รายงานวิจัยฉบับสมบูรณ์

สารต้านจุลินทรีย์จากเชื้อราเอนโดฟท์ที่คัดเลือกจากหญ้าทะเลและพืชป่าชายเลน

โดย

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9 กันยายน 2554

สัญญาเลขที่ DBG5280020

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สนับสนุนโดยสำนักงานกองทุนสนับสนุนการวิจัย

(ความเห็นในรายงานนี้เป็นของผู้วิจัย

สกว. ไม่จำเป็นต้องเห็นด้วยเสมอไป)

บทคัดย่อ

จากการศึกษาราเอนโดไฟท์จำนวน 4 ไอโซเลทซึ่งแยกจากพืชป่าชายเลนและหญ้าทะเล สามารถแยกสารใหม่และสารที่มีการรายงานโครงสร้างแล้วหลากหลายประเภทจำนวน 12 และ 25 สาร ตามลำดับ โดยพบว่าราเอนโดไฟท์ *Acremonium sp.* PSU-MA70 จากกิ่งโกงกางเล็ก (*Rhizophora apiculata*) ผลิต (+)-brefeldin A และ 8-deoxytrichothecin แสดงฤทธิ์ต่อเชื้อรา *Candida albicans* ในระดับปานกลางด้วยค่า MIC 32 และ 16 μg/mL ตามลำดับ ขณะที่ trichodermol แสดงฤทธิ์ต่อเชื้อรา *Cryptococcus neoformans* ในระดับปานกลางเช่นกันด้วยค่า MIC 32 μg/mL นอกจากนี้ 2-chloro-5-methoxy-3-methylcyclohexa-2,5-diene-1,4-dione ที่แยกได้จากราเอนโดไฟท์ *Xylaria cubensis* PSU-MA34 จากกิ่งถั่วดำ (*Bruguiera parvifolia*) แสดงฤทธิ์ในระดับต่ำต่อเชื้อแบคทีเรีย *Staphylococcus aureus* และ methicillin-resistant *S. aureus* ด้วยค่า MIC เท่ากัน คือ 128 μg/mL สำหรับราเอนโดไฟท์ *Fusarium* sp. PSU-ES73 แยกจากหญ้าทะเล *Thalassia hemprichii* ผลิต zearalenone ซึ่งยับยั้งการเจริญเติบโตของ *S. aureus*, methicillin-resistant *S. aureus* และ *C. neoformans* ในระดับต่ำมาก จากการศึกษานี้สามารถสรุปได้ว่า ราเอนโดไฟท์จากพืชป่าชายเลนและหญ้าทะเลสร้างผลิตภัณฑ์ธรรมชาติหลากหลายประเภท ซึ่งสารบางสารแสดงฤทธิ์ต้านเชื้อราในระดับที่น่าสนใจ

Abstract

The research project involved the investigation of antimicrobial substances from selected endophytic fungi isolated from mangrove plants and seagrasses. 12 New and 25 known metabolites were obtained from two mangrove-derived and two seagrass-derived endophytic fungi. (+)-Brefeldin A and 8-deoxytrichothecin from *Acremonium sp.* PSU-MA70 which was isolated from the mangrove *Rhizophora apiculata* showed moderate antifungal activity against *Candida albicans* with minimum inhibitory concentration (MIC) values of 32 and 16 μg/mL, respectively, whereas trichodermol moderately exhibited the growth of *Cryptococcus neoformans* with the MIC value of 32 μg/mL. *Xylaria cubensis* PSU-MA34 isolated from the mangrove *Bruguiera parvifolia* produced 2-chloro-5-methoxy-3-methylcyclohexa-2,5-diene-1,4-dione which displayed weak antibacterial activity against *Staphylococcus aureus* and methicillin-resistant *S. aureus* with equal MIC values of 128 μg/mL. Finally, zearalenone from *Fusarium* sp. PSU-ES73 isolated from the seagrass *Thalassia hemprichii* exhibited mild antibacterial and antifungal activities against both bacterial strains and *C. neoformans*. Results from this study indicated that mangrove- and seagrass-derived endophytic fungi are a good source of structurally diverse natural products with interesting antifungal activity.

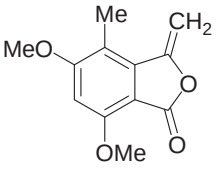
Executive Summary

งานวิจัยนี้ เป็นการแยกสารต้านจุลินทรีย์จากราเอนโดไฟท์ที่คัดเลือกจากพืชป่าชายเลนและหญ้าทะเลประเภทละ 2 ไอโซเลท สามารถแยกสารใหม่และสารที่มีการรายงานโครงสร้างแล้วจำนวน 12 และ 25 สาร ตามลำดับ ผลการศึกษาแบ่งออกเป็น 2 ส่วน สรุปผลงานในแต่ละส่วนดังนี้

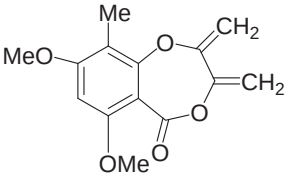
<u>ส่วนที่ 1</u> สารต้านจุลินทรีย์จากราเอนโดไฟท์จากพืชป่าชายเลน โดยแบ่งออกเป็น 2 ตอน

***ตอนที่ 1 เป็นการศึกษาราเอนโดไฟท์ Acremonium sp.* PSU-MA70** ที่แยกได้จากกิ่งโกงกางเล็ก (*Rhizophora apiculata*)

สามารถแยกสารบริสุทธิ์ได้จำนวน 19 สาร โดยจัดเป็นสารใหม่จำนวน 9 สาร (**1**-**9**) และสารที่มีการรายงานโครงสร้างแล้วจำนวน 10 สาร ได้แก่ (+)-brefeldin A (**10**), guangomide A (**11**), guangomide B (**12**), 8-deoxytrichothecin (**13**), trichodermol (**14**), 4-methyl-1-phenyl-2,3-hexanediol (**15**), (2*R*,3*R*)-4-methyl-1-phenyl-2,3-pentanediol (**16**), 5,7-dimethoxy-3,4-dimethyl-3-hydroxyphthalide (**17**), Sch 54794 (**18**) และ Sch 54796 (**19**) พบว่าสาร **10** และ **13** แสดงฤทธิ์ต่อเชื้อรา *Candida albicans* ในระดับปานกลางด้วยค่า MIC 32 และ 16 μg/mL ตามลำดับ ขณะที่สาร **14** แสดงฤทธิ์ต่อเชื้อรา *Cryptococcus neoformans* ในระดับปานกลางเช่นกันด้วยค่า MIC 32 μg/mL



1



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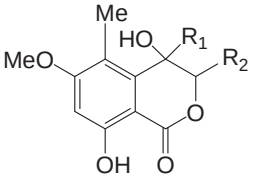
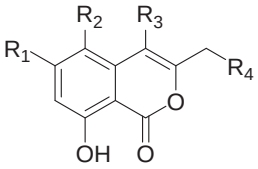


3: R_1 = COMe, R_2 = H

4: R_1 = CH_2OH, R_2 = Me

5: R_1 = CH(OH)Me, R_2 = H

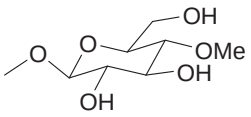


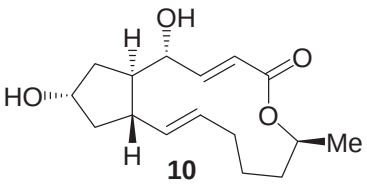
6: R_1 = OMe, R_2 = Me, R_3 = CH_2OH, R_4 = OH

7: R_1 = R_4 = OH, R_2 = Me, R_3 = CH_2OH

8: R_1 = R_4 = OH, R_2 = H, R_3 = Me

9: R_1 = OMe, R_2 = Me, R_3 = Me, R_4 =





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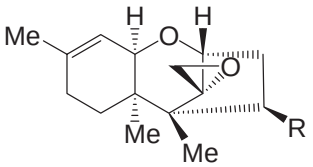
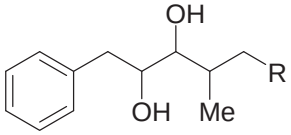
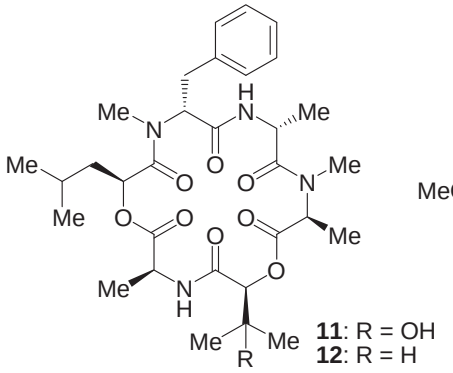


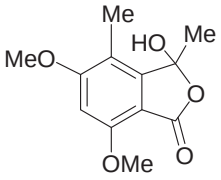
13: R = OCOCH$\overset{Z}{=}$CHMe
14: R = OH



15: R = Me
16: R = H



11: R = OH
12: R = H



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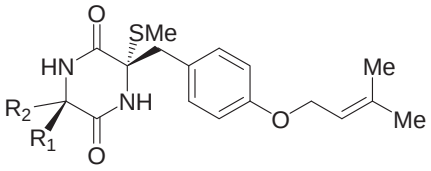
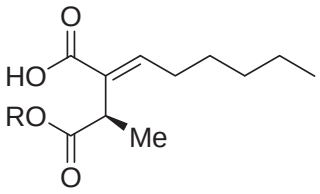


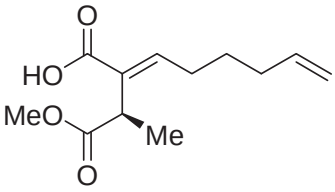
18: R_1 = H, R_2 = SMe
19: R_1 = SMe, R_2 = H

ตอนที่ 2 เป็นการศึกษาราแอนโดไฟท์ Xylaria cubensis PSU-MA34 ที่แยกได้จากกิ่งถั่วดำ (*Bruguiera parvifolia*)

สามารถแยกสารใหม่จำนวน 2 สาร (**20** และ **21**) และสารที่มีการรายงานโครงสร้างแล้วจำนวน 7 สาร ได้แก่ 2-hexylidene-3-methyl succinic acid 4-methyl ester (**22**), (*R*)-(-)-5-carboxymellein (**23**), (*R*)-(-)-5-methoxycarbonylmellein (**24**), (*R*)-(-)-mellein methyl ether (**25**), cytochalasin D (**26**), 2-chloro-5-methoxy-3-methylcyclohexa-2,5-diene-1,4-dione (**27**) และ isosclerone (**28**) จากการตรวจสอบฤทธิ์ต้านแบคทีเรีย พบว่าสาร **27** แสดงฤทธิ์ในระดับต่ำต่อเชื้อแบคทีเรีย *Staphylococcus aureus* และ methicillin-resistant *S. aureus* ด้วยค่า MIC เท่ากัน คือ 128 μg/mL นอกจากนี้สาร **26** แสดงความเป็นพิษต่อเซลล์มะเร็งช่องปากในระดับต่ำด้วยค่า IC_{50} 3.99 μg/mL



20: R = CH_2Me
22: R = Me



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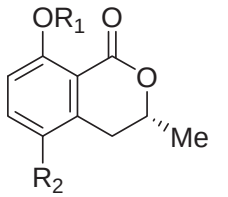
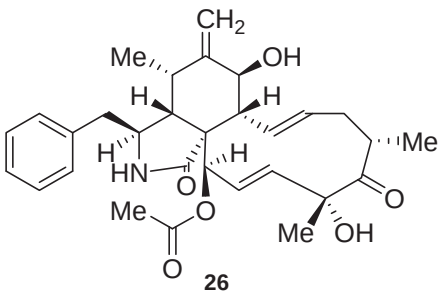


23: R_1 = H, R_2 = CO_2H
24: R_1 = H, R_2 = CO_2Me
25: R_1 = Me, R_2 = H



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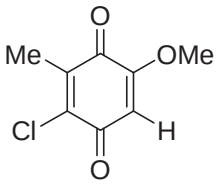

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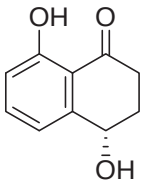

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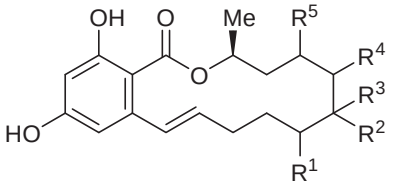
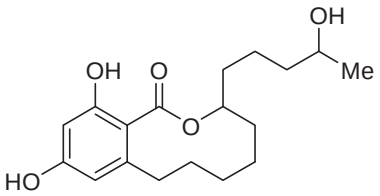
<u>ส่วนที่ 2</u> สารต้านจุลินทรีย์จากราเอนโดไฟท์จากหญ้าทะเล โดยแบ่งออกเป็น 2 ส่วน

***ตอนที่ 1 เป็นการศึกษาราเอนโดไฟท์ Fusarium* sp. PSU-ES73** แยกจากหญ้าทะเล *Thalassia hemprichii*

สามารถแยกสารบริสุทธิ์ประเภทอนุพันธ์ซีราลิโนนได้จำนวน 7 สาร โดยสาร **29** เป็นสารใหม่ และสารที่มีการรายงานโครงสร้างแล้ว ได้แก่ zearalenone (**30**), 8'-hydroxyzearalenone (**31**), 7'-dehydrozearalenone (**32**), β-zearalenol (**33**), 5'-hydroxyzearalenol (**34**), และ relgro (**35**) เมื่อทำการทดสอบฤทธิ์ทางชีวภาพของสารบริสุทธิ์ พบว่าสาร **30** ยับยั้งการเจริญเติบโตของ *S. aureus*, methicillin-resistant *S. aureus* และ *C. neoformans* ในระดับต่ำมาก



29: R^1 = β-OH, $R^2 + R^3$ = O, $R^4 = R^5$ = H
30: $R^1 = R^4 = R^5$ = H, $R^2 + R^3$ = O
31: $R^1 = R^4$ = H, $R^2 + R^3$ = O, R^5 = β-OH
32: R^1 = H, $R^2 + R^3$ = O, $R^4 + R^5$ = double bond
33: $R^1 = R^3 = R^4 = R^5$ = H, R^2 = β-OH
34: R^1 = β-OH, R^2 = α-OH, $R^3 = R^4 = R^5$ = H



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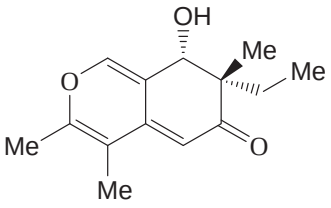
***ตอนที่ 2 เป็นการศึกษาราเอนโดไฟท์ Curvularia* sp. PSU-ES71** แยกจากหญ้าทะเล *Enhalus accoroides*

สามารถแยกสารบริสุทธิ์ที่มีการรายงานโครงสร้างแล้วจำนวน 2 สาร ได้แก่ pseudohalonectrin A (**37**) และ α-acetylorcinol (**38**) มีโครงสร้างดังแสดง เนื่องจากสารที่แยกได้มีปริมาณน้อย จึงไม่ได้ทดสอบฤทธิ์ต้านจุลินทรีย์



