The Antibacterial Activities of Mikanolide and its Derivatives

PC Facey, PC Peart, RBR Porter

ABSTRACT

Objective: *The sesquiterpene, mikanolide, was found to possess antibacterial activity. As a result, a structure-activity relationship study was carried out on mikanolide and eleven of its derivatives.*
Methods: *Mikanolide and two of its derivatives were isolated from organic extract of* Mikania micrantha *using chromatographic methods. Nine additional derivatives were synthesized and all were investigated for their antibacterial activity against the Gram positive pathogen* Staphylococcus aureus *and beta haemolytic* Streptococcus *group A (BHSA) as well as the Gram negative* Escherichia coli *using the disk diffusion assay.*
Results: *The investigation revealed that only four of the derivatives displayed antibacterial activity and only pathogens* Staphylococcus aureus *and beta haemolytic* Streptococcus *group A were susceptible at a concentration of 100 µg per disk. However, there was an increase in activity for three of the derivatives in comparison to mikanolide.*
Conclusion: *This study underscores the potential for phytochemicals from locally available plants to be further investigated and developed as antibacterial agents.*

Keywords: Antibacterial activity, *M. micrantha*, mikanolide, sesquiterpene

La Actividad Antibacteriana de la Mikanolida y sus Derivados

PC Facey, PC Peart, RBR Porter

RESUMEN

Objetivo: *Se halló que el sesquiterpene – mikanolida – posee actividad antibacteriana. Como resultado, se llevó a cabo un estudio de la relación estructura-actividad de la mikanolida y once de sus derivados.*
Métodos: *La mikanolida y dos de sus derivados fueron aislados a partir de un extracto orgánico de* Mikania micrantha, *mediante métodos cromatográficos. Se sintetizaron otros nueve derivados adicionales, y se investigó la actividad antibacteriana de todos sobre el patógeno Gram positivo* Staphylococcus aureus *y el* Streptococcus *beta hemolítico grupo A (BHSA) así como Escherichia coli Gram negativo, usando el ensayo de difusión en disco.*
Resultados: *La investigación reveló que sólo cuatro de los derivados mostraban actividad antibacteriana y sólo los patógenos Staphylococcus aureus* y *Streptococcus beta hemolítico grupo A eran susceptibles a una concentración de 100 µg por disco. Sin embargo, se produjo un aumento en la actividad de tres de los derivados en comparación con la mikanolida.*
Conclusión: *Este estudio subraya el potencial que poseen los fotoquímicos a partir de plantas localmente disponibles, para ser objeto de investigación ulterior y ser desarrollados como agentes antibacterianos.*

Palabras claves: Actividad antibacteriana, mikanolida, *M. micrantha*, sesquiterpene

West Indian Med J 2010; 59 (3): 249

From: Department of Chemistry, The University of the West Indies, Kingston 7, Jamaica, West Indies

Correspondence: Dr RBR Porter, Department of Chemistry, The University of the West Indies, Kingston 7, Jamaica, West Indies, Fax: (876) 977-1835, e-mail:roy.porter@uwimona.edu.jm

INTRODUCTION

Traditional medicine is still widely practised in rural and urban Jamaica and folklore suggests that there are a number of endemic plants that possess dramatic curative properties (1, 2). These plants offer a diversity of secondary metabolites which may possess biological properties. The increase in antibacterial resistance and the widespread emergence of multi-drug resistant pathogens such as methicillin-resistant *Staphylococcus aureus* pose great therapeutic challenges (3, 4). As a result, there is an urgent need for new classes of antibacterial agents. Preliminary antibacterial screening of 100 Jamaican plants revealed that *Mikania micrantha* possessed high activity and as a result, this plant was selected for activity guided isolation studies (5).

Commonly known as "guaco", the plant *Mikania micrantha* is a branched, extensively scrambling and twining slender-stemmed vine (6). Its leaves are used to make a poultice for snake bites and scorpion stings. A decoction of the leaves is also used as a bath for rashes, skin itch, athlete's foot and wound dressings (1). In Jamaica, its most popular uses are for wound dressings and healing of sores. Work done in the Department of Chemistry, The University of the West Indies, Mona, by Minott and Lewis revealed that the aqueous extract of *Mikania micrantha* produced a sustained rise in the blood pressure of rats as well as increased contractions of the mammal's uterus (7).

