Bioactive Compounds Isolated from a Terrestrial *Streptomyces* sp. YT202

Yee Yee Thu[1], Mon Mon[2] and Kyaw Thu Ya Aung[3]

Abstract

A *Streptomyces* sp. YT202 was isolated from soil sample in the campus of Dagon University. The fermented broth of this strain indicated antimicrobial activities on nine test organisms and its fermentation studies were carried out at Department of Botany, University of Yangon. For isolation of the bioactive compounds, 15 L fermentation of *Streptomyces* sp. YT202 was carried out and the filtrate was applied on the Amberlite XAD-2 resin column that was washed with distilled water and followed by methanol. The bioactive compounds from the methanol extract were isolated on silica gel and Sephadex LH 20 gel columns with various solvent systems. The isolated compounds were characterized by ESI-MS, 1D-NMR and 2D-NMR spectra at Institute for Organic and Biomolecular Chemistry, Georg-August University, Goettingen, Germany. The two compounds: 'Trytophan-dehydrobutyrin-N-methyl-diketopiperazin' and 'Nargenicin A1' were isolated. The compound Nargenicin A1 exhibited good antimicrobial activities on *Agrobacterium* sp., *Bacillus subtilis*, *Escherichia coli*, *Micrococcus luteus*, *Staphylococcus aureus*, *Salmonella typhi* and *Xanthomonas* sp. Therefore, this compound could be applied to inhibit cholera, diarrhea, skin diseases and urinary infections on man as well as crown gall and leaf blight diseases on plants. These findings are very beneficial both for our health and for our farmers.

Keywords*:* Antimicrobial activity, Fermentation, Nargenicin A1, *Streptomyces* sp. YT202

Introduction

Isolation of microorganisms from the environment is the microbiologist's first step in screening for natural products such as secondary metabolites. Microorganisms, in particular the bacteria, have had a profound effect on the development of chemistry and upon medical science. Since the discovery of penicillin in 1929, intensive studies of mainly soil derived from bacteria and fungi have shown that the microorganisms are a rich source of structurally unique pharmaceutically important bioactive substances (Mann & Murder, 1994).

Antibiotics are defined as low-molecular weight (MW<2000 Dalton) secondary metabolites from natural sources including their chemically or biosynthetically produced derivatives. These show inhibition of the growth of higher organisms (e.g. tumour cells) or pathogens (e.g. bacteria, fungi, viruses) at low concentration, and subsequently can be used to cure infectious diseases (Fenical, 1993).

The use of microorganisms to produce natural products and processes that benefit and improve our socioeconomic lifestyles has been a part of human history since the days of early civilization. Many antibiotics have been isolated from various microorganisms like *Actinomycetes*, other bacteria, fungi, etc. Today over 100 microbial products continue to be used clinically as antibiotics, antitumor drugs, enzyme inhibition and agrochemicals (Berdy, 1989).

Nowadays, the search for new microbial drugs is aimed at those pharmacological agents. At the beginning of 21st century, the microbial secondary metabolism still appears as a promising field for both fundamental and applied research. It owes much of its attraction to its interdisciplinary character, providing

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numerous impulses to other scientific fields that will promote new approaches to new metabolites and new activities (Gräfe, 1992; Döhren & Gräfe, 1997).

The main goals of the present research are the isolation, purification and identification of the biologically active secondary metabolites from *Streptomyces* sp. YT202 isolated from the soil sample and antimicrobial activity of the isolated compounds.

Materials and Methods

Isolation of *Streptomyces* sp. from Soil Sample

The soil sample was collected in the campus of Dagon University for screening of antibiotic producing microorganisms. The isolation of soil strain can be carried out with the following schemes: (1) The soil sample 1.0 g was suspended in a definite amount of sterile water. The sample is vigorously agitated. (2) The supernatant was diluted 10^{-1}-10^{-10}. (3) One loop from each sample of the dilution series were plated on various culture media and then incubated at 27°C. (4) Single colonies from the plates were picked and purified by restreaking. (5) The pure strains were maintained as slant cultures in the test tubes (Nolan and Cross, 1988).