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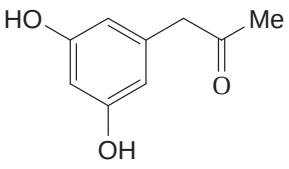

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เนื้อหางานวิจัย

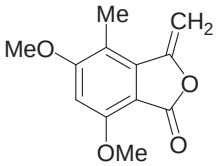
งานวิจัยนี้ เป็นการแยกสารต้านจุลินทรีย์จากราเอนโดไฟท์ที่คัดเลือกจากหญ้าทะเลและพืชป่าชายเลนประเภทละ 2 ไอโซเลท สามารถแยกสารใหม่และสารที่มีการรายงานโครงสร้างแล้วจำนวน 12 และ 25 สาร ตามลำดับ ผลการศึกษาแบ่งออกเป็น 2 ส่วน สรุปผลงานในแต่ละส่วนดังนี้

<u>ส่วนที่ 1</u> สารต้านจุลินทรีย์จากราเอนโดไฟท์จากพืชป่าชายเลน โดยแบ่งออกเป็น 2 ตอน

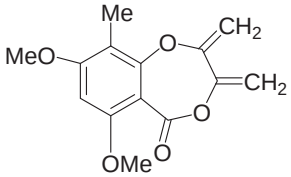
***ตอนที่ 1 เป็นการศึกษาราเอนโดไฟท์ Acremonium sp.* PSU-MA70** ที่แยกได้จากกิ่งโกงกางเล็ก (*Rhizophora apiculata*)

จากการศึกษาพบว่า ส่วนสกัดหยาบเอทิลอะซีเทตของน้ำเลี้ยงเชื้อแสดงฤทธิ์ต้านเชื้อรา *Candida albicans* และ *Cryptococcus neoformans* ด้วยค่า MIC ที่เท่ากัน คือ 128 μg/mL แต่ไม่แสดงฤทธิ์ต้านเชื้อแบคทีเรีย *Staphylococcus aureus*, *Pseudomonas aeruginosa* และ *Escherichia coli* ที่ความเข้มข้น 200 μg/mL

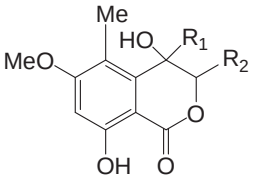
แยกส่วนสกัดหยาบข้างต้นด้วยตัวทำลายเมทานอลได้เป็น 2 ส่วน ส่วนที่ไม่ละลายเมทานอลเป็นสารบริสุทธิ์ มีลักษณะเป็นของแข็งใสไม่มีสี น้ำหนัก 893 มิลลิกรัม ซึ่งเป็นสารที่มีการรายงานโครงสร้างแล้ว คือ (+)-brefeldin A (**10**) เมื่อนำส่วนที่ละลายเมทานอลมาทำการแยกด้วยคอลัมน์โครมาโทกราฟี สามารถแยกเป็นส่วนย่อย 7 ส่วน นำส่วนย่อยที่ได้ไปทำให้บริสุทธิ์สามารถแยกสารบริสุทธิ์ได้จำนวน 19 สาร โดยจัดเป็นสารใหม่จำนวน 9 สาร(**1**-**9**) และสารที่มีการรายงานโครงสร้างแล้วจำนวน 9 สาร ได้แก่ guangomide A (**11**), guangomide B (**12**), 8-deoxytrichothecin (**13**), trichodermol (**14**), 4-methyl-1-phenyl-2,3-hexanediol (**15**), (2*R*,3*R*)-4-methyl-1-phenyl-2,3-pentanediol (**16**), 5,7-dimethoxy-3,4-dimethyl-3-hydroxyphthalide (**17**), Sch 54794 (**18**) และ Sch 54796 (**19**) มีโครงสร้างดังแสดง



1



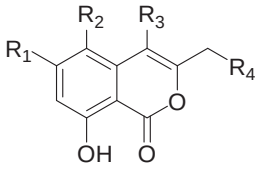
2



3: R_1 = COMe, R_2 = H

4: R_1 = CH_2OH, R_2 = Me

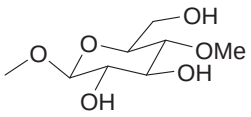
5: R_1 = CH(OH)Me, R_2 = H

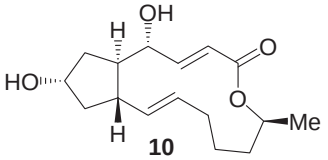


6: R_1 = OMe, R_2 = Me, R_3 = CH_2OH, R_4 = OH

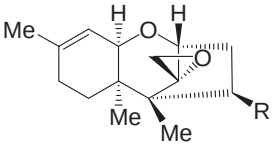
7: R_1 = R_4 = OH, R_2 = Me, R_3 = CH_2OH

8: R_1 = R_4 = OH, R_2 = H, R_3 = Me

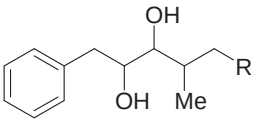
9: R_1 = OMe, R_2 = Me, R_3 = Me, R_4 = 



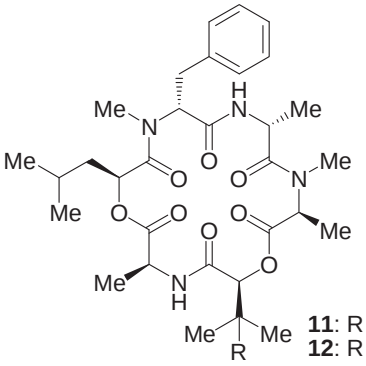
10



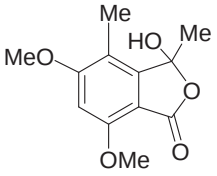
13: R = OCOCH=CHMe
14: R = OH



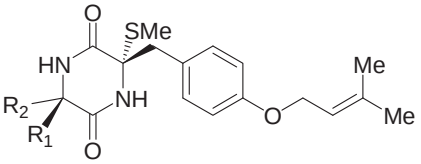
15: R = Me
16: R = H



11: R = OH
12: R = H



17



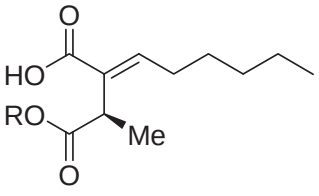
18: R_1 = H, R_2 = SMe
19: R_1 = SMe, R_2 = H

เมื่อนำสารเหล่านี้มาตรวจสอบฤทธิ์ต้านเชื้อรา *C. albicans* และ *C. neoformans* พบว่าสาร **10** และ **13** แสดงฤทธิ์ต่อ *C. albicans* ในระดับปานกลางด้วยค่า MIC 32 และ 16 μg/mL ตามลำดับ ขณะที่สาร **14** แสดงฤทธิ์ต่อ *C. neoformans* ในระดับปานกลางเช่นกันด้วยค่า MIC 32 μg/mL

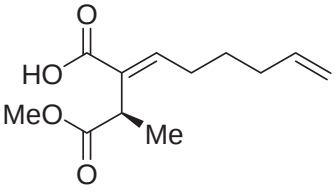
ผลงานวิจัยนี้ได้ส่งต้นฉบับเพื่อการตีพิมพ์ในวารสาร *Journal of Natural Products* รายละเอียดตามเอกสารแนบ

ตอนที่ 2 เป็นการศึกษาราเอนโดไฟท์ Xylaria cubensis PSU-MA434 ที่แยกได้จากกิ่งถั่วดำ (*Bruguiera parvifolia*)

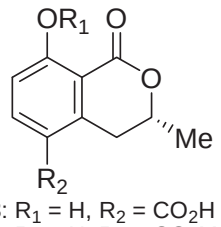
จากการศึกษาพบว่าส่วนสกัดหยาบเอทิลอะซีเทตของน้ำเลี้ยงเชื้อ แสดงฤทธิ์ต้านเชื้อแบคทีเรีย *Staphylococcus aureus* และ methicillin-resistant *S. aureus* ด้วยค่า MIC 200 μg/mL เท่ากัน นอกจากนี้ยังแสดงความเป็นพิษต่อเซลล์มะเร็งช่องปากด้วยค่า IC_{50} 2.79 μg/mL ทำการแยกส่วนสกัดหยาบนี้หนัก 2.08 กรัม ด้วยวิธีการทางโครมาโทกราฟี สามารถแยกเป็นส่วนย่อย 5 ส่วน นำส่วนย่อยที่ได้ไปทำให้บริสุทธิ์ แยกได้สารใหม่จำนวน 2 สาร (**20** และ **21**) และสารที่มีการรายงานโครงสร้างแล้วจำนวน 7 สาร ได้แก่ 2-hexylidene-3-methyl succinic acid 4-methyl ester (**22**), (*R*)-(-)-5-carboxymellein (**23**), (*R*)-(-)-5-methoxycarbonylmellein (**24**), (*R*)-(-)-mellein methyl ether (**25**), cytochalasin D (**26**), 2-chloro-5-methoxy-3-methylcyclohexa-2,5-diene-1,4-dione (**27**) และ isosclerone (**28**) มีโครงสร้างดังแสดง



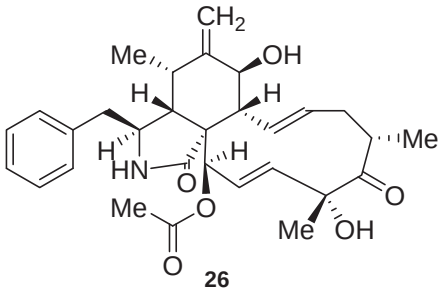
20: R = CH_2Me
22: R = Me



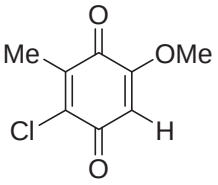
21



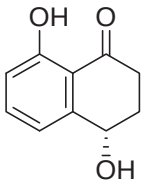
23: R_1 = H, R_2 = CO_2H
24: R_1 = H, R_2 = CO_2Me
25: R_1 = Me, R_2 = H



26



27



28

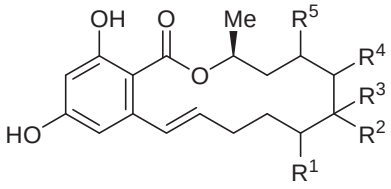
เมื่อนำสารเหล่านี้มาตรวจสอบฤทธิ์ฤทธิ์ต้านเชื้อแบคทีเรีย *S. aureus* และ methicillin-resistant *S. aureus* พบว่าสาร **27** แสดงฤทธิ์ในระดับต่ำต่อเชื้อแบคทีเรียทั้งสองสายพันธุ์ด้วยค่า MIC เท่ากัน คือ 128 μg/mL นอกจากนี้สาร **26** แสดงความเป็นพิษต่อเซลล์มะเร็งช่องปากในระดับต่ำด้วยค่า IC_{50} 3.99 μg/mL

ผลงานวิจัยนี้ได้รับการตอบรับตีพิมพ์ในวารสาร Archives of Pharmacal Research รายละเอียดตามเอกสารแนบ และนายศรัณยู ใคลคลาย นักศึกษาปริญญาเอกโครงการปริญญาเอกกาญจนาภิเษกได้รับรางวัลการนำเสนอโปสเตอร์ดีเด่น เรื่อง Cytochalasin, Quinone, Succinic acid and Tetralone Derivatives from the Mangrove-derived Fungus *Xylaria cubensis* PSU-MA34 ในการประชุม 7th IMT-GT UNINET and the 3rd Joint International PSU-UNS, 7-8 ตุลาคม 2553 ณ ศูนย์ประชุมนานาชาติฉลองสิริราชสมบัติครบ 60 ปี มหาวิทยาลัยสงขลานครินทร์

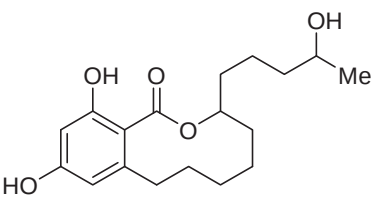
<u>ส่วนที่ 2</u> สารต้านจุลินทรีย์จากราเอนโดไฟท์จากหญ้าทะเล โดยแบ่งออกเป็น 2 ส่วน

***ตอนที่ 1 เป็นการศึกษาราเอนโดไฟท์ Fusarium* sp. PSU-ES73** แยกจากหญ้าทะเล *Thalassia hemprichii*

จากการศึกษาสามารถแยกสารบริสุทธิ์ประเภทอนุพันธ์ซีราลิโนนได้จำนวน 7 สาร โดยสาร **29** เป็นสารใหม่ และสารที่มีการรายงานโครงสร้างแล้ว ได้แก่ zearalenone (**30**), 8'-hydroxyzearalenone (**31**), 7'-dehydrozearalenone (**32**), β-zearalenol (**33**), 5'-hydroxyzearalenol (**34**), และ relgro (**35**) เมื่อทำการทดสอบฤทธิ์ทางชีวภาพของสารบริสุทธิ์ พบว่าสาร **30** ยับยั้งการเจริญเติบโตของ *S. aureus*, methicillin-resistant *S. aureus* และ *C. neoformans* ในระดับต่ำมาก



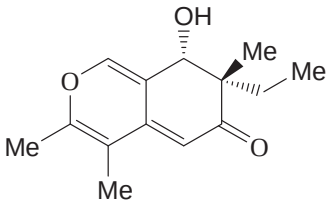
29: R^1 = β-OH, R^2 + R^3 = O, R^4 = R^5 = H
30: R^1 = R^4 = R^5 = H, R^2 + R^3 = O
31: R^1 = R^4 = H, R^2 + R^3 = O, R^5 = β-OH
32: R^1 = H, R^2 + R^3 = O, R^4 + R^5 = double bond
33: R^1 = R^3 = R^4 = R^5 = H, R^2 = β-OH
34: R^1 = β-OH, R^2 = α-OH, R^3 = R^4 = R^5 = H



35

ผลงานวิจัยนี้ได้รับการตอบรับตีพิมพ์ในวารสาร Archives of Pharmacal Research รายละเอียดตามเอกสารแนบ นอกจากนี้ นางสาวจิราพร อรุณพานิชเลิศ นักศึกษาปริญญาเอกทุนพัฒนาและส่งเสริมผู้มีความสามารถพิเศษทางวิทยาศาสตร์และเทคโนโลยี (พสวท.) ได้นำเสนอผลงานแบบโปสเตอร์ เรื่อง อนุพันธ์ซีราลิโนนจากเชื้อราของหญ้าทะเลที่ยังไม่ได้จำแนก PSU-ES73 ในการประชุมวิชาการวิทยาศาสตร์และเทคโนโลยีแห่งประเทศไทย ครั้งที่ 36 (วทท.36) วันที่ 26-28 ตุลาคม 2553 ณ ศูนย์นิทรรศการและการประชุมไบเทค กรุงเทพมหานคร

***ตอนที่ 2 เป็นการศึกษาราเอนโดไฟท์ Curvularia* sp. PSU-ES71** แยกจากหญ้าทะเล *Enhalus accoroides* แยกส่วนสกัดหยาบเอทิลอะซีเทตของน้ำเลี้ยงเชื้อนี้หนัก 0.71 กรัม ด้วยวิธีการทางโครมาโทกราฟี สามารถแยกสารบริสุทธิ์ที่มีการรายงานโครงสร้างแล้วจำนวน 2 สาร ได้แก่ pseudohalonectrin A (**37**) (Dong *et al*., 2006) และ α-acetylorcinol (**38**) (Nukina and Marumo, 1977) ดังแสดง เนื่องจากสารที่แยกได้มีปริมาณน้อย จึงไม่ได้ทดสอบฤทธิ์ต้านจุลินทรีย์



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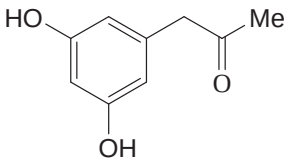

37

Dong, J., Zhou, Y., Li, R., Zhou, W., Li, L., Zhu, Y., Huang R. and Zhang, K. 2006. New nematicidal azaphilones from the aquatic fungus *Pseudohalonectria adversaria* YMF1.01019. FEMS. Microbiol. Lett. 264, 65-69.

