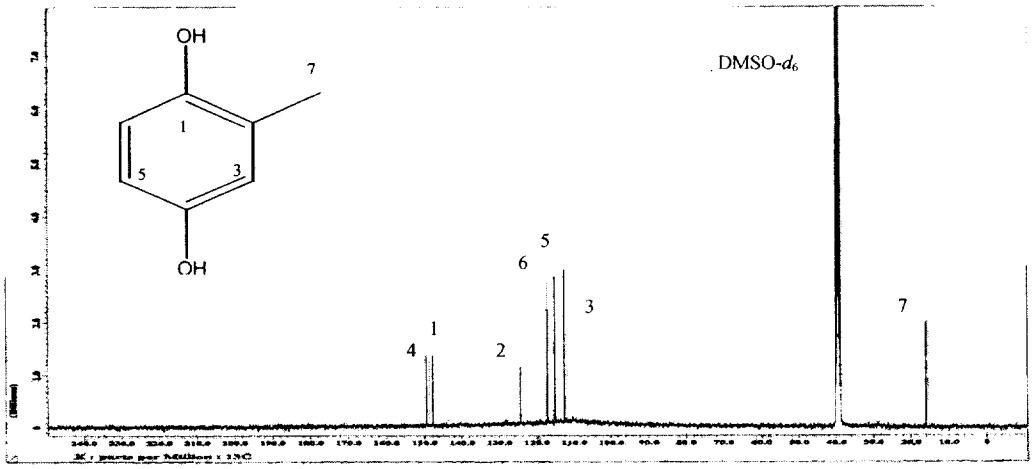


Fig. 59. ^{13}C NMR (DMSO-d_6) spectrum of 2-methylhydroquinone.

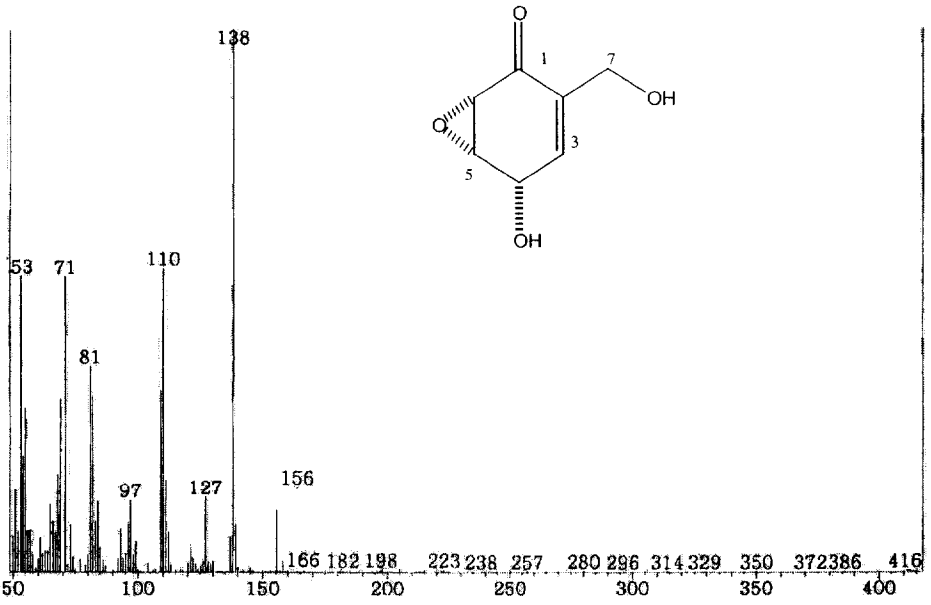


Fig. 60. LREIMS spectrum of (+)-epoxydon.

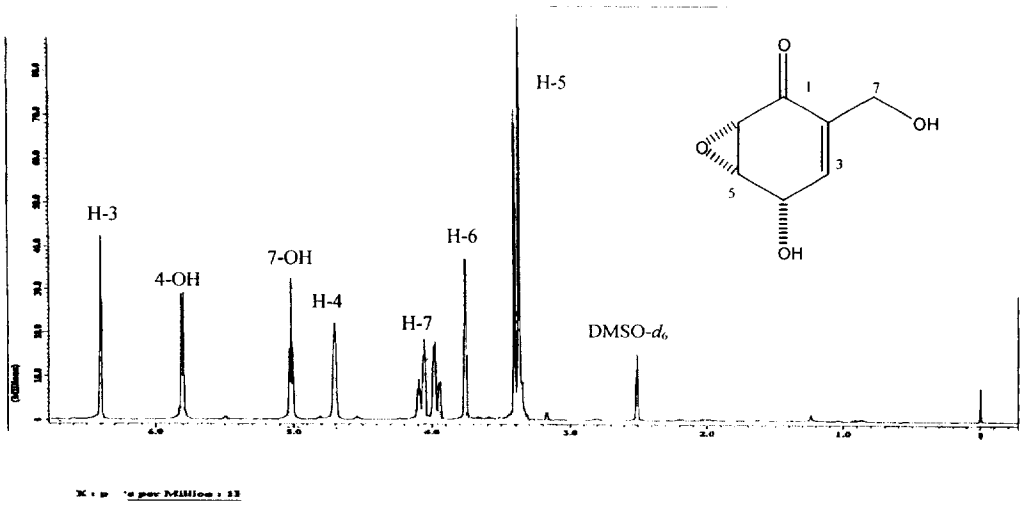


Fig. 61. ^{1}H NMR (DMSO-d_6) spectrum of (+)-epoxydon.

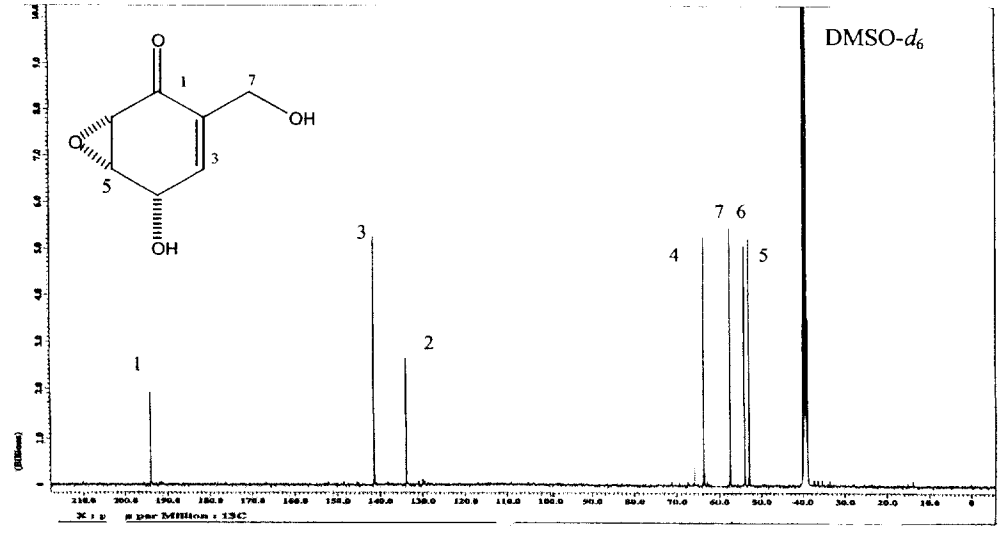


Fig. 62. ^{13}C NMR (DMSO-d_6) spectrum of (+)-epoxydon.

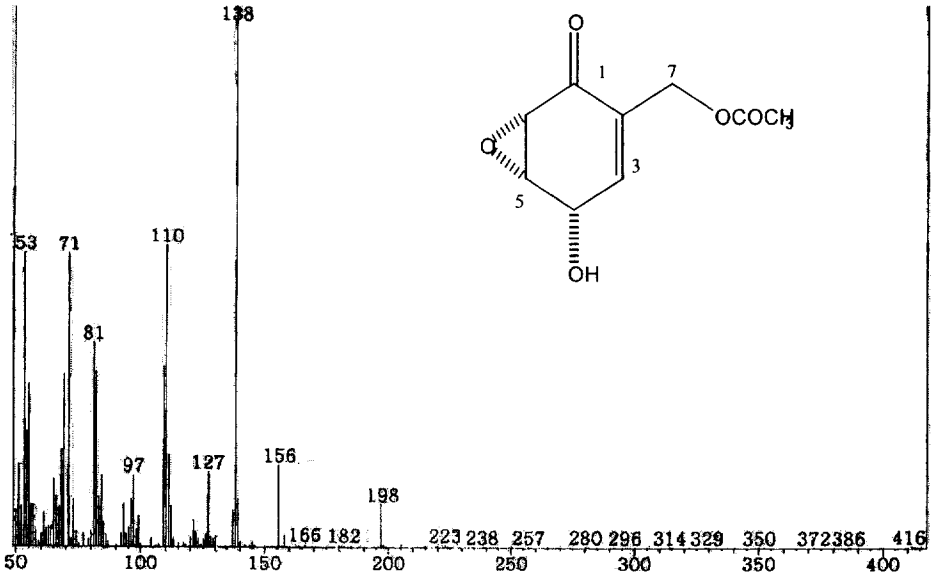


Fig. 63. LREIMS spectrum of (+)-epoxydon monoacetate.

