Proceedings of the First International Conference (Egyptian British Biological Society, EBB Soc)
Egyptian Journal of Biology, 2001, Vol. 3, pp 11-21

Isolation and characterisation of an antimicrobial substance produced by *Streptomyces violatus*

Moustafa Y. El-Naggar[1*] Maha A. Hassan[2], and Wafa Y. Said[1]

1. Botany & Microbiology Department, Faculty of Science Alexandria University, Alexandria, Egypt
2. Biology Department, Faculty of Education, Alexandria University, Alexandria, Egypt

ABSTRACT

Streptomyces violatus produced an antimicrobial substance (268 μg/ml) after 7 days incubation at 30°C in static cultures. Fifteen litres of the culture filtrate were extracted with ethyl acetate and concentrated till dryness. The oily extract was subjected to silica gel column chromatography. Gradient elution was carried out using n-hexane-ethyl acetate (4:1→1:14) and ethyl acetate-ethanol (7:3→1:10). The fractions showing the highest antimicrobial activity were pooled and subjected to a second silica gel column chromatography. The active fractions obtained from the second column were pooled and further purified using preparative high performance liquid chromatography (HPLC). The major peak was collected, concentrated and subjected to spectroscopic analysis (300 MHz ^{1}H-NMR, 2D COSY, 75 MHz ^{13}C-NMR, DEPT, UV, IR, Mass spectrometry, elemental analysis) in order to characterize the antimicrobial compound. ^{1}H-NMR spectra indicated the presence of a di-substituted benzene ring with two aliphatic side chains. The molecular formula of $C_{15}H_{23}NO_3$ with a molecular weight of 276 was estimated from the elemental analysis and mass spectra. Spectroscopic analysis of the purified fraction indicated that the antibiotic produced by *S. violatus* belongs to an aromatic family of antibiotics, but this compound was not identical to similar antibiotics described in the literature and was designated MSW2000. Gram-positive bacteria were more sensitive than Gram-negative to MSW2000. The antibiotic weakly affected the growth of *Saccharomyces cerevisiae* and *Mucor meihei*. X-ray microanalysis of *S. aureus* treated with the antibiotic MSW2000 showed a reduction in element levels in the cells, especially K, P and S, which may indicate that the antibiotic affects the cell membrane.

KEYWORDS: Antibiotic MSW2000, spectroscopy, *Streptomyces violatus*, antimicrobial activity, X-ray microanalysis.

INTRODUCTION

The genus *Streptomyces* exhibits a remarkable capacity for biochemical differentiation, producing a wide variety of secondary metabolites (Berdy 1984; Miyadoh 1993) which show diversity of chemical structures and biosynthesis (Arisawa *et al*. 1996). Many of these compounds have important applications in human medicine as antibacterial, antitumor and antifungal agents and in agriculture as growth promoters, agents for plant protection, antiparasitic agents and herbicides.

Methodology of isolation and purification of antibiotics to a large extent depends on the properties of the antibiotic, its chemical nature and the environment in which it is accumulated (Waksman & Lechevalier 1962). Separation methods have been developed, and without doubt natural chemistry has greatly stimulated to the development of the refined techniques applied today. Chromatographic methods including thin layer chromatography (TLC), column chromatography, gas chromatography (GC) and high performance liquid chromatography (HPLC), have made it possible to isolate compounds present in extremely small quantities. Spectral analysis including infrared (IR), ultraviolet (UV), NMR (nuclear magnetic resonance) spectroscopy and mass spectroscopy are used in the identification of antibiotics (Williams & Fleming 1987; Hayakawa *et al*. 1994 & 1996). The data obtained from spectral analysis can be

* Address for Correspondence

interpreted to give the most probable structure of the antibiotic (El-Naggar 1991 & 1996; Hayashi *et al*. 1992; Hwang *et al*. 1994).

In the present work, the antimicrobial substance produced in *S. violatus* was extracted, isolated and purified. The purified substance was characterized in order to determine its possible structure. The antimicrobial spectrum of the isolated substance was also determined.

MATERIALS AND METHODS

Organism and cultivation

Streptomyces violatus was isolated from garden soil of the Faculty of Science (El-Shatby), Alexandria, Egypt. This strain was identified as *Streptomyces* according to the International Streptomyces Project (ISP) Scheme as described by Shirling & Gottlieb (1966) and the diagnostic key of Szabo (1975). It was maintained on starch-casein agar slants and kept in a refrigerator at 4^{o}C until further use.

For antibiotic production, a medium was used composed of (g/l): glycerol, 12.5, $NaNO_3$, 2.5, K_2HPO_4, 1.0, KH_2PO_4,1.0 , $MgSO_4.7H_2O$, 0.5, KCl, 0.5, trace salt solution 1.0 ml ($CuSO_4.5H_2O$ (0.64 g/l), $FeSO_4.7H_2O$ (0.11 g/l), $MnCl_2.4H_2O$ (0.79 g/l) and $ZnSO_4.7H_2O$ (0.15 g/l), distilled water,1.0 litre. The pH of the medium was adjusted to 7.0 before autoclaving using 0.1N NaOH or 0.1 N HCl solution.

Target organisms

The following test organisms were used for the determination of minimum inhibitory concentration (MIC) of the antibiotic: *Staphylococcus aureus* (209 P FDA), *Sarcina leutea* (NCIB 495), *Bacillus subtilis* (ATCC 6051), *Escherichia coli* (NCIB 1186), *Klepsiella pneumonia* (Local isolate), *Pseudomonas fluorescens* (ATCC 13525), *Enterobacter cloacae* (ATCC 13047), *Aspergillus terreus* (Tü 155), *Mucor miehei* (Tü 284), *Saccharomyces cerevisiae* (Local isolate) and *Candida albicans* (Local isolate).

