Arch Pharm Res Vol 34, No 1, 71-77, 2011
DOI 10.1007/s12272-011-0108-1

Archives of Pharmacal Research
www.springer.com/12272

Effect of the Derivatives of Andrographolide on the Morphology of *Bacillus subtilis*

Chantana Aromdee[1], Nongluksna Sriubolmas[2], Suthep Wiyakrutta[3], Supawadee Suebsasna[1], and Watcharee Khunkitti[1]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen, Thailand, [2]Department of Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand, and [3]Department of Microbiology, Faculty of Science, Mahidol University, Bangkok, Thailand

(Received August 3, 2009/Revised December 30, 2009/Accepted July 16, 2010)

Andrographis paniculata has been reported to have antiviral, antipyretic and anticancer activities. Andrographolide, an *ent*-labdane diterpene, is an active constituent in this plant. In this study, andrographolide (1) and its natural derivative 14-deoxy-11,12-didehydroandrographolide (2) and 5 other semisynthetic derivatives were tested for their activity against Gram-positive and Gram-negative bacteria and *Candida albican*s. Only derivatives bearing a 14-acetyl group showed activity, and this activity was only against Gram-positive bacteria. 14-Acetylandrographolide showed the highest potency against *Bacillus subtilis*; the other 14-acetylandrographolides with additional substitution at the 3- and 19-hydroxyl groups showed lower activity against Gram-positive bacteria. The morphology of *B. subtilis* after being treated with 14-acetylandrographolide was investigated with TEM. This is the first report on 14-acetylandrographolide's quantified antibacterial activity, and the crucial functional group of this *ent*-labdane that plays an important role in perturbing the morphogenesis of *B. subtilis* leading to cell death.

Key words: Acetylated andrographolides, Antibacterial, *B. subtilis,* Morphological change

Selected by Editors

INTRODUCTION

Andrographis paniculata (Burm. f.) Wall. ex. Nees (Acanthaceae) is a traditional medicine widely used in Asian countries for protozoacidal, antihepatotoxic, anti-HIV, immunostimulant, anticancer, hypoglycemic and hypotensive activities (Nanduri et al., 2004). Andrographolide is an *ent*-labdane diterpene, a bicyclic hydrocarbon with a γ-lactone. Andrographolide (1) and its 22 natural derivatives isolated from *A. paniculata* were qualitatively evaluated for their antimicrobial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Micrococcus luteus*, *Candida albicans*, *Candida sake* and *Aspergillus niger* (Shen et al., 2006). Andrograpanin, 14-deoxy-11,12-didehydroandrographolide, 14-deoxy-12-hydroxyandrographolide and isoandrographolide at a concentration of 10 μg/mL were found to inhibit *B. subtilis* with clear inhibition zones of 7-8 mm. The other compounds were inactive against all of the organisms tested.

Fan et al. (2006) investigated the activity of acetylandrographolide on some bacteria, mildews, yeasts and phytopathogens. They found that acetylandrographolide was a stronger antimicrobial agent than (1) against a majority of the tested microbes, and particularly on *B. subtilis*, but the extract could not inhibit or kill all *S. aureus*.

Some other bicyclic diterpenoids with or without a γ-lactone ring were found to have activity against Gram-positive bacteria (Habibi et al., 2000; Hanson, 2002). Until now, it has been found that all bacteria sensitive to labdane diterpenes were Gram-positive (Habibi et al., 2000; Hanson, 2002; Fan et al., 2006; Shen et al., 2006).

Correspondence to: Chantana Aromdee, Faculty of Pharmaceutical Sciences, Khon Kaen University, Mitraparp Road, Khon Kaen, 40002, Thailand
Tel: 6643362095, Fax: 6643202379
E-mail: chaaro@kku.ac.th

In this work, two natural compounds, andrographolide (1) and 14-deoxy-11,12-didehydroandrographolide (2), as well as the semisynthetic derivatives 3,19-isopropylideneandrographolide (3), 14-acetyl-3,19-isopropylideneandrographolide (4), 14-acetylandrographolide (5), 3,14,19-triacetylandrographolide (6) and 14-deoxy-11,12-didehydro-3,19-diacetylandrographolide (7), see Fig, 1, were tested for antimicrobial activity against *S. aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *B. subtilis* ATCC 6633, *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *C. albicans* ATCC 90028. Compound(s) showing the strongest antimicrobial activity were selected for examination of their effect on the microbial cellular morphology by transmission electron microscopy. Thus, postulation of the mode or site of action of the *ent*-labdanes could be established, and the functional groups that play important roles in the activity could be recognised. Utilisation of this structure activity relationship (SAR) could be applied in the future development of antimicrobial compounds against other related microorganisms for therapeutic or other purposes.