Fermentation Studies of *Streptomyces* sp. YT202

(1) Utilization of carbon and nitrogen sources

In this study carbon sources: glucose, sucrose, glycerol, fructose, dextrin, maltose, lactose, mannitol and potato starch were utilized, whereas nitrogen sources: yeast extract, corn steep powder, soy bean, polypeptone from meat, polypeptone from casein, meat extract, hafer extract and malt extract were also employed for media optimization. The basal media for carbon sources were yeast extract 0.3%, K_2HPO_4 0.01%, $MgSO_4$ 0.01%, $CaCO_3$ 0.01% while those for nitrogen sources were glycerol 1.0%, K_2HPO_4 0.01%, $MgSO_4$ 0.01%, $CaCO_3$ 0.01% (Monaghan *et al*., 1999).

(2) Size of inoculum

The proper cultivation and transfer (size of inoculum) are essential for the production of bioactive metabolites. Optimal fermentation conditions are very important for maximal productivity of compounds. A loop from the plate culture of YT202 grown for 3 days was inoculated into 300 ml of conical flasks containing 100 ml of the seed medium (SM) and the flask was incubated at 28°C for 2 days. After 2 days, the seed culture (0.5%, 1.0%, 1.5%, 2%, 2.5% and 3.0%) was transferred into six of 300 ml conical flasks containing 100 ml of fermentation medium in each. The fermentation was carried out for 5 days (Monaghan *et al*., 1999).

(3) Fermentation

After autoclaving, one loop of strain YT202 grown on the plate culture was inoculated into the seed culture flask containing 400 mL seed medium (soy bean 20 g/L, mannitol 20g/L). Then, the flask was incubated at 28°C for 2 days on the shaker at 180 rpm. After two days, the seed culture 375 mL was transferred into the 30 L fermenter containing 15 L of the fermentation medium (soy bean/mannitol). The aeration and agitation of the fermentation were maintained at 15 L per minute and 300 rpm at 28°C for 80 hours (Strobel & Sullivan, 1999).

Extraction and Isolation of Bioactive Compounds

The fermentation broth (15 L) was centrifuged at 4500 rpm for 20 minutes. The supernatant was applied on an Amberlite XAD-2 resin column. The column was washed with water followed by 10 L of MeOH. The extracted samples were concentrated by using a rotary evaporator and lyophilized. The bioactive compounds from *Streptomyces* sp. YT202 were isolated and purified by using various solvent systems on silica gel column and Sephadex LH 20 column. The isolated compounds

were characterized and identified by EI-, ESI-MS, 1D-NMR (^{1}H- and ^{13}C-NMR) and 2D-NMR (COSY, HMBC, HSQC, etc.), UV and IR spectra at Institute for Organic and Biomolecular Chemistry, Georg-August University, Goettingen, Germany (Grabley *et al.*, 1999).

Antimicrobial Activity of Isolated Compounds from *Streptomyces* sp. YT202

The isolated compounds were evaluated their antimicrobial activities by paper disc diffusion assay on nine test organisms: *Agrobacterium* sp., *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Malassezia furfur*, *Micrococcus luteus*, *Staphylococcus aureus*, *Salmonella typhi* and *Xanthomonas* sp. at Department of Botany, University of Yangon (David and Stout, 1971).

Results

Fermentation Studies of *Streptomyces* sp. YT202

(1) Utilization of carbon and nitrogen sources

Glucose, sucrose, maltose and mannitol were the best carbon sources whereas glycerol, fructose, dextrin and lactose were poor carbon sources. The surface colour of *Streptomyces* sp. on sucrose, fructose and minnitol were yellow while that on the other carbon sources were pale yellow.

Yeast extract, corn steep powder, soy bean, polypeptone from meat, meat extract and hafer were moderate nitrogen sources for fermentation while polypeptone from casein, malt extract and NH_4SO_4 were poor sources. The surface colour of *Streptomyces* sp. on yeast extract, soy bean, polypeptone from meat, meat extract and hafer were yellow but that on the other nitrogen sources were pale yellow.

(2) Size of inoculum

In the study of inoculum optimization, among the seed cultures (0.5%, 1.0%, 1.5%, 2%, 2.5% and 3.0%) 2.5% of the seed culture at fourth day fermentation was suitable for the production of bioactive metabolites. Clear zones against *E. coli* and *S. aureus* were showed in Tables 1 and 2.