Antibiotic Bioassay

This was carried out using paper disc diffusion method and Mueller-Hinton agar as an assay medium and *S. aureus* as a test organism. The Mueller-Hinton agar (45^{o}C) was poured in sterile Petri dishes (9-cm diameter) and allowed to solidify. 0.1 ml bacterial suspension (3 x 10^6 cells) of the test organism was inoculated into the agar surface. Sterile paper discs (6.0 mm diameter, Whatman antibiotic assay discs), were placed on the dried surface of the medium using alcohol-flame-sterilized forceps. Each disc received 20 µl of the antibiotic preparation. Petri dishes were kept in a refrigerator for 2 hours to allow for the diffusion of the antibiotic. Petri dishes were then incubated inverted for 18-24 hours at 37^{o}C. The inhibition zone was measured in mm (Amade *et al*. 1994).

Extraction of the antibiotic

Culture medium (15 litre) was inoculated (4%) and incubated at 30^{o}C for 7 days in a Köttermann incubator. After the incubation period, the culture filtrate was separated from the mycelial cake using Chilspin MSE Fisons centrifuge at 4^{o}C, 5000 rpm for 15 minutes. In this part of the work, it was aimed to select the best solvent for the extraction of the antibiotic from the culture medium. Three solvents were examined including petroleum ether, ethyl acetate and chloroform. After measuring the antibiotic activity (inhibition zone diameter) and ^{1}H-NMR spectra for the petroleum ether, ethyl acetate and chloroform extracts, it was found that ethyl acetate was the best solvent and the culture filtrate was extracted with an equal volume of pre-distilled ethyl acetate (BDH, Analar grade). The organic layer was separated and dried over anhydrous sodium sulphate, filtered and concentrated in a rotary evaporator (Büchi RE-114 rotary evaporator,

Switzerland, water bath B-480) at 40°C till dryness. The crude residue (350-mg) was then dissolved in a minimum amount of pre-distilled ethyl acetate and transferred into a dry and clean glass vial and kept in a dessicator for 4 days. The antibiotic activity of the crude extract was then determined using *S. aureus* as a test organism.

Purification of the antibiotic

The crude residue (350 mg) was dissolved in a minimum amount of ethyl acetate (BDH) and applied to a silica gel column chromatography (Sorbisil 60 mesh; column dimensions 2.5 cm inner diameter x 30 cm length). The column was eluted with n-hexane:ethyl acetate (4:1→1:14). Seventy-six fractions (30 ml each) were obtained. All fractions were then tested for their complexity using thin layer chromatography (TLC) plates (Merck, Kiesel gel 60 F_{254}, 20 x 20cm, 0.2 mm thickness). Based on the complexity of each fraction, the fractions were regrouped to make 18 fractions. The activity of these fractions was assessed against *S. aureus* as a test organism. Fractions 1-8 (250 mg) were red in colour and showed the highest activity (28 mm inhibition zone diameter); fractions 9-16 (35 mg) were dark violet with lower activity (12 mm inhibition zone diameter) and fractions No. 17 & 18 (30 mg) were mauve in colour and showed no activity. The major (red) fraction (1-8) was then subjected to the second round of purification. The semi-purified red fraction (250 mg) was loaded on the silica gel column (2cm inner diameter x 20 cm length) and eluted with n-hexane: ethyl acetate (2:1→1:19), ethyl acetate (100%), ethanol: ethyl acetate (1:9 → 9:1) and ethyl alcohol (100%). Fifteen sub-fractions (10 ml each) were obtained and the activity and complexity of each fraction were determined as previously described. Sub fractions1-3 (A_1) were complex (2 spots on TLC plates) with low activity (12 mm inhibition zone diameter) while fractions 4-10 (A2) contained only one spot (28 mm inhibition zone diameter) with the same R_f value (0.53) using iodine vapour and UV-detection at 230nm. Fractions No. 11-15 (A_3) gave the lowest value of inhibition zone diameter (9mm). The A_2 fraction was evaporated at 40°C in a rotary evaporator till dryness (150 mg) and then subjected to analytical high performance liquid chromatography (HPLC) using a Spherisorb C18 column (0.46cm i.d.x25cm). The column was run with a flow rate of 1ml/min with ultra-violet detection. The HPLC trace for A_2 showed one major peak very close to one shoulder. The major peak was successfully separated from the shoulder using an HPLC preparative column (0.80 cm i.d. x 25 cm). The major peak was collected and concentrated (70 mg, 30mm inhibition zone diameter) and then submitted for spectrosopic analyses: 300 MHz ^{1}H-NMR (Proton-Nuclear Magnetic Resonance), 2D COSY (Two Dimentional Correlated Spectroscopy), 75 MHz ^{13}C-NMR (Carbon-Thirteen Magnetic Resonance), DEPT (Distortionless Enhancement by Polarization Transfer). All preparations were measured in $CDCl_3$ (Deuterated Chloroform) using a Varian Oxford 300 MHz NMR spectrophotometer. The flow diagram for antibiotic isolation is summarized in Figure 1.

Mass spectra (MS) were performed using Shimadzu GC MS-QP 1000 EX mass spectrometer at 70 eV. The analyses were carried out at the Magnetic Resonance Laboratory, Faculty of Science, Cairo University. Elemental analysis and Infra-Red Spectra (IR) were carried out at the Microanalytical Centre, Faculty of Science, Cairo University. UV absorption was carried out using Unicam 1750 ultraviolet spectrophotometer at the Central Laboratory, Faculty of Science, Alexandria University. X-ray microanalysis using X-ray Link AN 10000 analyzer was carried out at the Electron Microscope Unit, Faculty of Science, Alexandria University, Egypt.