MATERIALS AND METHODS

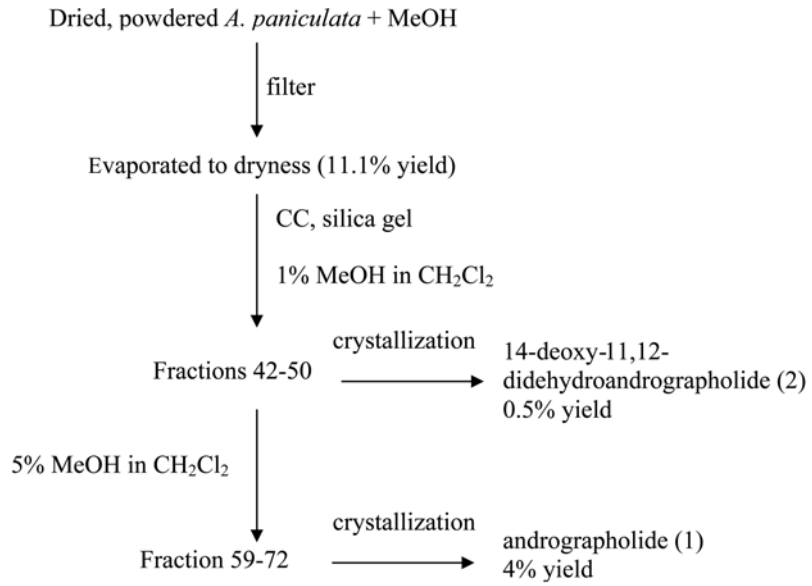
Andrographolide and 14-deoxy-11,12-didehydroandrographolide were isolated from dried and powdered *A. paniculata* purchased from an Ubon Ratchathani community agricultural agent. The plant's voucher number (ISB 003) was deposited at the Faculty of Pharmaceutical Sciences, Khon Kaen University, and had been compared to the authentic plant, DMSc Herbarium No. 821. The source of this material was ascertained to contain very high andrographolide content (Aromdee et al., 2005). Structures of all compounds were elucidated by NMR (Varian, Mercury 400, $CDCl_3$ or CD_3OD), IR (Perkin Elmer Series 1600, KBr Disc) and LCMS (Bruker Daltonics, Billerica). Melting temperatures were determined on a melting point apparatus (Electrothermal Engineering Ltd. IA9900 Series no. 9808). LCMS samples were introduced by flow injection and electrospray ionisation for positive mass and detected with a TOF detector from 50 m/z to 3000 m/z. Spectroscopic data of known compounds were compared with published data.

All andrographolide and andrographolide derivatives were detected by a thin-layer chromatography (TLC) system using silica gel GF_{254} precoated plates (Merck KGaA) as a stationary phase, and mixtures of methanol in dichloromethane as mobile phases. The plates were examined under ultraviolet (UV) light at 366 nm, and the existence of the intact γ-lactone was confirmed by spraying with the Kedde reagent. Reaction mixtures were partitioned in water and dichloromethane. The dichloromethane phase was dried with anhydrous sodium sulphate and chromatographed on a silica column eluted with mixtures of hexane and ethyl acetate.