From our investigation of organic extracts from this plant, the compound mikanolide was isolated as the active component. In this present study, the antibacterial activities of mikanolide and eleven of its derivatives (Fig. 1) were investigated for their structure-activity relationship against the pathogens *Staphylococcus aureus*, *Streptococcus* group A and *Escherichia coli* using the disk diffusion method (8–11).

MATERIALS AND METHODS

Plant Material

The aerial parts of *Mikania micrantha* were collected at Port Antonio, Portland. The plant material was authenticated (UWI accession # 34309) by Mr Patrick Lewis, of the Herbarium, Department of Life Science, UWI, Mona, as being genuine leaves and stems of *Mikania micrantha*.

Extraction and Activity-guided Isolation

The dried milled plant (7.83 kg) was exhaustively extracted with hexane followed by ethyl acetate: acetone (1:1) at room temperature. The resulting extracts were concentrated *in vacuo* to give brown and green gums respectively. The ethyl acetate: acetone extract (250.9 g) exhibited antibacterial activity when tested against *Staphylococcus aureus* and *Streptococcus* group A and hence was subjected to chromatographic analysis. Repeated Column Chromatography of the active fractions followed by Preparative Layer Chromatography (PLC) led to the isolation of the sesquiterpene lactones mikanolide (1) (21.0 mg), dihydromikanolide (2) (9.3 mg) and deoxymikanolide (3) (17.1 mg).

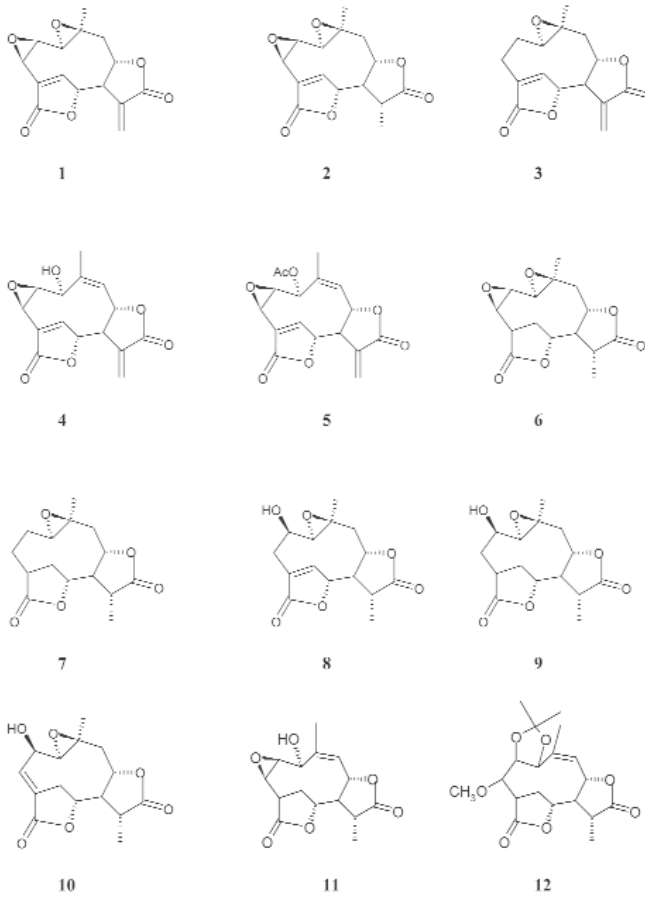

Fig. 1: Structures of mikanolide and its derivaties.

Preparation of Derivatives

Mikanolide (**1**) (55.2 mg, 0.19 mmol) was refluxed with 3 mL of a mixture of concentrated HCl (1.3 mL) and methanol (11 mL) for 1 hour on a steam bath. The mixture was evaporated to dryness repeatedly with methanol to remove the residual hydrogen chloride vapours (12). The brown solid obtained was chromatographed over silica gel to give the allylic alcohol (**4)** (18.0 mg) as a white solid.

A mixture of mikanolide (**1**) (80.1 mg, 0.28 mmol), *p*-toluenesulphonic acid (60 mg, 0,32 mmol) and acetic anhydride (4 mL) was refluxed for 1 hour. The brown mixture was taken to dryness *in vacuo*, dissolved in water (40 mL) and extracted with EtOAc (2 x 30 mL). The extract was washed with aqueous sodium bicarbonate ($NaHCO_3$) (2 x 20 mL) and then water (30 mL). The resulting solution was dried over anhydrous sodium sulphate (Na_2SO_4) and evapoarated *in vacuo* to give a brown gum (12). This was chromatographed over silica gel to give the monoacetate (**5**) (46.3 mg).