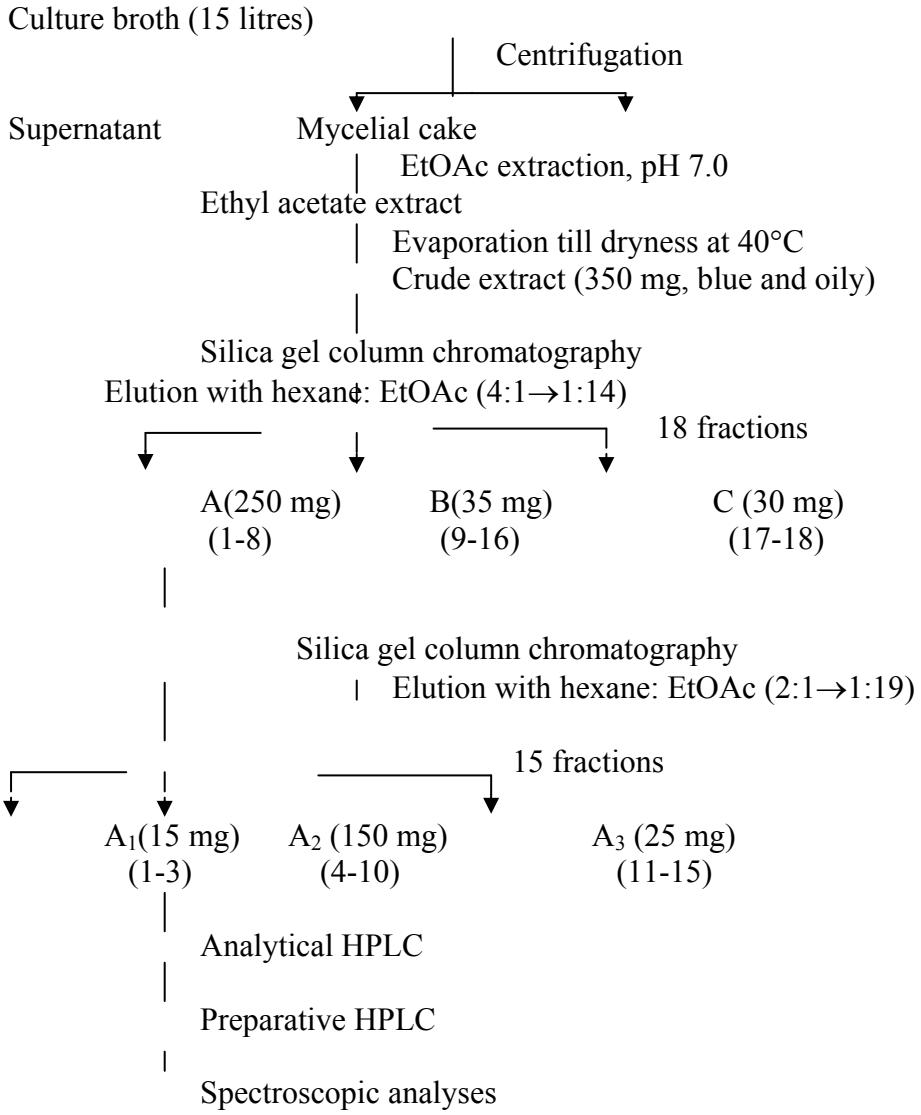


Figure 1. Schematic diagram shows the isolation procedure of the antibiotic MSW2000.

Determination of minimum inhibitory concentration (MIC)

The antimicrobial activity of the antibiotic was determined by 2-fold dilution method using Mueller-Hinton agar (Difco) and MICs were read in µg/ml after overnight incubation at 37°C (Omura *et al*. 1993).

Yeast extract-malt extract medium was used for yeast, which was incubated at 28°C and fungi at 30°C according to Balagurunathan & Subramanian (1993).

RESULTS

Characterization of the antibiotic

1- Ultraviolet (UV) Spectrum

The UV spectrum (Fig.2) indicates the presence of an aromatic ring with a maximum absorption at ε_{max} (EtOH) of 204.3 nm and a shoulder at 222.3 nm.

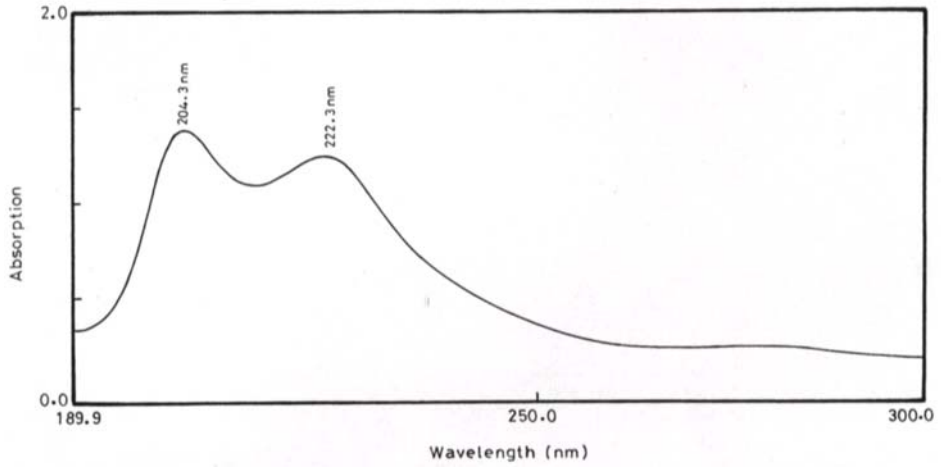


Figure 2. Ultraviolet (UV) spectrum of the purified antibiotic MSW2000.