Isolation and semisynthesis of andrographolide derivatives

Isolation of andrographolide (1) and 14-deoxy-11,12-didehydroandrographolide (2)

Dried powder of *A. paniculata* was macerated in methanol for 3 days. The extract was evaporated to dryness (11.1% yield) and chromatographed on a column



Scheme 1. Separation of andrographolide (1) and 14-deoxy-11, 12-didehydroandrographolide (2).

using dichloromethane and 1-5% methanol in dichloromethane as eluents (see Scheme 1). Eluates were continuously collected in 100-mL fractions and monitored by a TLC system using 10% methanol in dichloromethane as a mobile phase. Compound (1) was eluted in fractions 59-72; the yield was 4% - white or colourless crystals (CH_2Cl_2), mp 225-227°C, lit 218-221°C (Fujita et al., 1984).

Compound (2) was eluted in fractions 42-50; the yield was 0.5% - white or colourless crystals (CH_2Cl_2), mp 198-200°C, lit 203-204°C (Fujita et al., 1984).

3,19-Isopropylideneandrographolide (3)

As described by Nanduri et al. (2004), (3) was prepared from the reaction of (1) and dimethoxypropane using pyridinium *p*-toluenesulfonate as a catalyst. Compound (3) was obtained in 80% yield - a crystalline powder from hexane and ethyl acetate (1:1); mp 194-196°C.

14-Acetyl-3,19-isopropylideneandrographolide (4)

Compound (3) (0.1934 g, 0.495 mmole) in dichloromethane was stirred with acetic anhydride (2.5 mL, 26.4 mmole) and 2 mg of DMAP for 1 h at room temperature. Compound (4) and compound (5) were obtained in 30% and 60% yields, respectively. (4) - amorphous powder (CH_2Cl_2), hygroscopic, mp 58-59°C.

14-Acetyl andrographolide (5)

Compound (5) was obtained as described in the semisynthesis of (4). (5) - white powder (CH_2Cl_2); mp 168.29°C (onset at 163.65, end at 170.41°C), lit 168-170 °C (Jada et al., 2007).

3,14,19-Triacetyl andrographolide (6)

Andrographolide (1) (0.5368 g, 1.53 mmole) in DMSO and dichloromethane was reacted with 2.5 mL (26.4 mmole) of acetic anhydride and 5 mg of DMAP for 1 h at room temperature. The yield of (6) was 95% - crystalline powder (CH_2Cl_2), mp 110-111°C, lit 110-111°C (Jada et al., 2007).

14-Deoxy-11,12-didehydro-3,19-diacetyl andrographolide (7)

Andrographolide (1) (0.5004 g, 1.43 mmole) was mixed with acetic anhydride (0.33 mL, 3.23 mmole) and 5.0 mL of pyridine for 4 h at room temperature. Compound (7) was obtained in 50% yield - amorphous powder (CH_2Cl_2), mp 107-109°C. ^{1}H-NMR ($CDCl_3$) δ: 7.15 (1H, *s*, H-12), 6.91 (1H, *dd*, *J* = 10.1 and 15.6 Hz, H-12), 6.12 (1H, *d*, *J* = 15.8 Hz, H-14), 4.80 (2H, *s*, H-15), 4.79 (1H, *s*, 17a), 4.59 (1H, *t*, *J* = 8.4 and 7.9 Hz, H-3), 4.55 (1H, *s*, H-17b), 4.37 (1H, *d*, *J* = 11.4 Hz, H-19a), 4.14 (1H, *d*, *J* = 11.9 Hz, H-19b), 2.03 (6H, 2Ac), 1.02 (3H, *s*, H-20), 0.89 (3H, *s*, H-18). ^{13}C-NMR ($CDCl_3$) δ: 172.2 (C, C-16), 170.9 (C, Ac), 170.6 (C, Ac), 147.9 (C, C-8), 143.2 (CH, C-12), 135.6 (CH, C-11), 129.2 (C, C-13), 121.4 (CH, C-14), 109.3 (CH_2, C-17), 80.0 (CH, C-3), 69.6 (CH_2, C-15), 64.8 (CH_2, C-19), 61.7 (CH, C-9), 54.8 (CH, C5), 41.1 (C, C-4), 38.6 (C, C-10), 38.3 (CH_2, C-7), 36.7 (CH_2, C-1), 24.1 (CH_2, C-2), 23.9 (CH_2, C-6), 22.7 (CH_3, C-18), 21.1 (CH_3, 2Ac), 15.2 (CH_3, C-20). IR (KBr) cm^{-1}: 2933 (s), 1749, 1729 (s), 1247(s), 1037cm^{-1}. ESI *m/z*: 439.2054 $(M+Na)^+$.