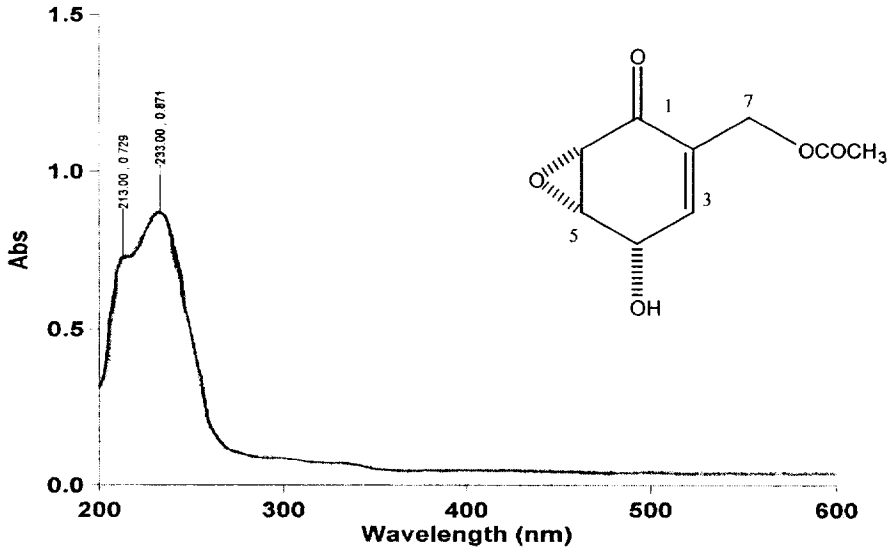


Fig. 64. UV spectrum of (+)-epoxydon monoacetate.

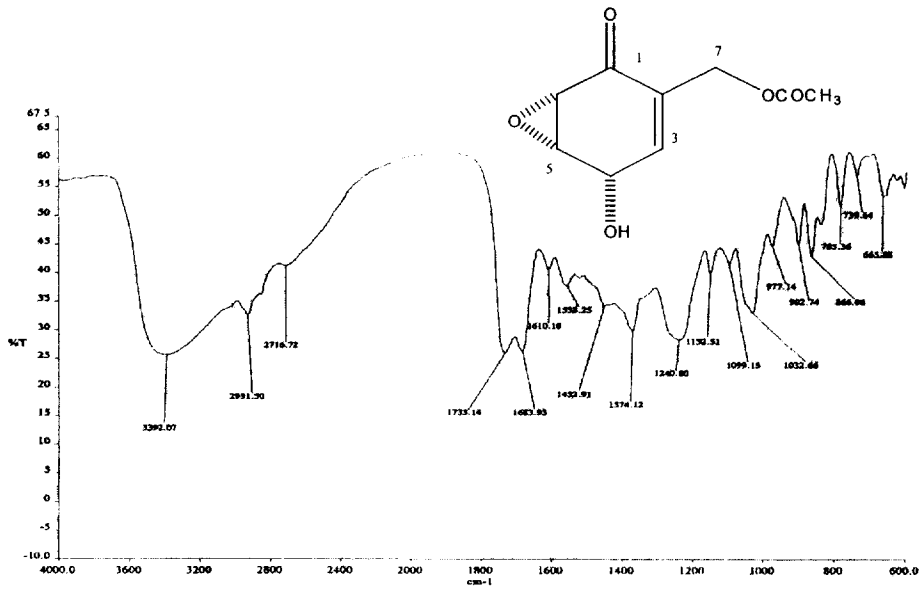


Fig. 65. IR spectrum of (+)-epoxydon monoacetate.

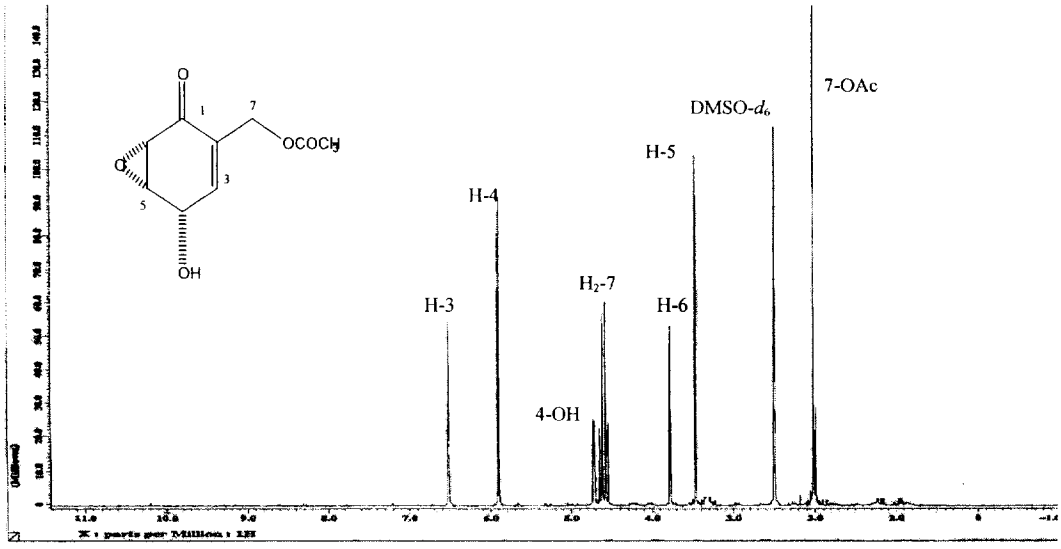


Fig. 66. ^{1}H NMR (DMSO-d_6) spectrum of (+)-epoxydon monoacetate.

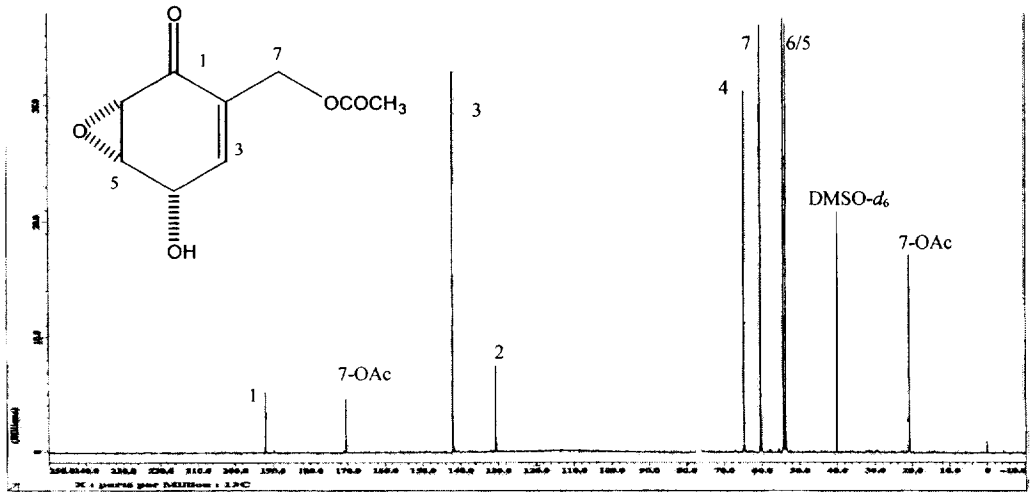


Fig. 67. ^{13}C NMR (DMSO-d_6) spectrum of (+)-epoxydon monoacetate.