2- Infrared (IR) Spectrum

The IR spectrum (Fig. 3) had a peak at about 1700 cm^{-1}, which indicates carbonyl function of an ester or an amide group. The peaks appearing at 3600-3200 cm^{-1} indicated the presence of hydroxyl group absorption. The peaks at 1382 cm^{-1} may be R-CO-NH_2 or R-CO-NH-R components and finally the peaks between 699-784 cm^{-1} can be assigned to -CH groups.

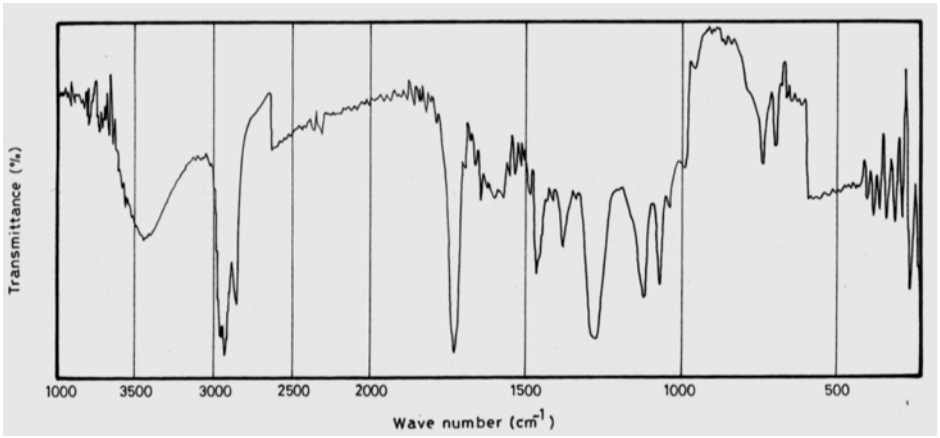


Figure 3. Infrared (IR) spectrum (KBr) of the purified antibiotic MSW 2000.

Magnetic Resonance (NMR) Spectra

a- Proton-NMR [(^{1}H-NMR) σ_H (300 MHz; $CDCl_3$)]

Proton nuclear magnetic resonance experiment (Fig. 4) showed that the peaks at 7.57 and 7.73 ppm indicate a di-substituted aromatic ring. There also appear to be a double doublet at 4.24 ppm which may belong to an -O-CH_2-group. The peaks at *ca.* 1.30 and 0.90 ppm may contain other dialiphatic groups and dimethyl groups. The exact linkage position of the partial structures was studied by ^{1}H NMR double reasonance experiments (COSY). It appears that the peak at 4.24 ppm is coupled to the peak cluster at 1.30 ppm.

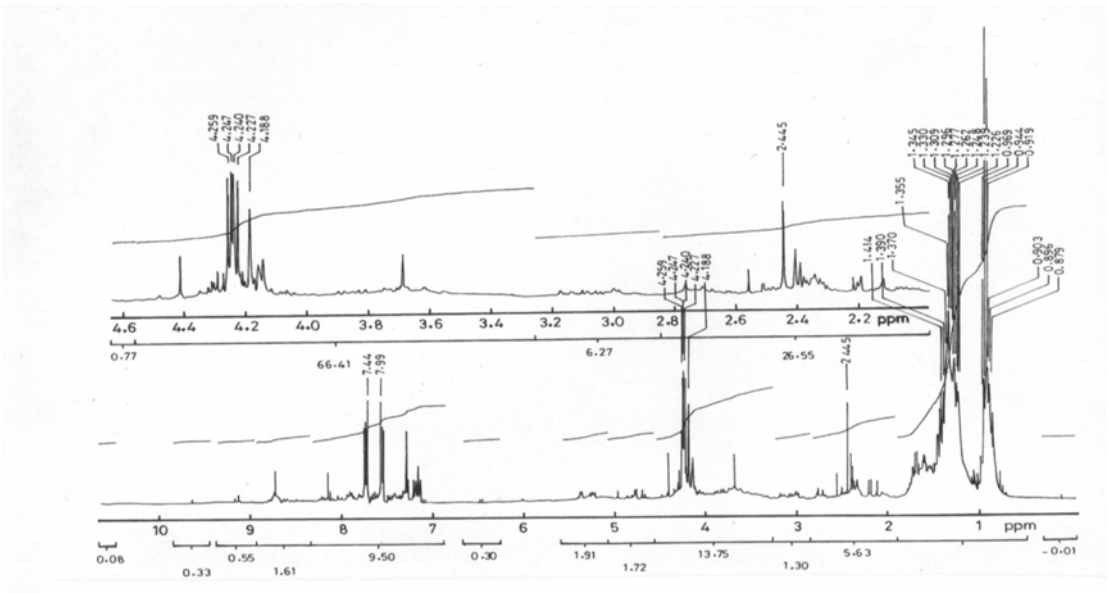


Figure 4. ^{1}H NMR spectrum ($CDCl_3$) of the purified antibiotic MSW 2000.

b- Carbon-13 NMR [(^{13}C-NMR) σ_C (75 MHz; $CDCl_3$)]

The ^{13}C-NMR (Fig. 5) spectrum showed two methyl groups at 6.66 and 9.78 ppm. There are also five CH_2 groups at 18.67, 19.37, 24.57, 25.98 and 63.73 ppm with the latter being in a completely different environment compared to the other four. This may mean that it is attached to an oxygen atom as a part of an ester group. There are also three H groups: the one at 34.31 ppm is probably an aliphatic CH group whilst those at 124.27 and 126.40 ppm belong to aromatic CH groups. The other peaks between 120-130 ppm (Fig.6), which disappear in Distortionless Enhancement by Polarization Transfer (DEPT) experiment are also aromatic carbons which carry no hydrogen atoms (which disappear in DEPT analysis). Finally, the peak at 167.59 ppm, which also disappears on DEPT analysis, is a C = O group belonging to either an amide or an ester function.