Antimicrobial assays

Determination of antibacterial activity

Broth microdilution was used to determine antibacterial activities of compounds, as described in the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) M7-A4 method (National Committee for Clinical Laboratory Standards, 1997). The test bacteria were *B. subtilis* ATCC 6633, *E. faecalis* ATCC 29212, *S. aureus* ATCC 25923, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853. Each bacterial strain suspension was prepared and diluted in Mueller-Hinton broth to obtain 10^6 CFU/mL. A 50-µL inoculum was dispensed into each well containing 50 µL of test compound. All compounds were tested in duplicate at a final concentration of 2 mM.

After incubation at 37°C for 24 h, a 20 µL aliquot of *p*-iodonitrotetrazolium (INT) solution (1 mg/mL) was added into each well. The assay plates were further incubated for 1 h. A violet colour developed in the well indicated growth of the test organism. No change in colour indicated no growth, and thus antibacterial activity of the test compound.

Compounds showing antibacterial activity were further tested for the minimum inhibitory concentrations (MICs) over a final concentration range of 3.9 µM to 2000 µM. Ampicillin was used to compare the potency of the active compounds. MIC was defined as the lowest concentration that inhibited the growth of test bacteria. The solution from each well that showed no growth was further inoculated onto Mueller-Hinton agar and incubated at 37°C for 24 h to determine the minimum bactericidal concentration (MBC). MBC was defined as the lowest concentration that killed test bacteria.

Determination of activity against *Candida albicans* ATCC 90028

Anti-*Candida albicans* activities of andrographolide and 14-acetylandrographolide were determined by the broth microdilution method as described in CLSI

M27-A2 (National Committee for Clinical Laboratory Standards, 2002). *C. albicans* ATCC 90028 suspension was prepared and diluted in RPMI-1640 medium to yield 1×10^3 - 5×10^3 CFU/mL. A 100-µL inoculum was dispensed into each well containing 100 µL of test compound.

All compounds were tested in duplicate at a final concentration of 2 mM. Sample solutions were prepared immediately before use. After incubation at 37 °C for 24 h, 20 µL of *p*-iodonitrotetrazolium (INT) solution (1 mg/mL) was added into each well. The assay plate was further incubated for 24 h.

Transmission electron microscopy

B. subtilis cells were grown in tryptic soy broth at 37°C for 2-3 h and further diluted in Mueller-Hinton broth to yield 10^6 CFU/mL. The 14-acetylandrographolide was added at a final concentration of 125 µM (4× MIC), and cells were harvested after 4 h of incubation in an incubator shaker at 37°C and 200 rpm. Untreated cells were grown and harvested under the same conditions. Bacterial pellets were washed in sterile normal saline solution and centrifuged at 1000 ×*g* for 10 min. Cells were fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) at 4°C overnight. After centrifugation, the pellets were washed three times with buffer only. The washed cell pellets were postfixed in 1% osmium tetroxide in 0.1 M cacodylate buffer (pH 7.4) for 1 h at 4°C. They were dehydrated in a graded ethanol series. After dehydration, the specimens were treated with propylene oxide for 20 min (2 times), an equal mixture of propylene oxide and resin for 1 h, a 1:2 mixture overnight, and finally with resin only overnight. They were embedded in resin by polymerisation at 45°C for 2 days and 60°C for 2 days. The embedded cells were sectioned with a diamond knife on an ultramicrotome. Thin sections were mounted on copper grids and stained with alcoholic saturated uranyl acetate and lead citrate. The stained sections were examined with a JEM 2100 transmission electron microscope (JEOL Ltd.) at 120 kV.