4) MFA936 (unidentified strain)

(1) Tryptamine acetate

N_b-acetyltryptamine was isolated as a yellow oil and was found to have an elemental composition $C_{12}H_{14}N_2O$ on the basis of HREIMS (Fig. 63) and ^{13}C NMR method (Fig. 67). The IR spectrum of it revealed absorption bands for amine (3400 cm^{-1}), and amide (1638 cm^{-1}) (Fig.65). The ^{1}H (Fig. 66) and ^{13}C NMR data for tryptamine acetate Fig.66), including results from DEPT, COSY, HMQC, and HMBC experiments, showed indol and ethylamine acetate moieties. These moieties were further supported by UV spectral data [222 (logε 4.0), 283 (2.3) nm] (Fig. 64), and by MS fragment *m/z* 143 $[M\text{-}CH_3CONH_2]^+$. The position of ethylamino acetate group was deduced by HMBC correlations from H-4 to C-3, and H_2-10 to C-2, C-3 and C-9, and by TOCSY correlation between H_2-10 and H-2, H_2-11 and H-12 (Table 17).

Based on all of the foregoing evidence, the structure of N_b-acetyltryptamine was determined to be 2-(3-indiyl) ethylamine acetate (Chart 17). N_b-acetyltryptamine has not previously been found to occur in nature but had been obtained as a biotransformed-metabolite derived from tryptamine by *Streptomyces staurosporeus*.

Tryptamine and its derivatives are widely distributed in animals, plants and fungi. Tryptamine has discovered to be present in several edible fruits, namely, tomato, plum, and eggplant, and also in traces in orange, *N,N*-dimethyltryptamine was first identified as a constituent of the seeds and pods during an attempt to isolate the hallucinogenic principles by certain American Indian tribes. [62]

In later investigations, *N,N*-dimethyltryptamine occurs more widely in nature, and is the simplest of several psychotomimetic activity. [65] and N_b-acetyltryptamine revealed the anticancer (HT 29) activity at the value of 46 % (cell viability, % of con., 50 μM).

On account of the activity of *N,N*-dimethyltryptamine, the physiological activity of N_b-acetyltryptamine in humans is of interest.

Table 17. Tabulated NMR data for tryptamine acetate[a]

Carbon no.	δ_H (mult, *J*)	δc (mult)	HMBC (H to C)
1	8.14 (br.s)		
2	7.04 (d, 2.2)	122.0 (d)	8, 9
3		113.1 (s)	
4	7.60 (d, 8.0)	118.7 (d)	9, 11
5	7.13 (dd, 8.0, 8.0)	119.5 (d)	7, 9
6	7.21 (dd, 8.0, 8.0)	122.2 (d)	4, 8
7	7.38 (d, 8.0)	111.2 (d)	5, 9
8		136.4 (s)	
9		127.3 (s)	
10	2.98 (t, 6.5)	25.3 (t)	2, 3, 9, 11
11	3.60 (dt, 6.5, 6.0)	39.8 (t)	3, 10, 13
12	5.53 (br,s)		
13		170.0 (s)	
14	1.92 (s)	23.4 (q)	11, 13

[a] Recorded in $CDCl_3$ at 400 MHz (^1H) and 100 MHz (^{13}C).

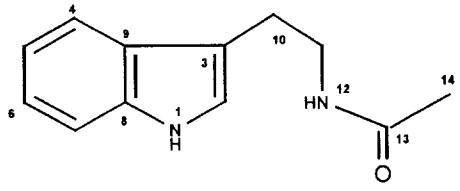


Chart 17. Chemical structure of tryptamine acetate.

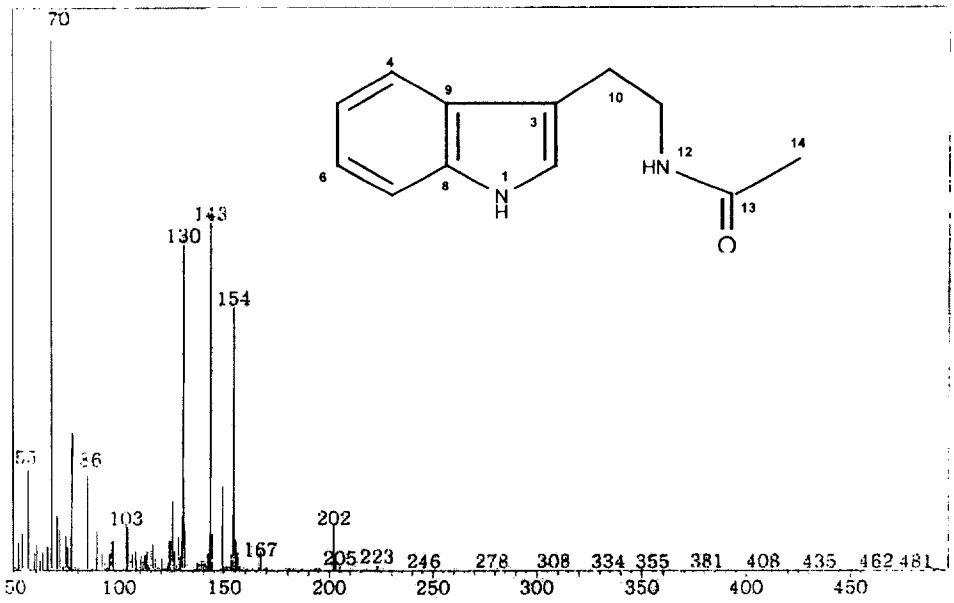


Fig. 63 LREIMS spectrum of tryptamine acetate.

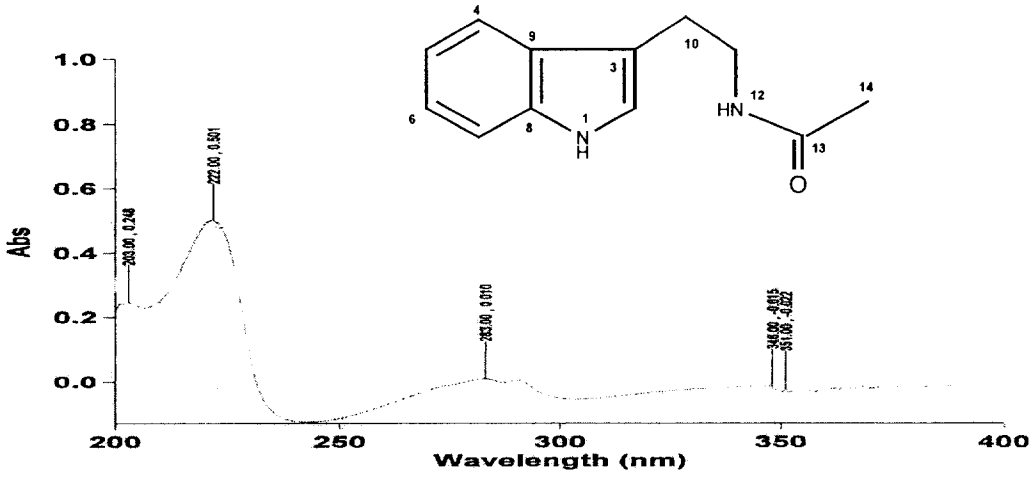


Fig. 64 UV spectrum of tryptamine acetate.

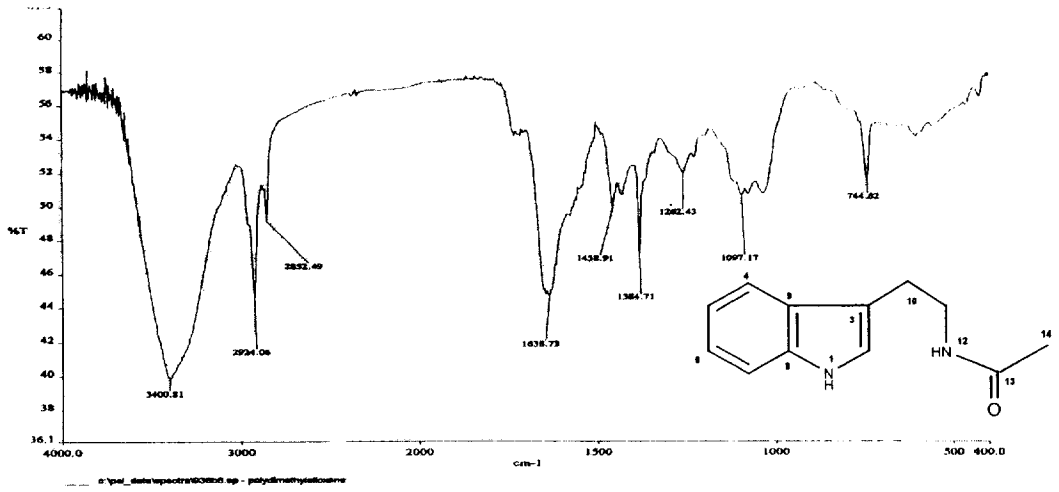


Fig. 65. IR spectrum of tryptamine acetate.