Table 1. Clear zone (mm) of the strain YT202 against *Escherichia coli* (disc size = 9mm)

Days	0.5%	1.0%	1.5%	2.0%	2.5%	3.0%
2	17.0	18.0	18.4	19.5	22.2	19.1
3	18.5	19.5	19.8	20.2	21.0	21.6
4	20.0	22.0	23.5	26.5	**28.2**	25.0
5	16.3	17.2	17.8	18.9	21.4	20.0

Table 2. Clear zone (mm) of the strain YT202 against *Staphylococcus aureus* (disc size = 9mm)

Days	0.5%	1.0%	1.5%	2.0%	2.5%	3.0%
2	15.0	16.0	16.8	17.4	19.0	17.1
3	16.0	16.6	17.9	18.1	21.0	19.6
4	19.2	21.1	23.0	25.0	**27.0**	25.0
5	15.3	16.0	18.0	20.0	19.5	18.0

Extraction and Isolation of Bioactive Compounds

The ten compounds from *Streptomyces* sp. YT202 were isolated and purified by using various solvent systems on silica gel and Sephadex LH 20 columns. Among

the ten isolated compounds, the isolation and purification procedures of the two compounds are shown in Figure 1.

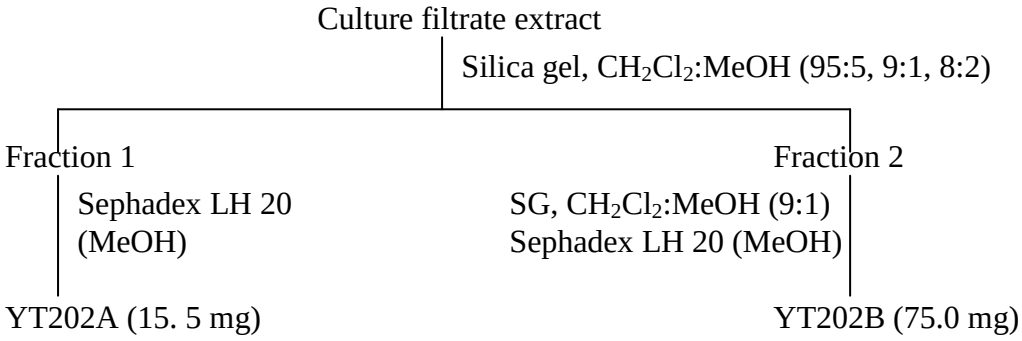
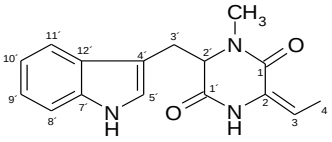


Figure 1. Isolation procedure of bioactive compounds

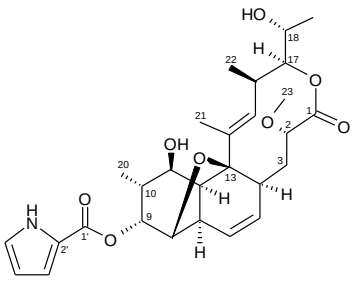
Physico-chemical Properties of Isolated Compounds

YT202A *α-N*-Methyltryptophan-dehydrobutyrin-diketopiperazin



ESI-MS (positive ions): m/z = 306.2 $[M+Na]^+$, 589.0 $[2M+Na]^+$
ESI-MS (negative ions): m/z = 282.1 $[M-H]^-$
IR (KBr) $\tilde{\nu}$: 3400, 3267, 3059, 2926, 1682, 1627, 1491, 1436, 1400, 1339,1265,1105,1031,742 cm^{-1}
UV (MeOH): λ_{max} (log ε) = 219 nm, (MeOH/HCl): λ_{max} (log ε) = 219 nm (MeOH/NaOH): λ_{max} (log ε) = 218 nm
CD (MeOH): λ_{max} ([Θ]) = 218 (-147900), 257 (39500), 292 (-12300) nm
$[\alpha]_D^{20}$ = - 5° (c = 1 in Methanol)
^{1}H NMR ($CDCl_3$, 600 MHz): δ_H = 0.96 (d, *J* = 7.5 Hz, 3H, 4-H_3), 3.10 (s, 3H, N-CH_3), 3.28 (dd, *J* = 15.0, 5.0 Hz, 1H, 3´-H_a), 3.57 (dd, *J* = 15.0, 3.0 Hz, 1H, 3´-H_b), 4.28 (dd, *J* = 7.0, 3.5 Hz, 1H, 2´-H), 5.49 (q, *J* = 7.5 Hz, 1H, 3-H), 6.82 (d, *J* = 2.0 Hz, 1H, 5´-H), 7.09 (t, *J* = 8.0 Hz, 1H, 10'-H), 7.17 (t, *J* = 8.0 Hz, 1H, 9´-H), 7.31 (d, *J* = 8.0 Hz, 1H, 8´-H), 7.60 (s, 1H, 2-NH), 7.64 (d, *J* = 8.0 Hz, 1H, 11´-H), 8.66 (s, 1H, 6'-NH)
^{13}C NMR ($CDCl_3$, 75.5 MHz): δ_C = 9.7 (q, C-4), 32.9 (q, N-CH_3), 27.6 (t, C-3´), 63.2 (d, C-2´), 107.7 (s, C-4´), 110.9 (d, C-8´), 112.7 (d, C-3), 118.6 (d, C-11´), 119.5 (d, C-10´), 122.1 (d, C-9´), 124.7 (d, C-5´), 126.2 (s, C-2), 127.4 (s, C-12´), 136.2 (s, C-7´), 160.4 (s, C-1), 166.7 (s, C-1´)

