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Two isomeric compounds from *Streptomyces* species and their antimicrobial activity

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Abstract

The chloroform extract of the culture filtrate of an isolated *Streptomyces* species upon chromatographic analysis had lead to the isolation of two isomeric compounds (I and II). The structure of the compounds was considered to be Stretomysone A (I) and Streptomysone B (II) by its spectral data. Both the compounds showed significant antimicrobial activity against tested pathogenic bacteria and fungus. The compounds seem to be first report of isomeric compound from *Streptomyces* species having antimicrobial activity.

Introduction

Microbial natural products still appear as the most promising source of the future antibiotics that society is expecting (Pelaez, 2006). Since the isolation of actinomycin in 1940 and streptomycin in 1944 by Waksman (Waksman and Woodruff, 1940; Schatz et al., 1944), the *Actinomycetes* have received tremendous attention of the scientists. Members of *Streptomyces* are a rich source of bioactive compounds, notably antibiotics, enzymes, enzyme inhibitors and pharmacologically active agents (Kazuki et al., 2005). About 75% of the known commercially and medically useful antibiotics are produced by *Streptomyces* (Sujatha et al., 2005). Waksman (Waksman, 1959) recognized the natural substrates that are ideal sources for the isolation of *Actinomycetes*. Among these, they are quite commonly found in soil, water and other environments (Ghanem et al., 2000). Owing to indiscriminate use of antibiotics and for various reasons the bacteria and other microorganisms are gaining resistance to the presently available antibiotics and pose a serious threat to the existence of human. Hence the search of new and more efficient antibiotics is a pressing need to time. As a part of our continuing studies of metabolites produced by microorganisms obtained soil samples collected throughout Bangladesh (Anisuzzaman, 2000), we isolated *Streptomyces* from soil sample and report herein the isolation of two isomeric compounds from the *Streptomyces* species and their antimicrobial activity.

Materials and Methods

Collection of organism

The organism was isolated from the soil sample, collected from the district of Pabna, Bangladesh at the depth of 0.5 meter using "crowded plate technique". The organism was identified as *Streptomyces* species (Anisuzzaman, 2000) by morphological and biochemical studies (Holt et al., 1994; Williams et al., 1983).

Production, isolation and purification of compounds

The organism was allowed to grow in a number of culture flasks of 500 mL capacity containing Czapek-

Dox broth alkaline medium at 37.5°C. The broth was separated from the mycelial mat on 8th day to get the maximum yield of antibacterial activity. The culture filtrate then subjected to repeated chloroform extraction and the extract was evaporated under reduced pressure. The crude antibiotic fraction was resolved by thin layer chromatography (TLC), preparative TLC (Stahl, 1969) using the solvent system, *n*-hexane, chloroform and methanol in a ratio of 7:5:1 (Stahl, 1969) and obtained on large scale on column chromatography (Beckett et al., 1986). For checking purity of the compound TLC was carried out using pre-coated silica gel 60 F254 plates (Merck) and detection was made by visualization under UV light (254 nm) and spraying with 0.1% vanillin sulfate spray reagent followed by heating.

Spectral measurement

UV spectra were recorded on a Beckman double beam spectrometer. IR spectra were obtained by a Perkin Elemer 1600 FTIR spectrometer. ^{1}H-(500 MHz) and ^{13}C (125 MHz) spectra were acquired on a JEOL JNM alpha spectrometer using TMS as internal standard.

Antimicrobial screening

Antimicrobial compound I and II (25 and 50 qg per disc, respectively) were determined against four Gram positive and five Gram negative bacteria, and four pathogenic fungus by standard disc diffusion method (Masako et al., 2004; Bauer et al., 1996). Amoxicillin disc (25 qg per disc) and griseofulvin (20 qg per disc) were used as standard for the comparison of antimicrobial activity for the bacteria and fungus, respectively. The test organisms were collected from the Department of Microbiology, University of Dhaka and antimicrobial activity was conducted at the Department of Pharmacy, Daffodil International University, Dhaka.

The minimum inhibitory concentration (MIC) values of the compounds were determined against Gram positive *(Bacillus subtilis, Streptococcus-β-hemolyticus)* and Gram negative (*Escherichia coli, Shigella dyscenteriae* and *Salmonella typhii A)* bacteria (10^7 cells/mL) by serial dilution technique (Reiner, 1982) in nutrient broth media.

Results and Discussion

The chloroform extract of the culture filtrate after resolution by conventional thin layer chromatographic technique yielded two compounds designed as I and II having Rf value 0.60 and 0.65, respectively in solvent system $CHCl_3$: CH_3OH (10:1). Both the compounds were crystalline and soluble in chloroform, ethyl acetate and methanol. Their structure was elucidated from the UV, IR, 1H-NMR, 13C-NMR and comparing the 13CNMR spectrum with the compounds, monocillinols 1 and 2 (Biswas et al., 2000).