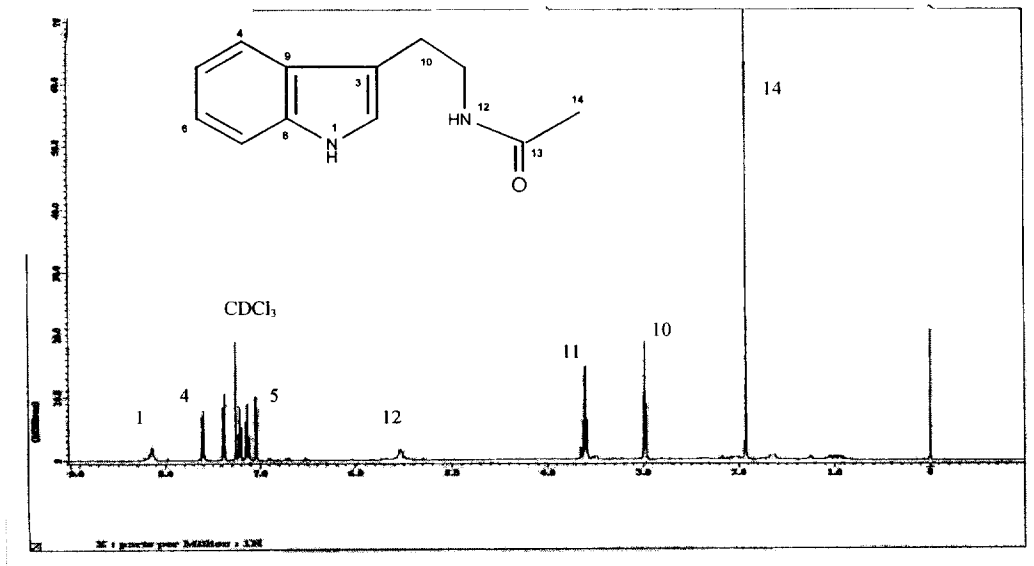


Fig. 66. ^{1}H NMR ($CDCl_3$) spectrum of tryptamine acetate.

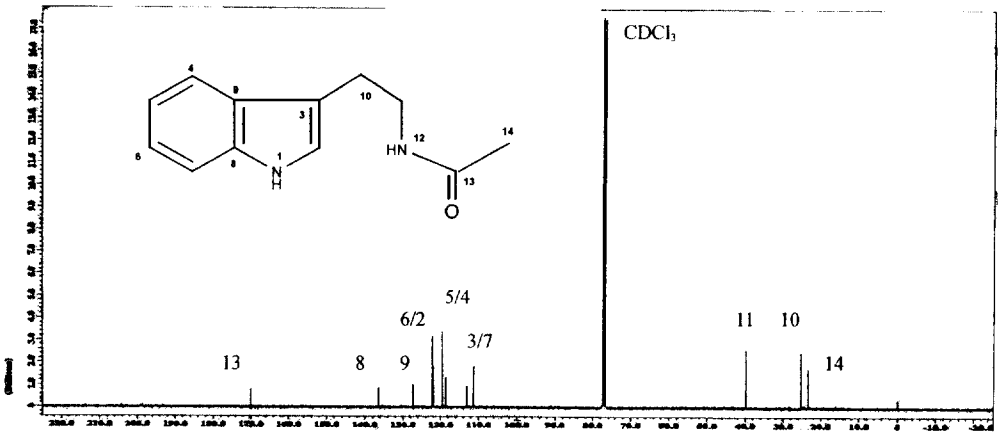


Fig. 67. ^{13}C NMR ($CDCl_3$) spectrum of tryptamine acetate.

5) MFA552 (unidentified strain)

(1) (+)-Brefeldin A

(+)-Brefeldin A was isolated as a white needle which was supposed to have a molecular composition of $C_{16}H_{24}O_6$ from the LREIMS and ^{13}C NMR data (Fig. 68, 70). (+)-Brefeldin A showed five unsaturations on account of the molecular formula. (+)-Brefeldin A implied that it contained one carbonyl, two double bonds and two rings.

In the ^1H NMR spectrum (Fig. 69), two protons were exchanged by D_2O, suggesting that it has two hydroxyl protons [δ 5.10 (1H, d, J = 5.6 Hz, 4-OH), 2.29 (1H, dd, J = 4.8, 4.3 Hz, 7-OH)]. Detailed analyses of the ^1H and ^{13}C NMR spectra of (+)-brefeldin A, including the results from distortionless enhancemen by polarization transfer (DEPT), revealed signals ascribable to a methyl group [δ 1.18 (3H, d, J = 6.4 Hz, H_3-16)], one carbonyl group [δ 165.6 (C-1)], five methylene protons [δ 1.65 (2H, m, H_2-6), 1.80 (2H, m, H_2-8), 1.96 (2H, m, H_2-12), 1.29, 0.75 (1H, m, H_2-13, each), 1.49, 1.60 (1H, m, H_2-14, each)], four olefenic methyline [δ 5.72 (1H, m, H-2), 7.32 (1H, ddd, J = 12.6, 2.9, 2.1 Hz, H-3), 5.20 (1H, dt, J = 9.6, 5.3 Hz, H-10), 5.68 (1H, m, H-11)], five methyline groups [δ 4.48 (1H, d, J = 3.2 Hz, H-4), 1.75 (1H, m, H-5), 4.04 (1H, m, H-7), 2.31 (1H, ddd, J = 8.8, 8.6, 8.3 Hz, H-9), 4.70 (1H, dd, J = 6.4, 4.8 Hz, H-15)]. ^{13}C NMR spectra of (+)-brefeldin A showed sixteen carbon atoms, which are elucidated as 165.6 (C-1), 116.2 (C-2), 154.3 (C-3), 74.3 (C-4), 51.7 (C-5), 31.4 (C-6), 70.5 (C-7), 40.9 (C-8), 43.3 (C-9), 138.1 (C-10), 129.2 (C-11), 33.4 (Cc-12), 26.4 (C-13), 43.0 (C-14), 70.8 (C-15), 20.7 (C-16) (Table18).

(+)-Brefeldin A showed spectral data virtually identical to those reported in the literature. Based on the above evidence, the stereochemistry of (+)-brefeldin A has been determined (Chart 18). [66]

Due to the wide range of biological activities and well-functionalized macrolide structure of (+)-brefeldin A, its biological mode of action has been disclosed by a number of important discoveries. Especially, (+)-brefeldin A is known as a disassembler of the Golgi apparatus because of blocking protein transport.[66]

Table 18. Tabulated NMR data for (+)–brefeldin A [a]

Carbon no.	δ_H (mult, *J*)	δ_C (mult)	Carbon no.	δ_H (mult, *J*)	δ_C(mult)
1		165.6 (s)	11	5.68 (m)	129.2 (d)
2	5.72 (m)	116.2 (d)	12	1.96 (m)	33.4 (t)
3	7.32 (ddd, 12.6, 2.9, 2.1)	154.3 (d)	13	1.29 (m)	26.4 (t)
4	4.48 (d, 3.2)	74.3 (d)		0.75 (m)	
5	1.75 (m)	51.7 (d)	14	1.49 (m)	43.0 (t)
				1.60 (m)	
6	1.65 (m)	31.4 (t)	15	4.70 (dd, 6.4, 4.8)	70.8 (d)
7	4.04 (m)	70.5 (d)	16	1.18 (d, 6.4)	20.7 (q)
8	1.80 (m)	40.9 (t)	4-OH	5.10 (d, 5.6)	
9	2.31 (ddd, 8.8, 8.6, 8.3)	43.3 (d)	7-OH	2.29 (dd, 4.8, 4.3)	
10	5.20 (dt, 9.6, 5.3)	138.1 (d)			

[a] Recorded in DMSO-d_6 at 400 MHz (^{1}H) and 100 MHz (^{13}C).

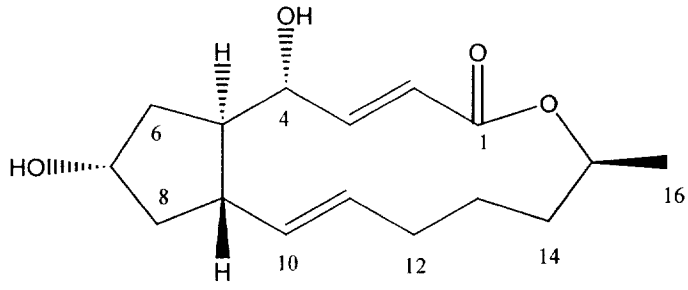


Chart 18. Chemical structure of (+)-brefeldin A.