Nukina, M. and Marumo, S. 1977. α-Acetylorcinol from *Cochliobolus lunata*. Agric. Biol. Chem. 41, 717.

Output

1. ผลงานที่ได้รับการตอบรับตีพิมพ์แล้วจำนวน 2 เรื่อง (*ภาคผนวก-accepted manuscript*)
    1.1 J. Arunpanichlert, V. Rukachaisirikul, Y. Sukpondma, S. Phongpaichit, O. upaphon, and J. Akayaroj, A β-resorcylic macrolide from the seagrass-derived fungus *Fusarium* sp. PSU-ES73, *Arch. Pharm. Res.*, accepted.
    1.2 S. Klaiklay, V. Rukachaisirikul, Y. Sukpondma, S. Phongpaichit, J. Buatong and B. Bussaban, Metabolites from the mangrove-derived fungus *Xylaria cubensis* PSU-MA34, *Arch. Pharm. Res.*, accepted.
2. ต้นฉบับผลงานจำนวน 1 เรื่อง (*ภาคผนวก-manuscript*)
    2.1 V. Rukachaisirikul, A. Rodglin, Y. Sukpondma, S. Phongpaichit, J. Buatong and J. Sakayaroj, Phthalide and Isocoumarin Derivatives from *Acremonium* sp. PSU-MA70, a Mangrove-derived Fungus from *Rhizophora apiculata*
3. ผลงานที่ได้นำเสนอในที่ประชุมวิชาการจำนวน 2 เรื่อง (*ภาคผนวก- presentation*)
    3.1 **จิราพร อรุณพานิชเลิศ** วัชรินทร์ รุกขไชยศิริกุล เสาวลักษณ์ พงษ์ไพจิตร และ อรทัย ศุภพล อนุพันธ์ซีราลิโนนจากเชื้อราของหญ้าทะเลที่ยังไม่ได้จำแนก PSU-ES73 การประชุมวิชาการวิทยาศาสตร์และเทคโนโลยีแห่งประเทศไทย ครั้งที่ 36 (วทท.36) วันที่ 26-28 ตุลาคม 2553 ณ ศูนย์นิทรรศการและการประชุมไบเทค กรุงเทพมหานคร (แบบโปสเตอร์)
    3.2 **ศรัณยู ใคลคลาย** วัชรินทร์ รุกขไชยศิริกุล เสาวลักษณ์ พงษ์ไพจิตร และจิรายุ บัวทอง Cytochalasin, Quinone, Succinic acid and Tetralone Derivatives from the Mangrove-derived Fungus Xylariales PSU-MA34 การประชุม 7th IMT-GT UNINET and the 3rd Joint International PSU-UNS, 7-8 ตุลาคม 2553 ณ ศูนย์ประชุมนานาชาติฉลองสิริราชสมบัติครบ 60 ปี มหาวิทยาลัยสงขลานครินทร์ (แบบโปสเตอร์)
4. นายศรัณยู ใคลคลาย ได้รับรางวัลการนำเสนอโปสเตอร์ดีเด่น จากการประชุม 7th IMT-GT UNINET and the 3rd Joint International PSU-UNS, 7-8 ตุลาคม 2553 ณ ศูนย์ประชุมนานาชาติฉลองสิริราชสมบัติครบ 60 ปี มหาวิทยาลัยสงขลานครินทร์ เรื่อง Cytochalasin, Quinone, Succinic acid and Tetralone Derivatives from the Mangrove-derived Fungus *Xylaria cubensis* PSU-MA34
5. ความร่วมมือในการทดสอบฤทธิ์ทางชีวภาพกับ Professor Dr. Xu Shen, Shanghai Institute of Materia Medica สาธารณัฐประชาชนจีน โดยมีผลงานตีพิมพ์ร่วมกัน 1 เรื่อง X. Tang, H. Shen,

J. Chen, X. Wang, Y. Zhang, L-L. Chen, V. Rukachaisirikul, H-L. Jiang and X. Shen, Activating Transcription Factor 6 Protects Insulin Receptor from ER Stress-Stimulated Desensitization via p42/44 ERK Pathway, *Acta Pharmacologica Sinica*, 2011, 32: 1138–1147.

Metabolites from the Mangrove-derived Fungus *Xylaria cubensis* PSU-MA34

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Two new succinic acid derivatives, xylacinic acid A (**1**) and B (**2**), along with seven known compounds, including one succinic acid derivative (**3**), three mellein derivatives (**4**-**6**), cytochalasin D (**7**), 2-chloro-5-methoxy-3-methylcyclohexa-2,5-diene-1,4-dione (**8**) and isosclerone (**9**), were isolated from the mangrove-derived fungus *Xylaria cubensis* PSU-MA34. Their structures were established by spectroscopic evidence. They were evaluated for cytotoxicity against KB cells and antibacterial activity against *Staphylococcus aureus* ATCC 25923 and methicillin-resistant *S. aureus*.

Keywords: *Xylaria cubensis*; mangrove-derived fungus; succinic acid; mellein; cytotoxicity; antibacterial activity

INTRODUCTION

A large variety of new bioactive compounds have recently been isolated from different organisms. Mangrove fungi have yielded various new bioactive compounds (Xu et al., 2008). The genus *Xylaria* is a rich source of biologically active secondary metabolites including cytotoxic cytochalasins (Pongcharoen et al., 2007), antimalarial benzoquinones (Tansuwan et al., 2007) and antifungal lactones (Boonphong et al., 2001). As a part of our search for biologically active compounds from bioresources in Thailand, we investigated the constituents of the mangrove-derived fungus *Xylaria cubensis* PSU-MA34. The crude extract from the culture broth exhibited cytotoxic activity against KB cells derived from epidermoid carcinoma of the oral cavity, with an IC_{50} value of 2.79 μg/mL, and antibacterial activity against *Staphylococcus aureus* ATCC 25923 and methicillin-resistant *S. aureus* (MRSA) with equal MIC values of 200 μg/mL. The isolated substances were cubensic acid, a tetraenoic acid, and cytochalasin D. We report herein the isolation of two new succinic acid derivatives, xylacinic acids A (**1**) and B (**2**), together with seven known compounds from *X. cubensis* PSU-MA34. Antibacterial activity against *S. aureus* 25923 and MRSA, and cytotoxicity against KB cells were determined. So far, there has been only one report on isolation of metabolites from *X. cubensis* (Edwards et al., 1991).

MATERIALS AND METHODS

General experimental procedures

Infrared (IR) spectra were recorded neat using a Perkin-Elmer 783 FTS165 FT-IR spectrometer. The ultraviolet (UV) absorption spectra were measured on a Shimadzu UV-1600 spectrophotometer. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a 300 or 500 MHz Bruker FTNMR Ultra Shield spectrometer using tetramethylsilane (TMS) as an internal standard. The electron ionization (EI) and high resolution electron ionization (HREI) mass spectra (MS) were obtained on a MAT 95 XL Mass Spectrometer (Thermofinnigan). ESITOF MS were determined using a LCT liquid chromatogram-mass spectrometer (Micromass). Optical rotation was measured on a JASCO P-1020 polarimeter. Thin-layer chromatography (TLC) and preparative TLC were performed on silica gel 60 GF_{254} (Merck). Column chromatography (CC) was carried out on Sephadex LH-20 with methanol (MeOH), silica gel (Merck) type 100 (70-230 mesh ASTM) with a gradient of MeOH-CH_2Cl_2 or reverse phase C-18 silica gel with a gradient of MeOH-H_2O, or otherwise stated. Light petroleum has a boiling point of 40–60 ^{o}C.

Fungal material

The mangrove-derived fungus *X. cubensis* PSU-MA34 was isolated from a branch of *Bruguiera parviflora*, a mangrove plant, which was collected in Suratthani province, Thailand, in 2005. The medium used for the isolation of the fungal strain was cornmeal agar supplemented with antibiotics as previously described (Phongpaichit et al., 2006). The isolate was deposited as fungus PSU-MA34 at the Department of Microbiology, Faculty of Science, Prince of Songkla University and in the BIOTEC Culture Collection as BCC42022.

The mangrove fungal endophyte PSU-MA34 was identified based on morphological characteristics. Colonies grow well on potato dextrose malt extract agar at

25 ^{o}C. It produces sterile cylindrical and salmon-colored stromata with black base within 2 weeks. It also produces conidia in white and powdery masses. Conidia are 4.5-5.8 x 1.5-2.0 μm, clavate, colorless and smooth with a flat and refractive basal abscission scar. These are characteristics of *X. cubensis* as previously described (Rogers, 1984).

Fermentation and isolation

The broth extract of PSU-MA34 was prepared using the same procedure as described for Diaporthaceous fungus PSU-H2 (Sommart et al., 2009). The crude extract (2.08 g) was subjected to CC over Sephadex LH-20 to obtain five fractions (A-E). Fraction B (342.9 mg) was further purified by CC over silica gel to give **7** (27.2 mg). Fraction C (101.4 mg), upon CC over silica gel, gave six subfractions (C1-C6). Subfraction C4 (283.9 mg) was further subjected to CC over silica gel to yield five subfractions (C4A-C4E). Subfraction C4D (30.1 mg) was purified by CC over reverse phase C-18 silica gel to yield **1** (1.4 mg), **2** (5.9 mg) and **3** (13.4 mg). Fraction D (339.7 mg) was subjected to CC over silica gel to yield five subfractions (D1-D5). Subfraction D1 (8.0 mg) was purified by preparative TLC using 5% ethyl acetate (EtOAc)-light petroleum (three runs) as a mobile phase to yield **8** (3.0 mg). Compound **5** (15.6 mg) was obtained from subfraction D2. Subfraction D3 (19.2 mg) was purified by CC over Sephadex LH-20 using a solution of 50% MeOH-CH_2Cl_2 to give four fractions (D3A-D3D). Subfraction D3A (3.0 mg) was subjected to preparative TLC using 25% EtOAc-light petroleum (2 runs) as a mobile phase to yield **6** (1.6 mg). Subfraction D3C (9.0 mg) was purified by preparative TLC using 5% EtOAc-CH_2Cl_2 (4 runs) as a mobile phase to provide **9** (4.1 mg). Fraction E (237.3 mg) was purified by CC over reverse phase C-18 silica gel to give **4** (7.4 mg).

Xylacinic acid A (1)

Colorless gum; $[\alpha]_D^{25}$ -117.2 ($CHCl_3$; c 0.50); UV (MeOH) λ_{max} nm (log ε): 204 (3.31), 220 (3.20); IR (neat): 3449, 1718, 1703 cm^{-1}; HREIMS *m/z* 242.1511 $[M]^+$ (calcd. for $C_{13}H_{22}O_4$, 242.1518); ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$): see Table I.

Xylacinic acid B (2)

Colorless gum; $[\alpha]_D^{25}$ -112.4 ($CHCl_3$; c 0.50); UV (MeOH) λ_{max} nm (log ε): 218 (2.91); IR (neat): 3443, 1717, 1707 cm^{-1}; ESITOF MS *m/z* 249.1111 $[M+Na]^+$ (calcd. for $C_{12}H_{18}O_4$+Na, 249.1103); ^{1}H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$): see Table I.

2-Hexylidene-3-methyl succinic acid 4-methyl ester (3)

Colorless gum; $[\alpha]_D^{25}$ -108.1 ($CHCl_3$; c 0.50); UV (MeOH) λ_{max} nm (log ε): 205 (3.01), 229 (2.92); IR (neat): 3390, 1719, 1705 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.99 (1H, t, *J* = 7.5 Hz, H-1′), 3.66 (3H, s, H-6), 3.59 (1H, q, *J* = 7.2 Hz, H-3), 2.21 (2H, m, H-2′), 1.48 (2H, m, H-3′), 1.35 (3H, d, *J* = 7.2 Hz, H-7′), 1.31 (4H, m, H-4′, 5′), 0.90 (3H, t, *J* = 6.6 Hz, H-6′); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 174.1 (s, C-4), 171.5 (s, C-1), 146.5 (d, C-1′), 131.5 (s, C-2), 52.0 (q, C-6), 37.4 (d, C-3), 31.5 (t, C-4′), 28.7 (t, C-2′), 28.1 (t, C-3′), 22.4 (t, C-5′), 15.7 (q, C-7′), 13.9 (q, C-6′).

(*R*)-(-)-5-Carboxymellein (4)

Colorless gum; $[\alpha]_D^{26}$ -51.2 (EtOH; c 0.02); UV (MeOH) λ_{max} nm (log ε): 204 (3.91), 218 (3.20), 248 (3.20), 318 (2.90); IR (neat): 3086, 1716, 1686 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$+CD_3OD) δ: 11.81 (1H, brs, OH-8), 8.18 (1H, d, *J* = 9.0 Hz, H-6), 6.94 (1H, d, *J* = 9.0 Hz, H-7), 4.68 (1H, m, H-3), 3.92 (1H, dd, *J* = 18.0, 3.5 Hz, H-4a), 3.04 (1H, dd, *J* = 18.0, 11.5 Hz, H-4b), 1.56 (3H, d, *J* = 7.0 Hz, H-9); ^{13}C NMR (75 MHz, $CDCl_3$+CD_3OD) δ: 170.0 (s, C-1), 168.0 (s, C-10), 165.4 (s, C-8), 143.7 (s, C-4a), 139.2 (d, C-6), 119.2 (s, C-5), 116.2 (d, C-7), 109.0 (s, C-8a), 75.8 (d, C-3), 32.8 (t, C-4), 20.8 (q, C-9).

(*R*)-(-)-5-Methoxycarbonylmellein (5)

Colorless gum; $[\alpha]_D^{27}$ -144.3 ($CHCl_3$; c 1.00); UV (MeOH) λ_{max} nm (log ε): 203 (3.81), 227 (3.20), 310 (2.96); IR (neat): 3244, 1717, 1676 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ: 11.83 (1H, s, OH-8), 8.13 (1H, d, *J* = 9.0 Hz, H-6), 6.94 (1H, d, *J* = 9.0 Hz, H-7), 4.68 (1H, m, H-3), 3.88 (1H, dd, *J* = 17.7, 3.0 Hz, H-4b), 3.86 (3H, s, H-11), 3.05 (1H, dd, *J* = 17.7, 12.0 Hz, H-4a), 1.57 (3H, d, *J* = 6.3 Hz, H-9); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.0 (s, C-1), 166.2 (s, C-10), 165.5 (s, C-8), 143.5 (s, C-4a), 138.5 (d, C-6), 118.6 (s, C-5), 116.2 (d, C-7), 108.9 (s, C-8a), 75.6 (d, C-3), 52.0 (q, C-11), 32.6 (t, C-4), 20.8 (q, C-9).