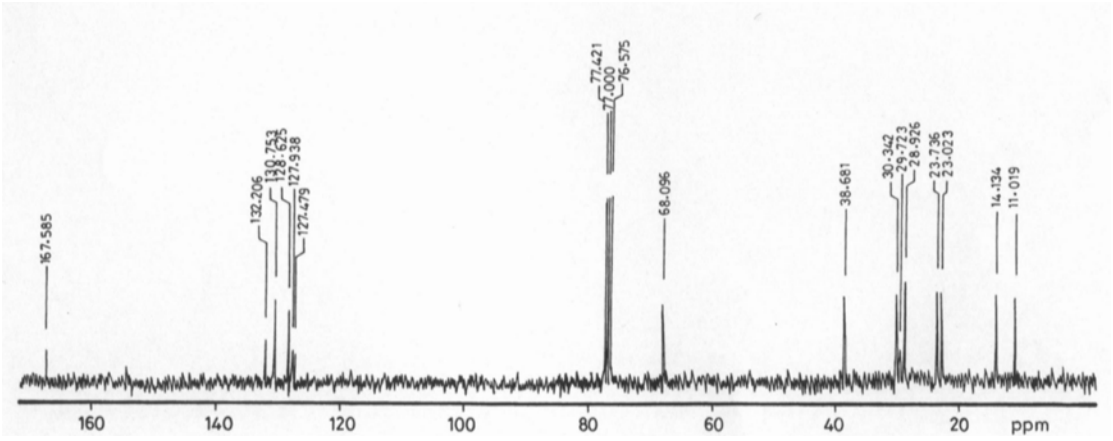


Figure 5. ^{13}C NMR spectrum ($CDCl_3$) of the purified antibiotic MSW 2000.

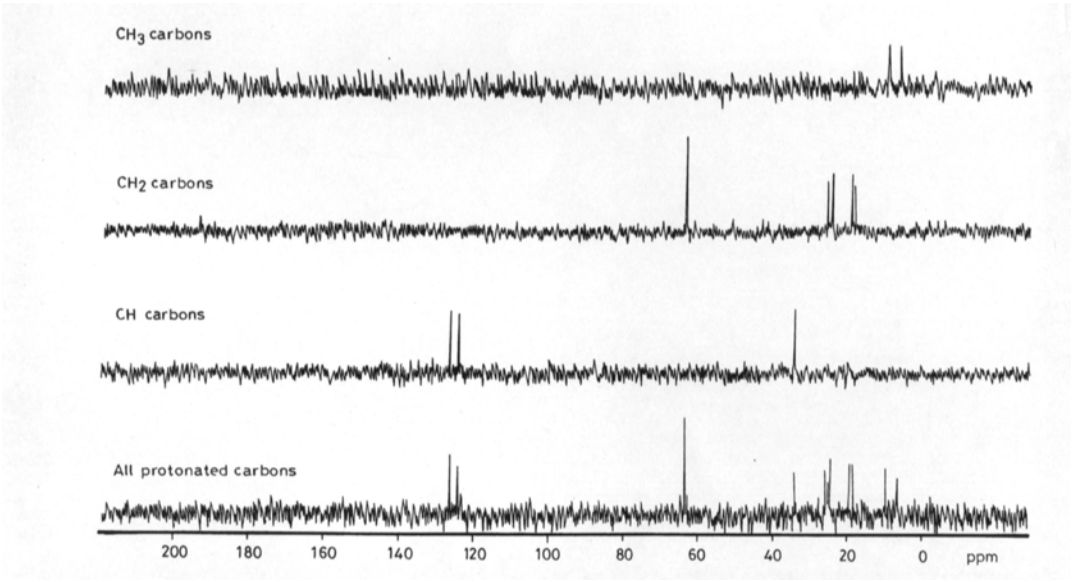


Figure 6. DEPT ^{13}C NMR spectrum ($CDCI_3$) of the purified antibiotic MSW 2000.

4- Mass Spectra

Since the electron impact mass spectrum (EI-MS) did not show a definite molecular ion, the molecular formula $C_{15}H_{23}NO_3$ of the antibiotic was estimated from the elemental analysis (Anal.Calcd. for $C_{15}H_{23}NO_3$: C 67.34, H 9.95. N 5.24; Found C 66.34, H 9.23, N 4.99) in accordance with the number of carbon atoms observed in the ^{13}C NMR spectrum (multiplicity assigned by DEPT).

According to the described chemical assignments obtained from the UV, IR, NMR, MS and elemental analysis, this compound is not identical with similar antibiotics described in literature. The antibiotic is therefore designated as MSW 2000 and its probable structure is given in Figure 7.

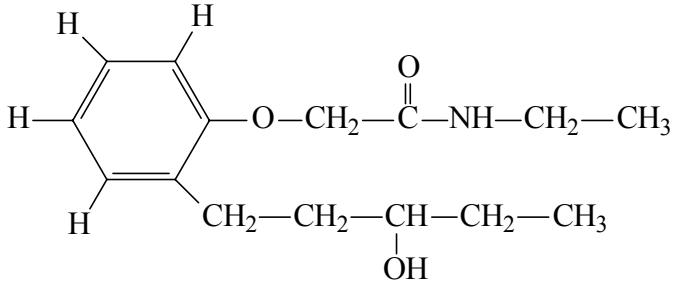


Figure 7. Molecular structure of the antibiotic MSW 2000.