Compound I: In UV spectrum, the strong absorption band at 209 nm indicated the presence of unsaturation. The IR spectrum revealed that the absorption band at 1760 cm-1 and 1420 cm-1 which demonstrative of carbonyl group (>C=O) in six membered lactone ring and >C=C< stretching in aromatic compound, respectively. While the absorption band at 1000 cm-1 and 1210 cm-1 are indicative of C-N and >C=O stretching and 820 cm-1 for C-H stretching (Pavia et al., 1979).

The 1H-NMR spectrum exhibited signals for two aromatic olefenic proton at δ 8.8 (1H, d like) and 5.9 (1H, m like), two methine proton at δ 8.5 (1H, d like) and 4.93 (1H, d like), two methylene proton in cyclic system at δ 8.79 (1H, m) and 2.7 (1H, m) and two methine proton at δ 2.51 (1H, m) and 2.0 (1H, m). The spectrum also showed signals for two methoxy methyl proton at δ 2.1 (3H, s) and 1.96 (3H, s). In addition for tertiary methyl, secondary methyl and primary methyl proton signal at δ 0.95 (3H, s) 1.18 (3H, d like) and 1.3 (3H, s like), respectively were also evident.

The 13C-NMR spectrum (Table I) of the compound I exhibited signals for three carbonyl carbon at δ 179.0, 172.77 and 168.0, a signal for a double bonded carbon attached with oxygen at δ 157.1, for two olefenic carbon at 132.9 and at δ 127.0, for olefinic tertiary carbon at δ 130.0 and two omethoxy carbon at δ 52.02 and 49.39, for tertiary carbon attached with electronegative atom or group at δ 47.18, for methylene group in cyclic ring system at δ 38.12 and for carbon attached with oxygen at δ 66.0.

From the IR and NMR spectra, the compound I was supposed to contained groups: One six-membered lactone ring, two methoxy group, two methyl group, two methylene group, three carbonyl group and two methene group.

Comparison of the 13C-NMR spectrum revealed that the compound I those of monocillinol 1 and was similar to those except at C-2 (157.1), C-5 (47.18) and C-6 (76.39). In addition compound I contained signals at δ 49.39 for OCH_3, 20.48 for CH_3 and at 168, 47.18 & 20.67 for (>$COCH_2$-CH_3), respectively.

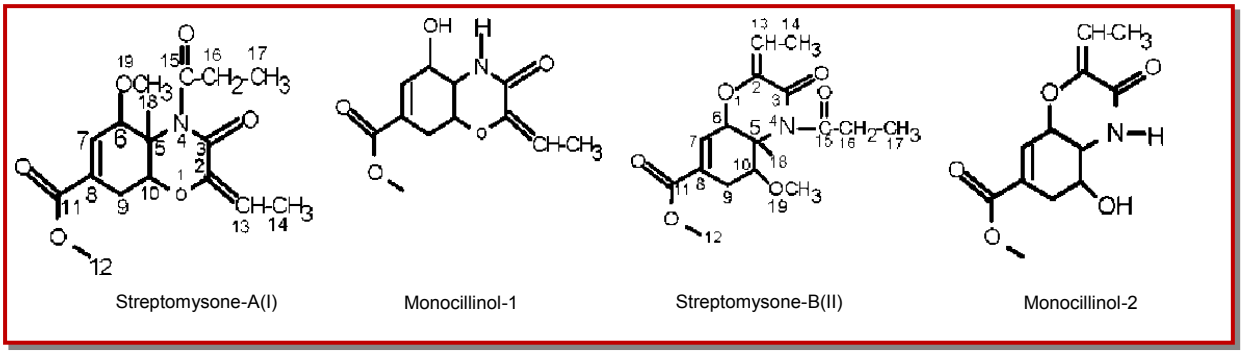
The shift of signal 58.1 to 47.18 at C-5 of monocillinol 1 could be explained by the presence of methyl group attached at C-5 position. Again the attachment of methyl group at C-13 could be explained considering the shift of signal from 98.7 to 127.0. The remaining signals –CO-CH_2-CH_3 and –OCH_3 were could assign at position 4 and 19 respectively.

Compound II: In UV spectrum, the strong absorption band at 215 nm indicates the presence of unsaturation. In IR spectrum, the absorption band at 1750 cm-1 and 1470 cm-1 are characteristics of carbonyl group (>C=O) of six membered lactone ring and >C=C< stretching in aromatic compound, respectively. The absorption band

Table I

^{1}H-NMR and ^{13}C-NMR spectral data (500 MHz, $CDCl_3$) for compound I and II $CDCl_3$) for compound I and II

Position of carbon	^{13}C-NMR of 5 in ppm			
	Monocillinol 1	Streptomysone A (I)	Monocillinol 2	Streptomysone B (II)
1	-	-	-	-
2	157.1	153.0	-	-
3	168.8	172.8	-	-
4	-	-	-	-
5	58.1	47.2	59.0	46.5
6	75.7	76.9	76.7	70.8
7	134.4	132.9	127.5	127.0
8	130.9	130.0	141.1	147.6
9	35.0	38.1	30.2	29.6
10	67.0	66.0	72.6	73.7
11	166.0	168.0	166.3	170.1
12	52.1	52.0	51.9	52.6
13	98.7	127.0	98.5	122.0
14	-	20.5	-	20.5
15	-	179.0	-	179.0
16	-	17.2	-	47.2
17	-	20.7	-	20.7
18	-	13.1	1.0	17.7
19	-	49.4	2.0	49.4



at 1260 cm-1 and 1300 cm-1 are indicative of C-N and >C=O stretching and at 820 cm-1 for C-H stretching (Pavia et al., 1979).