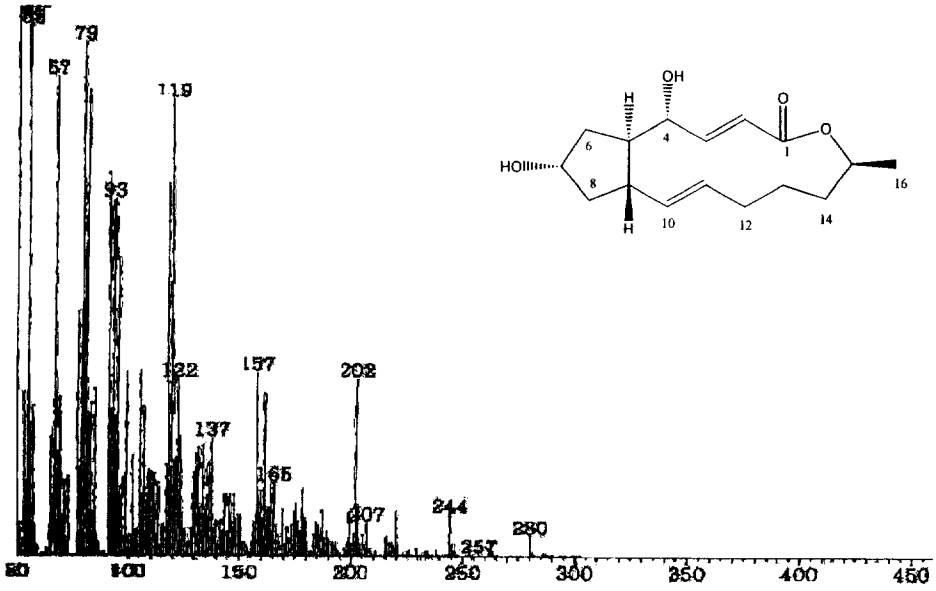


Fig. 68 LREIMS spectrum of (+)-brefeldin A.

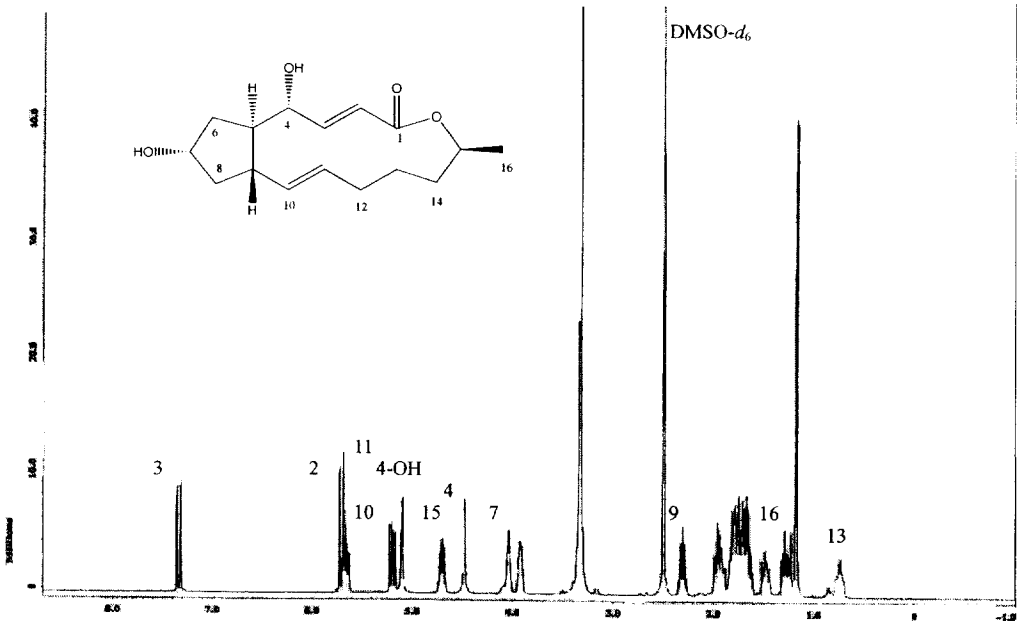


Fig. 69 ^{1}H NMR (DMSO-d_6) spectrum of (+)-brefeldin A.

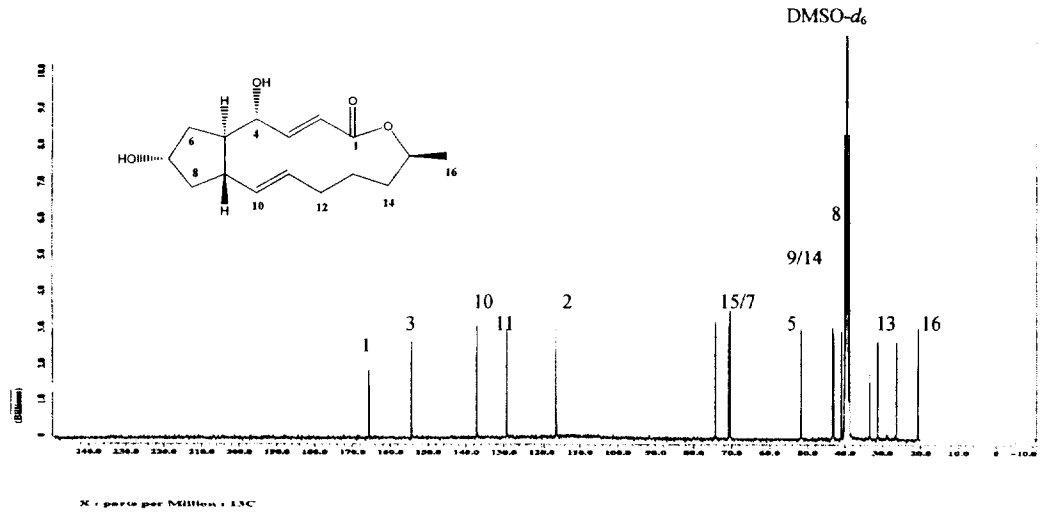


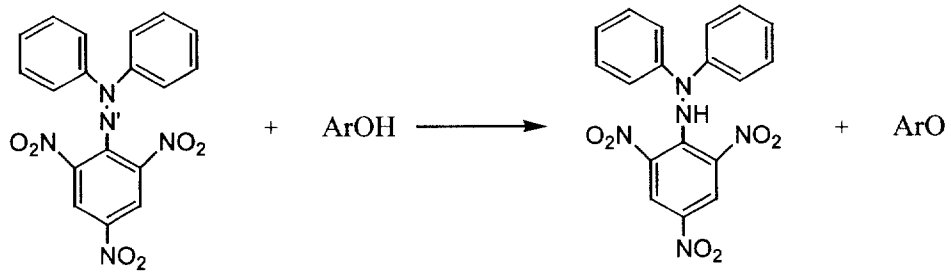
Fig. 70 ^{13}C NMR (DMSO-d_6) spectrum of (+)-brefeldin A.

2. Biological activity of the secondary metabolites

1) Free radical scavenging activity [DPPH (1,1-diphenyl-2-picrylhydrazyl)]

Free radical scavenging assay has been employed since 1958 as an effective method to screen antioxidant substances.[23, 24] At present, 1,1-diphenyl-2-picrylhydrazyl (DPPH) was used as the indicator of free radical, and vitamin C used as standard sample. Free radical scavenger is a preventive antioxidant. The term antioxidant is defined as any substance that, when present at low concentrations compared to that of an oxidizable one, significantly delays or inhibits oxidation of that substrate. Antioxidants can act at different levels in an oxidative sequence. This illustrated by considering one of the many mechanisms by which oxidative stress can cause damage by stimulating the free radical chain reaction of lipid peroxidation. Free radical chain reactions within a material inhibited either by adding chemicals that retard the formation of free radicals or by introducing substances that compete for the existing radicals and remove them from the reaction medium.[24]

Free radical scavenging activity [DPPH(1,1-diphenyl-2-picryhydrazyl)] is known as a practical method for anti-oxydation, especially for phenol and aromatic amine compounds. DPPH, which used as dye, showes characteristic absorption band at 520 nm, when DPPH is mixed with electron donor, for example, phenolic, DPPH will react with it and change into phenoxy radical immediately irreversibly. The character absorption band at 520 nm will disappear by this time, its color will change from purple into light yellow also (Scheme 14).