(*R*)-(-)-Mellein methyl ether (6)

Colorless gum; $[\alpha]_D^{26}$ -241.6 ($CHCl_3$; c 0.50); UV (MeOH) λ_{max} nm (log ε): 208 (3.72), 243 (3.18), 306 (2.99); IR (neat): 1691 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ: 7.45 (1H, dd, *J* = 8.7, 7.2 Hz, H-6), 6.92 (1H, d, *J* = 8.7 Hz, H-7), 6.79 (1H, d, *J* = 7.2 Hz, H-5), 4.56 (1H, qt, *J* = 6.3, 4.2 Hz, H-3), 3.95 (3H, s, H-10), 2.87 (2H, d, *J* = 4.2 Hz, H-4), 1.48 (3H, d, *J* = 6.3 Hz, H-9); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 162.6 (s, C-1), 161.3 (s, C-8), 142.0 (s, C-4a), 134.4 (d, C-6), 119.2 (d, C-5), 113.9 (s, C-8a), 110.9 (d, C-7), 74.1 (d, C-3), 56.2 (q, C-10), 36.1 (t, C-4), 20.7 (q, C-9).

Cytochalasin D (7)

White solid; mp. 252-255; $[\alpha]_D^{28}$ -17.7 ($CHCl_3$; c 0.55); UV (MeOH) λ_{max} nm (log ε): 204 (3.99), 282 (3.13); IR (neat): 3320, 1739, 1732, 1692 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ: 7.32 (1H, m, H-27), 7.28 (2H, m, H-26, 28), 7.14 (2H, dd, *J* = 8.1, 1.5 Hz, H-25 Hz, 29), 6.12 (1H, dd, *J* = 15.6, 2.7 Hz, H-20), 5.69 (1H, dd, *J* = 15.6, 9.9 Hz, H-14), 5.63 (1H, t, *J* = 2.4 Hz, H-21), 5.59 (1H, brs, NH-2), 5.34 (1H, ddd, *J* = 15.6, 10.5, 5.1 Hz, H-13), 5.30 (1H, brs, H-12b), 5.14 (1H, dd, *J* = 15.6, 2.4 Hz, H-19), 5.09 (1H, brs, H-12a), 3.81 (1H, d, *J* = 10.2 Hz, H-7), 3.24 (1H, mt, *J* = 4.5 Hz, H-3), 2.76 (5H, m, H-4, 8, 10, 16), 2.51 (1H, dd, *J* = 12.6, 11.1 Hz, H-15b), 2.27 (3H, s, H-31), 2.15 (1H, dd, *J* = 5.1, 3.6 Hz, H-5), 2.03 (1H, m, H-15a), 1.51 (3H, s, H-23), 1.20 (3H, d, *J* = 6.9 Hz, H-22), 0.94 (3H, d, *J* = 6.6 Hz, H-11); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 210.2 (s, C-17), 173.7 (s, C-1), 169.7 (s, C-30), 147.6 (s, C-6), 137.3 (s, C-24), 134.7 (d, C-13), 132.3 (d, C-20), 130.6 (d, C-14), 129.1 (d, C-25, 29), 128.9 (d, C-26, 28), 127.6 (d, C-19), 127.1 (d, C-27), 114.4 (t, C-12), 77.7 (d, C-21), 77.1 (s, C-18), 69.8 (d, C-7), 53.5 (s, C-9), 53.3 (d, C-3), 50.0 (d, C-5), 47.0 (d, C-4), 45.3 (t, C-10), 42.3 (d, C-16), 37.7 (t, C-15), 32.7 (d, C-8), 24.2 (q, C-23), 20.8 (q, C-31), 19.4 (q, C-22), 13.7 (q, C-11).

2-Chloro-5-methoxy-3-methylcyclohexa-2,5-diene-1,4-dione (8)
Pale yellow gum; UV (MeOH) λ_{max} nm (log ε): 204 (3.80), 279 (3.84), 362 (2.37); IR (neat): 1734, 1717 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 5.97 (1H, s, H-6), 3.78 (3H, s, H-8), 2.14 (3H, s, H-7); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 179.6 (s, C-4), 179.1 (s, C-1), 158.9 (s, C-5), 141.5 (s, C-2), 140.2 (s, C-3), 106.7 (d, C-6), 56.6 (q, C-8), 13.5 (q, C-7).

Isosclerone (9)
Colorless gum; $[\alpha]_D^{26}$ +24.4 ($CHCl_3$; c 0.03); UV (MeOH) λ_{max} nm (log ε): 213 (3.27), 235 (2.52), 258 (2.87); IR (neat): 3392, 1681 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 12.42 (1H, s, OH-8), 7.50 (1H, t, *J* = 7.8 Hz, H-6), 7.02 (1H, d, *J* = 7.5 Hz, H-5), 6.92 (1H, d, *J* = 7.5 Hz, H-7), 4.92 (1H, dd, *J* = 7.5, 3.9 Hz, H-4), 3.01 (1H, ddd, *J* = 17.7, 8.1, 4.8 Hz, H-2a), 2.65 (1H, ddd, *J* = 17.7, 5.4, 4.8 Hz, H-2b), 2.35 (1H, ddd, *J* = 13.2, 5.4, 4.5 Hz, H-3a), 2.20 (1H, ddd, *J* = 13.2, 8.1, 4.5 Hz, H-3b); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 204.3 (s, C-1), 162.7 (s, C-8), 145.8 (s, C-4a), 137.0 (d, C-6), 117.8 (d, C-7), 117.4 (d, C-5), 115.0 (s, C-8a), 67.7 (d, C-4), 34.6 (t, C-2), 34.2 (t, C-3).

Biological Assays

Antibacterial activity was evaluated against *S. aureus* 25923 and MRSA using a colorimetric broth microdilution test (CLSI, 2002; Drummond and Waigh, 2000). MICs were recorded by reading the lowest substance concentration that inhibited visible growth. Vancomycin, a positive control drug, exhibited a MIC value of 1 μg/mL. Cytotoxic assay against KB cells was conducted using a previously described method (O'Brien et al., 2000). The standard compound was ellipticine, exhibiting an IC_{50} value of 0.81 μg/mL against KB cells.

RESULTS AND DISCUSSION

The EtOAc extract from the culture broth of *X. cubensis* PSU-MA34, upon chromatographic purification, yielded two new succinic acid derivatives, xylacinic acids A (**1**) and B (**2**), one known succinic acid derivative, 2-hexylidene-3-methyl succinic acid 4-methyl ester (**3**) (Chinworrungsee et al., 2001), three mellein derivatives, (*R*)-(-)-5-carboxymellein (**4**) (Chen et al., 2006), (*R*)-(-)-5-methoxycarbonylmellein (**5**) (Sumarah et al., 2008) and (*R*)-(-)-mellein methyl ether (**6**) (Dimitriadis et al., 1997), cytochalasin D (**7**) (Xu et al., 2001), 2-chloro-5-methoxy-3-methylcyclohexa-2,5-diene-1,4-dione (**8**) (Tansuwan et al., 2007) and isosclerone (**9**) (Kokubun et al., 2003) (Fig. 1). Their structures were established on the basis of spectroscopic analyses, including two-dimensional nuclear magnetic resonance (2D NMR) data. For the known compounds, their NMR data were also compared with those previously reported. Their absolute configuration was assigned by comparison of their optical rotations with those previously reported.

Xylacinic acid A (**1**) with the molecular formula $C_{13}H_{22}O_4$ from HREIMS was obtained as a colorless gum with $[\alpha]_D^{25}$ -117.2 ($CHCl_3$; c 0.50). The ^{1}H NMR spectral data were similar to those of **3** (Chinworrungsee et al., 2001) except for the replacement of a

methoxyl group by an ethoxyl group (δ 4.13 and 1.21). This conclusion was supported by the HMBC correlation from H_2-6 (δ 4.13) to C-4 (δ 174.0) (Table I). Absorption peaks at 1718 and 1703 cm^{-1} in the IR spectrum and carbon resonances at δ 174.0 and 170.0 in the ^{13}C NMR spectrum confirmed the presence of ester and α,β-unsaturated carbonyl functionalities, respectively. In NOEDIFF experiment, irradiation of H-3 (δ 3.58) affected signal intensity of H_2-2′ (δ 2.21) but did not enhance signal intensity of H-1′ (δ 6.95), indicating that the configuration of C-2/C-1′ double bond was *E*. Therefore, **1** was assigned as a new succinic acid derivative. The absolute configuration at C-3 was proposed to have *R* configuration by comparing its optical rotation with that of (*R*)(-)-2-ethylidene-3-methylsuccinic acid 1-methyl ester (Matsumoto et al., 1973).

Xylacinic acid B (**2**) with the molecular formula $C_{13}H_{22}O_4$ from ESITOF MS was obtained as a colorless gum with $[\alpha]_D^{25}$ -112.4 ($CHCl_3$; c 0.50). The ^1H NMR spectral data (Table I) were similar to those of **3** (Chinworrungsee et al., 2001) except that signals for the pentyl unit in **3** were replaced by those for a 1-pentenyl unit: (δ 5.78, ddt, *J* = 16.8, 10.2 and 6.6 Hz; 5.06, ddt, *J* = 16.8, 1.8 and 1.8 Hz; 4.99, dm, *J* = 10.2 Hz; 2.21, m; 2.10, m; 1.59, quint, *J* = 7.2 Hz). The 1-pentenyl unit was established on the basis of the ^1H-^1H COSY data. In NOEDIFF experiment, signal enhancement of H_2-3′ (δ 1.59) upon irradiation of H-1′ (δ 6.98) indicated the *E* configuration of C-2/C-1′ double bond. Therefore, **2** was assigned as a new succinic acid derivative. The absolute configuration was proposed to be identical to that of **1** on the basis of the optical rotation.

All of isolated compounds except **1** were evaluated for cytotoxic activity against KB cells lines as well as antibacterial activity against both SA and MRSA. The results revealed that compound **7** displayed weak activity against KB cell lines with the IC_{50} value of 3.99 μg/mL whereas the other compounds showed no activity at the concentration of 50 μg/mL. Compounds **7** (Pongcharoen et al., 2007) and **8** (Tansuwan et. al., 2007) have been previously reported to exhibit strong cytotoxic activity against standard Vero cells. For antibacterial activity, compound **8** displayed weak antibacterial activity against both SA and MRSA with equal MIC values of 128 μg/mL while the remaining compounds were inactive at the concentration of 200 μg/mL.

ACKNOWLEDGEMENTS

V. Rukachaisirikul thanks the Office of Higher Education Commission and the Thailand Research Fund for the research grant (Grant No. DBG5280020). Finally, S. Klaiklay is grateful to the Royal Golden Jubilee Ph.D. Program of the Thailand Research Fund (Grant No. PHD/003/2552) and the Center for Innovation in Chemistry (PERCH-CIC) for a scholarship and to Prince of Songkla University for partial support.

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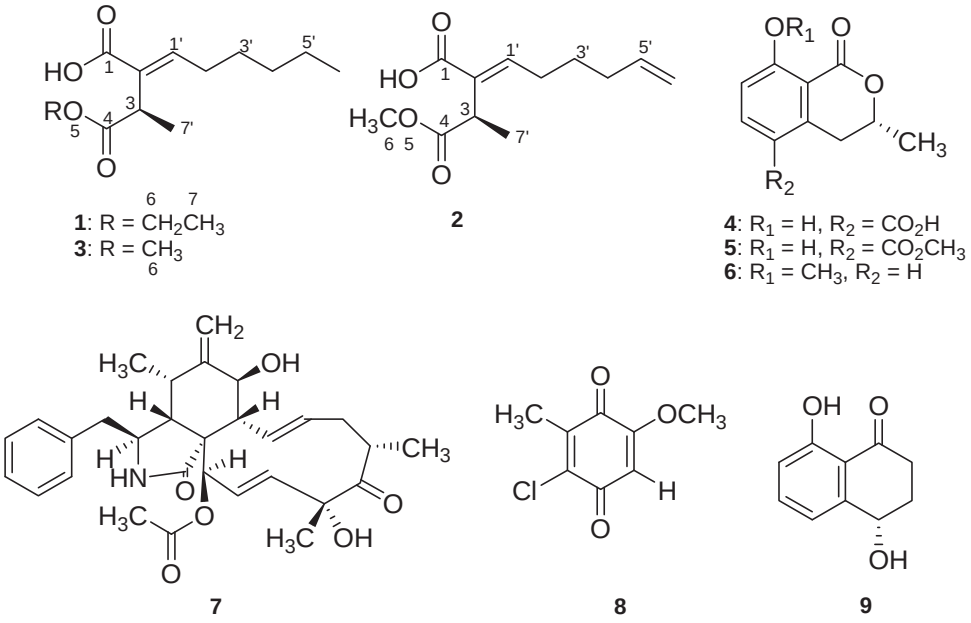

Fig. 1. Structures of compounds **1-9**.

Table I. ^{1}H, ^{13}C NMR and HMBC data of Xylacinic acids A (1) and B (2) in $CDCl_3$.

Position	**1**		**2**		**1** and **2**
	^{1}H	^{13}C	^{1}H	^{13}C	HMBC
	δ (mult., *J*, Hz)	δ (mult.)	δ (mult., *J*, Hz)	δ (mult.)	
1	-	170.0 (s)	-	171.0 (s)	-
2	-	131.4 (s)	-	131.8 (s)	-
3	3.58 (q, 7.0)	37.6 (d)	3.59 (q, 7.2)	37.6 (d)	C-1, C-2, C-4, C-1′, C-7′
4	-	174.0 (s)	-	174.1 (d)	-
6	4.13 (q, 7.5)	60.8 (t)	3.66 (s)	52.0 (q)	C-4
7	1.21 (t, 7.5)	14.1 (q)	-	-	C-6
1′	6.95 (t, 7.5)	146.4 (d)	6.98 (t, 7.5)	146.2 (d)	C-1, C-3, C-2′, C-3′
2′	2.21 (m)	28.7 (t)	2.21 (m)	28.0 (t)	C-2, C-1′, C-3′, C-4′
3′	1.48 (m)	28.2 (t)	1.59 (quint, 7.2)	27.6 (t)	C-1′, C-2′, C-4′, C-5′
4′	1.33 (m)	31.5 (t)	2.10 (m)	33.2 (t)	C-5′
5′	1.33 (m)	22.4 (t)	5.78 (ddt, 16.8, 10.2, 6.6)	137.8 (d)	C-4′
6′	0.90 (t, 6.0)	13.9 (t)	a: 5.06 (ddt, 16.8, 1.8, 1.8) b: 4.99 (dm, 10.2)	115.3 (t)	C-4′, C-5′
7′	1.35 (d, 7.0)	15.7 (q)	1.35 (d, 7.2)	15.7 (q)	C-2, C-3, C-4

Arch Pharm Res Vol 00, No 0, 000-000, 0000
DOI

Archives of Pharmacal Research
www.springer.com/12272

A β-Resorcylic Macrolide from the Seagrass-derived Fungus *Fusarium* sp. PSU-ES73

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(Received February 8, 2011/Revised June 21, 2011/Accepte June 27, 2011)

A new β-resorcylic macrolide, 5'-hydroxyzearalenone (**1**), and six known β-resorcylic macrolides were isolated from the seagrass-derived fungus *Fusarium* sp. PSU-ES73. Their structures were established by analysis of spectral data. All of the isolated compounds were evaluated for their antibacterial activity against *Staphylococcus aureus*, both standard and methicillin-resistant strains, as well as their antifungal activity against *Cryptococcus neoformans*. Only the known compound zearalenone (**2**) displayed weak antibacterial and antifungal activity.