RESULTS

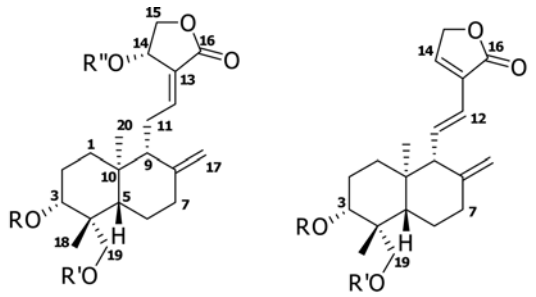
Antimicrobial activity

All compounds tested showed no detectable inhibitory activity against *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853 or *C. albicans* ATCC 90028. Some derivatives of andrographolide, however, displayed substantial activity against Gram-positive bacteria *B. subtilis* ATCC 6633, *E. faecalis* ATCC 29212 and *S. aureus* ATCC 25923 (Table I). Among the andrographolide derivatives tested in this study, 14-acetylandrographolide (5) was the most active compound against *B. subtilis* and *S. aureus*, with both MIC and MBC values of 31.25 and 62.5 µM, respectively. It was also relatively active against *E. faecalis* (MIC, MBC = 125 µM), slightly less active than 3,14,19-triacetyl andrographolide (6), which could inhibit and kill *E. faecalis* at 62.5 µM. However, (6) was less active against *S. aureus* and *B. subtilis*, having MIC and MBC values of 125 and 250 µM, respectively. Compared with (5) and (6), 14-acetyl-3,19-isopropylideneandrographolide (4) exhibited weaker antibacterial activity. It was the least active compound against *S. aureus* and *E. faecalis* (MIC, MBC = 250 and 1,000 µM, respectively). However, it was more active against *B. subtilis* (MIC, MBC = 62.5 µM) than compound (6), but not as good

Table I. Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of andrographolide and its acetyl derivatives against Gram-positive bacteria.

Compound	MIC, MBC (µM)		
	B. subtilis	*E. faecalis*	*S. aureus*
(1)	I	I	I
(2)	I	I	I
(3)	I	I	I
(4)	62.5, 62.5	1000, 1000	250, 250
(5)	31.25, 31.25	125, 125	62.5, 62.5
(6)	250, 250	62.5, 62.5	125, 125
(7)	I	I	I
Ampicillin	80, 80	5, 5	0.31, 0.31

I, Inactive.
Ampicillin was used to compare the potency of active compounds.



Andrographolide and derivatives
(1) R, R', R" = H
(3) Isopropylidene R, R' = *i*-Pr, R" = H
(4) R, R' = *i*-Pr, R" = Ac
(5) R, R' = H, R" = Ac
(6) R, R', R" = Ac

14-Deoxy11,12-didehydroandrographolide
(2) R, R' = H
(7) R, R' = Ac

Fig. 1. Structure of andrographolide and its derivatives.

as (5). The natural compounds andrographolide (1), 14-deoxy-11,12-didehydroandrographolide (2) and the semi-synthetic derivatives 3,19-isopropylideneandrographolide (3) and 14-deoxy-11,12-didehydro-3,19-diacetyl andrographolide (7) showed no activity against the three Gram-positive bacteria tested.

Transmission electron microscopy

Since (5) demonstrated the highest activity against *B. subtilis*, it was selected for further study of the morphological changes it induced using TEM. Treating *B. subtilis* with compound (5) at 4 times the minimum inhibitory concentration (125 μM) for 4 h resulted in the formation of large cytoplasmic aggregates, cell elongation with abnormal cell septation, cytoplasmic disintegration, and finally cell lysis (Fig. 2).