DPPH
520 nm(purple)

(DPPH)H
520 nm (yellow)

Scheme 14. DPPH radical scavenging action of antioxidants [ArOH].

The secondary metabolites, which were isolated from the marine-derived fungi *aspergillus* sp. (MFA212) and *microsporum* sp. (MFA212-1), showed significant activity compared with the positive control *L*-ascorbic acid (IC_{50}, 20μM), such as new compounds golmaenone, dihydroisoechinulin A, and asperflavin ribofuranoside with IC_{50} values of 20.2, 20.2, and 14.2 μM, respectively; together with the known compounds neoechinulin A & B, *L*-ananyl-*L*-tryptophan anhydride, echinulin, citrinin, flavoglaucin, and isodihydroauroglaucin with IC_{50} values of 23.4, 26.0, 20.7, 26.0, 48.5,11.3, and 11.5 μM, respectively. In addition, the strain MFA292 monitored by the AMT assay also provided some more interesting compounds with exciting activity in DPPH assay, gentisyl alcohol, 3-cholorogentisyl alcohol, 2-methylhydroquinone, (+)-epoxydon and (+)-epoxydon monoacetate with the IC_{50} values of 10.0, 3.5, 16.9, 17.9, 14.1 μM, respectively. All data were listed as well here. This means that marine-derived fungi are one of the interesting sources to explore and develop for searching for the bioactive components. The other compounds were tested by DPPH assay also, however, the results were not included in this table due to no activity.

Table 19. Radical scavenging activity of the secondary metabolites from the marine-derived fungi

Sample	DPPH radical scavenging activity	
	Inhibition (%)	IC_{50}: μg/mL (μM)
212ae	63	
212M		23.8
212B		6.6
Golmaenone		7.1 (20.2)
Dihydroisoechinulin A		8.5 (20.2)
Neoechinulin A		7.5 (23.4)
L-Ananyl-*L*-tryptophan anhydride		5.3 (20.7)
Echinulin		11.9 (26.0)
212-1ae	72	
212-1M		19.8
212-1B		21.6
Asperflavin ribofuranoside		5.9 (14.2)
Flavoglaucin		3.4 (11.3)
Isodihydroauroglaucin		3.4 (11.5)
Citrinin		12.1 (48.5)
Neoechinulin B		8.3 (26.0)
292ae	60.8	
292M		-[a]
292B		13.9
Gentisyl alcohol		1.4 (10.0)
3-Cholorogentisyl alcohol		0.6 (3.5)
2-Methylhydroquinone		2.1 (16.9)
(+)-Epoxydon		2.8 (17.9)
(+)-Epoxydon monoacetate		2.8 (14.1)
L-Ascorbic acid*		3.5 (20.0)

[a] no active.

* positive control.

2) Antimicrobial activity against methicillin-resisitant *Staphylococcus aureus* (MRSA) and multidrug-resistant *S. aureus* (MDRSA)

The development of resistance toward current antibiotics continues to be a significant problem in the treatment of infection's disease, and therefore the discovery and development of new antibiotics are evolving as a high priority in biomedical research. The classical agar diffusion methods have been used to isolate and identify new antibiotics.

In the recent years, there are a number of simple tests for antimicrobial activity that can be carried out with simple equipment and a minimum of microbiologic expertise. Here we selected the BHI dilution method, and the bacteria consisted of three pathogens such as *Staphylococcus aureus,* methicillin-resistant *S. aureus* and multidrug-resistant *S. aureus*. Oxacillin, which shows positive to *Staphylococcus aureus* except for the other pathogens, was used as standard sample. [67]

The marine-derived fungus *aspergillus* sp. (MFA292) produced some mild active compounds: 2-methylhydroquinone, gentisyl alcohol, (+)-epoxydon and (+)-epoxydon monoacetate and the new compound asperflavin ribofuranoside with the MIC_{50} values of 6.3, 12.5, 12.5, 12.5, 12.5, 50.0 μg/mL, respectively. This strain is been investigating for searching for some components with special structure and significant activity.

Table 20. Antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and multidrug-resistant *S. aureus* (MDRSA)

Sample	Antibacterial activity	
	% Inhibition	MIC: μg/mL (μM)
292ae	87.4	
292M		-[a]
292B		12.5
2-Methylhydroquinone		6.25 (50.4)
3-Cholorogentisyl alcohol		50.0 (287.3)
Gentisyl alcohol		12.5 (89.2)
(+)-Epoxydon monoacetate		12.5 (63.1)
(+)-Epoxydon		12.5 (80.1)
Asperflavin ribofuranoside		50.0 (118.7)
Oxacillin*		8.0 (18.9)

[a] no active.

* positive control.

3) Ultraviolet-A (UV-A) protecting assay

At present, it has been proved that more and more diseases are induced by the irradiation of UV-A (320-400 nm) and UV-B (290-320 nm), and the immunity of human body will be gradually deduced, some clinic cases had reveled that UV-A and UV-B also can result in the aging of skin, and so forth. Many scientists in all over the world are focusing on this field, namely, how to protect human from the damage of UV-A and UV-B. Now the mechanism of UV-B is already interpreted well, however, the mechanism of UV-A isn't discovered yet. As everybody knows, the ultraviolet radiation will make big trouble to human actions. The damaging effects of solar ultraviolet UV-B and UV-C radiation have been reported so well. However, as being a member UV-A (320-390 nm) is not taken systematic reported. Especially, the principle in photosynthesis is not well known till nowadays.[30, 31]

The marine-derived fungi own its unique ecological environment (high pressure, high concentration salt, and less oxygen) as well as its ecological advantage. In order to find active natural product against UV-A and UV-B, the marine-derived fungi are

increasingly regarded as a potential target. Hence, UV-A protecting activity assay is taken always for screening those secondary metabolites during our research.

Among these secondary metabolites, some of them showed mild activity against UV-A, even more than positive control (oxybenzone), for example, the new compounds dihydroxyisoechinulin A and golmaenone with the values of 130 μM and 90 μM, respectively.

Table 21. Ultraviolet-A (UV-A) protecting activity of the secondary metabolites from the marine-derived fungi

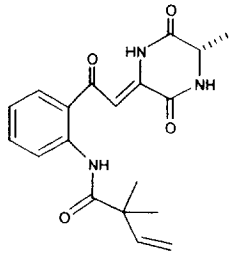
Sample	UV-A
	ED_{50}: μg/mL (μM)
Neoechiunulin A	55 (170)
Neoechinulin B	53 (165)
Dihydroxyisoechinulin A	55 (130)
Golmaenone	32 (90)
Oxybenzone*	80 (350)

*positive control.

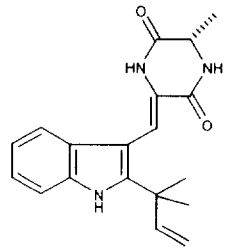
Part IV Conclusion

As part of an effort to discover biologically active natural products from marine organisms, we have investigated the bioactive constituents of the marine-derived algicolous fungi *Aspergillus* sp. (MFA212 and MFA292), *Microsporum* sp. (MFA212-1), and some unidentified fungi (MFA936 and MFA552), and isolated some new secondary metabolites together with interesting known compounds. The stereostructure of the secondary metabolites and their biological activity are as follows.