Key words: β-Resorcylic macrolide, *Fusarium* sp., Antibacterial activity, *Staphylococcus aureus*, Antifungal activity, *Cryptococcus neoformans*

INTRODUCTION

Marine organisms are an important source of various bioactive metabolites. Several seagrasses, a subset of marine plants, have produced interesting bioactive compounds, such as the antibacterial nodosal (Kontiza et al., 2008), the antifeedant apigenin (Qi et al., 2008) and the antilarval luteolin 4'-glucuronide (Qi et al., 2008). The seagrass *Thalassia hemprichii* (Ehrenb. ex. Solms) Asch. in the family Hydrocharitaceae is found throughout the shores of the Indian and the Western Pacific Oceans. Previous investigation of the chemical constituents of *T. hemprichii* led to the identification of sterols and fatty acids from leaves (Gillan et al., 1984) and roots (Nichols and Johns, 1985), while studies on secondary metabolites from the endophytic seagrass fungi led to the isolation of the cytotoxic cladionol A and sansalvamide (Folmer et al., 2010), the antimicrobial beauvericin (Xiao et al., 2004), and the antiviral halovir A (Fenical et al., 2000). During our continuing search for biologically active compounds from endophytic fungi isolated from seagrass, we found that an EtOAc extract of the culture medium of the fungus *Fusarium* sp. PSU-ES73, isolated from *T. hemprichii*, exhibited antibacterial activity against *Staphylococcus aureus* with an MIC value of 200 μg/mL. These results prompted us to isolate antibacterial compounds from this crude extract. Herein, we describe the isolation and structural elucidation of seven β-resorcylic macrolides, including one new compound, 5'-hydroxyzearalenone (**1**), and six known compounds, zearalenone (**2**) (Miles et al., 1996), 8'-hydroxyzearalenone (**3**) and 7'-dehydrozearalenone (**4**) (Bolliger and Tamm, 1972), β-zearalenol (**5**) (Burckhardt and Ley, 2002), 5'-hydroxyzearalenol (**6**) (Zhao et al., 2008), and relgro (**7**) (Kornblum and Stoopak, 1973). These compounds were tested for antibacterial and antifungal activities. All of the known metabolites had previously been shown to be produced by various species of *Fusarium* (Richardson et al., 1985). However, in this publication, we are the first to report the isolation of β-resorcylic macrolides from the seagrass-derived fungus

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Fusarium sp.

MATERIALS AND METHODS

General experimental procedures

Infrared spectra (IR) were obtained on a FTS165 FTIR spectrometer or a Perkin Elmer Spectrum GX FTIR system and recorded on wavenumber (cm^{-1}). Ultraviolet spectra (UV) were measured with a Shimadzu UV-1601 UV-Vis spectrophotometer. Principle bands (λ_{max}) were recorded as wavelengths (nm) and log ε in MeOH solution. Mass spectra were obtained on a MAT 95 XL mass spectrometer (Thermofinnigan). ^{1}H- and ^{13}C-NMR spectra were recorded on a 300 MHz or 500 MHz Bruker FTNMR Ultra Shield spectrometer using tetramethylsilane (TMS) as an internal standard. Optical rotations were measured on a JASCO P-1020 polarimeter. Solvents for extraction and chromatography were distilled at their boiling point prior to use, except for ethyl acetate and light petroleum, which were analytical grade reagents. Thin-layer chromatography (TLC) and precoated TLC (PTLC) were performed on silica gel GF_{254} (Merck). Column chromatography (CC) was carried out on silica gel (Merck) type 100 (70-230 Mesh ASTM) with a gradient system of MeOH-CH_2Cl_2, on Sephadex LH-20 with MeOH, or on reversed phase silica gel C-18 with a gradient system of MeOH-H_2O, or as otherwise stated.

Fungal material

The seagrass-derived fungus *Fusarium* sp. PSU-ES73 was isolated from leaves of *T. hemprichii* seagrass collected in Trang Province, Thailand, in 2008. This fungus was deposited as PSU-ES73 at the Department of Microbiology, Faculty of Science, Prince of Songkla University, and in the BIOTEC Culture Collection as BCC45411.

Identification of the fungus PSU-ES73

The endophytic fungus PSU-ES73 was identified based on analyses of its nuclear ribosomal internal transcribed spacer (ITS) regions. The ITS sequence of PSU-ES73 (GenBank accession number JN092584) had the highest similarity with fungal endophyte (FJ378078), *Fusarium equiseti* (EU595566, EUFJ459976, AY147366), *F. chlamydosporum* (AJ853773) and *F. incarnatum* (FN597588) with nucleotide identity of 99.8-100%. Thus, the endophytic fungus PSU-ES73 was identified as *Fusarium* sp.

Fermentation, extraction and isolation

The flask culture medium of the fungus PSU-ES73 (15 L) was filtered to separate the filtrate and wet mycelia. The filtrate was divided into three portions. Each portion was extracted twice with an equal volume of EtOAc (2 × 500 mL). The organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness under reduced pressure to afford a dark brown gum (1.12 g). It was fractionated by CC over Sephadex LH-20 to yield three fractions (A-C). Fraction C (880.7 mg) was further purified by CC over silica gel to give seven fractions (C1-C7). Separation of fraction C2 (246.9 mg) by CC over silica gel gave three fractions (C21-C23). The second fraction contained **2** (7.1 mg). The third fraction (204.4 mg) was subjected to CC over Sephadex LH-20 with 50% MeOH-CH_2Cl_2 followed by CC over silica gel, flash CC over silica gel and finally with PTLC using 1% MeOH-CH_2Cl_2 to obtain **4** (1.5 mg). Fraction C3 (220.4 mg) was separated by CC over silica gel to afford three fractions (C31-C33). Fraction C32 (65.1 mg) was then purified by CC over silica gel with a gradient of EtOAc-light petroleum, CC over reversed phase silica gel and PTLC using 5% acetone-$CHCl_3$ as a mobile phase to furnish **1** (8.0 mg). Compound **5** (8.0 mg) was obtained from fraction C33 (70.5 mg) after purification by CC over silica gel with a gradient of EtOAc-light petroleum. Fraction C4 (207.5 mg) was dissolved in ethyl acetate. Compound **3** (37.8 mg) was obtained from the insoluble part. The soluble part (167.0 mg) was fractionated by CC over silica gel and subsequent PTLC using 3% acetone-CH_2Cl_2 to yield **7** (1.0 mg). Fraction C6 (32.1 mg) was submitted to CC over Sephadex LH-20 with 50% MeOH-CH_2Cl_2 followed by CC over the reversed phase silica gel and finally PTLC using 1% MeOH-CH_2Cl_2 to obtain **6** (1.1 mg).

5'-Hydroxyzearalenone (1)

Colorless gum; $[\alpha]_D^{25}$ −29.5 (*c* = 1.00, acetone); UV λ_{max} (MeOH) nm (log ε) 231 (4.65), 271 (4.29), 308 (3.89). FT-IR (neat) ν_{max} 3361, 1709, 1644 cm^{-1}; HREIMS *m/z* 334.1410 $[M]^+$ (Calcd for $C_{18}H_{22}O_6$, 334.1416); ^{1}H- (500 MHz) and ^{13}C- (125 MHz) NMR data, see Table I.

Preparation of MTPA esters of 5'-hydroxyzearalenone (1)

Pyridine (100 μL) and (+)-(*S*)-MTPACl (40 μL) was added to a CH_2Cl_2 solution (300 μL) of 5'-hydroxyzearalenone (**1**, 2.2 mg). The reaction mixture was stirred at room temperature for 4 days. After removal of the solvent, the mixture was purified by PTLC using 50% $CHCl_3$-hexane to afford the (*R*)-MTPA ester (**1a**, 2.6 mg). Compound **1** (2.3 mg) was treated in a similar way with (-)-(*R*)-MTPACl and, after purification by PTLC, (*S*)-MTPA ester (**1b**, 2.0 mg) was obtained.

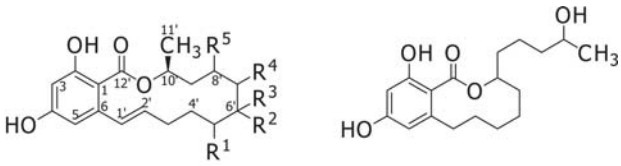
Antimicrobial assays

Antimicrobial activity was determined by standard method (Drummond and Waigh, 2000; Clinical and Laboratory Standards Institute, 2002a, 2002b). Vancomycin and amphotericin B were used as positive controls for bacteria and yeasts with MIC values of 0.69 and 0.54 μM, respectively.

RESULTS AND DISCUSSION

The crude EtOAc extract from the culture broth of the fungus PSU-ES73 was subjected to various chromatographic techniques leading to the isolation of one new (**1**) and six known (**2-7**) compounds. Their structures (Fig. 1) were elucidated from spectroscopic data, including IR, UV, NMR, and MS. The absolute configuration in **1** was determined using the modified Mosher's method (Ohtani et al., 1991). For known compounds, the structures were confirmed by comparison of the ^{1}H- and ^{13}C-NMR data with those previously reported. Their absolute configurations were determined by comparison of the optical rotations with those previously reported in the literature.

5'-Hydroxyzearalenone (**1**) was obtained as a colorless gum with $[\alpha]_D^{25}$ –29.5 (*c* = 1.00, acetone). The IR spectrum exhibited absorption bands at 3361, 1709 and 1644 cm^{-1} for hydroxyl, ester carbonyl and double bond functional groups, respectively. The molecular formula of $C_{18}H_{22}O_6$ was established by HREIMS at *m/z* 334.1410 $[M]^+$ (calcd. for $C_{18}H_{22}O_6$, 334.1416), implying the presence of eight degrees of unsaturation. The ^{1}H- and ^{13}C-NMR spectral data (Table I) were similar to those of zearalenone (**2**) except that the proton signals of one methylene group in **2** were replaced by an oxymethine proton (δ 4.29, t, *J* = 5.0 Hz) in **1**. This was supported by the presence of two oxymethine (δ 75.4 and 73.6) and five methylene (δ 38.1, 34.5, 31.6, 28.0 and 20.5) carbons in the ^{13}C-NMR spectrum of **1** instead of one oxymethine and six methylene



1: R^1 = α-OH, $R^2 + R^3$ = O, $R^4 = R^5$ = H
2: $R^1 = R^4 = R^5$ = H, $R^2 + R^3$ = O
3: $R^1 = R^4$ = H, $R^2 + R^3$ = O, R^5 = β-OH
4: R^1 = H, $R^2 + R^3$ = O, $R^4 + R^5$ = double bond
5: $R^1 = R^3 = R^4 = R^5$ = H, R^2 = β-OH
6: R^1 = β-OH, R^2 = α-OH, $R^3 = R^4 = R^5$ = H

Fig. 1. Metabolites isolated from the seagrass-derived fungus *Fusarium* sp. PSU-ES73

Table I. The NMR data for 5'-hydroxyzearalenone (**1**) and (*R*)-MTPA (**1a**) and (*S*)-MTPA (**1b**) esters in acetone-d_6

Position	**1**		HMBC (H→C)	**1a**	**1b**
	δ_H (mult., *J* in Hz)	δ_C (mult.)		δ_H (mult., *J* in Hz)	δ_H (mult., *J* in Hz)
1	-	104.8 s	-	-	-
2-OH	11.18 s	163.9 s	C-1, C-2, C-3	-	-
3	6.30 d (2.5)	101.7 d	C-1, C-2, C-4, C-5	7.29 d (2.1)	7.28 d (2.1)
4-OH	9.31 s	162.1 s	C-3, C-4, C-5	-	-
5	6.49 d (2.5)	107.6 d	C-3, C-4, C-1', C-12'	7.51 d (2.1)	7.52 d (2.1)
6	-	142.6 s	-	-	-
1'	6.87 d (15.5)	130.6 d	C-1, C-5, C-6, C-3'	6.55 d (16.2)	6.56 d (16.2)
2'	6.07 dt (15.5, 6.5)	131.7 d	C-6, C-1', C-3', C-4'	6.45 dt (16.2, 6.0)	6.50 dt (16.2, 6.6)
3'	2.33 m	28.0 t	C-1', C-2', C-4', C-5'	2.23 m	2.28 m
	2.18 m		C-1', C-2', C-5'		
4'	2.02 m	31.6 t	C-2', C-3', C-5', C-6'	2.40 m	2.46 m
	1.95 m		C-2', C-3', C-5', C-6'		
5'	4.29 t (5.0)	75.4 d	C-3', C-4', C-6'	5.51 t (4.8)	5.54 t (5.1)
6'	-	212.5 s	-	-	-
7'	2.80 m	38.1 t	C-6', C-8', C-9'	2.62 m	2.51 m
	2.49 m		C-6', C-8', C-9'		
8'	1.80 m	20.5 t	C-10'	1.83 m	1.84 m
9'	1.88 m	34.5 t	C-7', C-8', C-10', C-11'	1.78 m	1.71 m
	1.82 m		C-7', C-10'		
10'	5.11 m	73.6 d	C-9', C-12'	5.21 m	5.02 m
11'	1.38 d (6.5)	19.3 q	C-9', C-10'	1.19 d (6.0)	1.05 d (6.0)
12'	-	171.0 s	-	-	-

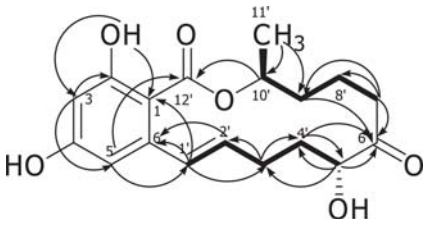
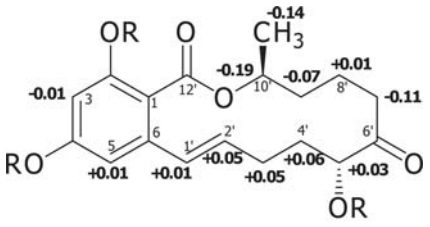


Fig. 2. Key ^{1}H-^{1}H COSY (▬) and HMBC (↷) correlations of **1**

carbons in **2**. These results indicated that **1** possessed a 1,2,3,5-tetrasubstituted benzene ring, identical to **2**. This assignment was confirmed by the HMBC correlations of 2-OH, H-3 and H-5 (Fig. 2). The ^{1}H-^{1}H COSY correlations revealed the connections of two structural units (C-1' to C-5') and (C-7' to C-11') that further established the location of the oxymethine proton (δ 4.29) at C-5' (δ 75.4). The location of this proton was confirmed by the HMBC correlations of H-5' with C-3' (δ 28.0), C-4' (δ 31.6) and C-6' (δ 212.5). The stereochemistry of the double bond was *E* according to the coupling constant of 15.5 Hz between the olefinic protons. The absolute configuration at C-5' was *R* on the basis of Mosher's method using the (*S*)- and (*R*)-MTPA esters (Fig. 3). Since 5'-hydroxyzearalenone (**1**) was co-produced with the zearalenone series (**2-6**), the absolute configuration of C-10' was proposed to be *S* on the basis of the known absolute configuration of the corresponding carbon in the naturally occurring zearalenone series (**2-6**) (Taub et al., 1968; Zinedine, et al., 2007). Consequently, 5'-hydroxyzearalenone (**1**) was a new β-resorcylic macrolide, possessing 5'*R* and 10'*S* configurations.