YT202B Nargenicin A1



ESI-MS (positive ions): m/z = 538.4 $[M+Na]^+$, 1053.1 $[2M+Na]^+$
ESI-MS (negative ions): m/z = 514.6 $[M-H]^-$
IR (KBr): $\tilde{\nu}$ = 3420, 2971, 2934, 2880, 1698, 1654, 1556, 1453, 1410, 1311, 1272, 1113, 1030, 970, 751 cm^{-1}

UV (MeOH): λ_{max} (log ε) = 227 (3.68), 265 (3.90) nm; (MeOH/HCl): λ_{max} (log ε) = 231 (3.64), 265 (3.89) nm; (MeOH/NaOH): λ_{max} (log ε) = 221 (3.72), 265 (3.91) nm
CD (MeOH): λ_{max} ([Θ]) = 204 (51800), 222 (-1330)

$[\alpha]_D^{20}$ = +48° (c = 1 in Methanol)

^{1}H NMR (CD_3OD, 300 MHz): 0.93 (d, *J* = 7.0 Hz, 3H, 20-H_3), 1.25 (d, *J* = 7.0 Hz, 3H, 22-H_3), 1.21 (d, *J* = 6.0 Hz, 3H, 19-H_3), 1.35 (m, 1H, 3-H_a), 1.82 (s, 3H, 21-H_3), 2.30 (m, 1H, 12-H), 2.34 (m, 1H, 10-H), 2.47 (m, 1H, 12-H), 2.48 (ddd, *J* = 15.0, 11.0, 4.0 Hz, 1H, 3-H_b), 2.61 (d, *J* = 7.0 Hz, 1H, 7-H), 3.08 (m, 1H, 16-H), 3.28 (s, 3H, 23-H_3), 3.69 (dd, *J* = 11.0, 3.0 Hz, 1H, 11-H), 3.72 (dd, *J* = 11.0, 4.5 Hz, 1H, 2-H), 3.99 (dq, *J* = 9.0, 6.0 Hz, 1H, 18-H), 4.12 (d, *J* = 5.0 Hz, 1H, 8-H), 5.03 (t, *J* = 5.0 Hz, 1H, 9-H), 5.14 (dd, *J* = 8.5, 6.0 Hz, 1H, 17-H), 5.44 (dd, *J* = 7.0, 1.0 Hz, 1H, 15-H), 5.60 (dd, *J* = 9.5, 3.0 Hz, 1H, 5-H), 5.90 (ddd, *J* = 9.0, 7.0, 2.0 Hz, 1H, 6-H), 6.20 (dd, *J* = 4.0, 2.5 Hz, 1H, 4'-H), 6.87 (dd, *J* = 4.0, 1.5 Hz, 1H, 3'-H), 6.99 (dd, *J* = 2.5, 1.5 Hz, 1H, 5'-H).