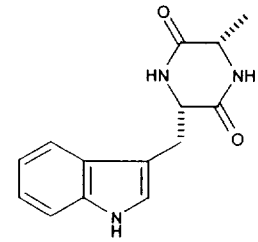
1. A new diketopiperazine alkaloid, golmaenone (**1**) and related alkaloids, neoechinulin A (**2**) and *L*-alanyl-*L*-tryptophan anhydride (**3**), have been isolated from the culture broth of the marine-derived fungus *Aspergillus* sp. The structure and absolute stereochemistry of the compounds were assigned by spectroscopic methods and the advanced Marfey's method. Compounds **1** and **2** exhibited a significant radical scavenging activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH) with IC_{50} values of 20 and 24 μM, respectively, which were similar to the positive control, ascorbic acid (IC_{50}, 20 μM). They also showed an ultraviolet-A (UV-A) (320-390 nm) protecting activity with ED_{50} values of 90 and 170 μM, respectively, which were more active than oxybenzone (ED_{50}, 350 μM) currently being used as sunscreen.



golmaenone (**1**)

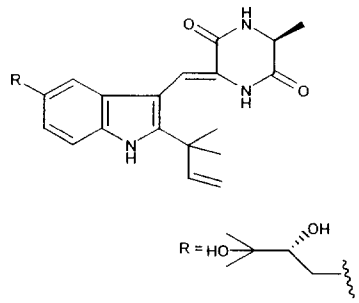


neoechinulin A (**2**)

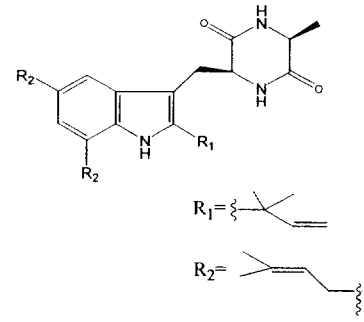


L-alanyl-*L*-tryptophan anhydride (**3**)

2. A more polar active fraction of *Aspergillus* sp. yielded a new metabolite, dihydroxyisoechinulin A (**4**), and related echinulin (**5**). The planar structures were elucidated on the basis of COSY, HMQC, HMBC, and NOESY correlations. The stereochemistry of **4** was carried out using the advanced Marfey's and Horeau's methods. Compound **4** exhibited a potent radical scavenging activity against DPPH (IC_{50}, 20 μM), and also showed an ultraviolet-A protecting activity with ED_{50} value of 130 μM, which was more active than oxybenzone (ED_{50}, 350 μM).



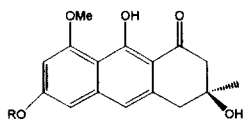
dihydroxyisoechinulin A (**4**)



echinulin (**5**)

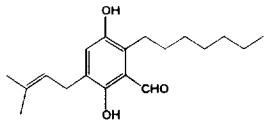
3. Bioassay-guided fractionation of the secondary metabolites from the marine-derived fungus *Microsporum* sp. led to the isolation of a new 6-*O*-α-*D*-asperflavin ribofuranoside (**6**), as well as the polyketides, flavoglaucin (**7**), isodihydroauroglaucin (**8**), citrinin (**9**), and neoechinulin B (**10**). The structures of **6-10** were elucidated on the basis of spectroscopic method, including 1D and 2D NMR comprehensive analyses. These compounds (**6-10**) showed the DPPH scavenging activity with IC_{50} values of 14.2, 11.3, 11.5, 48.5, and 26.0 μM, respectively, and **6** also exhibited mild *in vitro* antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and

multidrug-resistant *S. aureus* (MDRSA) with MIC values of 50 μM, respectively. Furthermore, compound (**10**) exhibited an ultraviolet-A (UV-A) protecting activity with ED_{50} value of 165 μM.

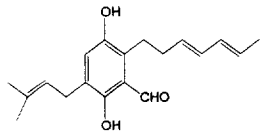


asperflavin ribofuranoside (**6**):
R=α-D-ribofuranoside

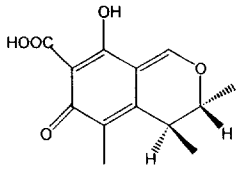
asperflavin (**6a**) : R=H



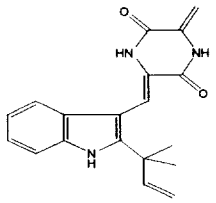
flavoglaucin (**7**)



isodihydroauroglaucin (**8**)

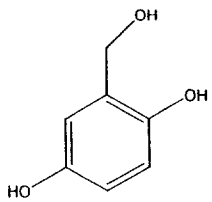


citrinin (**9**)

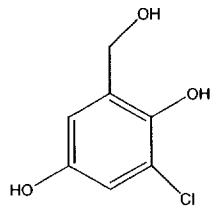


neoechinulin B (**10**)

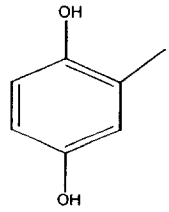
4. A series of bioactive compounds were isolated from the culture broth of the marine-derived fungus of *Aspergillus*, which had been isolated from the surface of the marine red alga *Hypnea saidana*. On the basis of the comprehensive analyses of spectroscopic data, their structures were established as gentisyl alcohol (**11**), 3-chlorogentisyl alcohol (**12**), 2-Methylhydroquinone (**13**), (+) epoxydon (**14**), and (+)-epoxydon monoacetate (**15**), respectively. The biological activity of **11-15** showed a mild activity *in vitro* antibacterial activity against MRSA and MDRSA with the MIC values (μ g/mL) of 12.5, 50.0, 6.25, 12.5, 12.5, respectively.



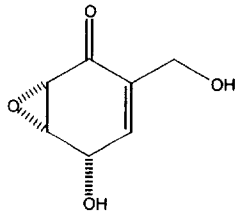
gentisyl alcohol (**11**)



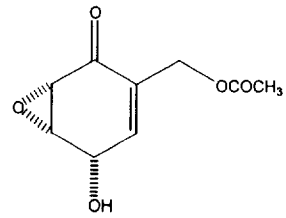
3-chlorogentisyl alcohol (**12**)



2-methylhydroquinone (**13**)

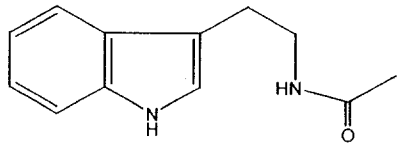


(+)- epoxydon (**14**)



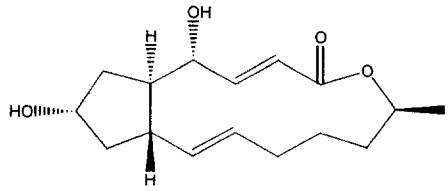
(+)-epoxydon monoacetate (**15**)

5. An indolyl alkaloid derivative, N_b-acetyltryptamine (**16**) was isolated from an unidentified marine-derived fungus (MFA936), which had been separated from the red alga *Gracilaria verrucosa*. The structure of N_b-acetyltryptamine was firmly determined to be 2-(3-indolyl)ethylamine acetate (**16**) from the physicochemical analyses.



N_b-acetyltryptamine (**16**)

6. (+)-Brefeldin A (**17**) was obtained from an unidentified marine-derived fungus (MFA552). The unique structure of (+)-brefeldin A was established on the basis of comprehensive spectroscopic analyses. Due to its wide range of biological activities and well-functionalized macrolide structure, its biological mode of action has been disclosed by a number of important discoveries. Especially, brefeldin A is known as a disassembler of the Golgi apparatus because of blocking protein transport.



(+)-brefeldin A (**17**)

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해양균류 2차대사성분에 대한 화학적연구와 생물활성

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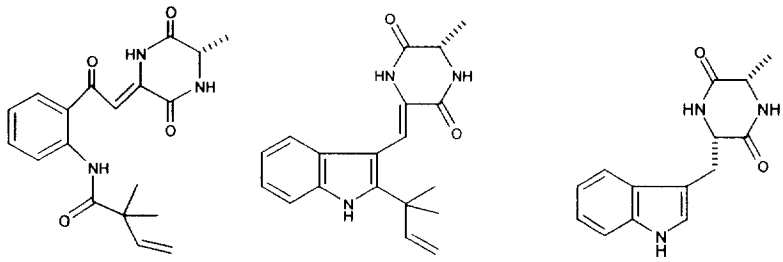
개 요

70여년 동안의 연구발전을 통하여 매우 많은 흥미로운 천연물이 미생물로부터 분리되고있다. 해양균류는 매우 중요한 하나의 미생물로서 생물활성을 갖고있는 2차대사산물의 새로운 자원으로 간주되고 있다. 해양 균류에 대한 연구는 화학, 생화학, 약학, 해양생태학 뿐 만 아니라, 많은 각종 응용 등 거의 모든 연구분야와 관련되어 있다. 특히 지난 5년 동안의 연구 보고들에 따르면 해양 균류는 그들의 독특한 생태 환경으로 말미암아 특이한 화학구조와 생물 활성을 가지고 있는 것으로 보고 되었다. 해양 대형 생물에 비해 해양미생물은 현저한 장점들을 갖고 있다. 첫째, 생태학적 고려의 필요 없이 생물 공학적인 배양이 가능하다. 둘째, 계속적인 배양을 통하여 2차대사성분들을 대량으로 획득할 수 있다. 셋째, 미생물들을 보다 쉽게 유전학적으로 조작 할 수 있다.

해양 균류는 신약 개발의 독특하고 새로운 자원으로서 날 따라 신비한 우수성을 나타내고 있다.