To a solution of the mikanolide and dihydromikanolide mixture (0.50 g) in EtOAc (100 mL) was added 5% palladium/carbon (0.50 g). The reactants were shaken under

hydrogen using a Parr apparatus operating at a pressure of 20 psi for 2 hours (12). The mixture was then filtered through celite and concentrated to give a white solid (0.49 g). The solid was chromatographed over silica gel to give **6** (225.7 mg), **7** (7.7 mg), **8** (5.9 mg) and **9** (132.5 mg).

To a solution of the mikanolide and dihydromikanolide mixture (0.98 g) in EtOAc (150 mL) was added 10% palladium/carbon (0.20 g). The reactants were shaken under hydrogen (Parr apparatus) at a pressure of 15 psi for 2 hours (12). The mixture was then filtered through celite and the filtrate concentrated to give a white solid (0.85 g). The solid was chromatographed over silica gel to give **10** (401.9 mg) as white needles in addition to **8** and **9**.

Compound **6** (227.4 mg, 0.76 mmol) was added to a mixture of concentrated HCl (1.3 mL) and methanol (11.0 mL). The solution was refluxed for 1.5 hours on a steam bath. The mixture was repeatedly taken up in acetone and then evaporated to dryness to remove the residual hydrogen chloride vapours (12). The brown solid (205.7 mg) obtained was chromatographed over silica gel **11** (8.2 mg) and **12** (22.9 mg) as white solids.

Antibacterial Assay

The disk diffusion method was employed to investigate the antibacterial activities of mikanolide and its derivatives. Concentration disks (100 µg per disk) were prepared in triplicate from blanks and these were tested against isolates of *Staphylococcus aureus*, *Streptococcus* group A and *Escherchia coli*. Standardized culture suspensions using the 0.5 McFarland Standard (1.5×10^8 colony forming units) were used for each inoculum. The plates were then incubated at 37–38° for 18–24 hours. Zones of inhibition of growth surrounding each disk were then measured and the average diameters noted (8–11). The antibiotic augmentin (30 µg) was used as the standard.

RESULTS

The antibacterial activity of mikanolide and eleven of its derivatives were investigated to identify the functionalities which produced maximum potency. The Gram positive pathogens, *Staphylococcus aureus* and beta-haemolytic *Streptococcus* group A were most susceptible to the derivatives of mikanolide. None showed any activity against the Gram-negative *E coli* at the concentrations tested. Deoxymikanolide (**3**) exhibited the greatest activity with zones of 20 mm and 25 mm against *Staphylococcus aureus* and *Streptococcus* group A respectively (Table).

On observation of the structure of the derivatives used in the study, it was evident that removal of the double bonds in the *αβ*-unsaturated lactone moieties made the compounds inert as only compounds 1–5 exhibited activities against the organisms tested. It is also noteworthy that removal of *trans* epoxide in compounds 4 and 5 produced a slight increase in the activity in comparision to mikanolide (1); however, retaining the *trans* epoxide and removing the *cis* epoxide, as in deoxymikanolide (3), seem to enhance the antibacterial activity even more (Fig. 2).

Table: The antibacterial activities of mikanolide and its derivatives against *Staphylococcus aureus* and *Streptococcus* group A

Compounds (100 µg)	Zone of Inhibition of Growth (mm) *Staphylococcus aureus*	*Streptococcus* group A
1	14.0	13.0
2	11.5	–
3	20.5	25.0
4	17.0	18.0
5	14.0	16.0
6	–	–
7	–	–
8	–	–
9	–	–
10	–	–
11	–	–
12	–	–
Augmentin (30 µg)	32.0	40.0

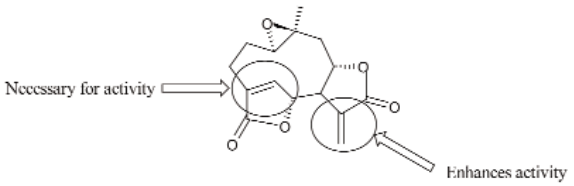

Fig. 2: Structural moieties of the sesquiterpenoid which increase antibacterial activity.