^{13}C NMR (CD_3OD, 75.5 MHz): 13.3 (q, C-20), 15.9 (q, C-22), 17.7 (q, C-21), 21.3 (q, C-19), 34.0 (d, C-16), 35.5 (d, C-10), 36.0 (t, C-3), 40.4 (d, C-7), 44.1 (d, C-4), 50.8 (d, C-12), 58.0 (q, C-23), 66.6 (d, C-18), 74.7 (d, C-9), 76.4 (d, C-11), 80.0 (d, C-17), 82.7 (d, C-8), 84.0 (d, C-2) , 90.6 (s, C-13), 110.8 (d, C-4'), 116.9 (d, C-3'), 123.1 (s, C-2'), 125.1 (d, C-5'), 128.9 (d, C-6), 132.3 (d, C-15), 133.9 (d, C-5), 136.2 (s, C-14), 161.9 (s, C-1'), 174.4 (s, C-1)

Identification of Isolated Compounds

Compound YT202A

Trytophan-dehydrobutyrin-diketopiperazin (YT202A) was isolated from the fraction 1 as an UV absorbing band at 254 nm and has R_f 0.61 (chloroform/methanol, 9:1). The molecular weight (283 g/mol) was determined by ESI-MS. It showed orange colour with orcin reagent and turned to violet with anisaldehyde/sulphuric acid reagent. This substance is good soluble in chloroform, dichloromethane and methanol.

In its FT-IR spectrum, NH (amine group) and O-H (phenolic group) were observed at 3400 and 3259 cm^{-1}. The band for 2926 cm^{-1} was due to the presence of C-H vibration of methyl and methylene groups. C=O stretching vibration (ketone) was showed at 1682 cm^{-1}. The bands at 1627, 1491 and 1436 cm^{-1} were for C=C aromatic group. C-H bending vibrations of methyl and methylene groups were showed at 1400, 1339 and 1265 cm^{-1}. C-C stretching vibrations were observed at 1105 and 1031 cm^{-1} while C-H out of plane bending was found at 724 cm^{-1}.

According to its ^{1}H-NMR spectrum, NH protons were found as singlet (s) at δ_H 8.66 ppm and as singlet (*s*) at δ_H 7.60 ppm. Aromatic protons were observed as doublets (*d*) at δ_H 7.64 ppm, 7.31 and 6.82 ppm, as triplet (*t*) at δ_H 7.17 and 7.09 ppm. Olefinic protons (C=CH) were found as quantet (*q*) at δ_H 5.49 ppm and as doublet of doublets (*dd*) at δ_H 3.28 ppm. CH_2 protons were as doublet of doublets (*dd*) at δ_H 4.28 ppm, as quantet (*q*) at δ_H 3.8 ppm and as doublet of doublets (*dd*) at δ_H 3.57 ppm, N-CH_3 was observed as singlet (s) at δ_H 3.10 and –CH_3 peak was found doublet (*d*) at δ_H 0.96 ppm in this compound as shown in Figure 2.

In the ^{13}C-NMR spectrum, the groups of carbon (C=O) ketone were found at δ_C 166.7 and 160.4 ppm. Aromatic carbons were observed at δ_C 136.2, 127.4, 122.1, 119.5, 118.6 and 110.9 ppm while olefinic carbons (C=C) were found at δ_C 124.7, 126.2, 112.7 and 107.7 ppm. The methine (CH) groups were observed at δ_C 126.2, 112.7 and 63.2 ppm. Methylene group (CH_2) was found at δ_C 27.6 ppm while the group of methyl (CH_3) was at δ_C 9.7 ppm and N-CH_3 group was at 32.9 ppm in this compound as shown in Figure 3.

According to its UV, CD, FT-IR spectral data, ^{1}H-NMR and ^{13}C-NMR spectral data, the compound YT202A was identified as α-N-Methyltryptophan-dehydrobutyrin-diketopiperazin. Its molecular formula is $C_{16}H_{17}N_3O_2$ and molecular weight is 283 g/mol.