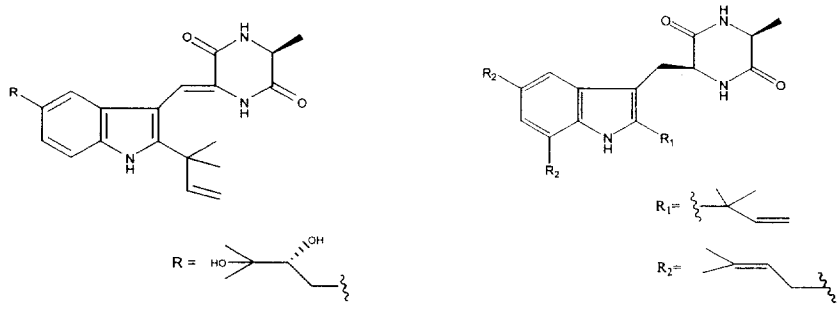
해양생물유래의 생물활성물질의 화학적연구와 일환으로서 해양숙주재료에서 분리한 해양균류를 대상으로 화학적연구를 행하여 2차대사성분들을 분리하고 그들의 생물활성을 측정하였으며, 그 결과를 요약하면 다음과 같다.

1. 해양 균류 *Aspergillus* sp. 배양액으로부터 새로운 diketopiperazine 알칼로이드, golmaenone (1) 및 이와 관련된 알칼로이드, neoechinulin A (2) 와 *L*-alanyl-*L*-tryptophan anhydride를 분리하였으며, 분광학적분석 및 Marfey's 방법을 이용하여 이들 화합물들의 입체구조를 결정하였다. 화합물 1과 2는 1,1-diphenyl-2-picrylhydrazyl (DPPH)에 대해 현저한 라디칼 소거활성을 나타냈으며 IC_{50} 값은 각각 20과 24 μM으로서 이것은 ascorbic acid (IC_{50}, 20 μM)와 비슷하였다. 뿐만 아니라 이 두 화합물들은 자외선 (UV-A, 320-390 nm) 차단 활성도 나타냈으며 ED_{50} 값은 각각 90과 170 μM으로서, 이것은 현재 사용되고 있는 자외선 차단제 oxybenzone (ED_{50}, 350 μM) 보다 더 좋은 활성을 발현하였다.



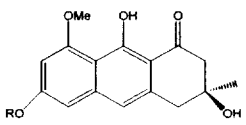
golmaenone (**1**)　　neoechinulin A (**2**)　　*L*-alanyl-*L*-tryptophan anhydride (**3**)

2. *Aspergillus* sp. 의 더 큰 극성 분획으로부터 라디칼 소거활성을 가진 새로운 대사성분 dihydroxyisoechinulin A (**4**) 및 이와 관련된 echinulin (**5**) 을 분리하였다. 이들의 평면 구조는 COSY, HMQC, HMBC와 NOESY 방법들을 이용하여 결정하였다. 개선된 Marfey's 법과 Horeau's 법을 이용하여 화합물 **4**의 입체 구조를 결정하였다. 화합물 **4**는 강한 라디칼 소거활성 (IC_{50}, 20 μM)을 나타내었을 뿐 만 아니라, 자외선-A (UV-A)차단활성 (ED_{50}, 130 μM)도 oxybenzone (ED_{50}, 350 μM) 보다 강한 활성이 관찰되었다.

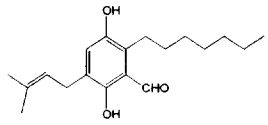


dihydroxyisoechinulin A (**4**)　　echinulin (**5**)

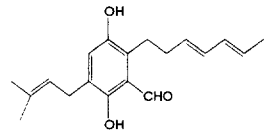
3. 생물활성을 지표로 한 분리정제로 해양균류 *Microsporum* sp.로부터 새로운 2차 대사성분, 6-*O*-α-*D*-asperflavin ribofuranoside (**6**)외에 기지의 polyketides, flavoglaucin (**7**), isodihydroauroglaucin (**8**), citrinin (**9**) 및 neoechinulin B (**10**) 들을 분리하였으며, 물리화학적성질 및 분광학적 분석 등에 의해 전 화학구조를 결정하였다. 이들 화합물 (**6**-**10**)은 모두 라디칼 소거활성을 나타냈으며, IC_{50} 값은 각각 14.2, 11.3, 11.5, 48.5 와 26.0 μM이었다. 화합물 **6**은 methicillin-내성 *Staphylococcus aureus* (MRSA) 와 다약제 내성 *S. aureus* (MDRSA) 에 대해 각각 약간의 항균활성을 나타냈다 (MIC, 50 μM). 또한, 화합물 **10**은 자외선 (UV-A) 차단 활성을 나타냈다 (ED_{50}, 165 μM).



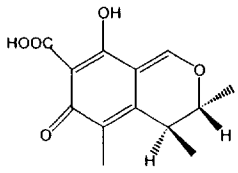
asperflavin ribofuranoside (**6**):
R=α-*D*-ribofuranoside
asperflavin (**6a**) : R=H



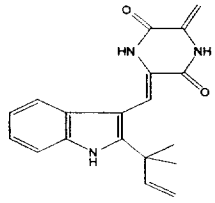
flavoglaucin (**7**)



isodihydroauroglaucin (**8**)

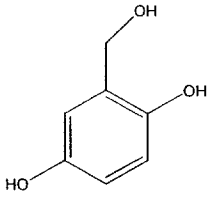


citrinin (**9**)

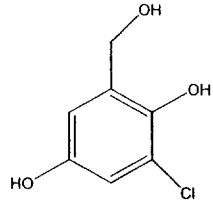


neoechinulin B (**10**)

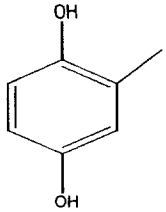
4. 해양균류*Aspergillus* sp.로부터 일련의 생물활성 화합물, gentisyl alcohol (**11**), 3-chlorogentisyl alcohol (**12**), 2-methylhydroquinone (**13**), (+)epoxydon (**14**) 및(+)-epoxydon monoacetate (**15**)를 분리하였으며, 물리화학적성질과 분광학적 분석 및 문헌치와 비교 검토하여 이들의 전 화학구조를 동정하였다. 이들 화합물 (**11**-**15**)은 모두 methicillin-내성 *Staphylococcus aureus* (MRSA)와 다약제 내성 *S. aureus* (MDRSA)에 대해 항균활성을 나타냈다 (MIC, 12.5, 50.0, 6.25, 12.5, 12.5 μg/mL).



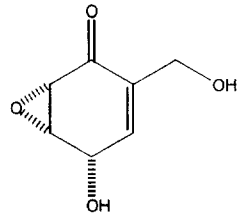
gentisyl alcohol (**11**)



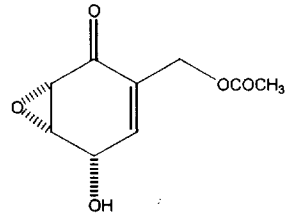
3-chlorogentisyl alcohol (**12**)



2-methylhydroquinone (**13**)

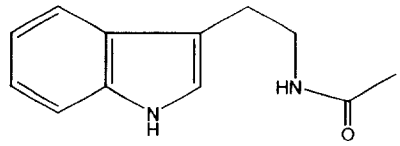


(+) epoxydon (**14**)



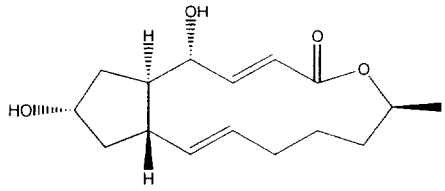
(+)-epoxydon monoacetate (**15**)

5. 미 동정 해양균류 MFA936으로부터 indolyl alkaloid, N_b-acetyltryptamine (**16**)을 분리하였으며, 물리화학적성질 및 분광학적 분석에 의해 전 화학구조를 2-(3-indolyl)ethylamine acetate로 동정하였다.



N_b-acetyltryptamine (**16**)

6. (+)-Brefeldin A (**17**)는 미 동정 해양균류 MFA552로부터 분리하였으며, 물리화학적 성질 및 분광학적 분석에 의해 전 화학구조를 (+)-brefeldin A로 동정하였다. 이 물질은 광범위한 생물 활성과 우수한 항균활성 기능구조로 하여 이미 많은 중요한 발견들에 의해 생물 기능 모형이 제기 되였다. 특히, brefeldin A는 단백질의 수송을 방해 함으로써 Golgi 기관을 분해하는 것으로 알려져 있다.



(+)-brefeldin A (**17**)

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