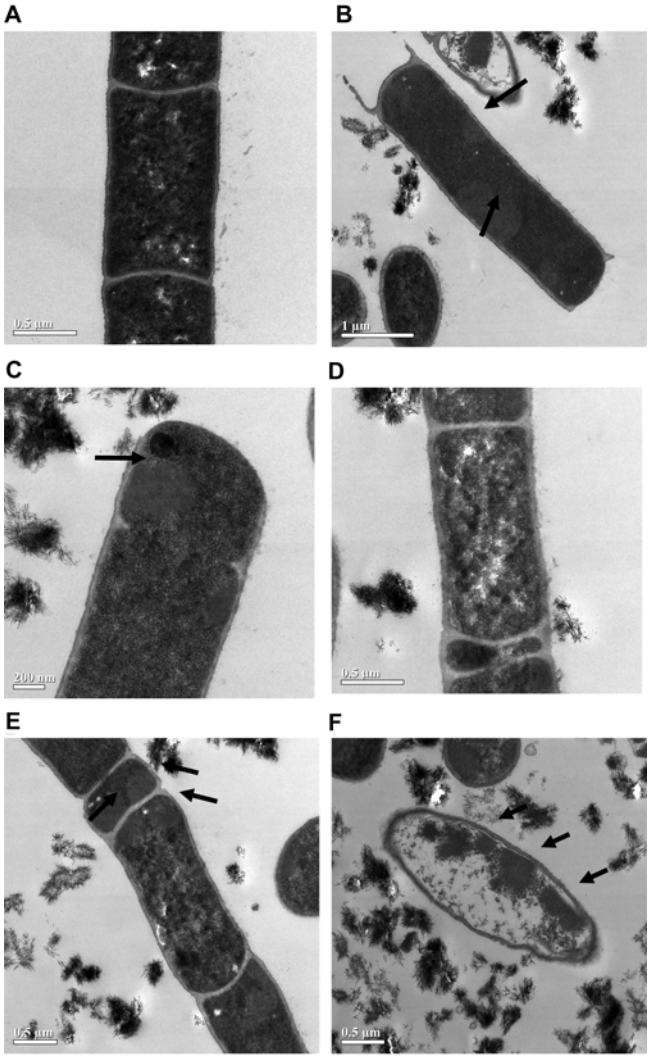


Fig. 2. Transmission electron micrographs showing effects of compound (5) on morphogenesis of *B. subtilis* ATCC 6633. Untreated cells (**A**). Cells after treating with compound (5) at 125 μM (4 × MIC) for 4 h (**B**-**F**). Abnormal cytoplasmic aggregates (**B**, **C**, **E**, **F**, arrow). Elongated cells (**B**-**E**). Abnormal polar septation (**C**-**E**).

DISCUSSION

Andrographolide (1) showed no activity against the tested bacteria and *C. albican*s. Acetylation of the 14-OH group rendered it active against *B. subtilis*, *S. aureus* and *E. faecalis* (see compound (5), Table I). Interestingly, compound (5) was more potent than ampicillin at killing *B. subtilis*. Further acetylation of the remaining two hydroxyl groups (3-OH, 19-OH) improved the activity against *E. faecalis*, but slightly weakened the activity against *S. aureus* and, to a greater extent, the activity against *B. subtilis* (see compound (6), Table I). Thus, differential acetylation of andrographolide could create antibacterial activity in an organism-selective manner. The three andrographolide derivatives (4), (5) and (6) acted on Gram-positive bacteria with MIC values equal to the MBC values, which suggested that their activities were bactericidal. It would be interesting to further investigate the 3-acetyl, 19-acetyl, 3,19-diacetyl, 3,14-diacetyl and 14,19-diacetyl derivatives of andrographolide, which has not been done in this study.

Modification of andrographolide by introducing the isopropylidene group to the 3-OH and 19-OH did not impart any antimicrobial activity to the molecule (see compound (3) Table I). Addition of isopropylidene groups at the 3-OH and 19-OH of compound (5) rendered the molecule less active against all three Gram-positive bacteria (see compound (4), Table I), indicating the negative effect of having isopropylidene at these positions.

14-Deoxy-11,12-didehydroandrographolide (2) exhibited no antimicrobial activity. Acetylation at position 14, which had been shown to be essential for the antibacterial activity of andrographolide, was not applicable since (2) is devoid of the 14-OH. Acetylation at the remaining 3-OH and 19-OH groups created no antimicrobial activity.