All of the isolated compounds were evaluated for antimicrobial activity against *S. aureus* ATCC25923, methicillin-resistant *S. aureus* SK1 and *Cryptococcus neoformans* ATCC90113. Compound **2** exhibited very weak antibacterial activity against both strains, with equal MIC values of 400 μM. In terms of antifungal activity, **2** was mildly active against *C. neoformans*



1a: R = (*R*)-MTPA
1b: R = (*S*)-MTPA

Fig. 3. Δδ values [Δδ (in ppm) = $\delta_S - \delta_R$] obtained from the (*R*)- and (*S*)-MTPA esters (**1a** and **1b**, respectively) of 5'-hydroxyzearalenone (**1**)

with an MIC value of 50.26 μM. The remaining compounds were inactive. These results indicated that the ketone functional group at C-6' was probably responsible for these activities. Moreover, the presence of a hydroxyl group at either C-5' in **1** or C-8' in **3** significantly decreased the activity.

ACKNOWLEDGEMENTS

V. Rukachaisirikul thanks the Thailand Research Fund for a Research grant (Grant No. DBG5280020). J. Arunpanichlert thanks the Development and Promotion of Science and Technology Talents Project for a scholarship. Also, the Center for Innovation in Chemistry (PERCH-CIC) and Prince of Songkla University are gratefully acknowledged for their partial support. We also thank Dr. Brian Hodgson for checking the English.

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***Manuscript* จำนวน *1* เรื่อง**

Phthalide and Isocoumarin Derivatives from *Acremonium* sp. PSU-MA70, a Mangrove-derived Fungus from *Rhizophora apiculata*

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ABSTRACT: Nine new fungal metabolites, one phthalide derivative, acremonide (**1)**, and eight isocoumarin derivatives, acremonones A-H (**2**-**9**), were isolated from the mangrove-derived fungus *Acremonium* sp. PSU-MA70 together with ten known compounds. Their structures were determined by NMR analysis. Known 8-deoxytrichothecin and trichodermol exhibited moderate antifungal activity against *Candida albicans* and *Cryptococcus neoformanns*, respectively.

The genus *Acremonium* produces various bioactive compounds, such as antibacterial acremoxanthone A,[1] antifungal dihydroresorcylide,[2] antioxidant acremonin A,[3] cytotoxic awajanoran,[4] and anti-inflammatory ascofuranone.[5] During our ongoing search for bioactive metabolites from mangrove-derived fungi, we discovered that the crude extract from a culture broth of the endophytic fungus *Acremonium* sp. PSU-MA70 isolated from a branch of *Rhizophora apiculata*, a mangrove plant, exhibited antifungal activity against standard *Candida albicans* NCPF3153, and *Cryptococcus neoformans* ATCC90113. We described herein the isolation and structural elucidation of nine new compounds including one phthalide derivative, acremonide (**1**)), and eight isocoumarin derivatives, acremonones A-H (**2-9**), together with ten known compounds, (+)-brefeldin A (**10**),[6] guangomide A (**11**),[7] guangomide B (**12**),[7] 8-deoxytrichothecin (**13**),[8] trichodermol (**14**),[9] 4-methyl-1-phenyl-2,3-hexanediol (**15**),[10] (2*R*,3*R*)-4-methyl-1-phenyl-2,3-pentanediol (**16**),[11] 5,7-dimethoxy-3,4-dimethyl-3-hydroxyphthalide (**17**),[12] Sch 54794 (**18**),[13] and Sch 54796 (**19**).[13] Their antifungal activity against CA and CN were evaluated.

All compounds (**1-19**) were isolated using chromatographic techniques and their structures were elucidated on the basis of UV, IR, NMR and MS analysis. For the known compounds, their ^{1}H and ^{13}C NMR data were compared with previously reported data. The absolute configuration of the known compounds was determined by comparison of their specific rotations with those previously reported.

Acremonide (**1**) was obtained as a white solid. The molecular formula $C_{12}H_{12}O_4$ was assigned by HREIMS. The IR spectrum exhibited an absorption band at 1760 cm^{-1} for a phthalide carbonyl group. Comparison of the ^{1}H, ^{13}C NMR (Table 1) and HMBC (see Supporting Information) data of **1** with those of **17** revealed the replacement of a methyl signal (H_3-10, δ_H 1.82; δ_C 25.7)[12] and a dioxyquaternary sp^3 carbon (C-3, δ_C 104.5) in **17** with two signals of *gem*-olefinic protons (H_2-10, δ_H 5.30 and 5.19, each 1H, d, J = 2.7 Hz; δ_C 95.2) and an oxyquaternary sp^2 carbon (C-3, δ_C 152.4) in **1**, respectively. The HMBC correlations from H_2-10 to C-3 (δ_C 152.4) and C-4 (δ_C 139.1) confirmed the location of the terminal double bond. Consequently, acremonide (**1**) was a dehydrate derivative of **17**.

Acremonone A (**2**)**,** a white solid, displayed the molecular formula $C_{14}H_{14}O_5$ from HREIMS, representing eight degrees of unsaturation. The IR spectrum exhibited an absorption band at 1745 cm^{-1} for a lactone carbonyl group. The ^{1}H NMR (Table 1) and HMBC (see Supporting Information) data indicated that it possessed a pentasubstituted benzene, similar to that of **1**. In addition, the ^{1}H NMR spectrum displayed signals for two sets of terminal olefinic protons, δ_H 5.16 and 5.11 (each 1H, d, J = 1.8 Hz) and 4.95 and 4.86 (each 1H, d, J = 1.5 Hz). Apart from the carbon resonances of the pentasubstituted benzene, the ^{13}C NMR spectrum (Table 1) consisted of one carbonyl carbon (δ_C 162.4) and four carbons of two *gem*-disubstituted alkenes (δ_C 156.2/106.6 and 148.9/97.3). All terminal olefinic protons, H_2-12 (δ_H 5.16 and 5.11) and H_2-13 (δ_H 4.95 and 4.86), exhibited the HMBC correlations with C-3 (δ_C 156.2) and C-4 (δ_C 148.9), constructing a 1,3-butadienyl unit with two oxysubstituents at C-3 and C-4. This unit was linked to C-1 and C-6 through ester and ether linkages, respectively, on the basis of the HMBC correlations of H_2-12/C-1 (δ_C 162.4), the chemical shift of C-6 (δ_C 153.3) and the degrees of unsaturation. Thus, the structure **2** was assigned for acremonone A.

Acremonone B (**3**) with the molecular formula $C_{13}H_{14}O_6$ from HREIMS was obtained as a pale yellow gum. The IR spectrum exhibited absorption bands at 3432, 1716 and 1673 cm^{-1} for hydroxyl, ketone carbonyl and conjugated lactone carbonyl groups, respectively. Detailed analysis of the ^{1}H, ^{13}C NMR (Table 1) and HMBC (Figure 1) data indicated that **3** has a pentasubstituted benzene similar to that in **1** but carried a chelated hydroxyl group (9-OH, δ_H 11.59, s) at a *peri* position to the lactone carbonyl group instead of a methoxyl group. In addition, the ^{1}H NMR spectrum (Table 1) displayed signals for one hydroxyl proton (δ_H 4.50, s), one set of nonequivalent oxymethylene protons (δ_H 4.39 and 4.19, each 1H, d, J = 12.0 Hz), and one methyl group (δ_H 2.26, s). The ^{13}C NMR spectrum (Table 1) showed resonances for one ketone carbonyl (δ_C 206.4), one lactone carbonyl (δ_C 169.4), one oxygenated quaternary (δ_C 76.1), one oxymethylene (δ_C 71.4), and one methyl (δ_C 25.4). The HMBC correlations of the nonequivalent oxymethylene protons (H_2-3, δ_H 4.39 and 4.19), the methyl protons (H_3-12, δ_H 2.26) and 4-OH (δ_H 4.50) (Figure 1) established a hydroisocoumarin derivative with a hydroxyl group and an

acetyl moiety at C-4. Irradiation of H_3-12 in the NOEDIFF experiment enhanced signal intensity of H_3-13 (δ_H 1.96), supporting above assignment. Thus, acremonone B had the structure **3**.

Acremonone C (**4**), a pale yellow gum, had the molecular formula $C_{13}H_{16}O_6$ by HREIMS. The IR spectrum was similar to that of **3** with the absence of an absorption band for a ketone functional group. The ^{1}H NMR data (Table 1) were almost identical to those of **3** except for the replacement of signals for H_2-3 and H_3-12 in **3** with those for a 1-substituted ethoxyl group (H-3, δ_H 4.44, q, J = 6.5 Hz, and H_3-11, δ_H 1.51, d, J = 6.5 Hz) and nonequivalent hydroxymethyl protons (H_2-12, δ_H 4.02 and 3.72, each 1H, d, J = 11.0 Hz). The HMBC correlations from H_2-12 to C-3 (δ_C 79.5), C-4 (δ_C 73.4) and C-5 (δ_C 141.0) and those from H-3 to C-1 (δ_C 169.9), C-5, C-11 (δ_C 14.7) and C-12 (δ_C 62.6) established an isochromanone derivative with the methyl group at C-3 and both hydroxy and hydroxymethyl groups at C-4. Irradiation of H_2-12 affected signal intensity of H_3-11 in the NOEDIFF experiment, indicating a *cis*-relationship between the hydroxymethyl and methyl groups. Therefore, acremonone C possessed the structure **4**.

Acremonone D (**5**) was obtained as a pale yellow gum and displayed the molecular formula $C_{13}H_{16}O_6$ by HRMS, indicating two mass units higher than that of **3**. A ketone carbonyl absorption band was absent in the IR spectrum of **5**. Analysis of the ^{1}H NMR data (Table 1) indicated the replacement of the acetyl signal (H_3-12, δ_H 2.26) in **3** with signals for a 1-substituted 1-hydroxyethyl unit (H-11, δ_H 4.17, q, J = 6.5 Hz and H_3-12, δ_H 1.20, d, J = 6.5 Hz). These results revealed that the acetyl group in **3** was reduced to a corresponding alcohol in **5**. The HMBC correlations of H-11 with C-3 (δ_C 70.4) and C-5 (δ_C 139.0) confirmed above conclusion. Irradiation of H_3-12 enhanced signal intensity of H_3-13 (δ_C 2.42) in the NOEDIFF experiment, supporting above assignment. Thus, acremonone D (**5**) was an 11-hydroxy derivative of **3**.

Acremonone E (**6**) with the molecular formula $C_{13}H_{14}O_6$ from HREIMS was obtained as a pale yellow gum. The IR spectrum exhibited absorption bands similar to those of **4** and **5**. The ^{1}H**,** ^{13}C NMR (Table 2) and HMBC (Figure 1) data indicated that **6** possessed a pentasubstituted benzene, identical to that of compounds **3**, **4** and **5**. In addition, the ^{1}H NMR spectrum consisted of signals for two sets of hydroxymethyl protons, (δ_H 4.76, d, J = 4.5 Hz, 2H and 4.49, br s, 1H, and δ_H 4.60, d, J = 5.5 Hz, 2H and 4.94, br s, 1H). The ^{13}C NMR spectrum contained, apart from the ^{13}C signals of the pentasubstituted benzoyl moiety, two quarternary sp^2 (δ_C 156.8 and 116.8) and two oxymethylene (δ_C 60.3 and 57.6) carbons. The hydroxymethyl protons (H_2-11, δ_H 4.60) exhibited the HMBC correlations with C-3 (δ_C 156.8), C-4 (δ_C 116.8) and C-12 (δ_C 57.6) while the other hydroxymethyl protons (H_2-12, δ_H 4.76) displayed the same correlations with C-3, C-4 and C-5 (δ_C 137.7). These data established an isocoumarin unit having the hydroxymethyl substituents at both C-3 and C-4. Signal enhancement of H_2-11 and H_3-13 (δ_H 2.56) upon irradiation of H_2-12 in the NOEDIFF experiment confirmed the assignment. Therefore, acremonone F had the structure **6**.

Acremonone F (**7**) with the molecular formula $C_{12}H_{12}O_6$ from HREIMS was obtained as a pale yellow gum. The IR spectrum exhibited absorption bands similar to those of **6**. The ^{1}H NMR data (Table 2) indicated that the methoxyl group in **6** was replaced by a hydroxy group. The chemical shift of C-7 (δ_C 165.4) confirmed the attachment of the hydroxy group at this carbon. Thus, acremonone F (**7**) was identified as a demethylated derivative of **6**.

Acremonone G (**8**) was obtained as a pale yellow gum. The molecular formula $C_{11}H_{10}O_5$ was assigned by HREIMS. The IR spectrum exhibited absorption bands similar to those of **7**. The ^{1}H NMR data (Table 2) differed from those of **7** in the presence of signals for two *meta*-coupled aromatic protons (δ_H 6.56 and 6.46, both as d, J = 2.0 Hz) and one hydroxylmethyl group (δ_H 4.51, d, J = 5.5 Hz, 2H and 4.65, br s, 1H). The *meta*-coupled aromatic protons resonating at δ_H 6.56 and 6.46 were attributed to H-6 and H-8, respectively, on the basis of their HMBC correlations (see Supporting Information), thus revealing that the methyl group at C-6 in **7** was replaced by the *meta*-coupled aromatic proton in **8**. The methyl group in **8** resonating at δ_H 2.19 was placed at C-4 (δ_C 111.8) on the basis of the HMBC correlations of H_3-12 with C-3 (δ_C 153.0) and C-5 (δ_C 142.0). Signal enhancement of H-6 and H_2-11 (δ_H

4.51) in the NOEDIFF experiment upon irradiation of H_3-12 confirmed the location of the methyl and the hydroxymethyl groups. Thus, acremonone G possessed the structure **8**.