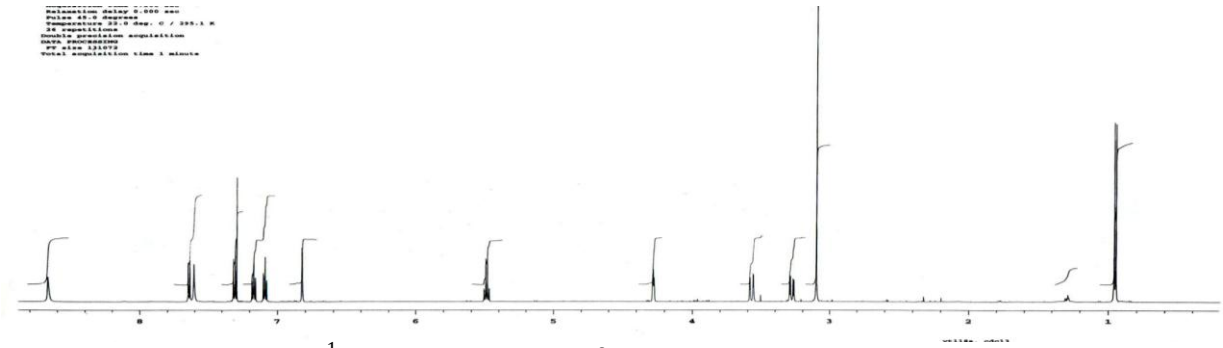


Figure 2. ^{1}H-NMR spectrum of the compound YT202A

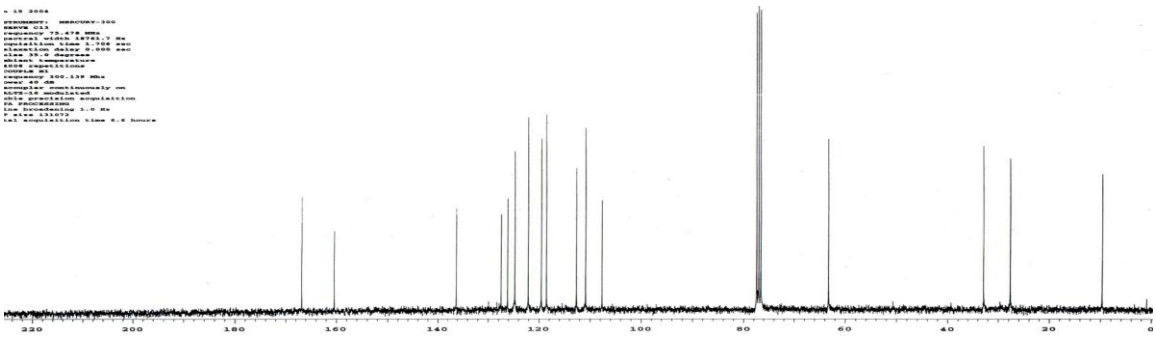


Figure 3. ^{13}C-NMR spectrum of the compound YT202A

Compound YT202B

Working up of the fraction 2 led to the compound YT202B as an UV absorbing band at 254 nm and has R_f 0.60 (chloroform/methanol, 9:1). The molecular weight (515 g/mol) was determined by ESI- and HR-ESI-MS. It showed brown colour with orcin reagent and turned to intensive brown with anisaldehyde/sulphuric acid reagent. This substance is good soluble in acetone, chloroform or dichloromethane.

In its FT-IR spectrum, OH (phenolic group) was observed at 3420 cm^{-1}. The bands for 2971, 2934 and 2880 cm^{-1} were due to the presence of C-H vibration of methyl and methylene groups. C=O stretching vibration (ketone) was showed at 1698 cm^{-1} while the bands at 1654, 1556 and 1453 cm^{-1} were for C=C aromatic group. C-H bending vibrations of methyl and methylene groups were showed at 1410, 1311 and 1272 cm^{-1}. C-C stretching vibrations were observed at 1113 and 1030 cm^{-1} while C-H out of plane bending vibrations were found at 970 and 724 cm^{-1}.

In its ^{1}H-NMR spectrum, the methine (-CH) protons were found as doublet of doublets (*dd*) at δ_H 5.14, 5.44, 5.60, 3.72, 3.69 and as (*ddd*) at δ_H 5.90 and 2.48 ppm, as triplet (*t*) at δ_H 5.03, as (*dq*) at 3.99 ppm, as double (*d*) at δ_H 4.12 and 2.61 ppm, as multiplet (*m*) at δ_H 3.08, 2.47, 2.34, 2.30 and 1.35 ppm. The (-CH) protons in sugar group were observed as doublet of doublets (*dd*) at δ_H 6.99, 6.87 and 6.20 ppm. Methyl protons (–CH_3) were found doublets (*d*) at δ_H 0.93, 1.25 and 1.21 ppm, as singlet (*s*) at δ_H 1.82 and 3.28 ppm in this compound as shown in Figure 4.