Previously, several antibacterial compounds from natural products had been reported to cause cell elongation or filamentation in *B. subtilis* (Beuria et al., 2005; De León et al., 2005; Jaiswal et al., 2007; De León and Moujir, 2008). Treating *B. subtilis* with a series of 6-oxophenolic triterpenoids (zeylasteral, demethylzeylasteral and zeylasterone) isolated from *Maytenus blepharodes* Lundell resulted in abnormally long cells (De León et al., 2005; De León and Moujir, 2008) and multiseptate filaments (De León et al., 2005). The compounds were found to damage the cytoplasmic membrane and compromise cell wall synthesis, leading to

loss of cytoplasmic material. Sanguinarine, a benzophenanthridine alkaloid derived from the rhizomes of *Sanguinaria canadensis*, blocked cytokinesis and induced filamentation in *B. subtilis* 168 by inhibiting Z-ring formation. However, nucleoid segregation and the cell membranes of treated bacteria were not affected by this compound (Beuria et al., 2005). More recently, totarol, a naturally occurring diterpenoid phenol extracted from *Podocarpus totara*, was found to induce filamentation in *B. subtilis* 168 without affecting the cell membrane. It perturbed the assembly dynamics of *Mycobacterium tuberculosis* FtsZ (*Mtb*FtsZ) protofilaments in the Z-ring and potently suppressed the GTPase activity of *Mtb*FtsZ (Jaiswal et al., 2007). Curcumin, a dietary polyphenolic compound isolated from the rhizomes of *Curcuma longa*, also induced filamentation in *B. subtilis* 168 without perturbing cell membrane structure. It inhibited FtsZ protofilament assembly and increased the GTPase activity of FtsZ (Rai et al., 2008). Nisin, a small cationic lanthionine antibiotic produced by *Lactococcus lactis*, was reported to cause abnormal morphogenesis in *B. subtilis* (Hyde et al., 2006). At lethal doses, nisin retarded bacterial cell elongation by interfering with cell wall synthesis, and accelerated cell division leading to cell length reduction and the formation of double or multiple septa in the midcell division region.

In contrast to the above reported antibacterials, treatment of *B. subtilis* with (5) caused cell elongation with normal cell diameters compared to control untreated cells (Fig. 2A-2E). A noticeable septation near the cell poles instead of the normal midcell division site was observed (Fig. 2C-2E). This suggested that (5) neither inhibits cell wall synthesis nor directly affects FtsZ assembly, but rather interferes with factors that regulate the position of normal septum formation. It is known that bacterial cell division depends on the FtsZ protein, which self-assembles into a membrane-associated ring structure that establishes the location of the nascent division site (Margolin, 2005). Division site selection in *B. subtilis* is controlled by a division inhibitor, MinCD, which prevents FtsZ assembly. The MinC and MinD proteins of *B. subtilis* are tethered to the cell poles by another protein, DivIVA, which binds strongly to the cell poles. In this way, MinCD-DivIVA prevents aberrant polar division of the cell (Marston et al., 1998). One possible explanation of polar septation observed in compound-(5)-treated *B. subtilis* is that the compound may interfere with the DivIVA protein. Inhibition of DivIVA might cause delocalisation of MinCD from the pole regions, leading to polar division and suppression of midcell septation.

Cytoplasmic membranes and cell walls of the treated cells were apparently intact until the late stages, suggesting that they might not be the primary antibacterial targets of (5). Subsequently, the affected cells lost their cytoplasmic contents, but the large aggregates formed from the action of (5) remained within ghost cells surrounded by an almost intact cell wall (Fig. 2F).

CONCLUSION

This study demonstrated that acetylation of andrographolide could create organism-selective antibacterial activity. The active derivatives were found to be bactericidal and appeared to have mechanism of action distinct from antibacterial drugs presently in clinical use. Our finding allows more understanding of the structure-activity relationship of this *ent*-labdane, which could be useful for the future development of new antibacterial agents.

ACKNOWLEDGEMENTS

We are grateful to the Department of Chemistry, Faculty of Science, Khon Kaen University, for acquiring NMR spectra. This work was financially supported by the Thailand Research Fund.

REFERENCES

Aromdee, C., Wichitchote, P., and Jantakun, N., Spectrophotometric determination of total lactones in *Andrographis paniculata* Nees. *Songklanakarin J. Sci. Technol.*, 27, 1227-1231 (2005).

Beuria, T. K., Santra, M. K., and Panda, D., Sanguinarine blocks cytokinesis in bacteria by inhibiting FtsZ assembly and bundling. *Biochemistry,* 44, 16584-16593 (2005).