Acremonone H (**9**) with the molecular formula $C_{20}H_{26}O_{10}$ from HREIMS was obtained as a white solid. The IR spectrum exhibited absorption bands similar to those of **6**. The ^{1}H NMR data (Table 2) were almost identical to those of **6**. The differences were the replacement of signals for one hydroxymethyl group in **6** with a methyl signal (δ_H 2.41, s) in **9** and additional signals for a *β*-glucopyranose unit [δ_H 4.43 (d, *J* = 8.0 Hz), 3.92 (m), 3.76 (br d, *J* = 12.0 Hz), 3.66 (t, *J* = 9.0 Hz), 3.41 (dd, *J* = 9.0 and 8.0 Hz), 3.36 (ddd, *J* = 9.0, 4.5, 3.0 Hz) and 3.24 (t, *J* = 9.0 Hz), each 1H][14,15] and a methoxyl group (δ_H 3.60, s). The large coupling constants ($J_{1',2'}$ = 8.0 Hz, and $J_{2',3'} = J_{3',4'} = J_{4',5'}$ = 9.0 Hz) as well as signal enhancement of H-3′ (δ_H 3.66) and H-5′ (δ_H 3.36) upon irradiation of H-1′ (δ_H 4.43) (Figure 1) supported the presence of the *β*-glucopyranose unit. The methoxyl protons (δ_H 3.60) exhibited the HMBC correlation with C-4′ (δ_C 79.1) (Figure 1), thus linking the methoxyl group at C-4′ of the sugar moiety. The attachment of the sugar moiety at C-11 (δ_C 66.5) was established on the basis of the HMBC correlation from H-1′ to C-11. The methyl group (H_3-12, δ_H 2.41) was located at C-4 (δ_C 115.3) according to the HMBC correlations of H_3-12 with C-3 (δ_C 147.0), C-4 and C-5 (δ_C 137.5) as well as signal enhancement of H_2-11 (δ_H 4.68 and 4.62, each 1H, d, *J* = 12.5 Hz) and H_3-13 (δ_H 2.38) upon irradiation of H_3-12. The specific rotation of **9**, $[\alpha]^{26}_D$ -46 (*c* 0.05, MeOH), was similar to that of 6,8-Dihydroxy-5-methoxy-3-methylisocoumarin 6-*O*-(4-*O*-methyl-*β*-D-glucopyranoside, $[\alpha]^{23}_D$ -55 (*c* 0.17, MeOH).[15] The absolute configuration of the sugar moiety was not determined due to the sample shortage. Consequently, acremonone H (**9**) was identified as a new isocoumarin 4-*O*-methyl-*β*-D-glucopyranoside.

The crude extract displayed weak antifungal activity against *C. albicans* NCPF3153 and *C. neoformans* ATCC90113 with equal MIC values of 128 *μ*g/mL. The isolated compounds **1**-**2**, **6**, **10**-**15**, **17** and **19**, which were obtained in sufficient amounts, were then tested against both human pathogenic fungi (Table 3). For *C. albicans*, **10** and **13** exhibited moderate activity with the MIC values of 32 and 16 *μ*g/mL, respectively. These compounds were much less active against *C. neoformans*. Compound **14** gave moderate effect against *C. neoformans* with the MIC value of 32 *μ*g/mL but twice less active against *C. albicans* (MIC value = 64 *μ*g/mL). Compounds **12** and **19** displayed very mild activity against *C. neoformans* and *C. albicans*, respectively. Compound **15** showed very mild activity against both pathogenic fungi whereas compounds **1**, **2**, **6**, **11** and **17** were inactive at the concentration of 200 *μ*g/mL.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a JASCO P-1020 polarimeter. Ultraviolet (UV) absorption spectra were measured in MeOH on a Shimadzu UV-1600 spectrophotometer. Infrared spectra (IR) were recorded on a Perkin-Elmer 783 FTS 165 FT-IR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on 300 and 500 MHz Bruker FTNMR Ultra Shield spectrometer. Mass spectra were measured on a MAT 95 XL mass spectrometer (Thermo Finnigan). Thin-layer chromatography (TLC) and precoated TLC (PTLC) were performed on silica gel GF_{256} (Merck). Column chromatography (CC) was carried out on silica gel (Merck) type 100 (70-230 Mesh ASTM) with a gradient system of MeOH-CH_2Cl_2, on Sephadex LH-20 with MeOH, or as otherwise stated.

Fungal Material. The mangrove-derived fungus *Acremonium* sp. PSU-MA70 was isolated from a branch of *R. apiculata*, collected from Satun province, Thailand in the year 2007. This fungus was deposited as PSU-MA70 (GenBank accession number GU592000 and GU592010) at the Department of Microbiology, Faculty of Science, Prince of Songkla University and as BCC 35914 at the National Center for Generic Engineering and Biotechnology (BIOTEC) Culture Collection, Thailand.

Fungal Identification. The fungus was identified based on the analyses of the LSU and ITS regions of their rDNA gene. Its LSU sequence (GU592010) matched with the closely related sequence of *Acremonium alternatum* FJ176883 with 82% bootstrap support. Moreover, its ITS sequence (GU592000) is well placed with Hypocreales species (EU164804), *Acremonium crotocinigenum*

DQ882846 and AJ621773 as sister taxa with high statistical support (98%) and sequence similarity between 91-100 %. The fungus PSU-MA70 was then identified as *Acremonium* sp.

Fermentation, Extraction and Isolation. The crude EtOAc extract (2.11 g, dark brown gum) from the culture broth of the fungus PSU-MA70 (15 L) was prepared using the same procedure as described previously.[16] It was dissolved with methanol to afford **10** (890 mg) and a methanol-soluble fraction (1.2 g). The methanol-soluble fraction was separated by CC over Sephadex-LH 20 to give six fractions (A-F). Fraction A (90.2 mg) was purified by CC over silica gel with 20% ethyl acetate in dichloromethane to yield three subfractions (A1-A3). Subfraction A3 (11.1 mg) was further subjected to CC over silica gel with 2% methanol in dichloromethane to afford **11** (5.4 mg). Fraction B (199.6 mg) was purified using the same procedure as subfraction A3 to afford four subfractions (B1-B4). Upon purification of subfraction B2 by CC over Sephadex-LH 20 afforded **12** (8.5 mg). Fraction C (794.7 mg) was separated by CC over silica gel using 1% methanol in dichloromethane to yield six subfractions (C1-C6). Purification of subfraction C3 (13.2 mg) by CC over silica gel with a solvent mixture of ethyl acetate, dichloromethane and petroleum ether in a ratio of 1:2:7 furnished **13** (4.0 mg). Subfraction C4 (156.4 mg) was purified by CC over Sephadex-LH 20 to give four subfractions (C41-C44). Subfraction C42 (37.7 mg) was purified using the same procedure as fraction A to afford **15** (17 mg). Subfraction C43 (39.7 mg) was subjected to CC over silica gel using 20% acetone in hexane followed by PTLC with 2% acetone in dichloromethane to give **16** (2.5 mg). Compound **14** (6.1 mg) was obtained from subfraction C5 (28.0 mg) upon purification on CC over silica gel with 30% ethyl acetate in petroleum ether. Subfraction C6 (258.6 mg) was purified by CC over silica gel with 40% acetone in hexane as a mobile phase to afford four subfractions (C61-C64). Subfraction C62 (115.0 mg) was further separated by CC over reverse phase silica gel using 50% methanol in water followed by PTLC with 40% ethyl acetate in petroleum ether to give **17** (3.7 mg). Subfraction C63 (14.1 mg) was further purified using the same procedure as subfraction A3 to yield **18** (1.5 mg) and **19** (5.7 mg). Fraction D (68.2 mg) was separated using the same procedure as subfraction C62 to give six subfractions (D1-D6). Subfraction D3 (15.3 mg) was purified using the same procedure as fraction C to yield five subfractions (D31-D35). Subfractions D31 and D32 were further purified by PTLC with 1% methanol in dichloromethane to give **3** (2.2 mg) and **4** (1.2 mg), respectively. Subfraction D34 was further separated by PTLC with 60% ethyl acetate in dichloromethane to furnish **9** (2.0 mg). Subfraction D5 (9.0 mg) was further purified by PTLC with 2% methanol in dichloromethane to give **1** (2.7 mg) and **2** (3.1 mg). Fraction E (48.7 mg) was separated using the same procedure as fraction C to yield six subfractions (E1-E6). Subfraction E4 (8.0 mg) was further purified by PTLC with 10% ethyl acetate in dichloromethane to afford **5** (0.7 mg). Subfraction E5 (9.7 mg) was further separated by PTLC with 30% ethyl acetate in petroleum ether to give **6** (3.7 mg). Fraction F (7.1 mg) was further purified using the same procedure as subfraction D5 to yield **7** (0.8 mg) and **8** (0.8 mg).

Acremonide (1): White solid; mp 167-169 ^{0}C; UV (MeOH) λ_{max} (log ε) 202 (3.55), 240 (3.63), 278 (3.06), 331 (2.93) nm; IR (neat) ν_{max} 1760 cm^{-1}; HREIMS *m/z* [M]$^+$ 220.0738 (calcd for $C_{12}H_{12}O_4$, 220.0736); ^{1}H NMR ($CDCl_3$, 300 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 1.

Acremonone A (2): White solid ; mp 184-186 ^{0}C; UV (MeOH) λ_{max} (log ε) 212 (3.67), 254 (3.08), 296 (2.93) nm; IR (neat) ν_{max} 1745 cm^{-1}; HREIMS *m/z* [M]$^+$ 262.0840 (calcd for $C_{14}H_{14}O_5$, 262.0481); ^{1}H NMR ($CDCl_3$, 300 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 1.

Acremonone B (3): Pale yellow gum; $[\alpha]^{25}_D$ -68 (*c* 1.0, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 217 (3.84), 268 (3.45), 315 (3.26) nm; IR (neat) ν_{max} 3432, 1716, 1673 cm^{-1}; HREIMS *m/z* [M]$^+$ 266.0787 (calcd for $C_{13}H_{14}O_6$, 266.0790); ^{1}H NMR ($CDCl_3$, 500 MHz), see Table 2; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2.

Acremonone C (4): Pale yellow gum; $[\alpha]^{25}_D$ -25 (*c* 0.04, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 203 (3.84), 217 (3.81), 268 (3.39), 312 (3.18) nm; IR (neat) ν_{max} 3394, 1670 cm^{-1}; HREIMS *m/z* [M]$^+$ 268.0958 (calcd for $C_{13}H_{16}O_6$, 268.0947); ^{1}H NMR ($CDCl_3$, 500 MHz), see Table 2; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2.

Acremonone D (5): Pale yellow gum; $[\alpha]^{25}_D$ -33 (*c* 1.0, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 204 (3.91), 217 (3.77), 230 (3.47), 270 (3.35), 314 (3.14) nm; IR (neat) ν_{max} 3370, 1665 cm^{-1}; HREIMS *m/z* [M]$^+$

268.0934 (calcd for $C_{13}H_{16}O_6$, 268.0947); ^{1}H NMR ($CDCl_3$, 500 MHz), see Table 2 ; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2.

Acremonone E ***(6):*** Pale yellow gum; UV (MeOH) λ_{max} (log ε) 202 (3.80), 249 (3.84), 333 (2.73) nm; IR (neat) ν_{max} 3352, 1666 cm^{-1}; HREIMS *m/z* $[M]^+$ 266.0789 (calcd for $C_{13}H_{14}O_6$, 266.0790); ^{1}H NMR (Acetone-d_6, 500 MHz), see Table 3 ; ^{13}C NMR (Acetone-d_6, 125 MHz), see Table 3.

Acremonone F ***(7):*** Pale yellow gum; UV (MeOH) λ_{max} (log ε) 200 (3.86), 247 (3.47) nm; IR (neat) ν_{max} 3356, 1674 cm^{-1}; HREIMS *m/z* $[M]^+$ 252.0642 (calcd for $C_{12}H_{12}O_6$, 252.0634); ^{1}H NMR (Acetone-d_6, 500 MHz), see Table 3 ; ^{13}C NMR (Acetone-d_6, 125 MHz), see Table 3.

Acremonone G ***(8):*** Pale yellow gum; UV (MeOH) λ_{max} (log ε) 201 (3.84), 219 (3.41), 246 (3.72), 328 (2.25) nm; IR (neat) ν_{max} 3356, 1674 cm^{-1}; HREIMS *m/z* $[M]^+$ 222.0539 (calcd for $C_{11}H_{10}O_5$, 222.0528); ^{1}H NMR (Acetone-d_6, 500 MHz), see Table 3 ; ^{13}C NMR (Acetone-d_6, 125 MHz), see Table 3.

Acremonone H ***(9):*** White solid; mp 180-182 ^{0}C; $[\alpha]^{26}_D$ -46 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 202 (3.86), 249 (3.75), 305 (2.33), 335 (2.85) nm; IR (neat) ν_{max} 3370, 1665 cm^{-1}; HREIMS *m/z* $[M]^+$ 426.1529 (calcd for $C_{20}H_{26}O_{10}$, 426.1526); ^{1}H NMR ($CDCl_3$, 500 MHz), see Table 4 ; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 4.

Antifungal Assays. Crude extracts (200 μg/mL) were preliminarily tested against all the test microorganisms by a colorimetric broth microdilution test.[17-19] The crude extract stock solutions (10 mg/mL) were diluted with MHB to 400 μg/mL and 50μL of each extract solution was pipetted into 3 wells of a 96 well plate. 50 μL of each inoculum was added to the test solution and incubated at 35 °C for 15 h (*C. albicans* NCPF3153) and 25 °C for 45 h (*C. neoformans* ATCC90113). 10 μL of 0.18% resazurin was added into each well and further incubated for another 2-3 h for yeast. Amphotericin B is used as a positive control. The color change was then observed visually. Any color changes from purple to pink or colorless were recorded as positive. The crude extracts that showed antifungal activity at 200 μg/mL were further assessed for their Minimum Inhibitory Concentrations (MICs). The MICs of extracts were tested over the concentration range of 0.25-128 μg/mL by the above colorimetric broth microdilution test. The lowest concentration at which color change occurred (2 to 3 wells) was taken as the MIC value.

ASSOCIATED CONTENT

Supporting Information. ^{1}H and ^{13}C NMR spectra for new compounds (**1-9**). This material is available free of charge via the Internet at http://pubs.acs.org.

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ACKNOWLEDGMENT

V. Rukachaisirikul thanks the Office of Higher Education Commission and the Thailand Research Fund for the research grant (Grant No. DBG5280020). A. Rodglin is grateful to the Center for Innovation in Chemistry (PERCH-CIC) for a scholarship. Finally, the Graduate School, Prince of Songkla University, is gratefully acknowledged for partial support.