In the ^{13}C-NMR spectrum, the groups of carbon (C=O) ketone were found at δ_C 174.4 and 161.9 ppm. The methine (-CH) groups were found at δ_C 133.9, 132.3, 128.9, 84.0, 82.7, 76.4, 74.7, 66.6, 35.5 and 34.0 ppm. The methine (-CH) groups in sugar were observed at δ_C 125.1, 123.1, 116.9 and 110.8 ppm. Methylene group (-CH_2) was found at δ_C 36.0 ppm whereas the methyl groups (–CH_3) were found at δ_C 58.0, 21.3, 17.7, 15.9 and 13.3 ppm. The quaternary carbon atoms were found at δ_C

136.2, 90.6, 80.0, 50.8, 44.1 and 40.4 ppm. The two hydroxyl groups (-OH) were substituted at C-11 and C-18 in this compound as shown in Figure 5.

According to ist UV, CD, FT-IR, ^{1}H-NMR and ^{13}C-NMR spectral data, the compound YT202B was identified as nargenicine A1. Its molecular formula is $C_{28}H_{37}NO_8$ and molecular weight is 515 g/mol.

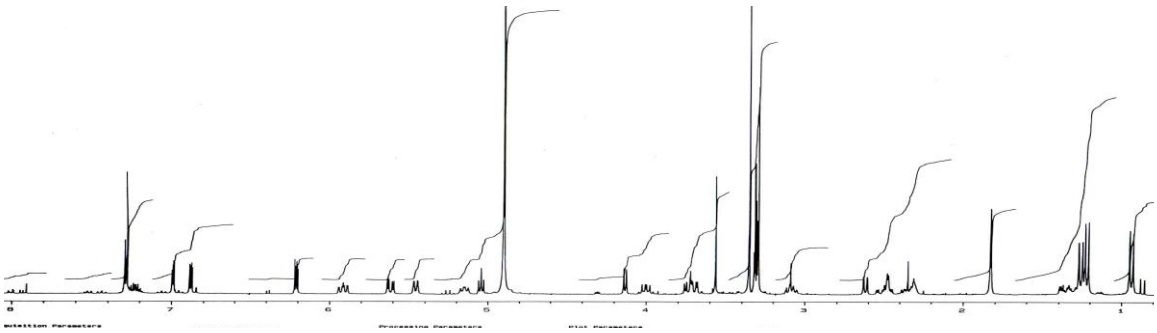


Figure 4. ^{1}H-NMR spectrum of the compound YT202B

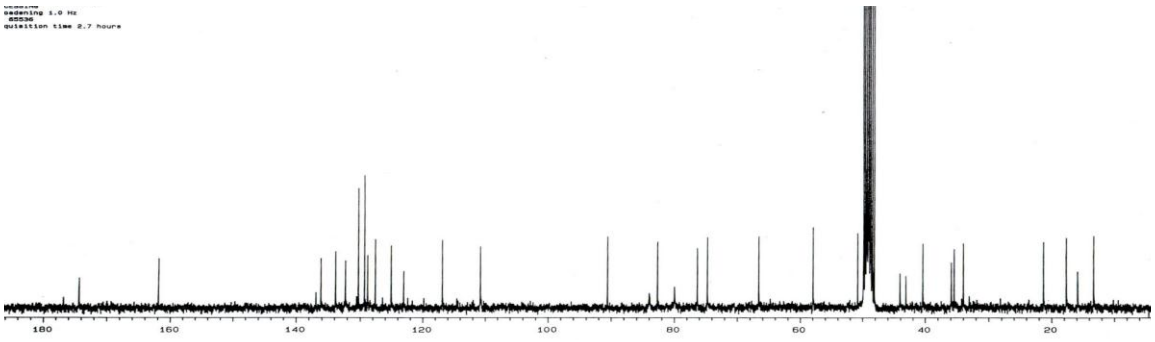


Figure 5. ^{13}C-NMR spectrum of the compound YT202B

Antimicrobial Activity

The compound YT202A exhibited weakly antibacterial activity while the compound YT202B indicated highly antibacterial activities on *Agrobacterium* sp., *Bacillus subtilis*, *Escherichia coli*, *Micrococcus luteus*, *Staphylococcus aureus*, *Salmonella typhi* and *Xanthomonas* sp. in 20 μg/disc.

Discussion and Conclusion

Streptomyces sp. YT202 was isolated from the soil sample from the campus of Dagon University. Glucose, sucrose, maltose and mannitol were the best carbon sources whereas yeast, corn steep powder, soybean, polypeptone from meat, meat extract and hafer were suitable for fermentation. In inoculum optimization, among the seed culture (0.5%, 1.0%, 1.5%, 2%, 2.5% and 3.0%) 2.5% of seed culture at fourth day fermentation was suitable for the production of bioactive metabolites. Omura in 1985 stated that the optimal fermentation conditions were very important for the production of microbial metabolites.