De León, L., Beltrán, B., and Moujir, L., Antimicrobial activity of 6-oxophenolic triterpenoids. Mode of action against *Bacillus subtilis*. *Planta Med.,* 71, 313-319 (2005).

De León, L. and Moujir, L., Activity and mechanism of the action of zeylasterone against *Bacillus subtilis*. *J. Appl. Microbiol.*, 104, 1266-1274 (2008).

Fan, A. -L., Li, X. -M., Gao, J. -M., and Wand, J., Preparation of acetyl andrographolide and comparison of Its antimicrobial activities and allelopathic effects. *Acta Bot. Boreal.-Occid. Sin.*, 26, 1905 (2006).

Fujita, T., Fujitani, R., Takeda, Y., Takaishi, Y., Yamada, T., Kido, M., and Miura, I., On the diterpenoids of *Andrographis paniculata*: X-ray crystallographic analysis of andrographolide and structure determination of new minor diterpenoids. *Chem. Pharm. Bull.*, 32, 2117-2125 (1984).

Habibi, Z., Eftekhar, F., Samiee, K., and Rustaiyan, A., Structure and antibacterial activity of a new labdane diterpenoid from *Salvia leriaefolia*. *J. Nat. Prod.,* 63, 270-271

(2000).

Hanson, J. R., Diterpenoids. *Nat. Prod. Rep.*, 19, 125-132 (2002).

Hyde, A. J., Parisot, J., McNichol, A., and Bonev, B. B., Nisin-induced changes in *Bacillus* morphology suggest a paradigm of antibiotic action. *Proc. Natl. Acad. Sci. U.S.A.*, 103, 19896-19901 (2006).

Jada, S. R., Subur, G. S., Matthews, C., Hamzah, A. S., Lajis, N. H., Saad, M. S., Stevens, M. F., and Stanslas, J., Semi-synthesis and *in vitro* anticancer activities of andrographolide analogues. *Phytochemistry,* 68, 904-912 (2007).

Jaiswal, R., Beuria, T. K., Mohan, R., Mahajan, S. K., and Panda, D., Totarol inhibits bacterial cytokinesis by perturbing the assembly dynamics of FtsZ. *Biochemistry,* 46, 4211-4220 (2007).

Margolin, W., FtsZ and the division of prokaryotic cells and organelles. *Nat. Rev. Mol. Cell Biol.*, 6, 862-871 (2005).

Marston, A. L., Thomaides, H. B., Edwards, D. H., Sharpe, M. E., and Errington, J., Polar localization of the MinD protein of *Bacillus subtilis* and its role in selection of the mid-cell division site. *Genes Dev.*, 12, 3419-3430 (1998).

Nanduri, S., Nyavanandi, V. K., Thunuguntla, S. S., Kasu, S., Pallerla, M. K., Ram, P. S., Rajagopal, S., Kumar, R. A., Ramanujam, R., Babu, J. M., Vyas, K., Devi, A. S., Reddy, G. O., and Akella, V., Synthetic and structure-activity relationships of andrographolide analogues as novel cytotoxic agents. *Bioorg. Med. Chem. Lett.*, 14, 4711-4717 (2004).

National Committee for Clinical Laboratory Standards. Methods for Dilution: Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. 4th ed. NCCLS document M7-A4. National Committee for Clinical Laboratory Standards, Wayne, PA, (1997).

National Committee for Clinical Laboratory Standards. Reference Method for Broth Dilution: Antifungal Susceptibility Testing of Yeasts. 2nd ed. NCCLS document M27-A2. National Committee for Clinical Laboratory Standards, Wayne, PA, (2002).

Rai, D., Singh, J. K., Roy, N., and Panda, D., Curcumin inhibits FtsZ assembly: an attractive mechanism for its antibacterial activity. *Biochem. J.,* 410, 147-155 (2008).

Shen, Y. -H., Li, R. -T., Xiao, W. -L., Xu, G., Lin, Z. -W., Zhao, Q. -S., and Sun, H. -D., *ent*-Labdane diterpenoids from *Andrographis paniculata*. *J. Nat. Prod.,* 69, 319-322 (2006).