Antimicrobial Spectrum of the Antibiotic MSW2000

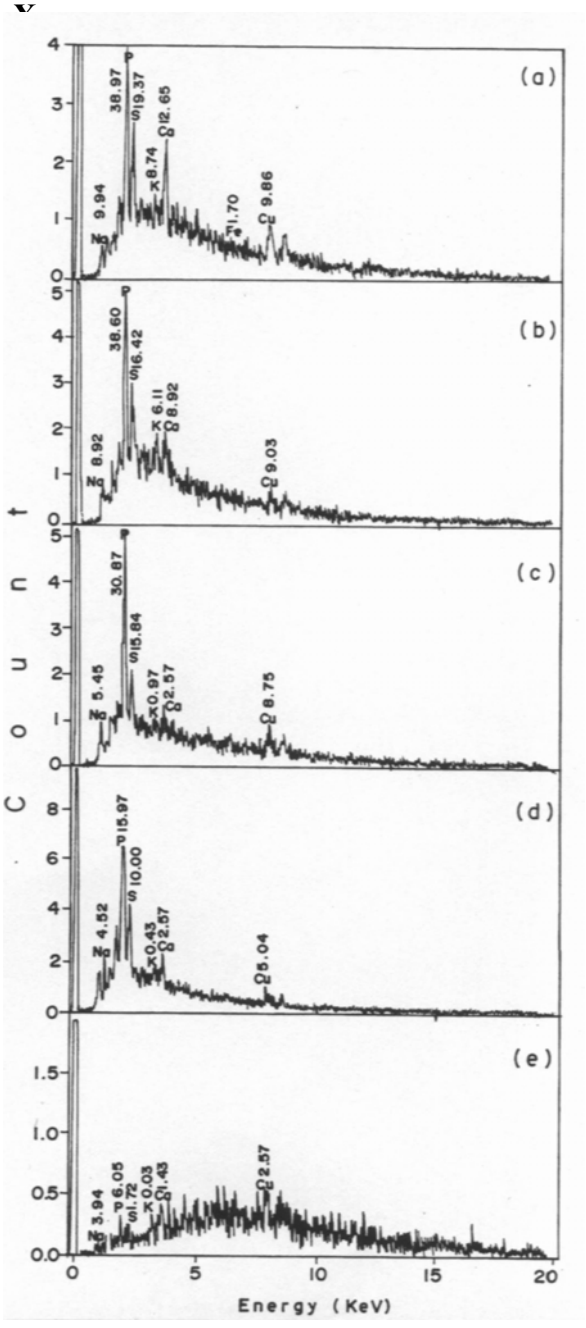
Minimum inhibitory concentrations (MICs) were determined using a two-fold dilution method. The assay media were Mueller-Hinton agar for the bacteria at pH 7.0, glucose-Peptone for fungi and yeast extract-malt extract for yeast. Bacteria were incubated at 37°C, fungi at 30°C and yeast at 28°C.

The MICs are shown in Table 1, It seems that the antibiotic is active against a number of Gram-positive and Gram-negative bacteria. It was noticed that, Gram-positive bacteria were more sensitive to the antibiotic than Gram-negative bacteria. An antibiotic concentration of 0.39 µg/ml completely abolished the growth of *S. aureus* and *B. subtilis*, and 0.78µg/ml for *S. leutea.* For Gram-negative bacteria, the MIC for *E. coli* and *Klepsiella peneumonia* was 1.56 µg/ml. *E. cloacae* and *P. fluoresens* had the same MIC (6.25 µg/ml).

On the other hand, the antibiotic has a weak effect on the growth of *S. cerevisiae* and *M. miehei* (MIC = 12.5 µg/ml), and *C. albicans* and *A. terreus* were ever more resistant with MIC of 50 µg/ml and 25 µg/ml, respectively.

Table 1: Antimicrobial spectrum of the antibiotic MSW2000 produced by *S. violatus*.

Target Organism	MIC (μg/ml)
Bacillus subtilis	0.30
Sarcina lutea	0.87
Staphyllococcus aureus	0.39
Escherichia coli	1.56
Klepsiella pneumonia	1.56
Enterobacter cloacae	6.25
Pseudomonas fluorescens	6.25
Candida albicans	50.0
Saccharomyces cerivisiae	12.50
Mucor miehei	12.50
Aspergillus terreus	25.0



eated with the antibiotic MSW 2000

In the present experiment, X-ray microanalysis was used to provide information on the elemental composition of *S. aureus* before (control) and after its treatment with antibiotic.

X-ray emission spectra from control cells of *S. aureus* and those which were treated individually with four concentrations (3, 6, 12 and 24 µg/ml) of the pure antibiotic MSW 2000 typically had clear peaks of Na, P, S, K, Ca and Cu. In addition to these elements Fe was only confined at low levels (1.7%) to the control (untreated) cells.

The data shown in the X-ray trace (Fig. 8) clearly showed that the antibiotic could reduce the elemental levels of *S. aureus* cells upon increasing the antibiotic concentration. The maximum reductive effect of the antibiotic occurred for K where the highest percentage reduction was recorded (30.1% at 3 µg/ml, 88.9% at 6 µg/ml, 95.1% at 12 µg/ml and 99.7% at 24 µg/ml). Phosphorous represented the highest occurrence percentage in the control (39 %) and this was reduced by 1.0, 20.8, 59.9 and 84.5% at 3, 6, 12 and 24 µg/ml, respectively.