In this research, the two known compounds: YT202A "*α-N*-methyl tryptophan-dehydrobutyrin-diketopiperazin" and YT202B "Nargenicine A1" were isolated from 15 L fermentation. The compound YT202A showed antibacterial activity. In 1974 this compound was firstly isolated from *Streptomyces spectabilis* by Kakinuma and Rinehart who reported that it showed weak activity against RNA directed DNA polymerase.

The next compound YT202B (Nargenicin A1) is the antibiotic that indicated the highly antibacterial activity in this research. It was firstly isolated from the cultures of *Nocardia argentinensis* Huang ATCC 31306 by Celmer *et al.* (1979). Celmer *et al.* (1992) and Cane *et al.* (1993) also reported that it showed the highly antibacterial activity on *S. aureus*.

In conclusion, it is essential to produce antibiotics that can fight serious microbial diseases because nowadays life-threatening fungal and bacterial diseases are strongly increasing. In this strategic research, the isolated compound B showed good antibacterial activities on seven test organisms. Therefore, the active compound (Nargenicin A1) from *Streptomyces* sp. YT202 can effectively be used in the field of medicine to inhibit cholera, diarrhea, skin diseases and urinary infections on man as well as crown gall disease and leaf blight diseases on plants. These findings are very beneficial not only for our health but also for our farmers.

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References

Berdy J. 1989. **Bioactive Metabolites from Microorganisms**, in: *Progress in Microbiology*, Bushell M.E. and Gräfe, U. (Eds.), *Elsevier*, Amsterdam, *27*, 3-25.

Cane D.E., Tan W. and Ott W. R. 1993. **Narginisin Biosynthesis. Incorporation of Polyketide chain Elongation Intermideates and Support for a proposed Intramolecular Diels-Alder Cyclization**, J. Am. Chem. Soc., 115, 527-535.

Celmer W. D., Chmurny G. N., Moppett C. E., Ware R. S., Watts P. C., Whipple E. B. 1979. J. Am. Chem. Soc., 102, 4203.

Celmer W. D., Ware R. S, Whipple E. B. 1992. **The search of bioactive metabolites**, Springer, Berlin Heidelberg New York, p 459.

Davis, W. W and T.R. Stout. 1971. **Disc Plate Method of Microbiological Antibiotic assay**. Applied Microbiology. Vol. 22, No. 4.

Döhren H., Gräfe U. 1997. **General aspects of secondary metabolism**. In: Rehm HJ, Reed G, Pühler A, Stadler P (eds) Biotechnology, 2nd edn, vol. 7: Kleinkauf 11, von Döhren 11 (eds) Products of secondary metabolism.VCH Weinheim, p 1

Fenical W. 1993. **Biotechnology of antibiotics and other bioactive microbial metabolite**. *Chem. Rev*., *93*, 1673-1683.

Grabley S., Thiericke R. & Zeeck A. 1999. **The Chemistry Screening Approach**, In Drug Discovery from Nature; Springer-Verlag, Berlin, Heidelberg, New York, p 125-148.

Gräfe U. 1992. ***Biochemie der Antibiotika***, Spektrum Akad. Verlag, Heidelberg, Berlin, New York, p 42.

Kakinuma K. and Rinehart K. L. 1974. **Tryptophan-Dehydrobutyrine Diketopipera- zine, A Metabolite of Streptomyces spectabilis**, The Journal of Antibiotics, Vol. XXVII, No. 10, 731-737.

Mann J. and Murder. 1994. ***Magic and Medicine***, Oxford University Press, New York, p 5-14.

Nolan R. D., Cross T. 1988. **Isolation and screening of actinomycetes**. In: Goodfellow M, Williams S.T., Mordarski M. (eds) Actinomycetes in biotechnology. Academic Press, New York, p 1-12.

Monaghan R. L., Gagliardi M. M., and Streicher S. L. 1999. **Culture preservation and inoculum develpoment, Manual of Industrial Microbiology and Biotechnology**, Second edition, p 29-48.

Omura, S. 1984. **Microbial growth kinetics and secondary metabolites, J. Fermentation Technology**, 46: 134-140

Stroble R. J. and Sullivan G. R. **1999**. **Experimental Design for improvement of fermentations**, Manual of Industrial Microbiology and Biotechnology, Second edition, p 80-102.