DISCUSSION

The presence of the *αβ*-unsaturated lactones in the examined sesquiterpenoids seemed to contribute to the antibacterial activity although these observations cannot be explained simply by the presence or absence of this functionality. However, these factors do affect the shape of the molecule and hence its ability to fit into binding sites also play an important role. From the present study, it was observed that the "flattened" or more planar molecules (*ie* those with the double bonds) were more active than those with sp^3 hybridised carbons (single bonds).

The mechanism of action of sesquiterpenoids against Gram positive bacteria such as *S aureus* is not fully understood but it is speculated to involve membrane disruption by lipophilic compounds. Also it has been postulated that since many sesquiterpene lactones contain electrophilic functional groups, it is likely that they react with cellular nucleophiles in DNA or enzymes (13, 14). One study showed that some sesquiterpene lactones inhibited the bacterial enzyme MurA which is responsible for the first step in the cytoplasmatic biosynthesis of peptidoglycan precursor molecules (13). However, no definite target structure relevant for the antibiotic activity of this class of compounds has been reported.

Work done using monoterpenes and diterpenes revealed that their antibacterial activities were related to the relative lipophilicity and water solubility of the compounds examined. As a result, the researchers were led to speculate that the antibacterial effect may have resulted at least partially from the perturbation of the lipid fraction of the bacteria plasma membrane resulting in alterations of membrane permeability and in leakage of intracellular materials (15).

It is evident from this study that the antibacterial activities observed for mikanolide and its derivatives explains the ethnomedicinal use of *Mikania micrantha* for wound dressings and healing of sores. It also provides opportunity for the development of new classes of antibiotics which are needed to build up the arsenal against these pathogens.

ACKNOWLEGEMENT

The authors wish to thank the University of the West Indies (UWI), Mona, for funding this work. Gratitude to the staff of the Department of Microbiology, University Hospital of the West Indies for their assistance and Mr Patrick A Lewis for authenticating the plant material.

REFERENCES

1. Ayensu ES. Medicinal Plants of the West Indies. Reference Publication. Inc, USA. 1981;
2. Huang H, Ye W, Wu P, Lin L, Wei X. New Sesquiterpene Dilactones from *Mikania micrantha*. J Nat Prod 2004; **67:** 734–6.
3. Stinson SC. Drug firms restock antibacterial arsenal. Chem Eng News, American Chemical Society, 1996; **23:** 75–99.
4. Henry CM. Chem Eng News, American Chemical Society 2000; **6:** 41–58.
5. Facey PC, Porter RB, Pascoe KO, Jones A. Investigation of Jamaican folk-medicine for antibacterial activity. J Pharm Pharmacol 1999; **51:** 1455–60.
6. Adams CD. Flowering plants of Jamaica. The University Press, Glasgow, 1972; 771.
7. Minott D, Lewis D. Chemistry and pharmacology of *Mikania micrantha*. Department of Chemistry, UWI Mona.
8. Boyd RF. General Microbiology, Times Mirror/Mosby College, St. Louis, 1984; 608–9.
9. Ericsson HM, Sherris JC. Acta Pathol Microbiol Scand, 1971, Section B, (Suppl. 217); 1–90.
10. National Committee for Clinical Laboratory Standards. Performance Standards for Antimicrobial Disk Susceptibility Tests – Fifth edition. Approved Standard, M2-A5, Volume 13, No. 24. National Committee for Clinical Laboratory Standards, Villanova, PA, 1993.
11. National Committee for Clinical Laboratory Standards. Performance Standards for Antimicrobial Disk Susceptibility Tests- Fifth Informational Supplement, M100-S5, Volume 14, No.16. National Committee for Clinical Laboratory Standards, Villanova, PA, 1994.
12. Hertz W, Subramaniam PS, Santhanam P S, Aota K, Hall AL. Structure elucidation of sesquiterpene dilactones from *Mikania scandens*. J Org Chem 1970; **35:** 1453–65.
13. Bachelier A, Mayerb R, Klein CD. Sesquiterpene lactones are potent and irreversible inhibitors of the antibacterial target enzyme MurA. Bioorg Med Chem Lett 2006; **16:** 5605–9.
14. Habtemariam S, Gray AI, Waterman PG. A New Antibacterial Sesquiterpene from *Premna oligotricha*. J Nat Prod 1993; **56:** 140–3.
15. Trombetta D, Castelli F, Sarpietro MG, Venuti V, Cristani M, Daniele C et al. Mechanisms of antibacterial action of three monoterpenes. Antimicrob Agents Chemother 2005; **49:** 2474–8.