Figure 8. X-ray emission spectra from the whole *Staphyllococcus aureus* cells. (a) Control (untreated) cells. Cells treated with 3µg/ml (b), 6 µg/ml (c), 12 µg/ml (d) and 24 µg/ml (e) of the purified antibiotic MSW 2000.

Sulphur was the second highest percentage in the control sample (19%) and this showed lowering at 3µg/ml (15.3%), 6 µg/ml (18.2%), 12 µg/ml (48.4%) and at 24 µg/ml (91.1%). On the other hand, Na and Cu recorded closely similar levels in the control sample. Compared to Cu, the reduction percentage of Na was higher at the first three concentrations (1.9, 33.9 and 5.6%, for 3, 6 and 12µg/ml, respectively).

Calculation of correlation coefficients between the mean concentrations of elements in the control cells showed a clear indication of elemental associations in the cell. Positive correlation (correlation coefficient 0.76 or greater) occurred between K-P (0.82), P-Ca (0.81), Ca-S (0.76), P-S (0.97) and K-Ca (1.00), with negative correlation between Na-S (-0.82).

DISCUSSION

Bioassay-directed fractionation of ethyl acetate extract by silica gel semi-preparative TLC gave an active fraction (A_2) which, on further purification by reversed-phase HPLC, yielded a red compound. This red compound is a pH indicator having a red colour at a neutral and acidic pH range, but purple at alkaline pH values. Identification of this compound was based on elemental analysis combined with NMR techniques, mass, IR and UV spectral analysis.

Spectroscopic analysis of fraction A_2 indicated that the antibiotic belongs to an aromatic family of antibiotics. The IR absorption spectra indicated the presence of a carbonyl group of an ester or amide (1700 cm^{-1}), hydroxyl group absorption (3600-3200 cm^{-1}) and R-CONH-R at 1382 cm^{-1}. The UV spectrum featured absorption at 204.3 nm and a shoulder at 222.3 nm-this confirmed the presence of an aromatic ring in the structure. Proton nuclear magnetic resonance (^{1}H-NMR) mainly indicated the presence of a di-substituted benzene ring with two aliphatic side chains. Carbon-13 nuclear magnetic resonance (^{13}C-NMR) revealed the presence of five CH_2 and two CH_3 groups together with the quaternary carbon as well as aromatic carbon (CH). On the other hand, mass spectra gave a 100% signal at m/e=276 corresponding to $C_{15}H_{23}NO_3$. A search with AntiBase (Laatsch 1994) was performed and the structure proved to be not the same as other antibiotics described in the literature. Consequently, the antibiotic is designated as MSW 2000.

The antimicrobial activity of the antibiotic MSW 2000 against a number of clinical strains indicated the broad-spectrum nature of the antibiotic, since it is active against a number of Gram-positive and Gram-negative bacteria. On the other hand, yeasts and fungi were less susceptible to the antibiotic treatment.

Electron-probe X-ray microanalysis has two major advantages in studies of the elemental composition (Sigee *et al*. 1993): high spectral resolution (allowing study of single cells and sub-cellular components) and simultaneous monitoring of a wide range of detectable elements (using energy-depressive x-ray microanalysis). Using this technique on the whole-cell preparations of *S. aureus* revealed that the antibiotic disturbed the element composition of this bacterium. Increasing antibiotic concentration resulted in a decrease in the levels of many elements. Positive correlation among these elements indicated that they may occupy the same binding site (K-P, P-Cu, Ca-S, P-S and K-Ca), whereas negative correlation imply only that Na and S are antagonistic and may compete for the same binding site. These changes may indicate that this antibiotic affects the membrane potential as a possible target.

Acknowledgement: Authors are indebted to Dr. Clive M. Raynor, Chemistry Department, Faraday Building, UMIST, Manchester, United Kingdom for his valuable and critical revision of the assignments of the spectroscopic analysis.

REFERENCES

Amade P, Mallea M & Bouaicha N (1994) Isolation, structural identification and biological activity of two metabolites produced by *Penicillium olsoniibainier and Sartory*. *Journal of Antibiotics*. 47 (2): 201-207.

Arisawa A, Kawamura N, Narita T, Kojima I, Okumura K, Tsunekawa H, Yashioka T & Okamoto R (1996) Direct fermentative production of acyltylosins by genetically engineered strains of *Streptomyces faradiae*. *Journal of Antibiotics* 49: 349-354.

Balagurunathan R & Subramanian A (1993) Studies on marine *Streptomyces nigrifaciens* (P-9). I-Taxonomy and standardization of antibiotic production. *Ciencias Marinas* 19(4): 435-443.

Berdy J (1984) New ways to obtain antibiotics. *Journal of Antibiotics* 7: 272-290.

El-Naggar MYM (1991) Antibiotic Production by some mutants of *Streptomyces nasri.* M.Sc. Thesis, Faculty of Science, Alexandria University, Alexandria, Egypt.

El-Naggar, MYM (1996) Some studies on the antibiotic produced by *Streptomyces* strain YG62. Ph.D. Thesis, Faculty of Science, Alexandria University, Alexandria, Egypt.

Hayakawa Y, Ishigmi K, Shin-Ya K & Seto H (1994) Menoxymycins-A and -B, antitumor antibiotics generating active oxygen in tumor cells. *Journal of Antibiotics* 47(11): 1344-1347.

Hayakawa Y, Sohda K, Furihata K, Kuzuyama T, Shin-Ya K & Seto H (1996) Studies on new antitumor antibiotics, leptofuranins A, B, C and D.I. fermentation, isolation and biological activities. *Journal of Antibiotics* 49 (10): 974-979.