In 1H-NMR spectrum, the signals at δ 8.76 (1H, d like) and 5.86 (1H, m like) may be attributed to two aromatic olefinic proton. The signals at δ 8.52 (1H, d like) and 4.93 (1H, d like) may be due to two methine proton. The proton signals at δ 8.79 (1H, m) and 2.7 (1H, m) may be ascrible to two methylene proton in cyclic system and at δ 2.51 (1H, m) and 2.0 (1H, m) may be due to two methine proton. The proton signals at δ 2.1 (3H, s) and 1.96 (3H,s) may be due to two methoxy methyl proton. The proton signals at δ 0.95 (3H,s) 1.18 (3H, d like) and 1.3 (3H, s like) may be due to tertiary methyl, secondary methyl and primary methyl proton, respectively.

In ^{13}C-NMR spectrum (Table I) the compound II exhibited signal at δ 122 which may be due to the double bonded carbon attached with oxygen. The carbon signals at δ 127.0 for olefinic carbon and δ 147.64 for olefinic carbon attached with carbon containing oxygen. The signals at δ 52.61 and 49.31 for two omethoxy carbon, at δ 46.54 for tertiary carbon and at δ 39.64 for methelene carbon in cyclic system. Carbon signal at δ 73.73 for metheine carbon attached with oxygen and at δ 47.18 for methelene carbon.

From the IR and NMR spectra, the compound II is supposed to contain the following groups: One six

Table II

Antibacterial activity of the compound I and II

Test bacteria	Diameter of zone of inhibition (mm)				
	Compound I		Amoxicillin	Compound II	
	25 pg per disc	50 pg per disc	25 pg per disc	25 pg per disc	50 pg per disc
Gram positive					
Bacillus subtilis	17	25	29	15	20
Bacillus megatrium	18	25	27	12	16
Staphylococcus aureus	11	16	26	09	14
Streptococcusf-hemolyticus	12	16	27	10	14
Gram negative					
Escherichia coli	20	28	33	25	31
Pseudomonas aure-ginosae	12	17	28	18	25
Shigella dyscenteriae	16	24	33	22	28
Salmonella typhii A	11	19	29	20	29
Klebsiella sp.	14	22	25	17	26

Table III

Antifungal activity of the compound I and II

Test pathogen	Diameter of zone of inhibition (mm)		
	Compound I	Griseofulvin	Compound II
	25 pg/disc	20 pg/disc	100 pg/disc
Tinea pedis	21	16	9
Tinea corporis	16	17	12
Candida albicans	13	15	10
Rhizoctoni solani	14	19	9

membered lactone ring, two methoxy group, two methyl group, two methylene group, three carbonyl group and two methene group.

The ^{13}C-NMR spectrum of the compound II was compared with those of monocillinol 2 and was found to vary at C-2 (157.1), C-5 (46.5) and C-6 (70.8). In addition to the presence of the following signals at δ 49.39 (OCH_3) and carbon signal at δ 170.1, 47.18 and 20.68 for (>CO-CH_2-CH_3), respectively.

The signals of monocillinol 2 at C-5 are shifted from 59.0 to 46.5 may due to the presence of methyl group at compound II. Similarly the carbon signals at C-13 is shifted from 98.5 to 122.0 may be due to the methyl group at C-13 position. So there is possibility of arrangement of -CO-CH_2-CH_3 group with N (at position No. 4, instead of its proton).

Antimicrobial activities of the compounds: Both the compounds showed significant antimicrobial activity against the test pathogens (Table II). However, the compound I exhibited strong activity against *Bacillus subtilis, Escherichia coli* and *Klebselia* species and comparatively weak activity was observed against *Pseudomonas aureginosa, Salmonella typhii* A and *Shegella dyscenteriae*. While the compound II exhibited strong activity against Gram negative than Gram positive bacteria. The compounds are also active against tested pathogenic fungus. The compound I showed promising antifungal activity compared to the standard griseofulvin (Table III).

Minimum inhibitory concentrations of the compounds: The MIC values of the compound I against *Bacillus subtilis, Streptococcus-β-hemolyticus, Escherichia coli, Shigella dyscenteriae* and *Salmonella typhii* A were 16, 32, 16, 32 and 128 pg/mL, respectively and that for compound II were 32, 64, 16, 16 and 32 pg/mL, respectively. From the MIC values it was found that both the compounds were potent against *Bacillus subtilis* and

Escherichia coli.

Though the compounds are isomer each other, their antimicrobial spectrum are quite different. Thus, the finding of this investigation would give us valuable support to search more potent antagonistic microorganism from the soil of different regions of Bangladesh.

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