Hayashi K, Hashimoto M, Shigematsu N, Nishikawa M, Ezaki M, Yamashita M, Kiyoto S, Okuhara M, Kohsaka M & Imanaka H (1992) WS9326A, a novel tachykinin antagonist isolated from *Streptomyces violaceousniger* No. 9326 I. Taxonomy, fermentation, isolation, physico-chemical properties and biological activities. *Journal of Antibiotics* 45(7): 1055-1063.

Hwang DT, Nuremberg JB & Schreiber SL (1994) Distinct binding and cellular properties of synthetic (+) and (-) discodermolides. *Journal of Chemistry & Biology* 1(1): 67-71.

Laatsch H (1994) AntiBase, microbial metabolites database, Weinheim, Germany: Chemical concepts.

Miyadoh S (1993) Research on antibiotic screening in Japan over the last decade: a production microorganisms approach. *Actinomycetologica* 7: 100-106.

Omura S, Pyl DVD, Inokoshi J, Takahashi Y & Takeshima H (1993) Pepticinnamins, new farnesyl-protein transferase inhibitors produced by an actinomycete. I. Producing strain, fermentation, isolation and biological activity. *Journal of Antibiotics* 46(2): 222-228.

Said WY (2001) Production of antibiotics by immobilized *Streptomyces* strains. Ph.D. Thesis, Faculty of Science, Alexandria University, Egypt.

Shirling EB & Gottlieb D (1966) Methods for characterization of *Streptomyces* species. *International Journal of Syrtematic Bacteriology* 16(3): 313-340.

Sigee, DC, Morgan AJ, Sumner AT & Warley A (1993) X-ray microanalysis in biology: Experimental techniques and applications. Cambridge University Press, Cambridge, UK.

Szabo T, Martonn M & Feranavdez CA (1975) Diagnostie key for the identification of species of *Streptomyces & Streptoverticillium* included in the ISP. *Acta Botanica Tungaricae Tomus*, 21 (3-4): 367.

Waksman SA & Lechevalier HA (1962) The actinomycetes vol. III. Antibiotic of actinomycetes Baltimore: William's and Wilkin's Company.

Williams DH & Fleming I, Eds. (1987) Spectroscopic methods in organic chemistry. Fourth edition. Mc Graw-Hill Book Company. Maidenhead-Berkshire, England.

الملخص العربى

عزل وتوصيف المادة المضادة لنمو البكتريا المنتجة من مزارع ستربتوميسس فيولاتس

مصطفي يوسف النجار[1]-مها علي حسن[2]-وفاء يوسف سعيد[1]

1. قسم النبات والميكروبيولوجي-كلية العلوم-جامعة الإسكندرية-جمهورية مصر العربية
2. قسم التاريخ الطبيعي-كلية التربية-جامعة الإسكندرية-جمهورية مصر العربية

ينتج ستربتوميسس فيولاتس مادة مضاد ة لنمو البكتريا(268 ميكروجرام/مل) بعد فترة تحضين تبلغ 7 أيـام و عنـد درجة حرارة 30°م. يهتم البحث باستخلاص المضاد الحيوي للتعرف علي تركيبه الكيميائي و قد تم استخدام 15 لتـر من رشيح المزرعة لإتمام عملية التنقية. أجري استخلاص المضاد الحيوي باستخدام خلات الإيثيـل ثـم تـم تركيـز المستخلص وأجريت عملية تنقية للمستخلص بتمريره في عمود يحتوي علي هلام السيليكا باستخدام مزيج من الهكسـان وخلات الإيثيل ومزيج أخر من الإيثانول وخلات الإيثيل ثم تم جمع الأجزاء التي تحتوي علي أعلي نشاط مضاد لنمـو البكتيريا وتعريضها لعملية تنقية أخري باستخدام عامود ثاني يحتوي علي هلام السيليكا ثم أستخدم بها الكروماتوجرافيـا ذات الضغط العالي للحصول علي المضاد الحيوي النقي. أجريت علي المادة النقية قياسات الرنين المغناطيسي والأشعة تحت الحمراء وفوق البنفسجية للتعرف علي تركيبه الكيميائي. أظهر طيف الرنين المغناطيسـي أن المضـاد الحيـوي يحتوي علي حلقة بنزين وحيدة يتصل بها سلسلتين جانبيتين اليفاتيتين تحتوي إحداهما علي مجموعة أمينيـة و قانونـه الأساسي ك15يد23ى3أ ووزنه الجزيئي الذي تم تقديره من التحليل العنصري ومطياف الكتلة هو 267. ومما هو جدير بالذكر أن هذا المضاد الحيوي غير مطابق لأي من المضادات الحيوية المعروفة لذا تم إطلاق اسم جديد عليه هو إم إس دبليو 2000. تم إجراء بعض التجارب علي هذا المضاد الحيوي لاختبار مدي تأثيره فأظهر قدرة كبيرة كمثبط لأنـواع مختلفة من البكتيريا وكانت البكتيريا موجبة الجرام أكثر حساسية للمضاد الحيوي من سالبة الجرام بينما كـان تـأثير المضاد الحيوي أقل علي الخمائر وبعض الفطريات الممرضة. تمت أيضا دراسة تأثير المضاد الحيـوي النقـي علـي خلايا ستافيللوكوكس أورياس باستخدام أشعة إكس أظهرت النتائج أن نسبة العناصر المعدنية فـي الخلايـا المعاملـة تنخفض بازدياد تركيز المضاد الحيوي و كان أكثرها تأثرا البوتاسيوم والفوسفور والكبريت مما يشير إلى احتمال تأثير المضاد الحيوي علي الغشاء البلازمي للخلية.