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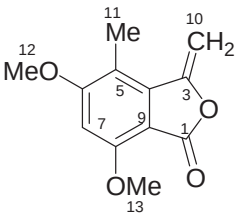

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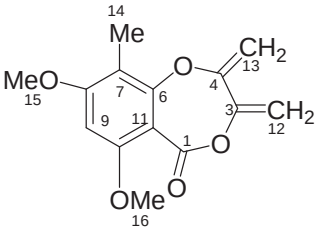

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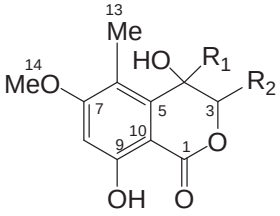
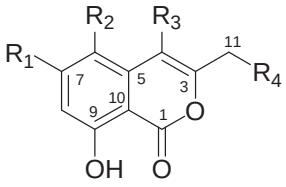


3: R_1 = ^{11}COMe, R_2 = H

4: R_1 = ^{12}CH$_2$OH, R_2 = ^{11}Me

5: R_1 = ^{11}CH(OH)^{12}Me, R_2 = H

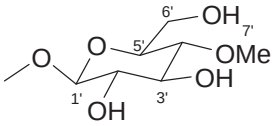


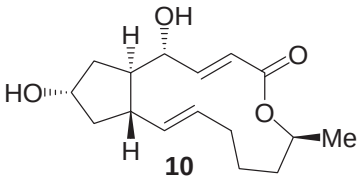
6: R_1 = ^{14}OMe, R_2 = ^{13}Me, R_3 = ^{12}CH$_2$OH, R_4 = OH

7: R_1 = R_4 = OH, R_2 = ^{13}Me, R_3 = ^{12}CH$_2$OH

8: R_1 = R_4 = OH, R_2 = H, R_3 = ^{12}Me

9: R_1 = ^{14}OMe, R_2 = ^{13}Me, R_3 = ^{12}Me, R_4 =





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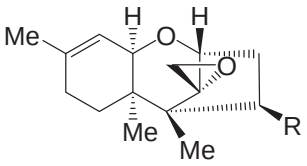
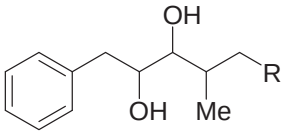
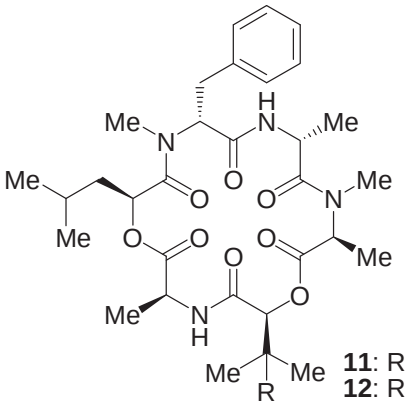


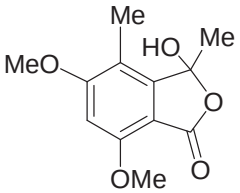
13: R = OCOCH=^{Z}CHCH$_3$
14: R = OH



15: R = Me
16: R = H



11: R = OH
12: R = H



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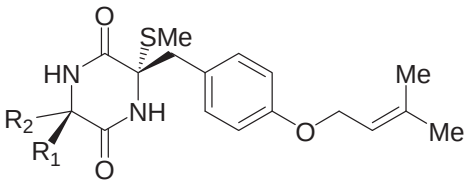


18: R_1 = H, R_2 = SMe
19: R_1 = SMe, R_2 = H

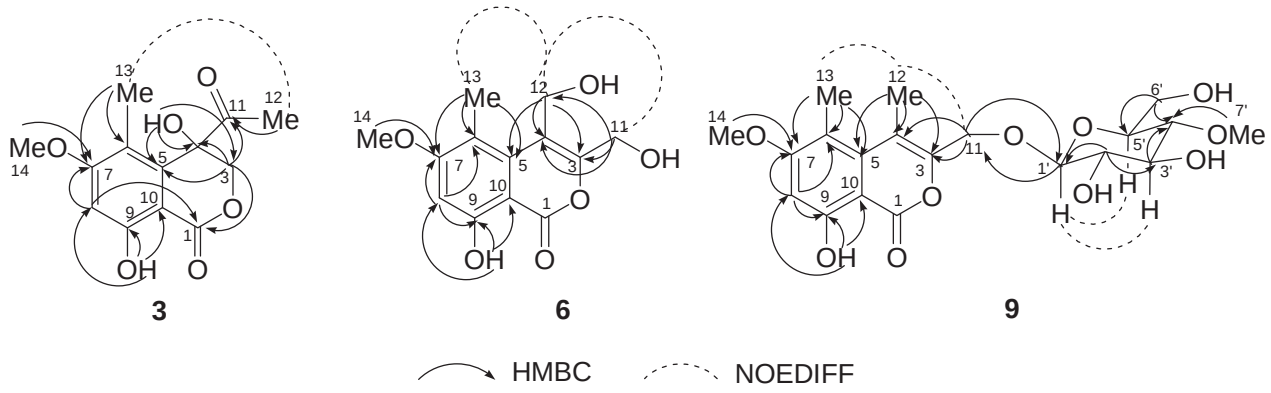


Figure 1. Selected HMBC and NOEDIFF data for compounds **3**, **6** and **9**

Table 1. ^{1}H and ^{13}C NMR Data for Acremonide (**1**) and Acremonone A (**2**), Acremonones B (**3**), C (**4**), and D (**5**) in $CDCl_3$

Position	**1**[a]		**2**[b]		**3**[c]		**4**[c]		**5**[c]	
	δ_C	δ_H m (J, Hz)	δ_C	δ_H m (J, Hz)	δ_C	δ_H m (J, Hz)	δ_C	δ_H m (J, Hz)	δ_C	δ_H m (J, Hz)
1	157.8, C		162.4, C		169.4, C		169.9, C		169.9, C	
3	152.4, C		156.2, C		71.4, CH_2	4.39, d (12.0)	79.5, CH	4.44, q (6.5)	70.4, CH_2	4.46, d (10.5)
						4.19, d (12.0)				4.14, d (10.5)
4	139.1, C		148.9, C		76.1, C		73.4, C		74.3, C	
4-OH						4.50, s		2.97, s		2.75, s
5	114.1, C				136.9, C		141.0, C		139.0, C	
6	164.3, C		153.3, C		118.3, C		117.3, C		117.9, C	
7	96.0, C	6.50, s	114.2, C		165.3, C		165.2, C		165.1, C	
8	157.8, C		162.3, C		99.9, CH	6.52, s	99.0, CH	6.39, s	98.7, CH	6.46, s
9	105.5, C		92.5, CH	6.31, s	164.4, C		163.4, C		163.5, C	
9-OH						11.59, s		11.52, s		11.64, s
10	95.2, CH_2	5.30, d (2.7)	158.9, C		100.2, C		100.3, C		99.6, C	
		5.19, d (2.7)								
11	11.0, CH_3	2.30, s	105.0, C		206.4, C		14.7, CH_3	1.51, d (6.5)	69.0, CH	4.17, q (6.5)
12	56.4, CH_3	3.96, s	106.6, CH_2	5.16, d (1.8)	25.4, CH_3	2.26, s	62.6, CH_2	4.02, d (11.0)	17.4, CH_3	1.20, d (6.5)
				5.11, d (1.8)				3.72, d (11.0)		
13	56.1, CH_3	4.01, s	97.3, CH_2	4.95, d (1.5)	11.4, CH_3	1.96, s	12.2, CH_3	2.27, s	12.3, CH_3	2.42, s
				4.86, d (1.5)						
14			7.9, CH_3	2.10, s	56.0, CH_3	3.87, s	55.9, CH_3	3.79, s	55.9, CH_3	3.88, s
15			55.8, CH_3	3.88, s						
16			56.4, CH_3	3.89, s						

[a]Data were measured at 300 MHz (^{1}H) and 125 MHz (^{13}C), [b]Data were measured at 300 MHz (^{1}H) and 75 MHz (^{13}C), [c]Data were measured at 500 MHz (^{1}H) and 125 MHz (^{13}C)

Table 2. ^{1}H and ^{13}C NMR Data for Acremonones E (**6**), F (**7**), G (**8**) and H (**9**) [a]

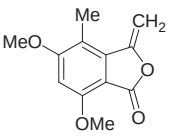
Position	**6** [b]		**7** [b]		**8** [b]		**9** [c]	
	δ_C	δ_H m (J, Hz)	δ_C	δ_H m (J, Hz)	δ_C	δ_H m (J, Hz)	δ_C	δ_H m (J, Hz)
1	167.3, C		167.0, C		167.0, C		167.0, C	
3	156.8, C		156.8, C		153.0, C		147.0, C	
4	116.8, C		117.0, C		111.8, C		115.3, C	
5	137.7, C		138.8, C		142.0, C		137.5, C	
6	114.8, C		113.9, C		102.2, CH	6.56, d, 2.0	114.5 C	
7	166.7, C		165.4, C		166.6, C		165.5, C	
8	99.1, CH	6.67, s	102.6, CH	6.58, s	102.7, CH	6.46, d, 2.0	98.8, CH	6.58, s
9	163.9, C		163.5, C		165.0, C		162.9, C	
9-OH		11.93, s		11.84, s		11.49, s		11.85, s
10	101.2, C		101.0, C		100.5, C		100.5, C	
11	60.3, CH_2	4.60, d, 5.5	60.4, CH_2	4.61, d, 5.5	59.6, CH_2	4.51, d, 5.5	66.5, CH_2	4.68, d (12.5)
								4.62, d (12.5)
11-OH		4.94, br, s		4.84, t, 5.5		4.65, br, s		
12	57.6, CH_2	4.76, d, 4.5	57.6, CH_2	4.78, d, 4.5	12.0, CH_3	2.19, s	17.2, CH_3	2.41, s
12-OH		4.49, br, s		4.38, t, 4.5				
13	12.1, CH_3	2.56, s	12.1, CH_3	2.58, s			13.7, CH_3	2.38, s
14	56.7, CH_3	3.98, s					56.0, CH_3	3.92, s
1′							102.1, CH	4.43, d (8.0)
2′							74.0, C	3.41, dd (9.0, 8.0)
3′							76.4, CH	3.66, t (9.0)
4′							79.1, CH	3.24, t (9.0)
5′							75.7, CH	3.36, ddd (9.0, 4.5, 3.0)
6′							62.0, CH_2	3.92, m
								3.76, br, d, (12.0)
7′							60.7, CH_3	3.60, s

[a]Data were measured at 500 MHz (^{1}H) and 125 MHz (^{13}C), [b]Data were measured in acetone-d_6, [c]Data were measured in $CDCl_3$

Table 3. Antifungal Activity of Some Metabolites

Compound/Extract	MIC (μg/mL)	
	C. albicans	*C. neoformans*
Crude extract	128	128
1	-[a]	-[a]
2	-[a]	-[a]
6	-[a]	-[a]
10	32	128
11	-[a]	-[a]
12	-[a]	200
13	16	64
14	64	32
15	200	200
17	-[a]	-[a]
19	200	-[a]
Amphotericin B	0.25	0.5

[a] Inactive at the concentration of 200 μg/mL.



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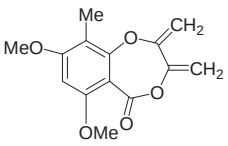

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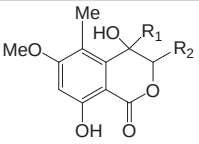
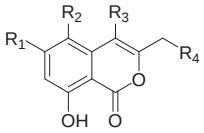
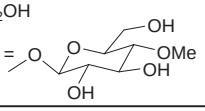


3: R_1 = COMe, R_2 = H
4: R_1 = CH_2OH, R_2 = Me
5: R_1 = CH(OH)Me, R_2 = H



6: R_1 = OMe, R_2 = Me, R_3 = CH_2OH, R_4 = OH
7: R_1 = R_4 = OH, R_2 = Me, R_3 = CH_2OH
8: R_1 = R_4 = OH, R_2 = H, R_3 = Me
9: R_1 = OMe, R_2 = Me, R_3 = Me, R_4 =



Presentation จำนวน 2 เรื่อง

Cytochalasin, Quinone, Succinic acid and Tetralone Derivatives from the Mangrove-Derived Fungus *Xylaria cubensis* PSU-MA34

Saranyoo Klaiklay[1], Vatcharin Rukachaisirikul[1], Souwalak Phongpaichit[2], Jirayu Buatong[2] and Boonsom Bussaban[3]

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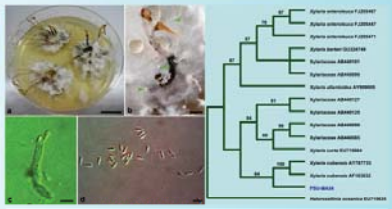
[3]Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand

Abstract: The ethyl acetate extract from the culture broth of the mangrove-derived fungus *Xylaria cubensis* PSU-MA34, upon chromatographic separation, afforded four known compounds: cytochalasin D (1), 2-hexylidene-3-methylsuccinic acid 4-methyl ester (2), 2-chloro-5-methoxy-3-methylcyclohexa-2,5-diene-1,4-dione (3) and isosclerone (4). Their structures were elucidated by analysis of spectral data, especially 1D and 2D NMR spectroscopic data, and comparison of the ^{1}H and ^{13}C NMR data with those previously reported.

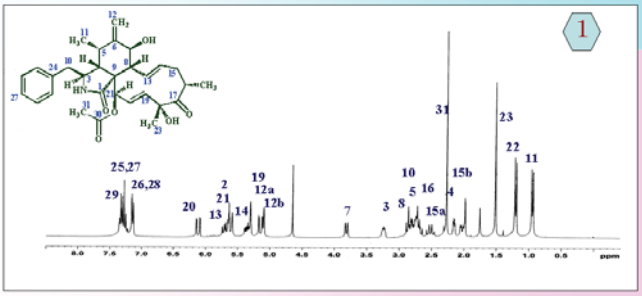
Introduction: The genus *Xylaria* is a rich source of biologically active secondary metabolites including antitumor cytochalasins, antiplasmodal benzoquinone and antioxidant tetralone. The fungus *X. cubensis* PSU-MA34 was isolated from the branches of *Bruguiera parviflora*. Its ethyl acetate extract displayed mild antibacterial activity against SA and MRSA with the same MIC values of >200 μg/mL. Thus, we were interested in the isolation and structural elucidation of metabolites from this fungus.

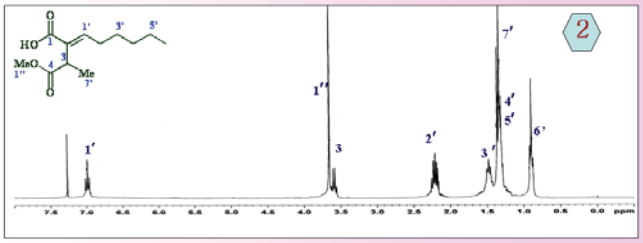
Bruguiera parviflora

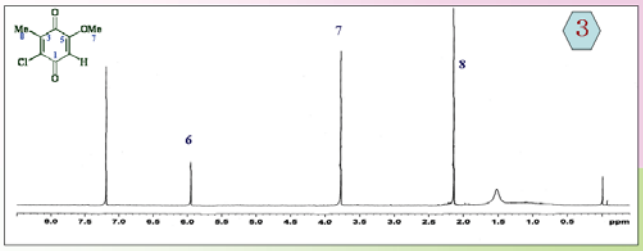


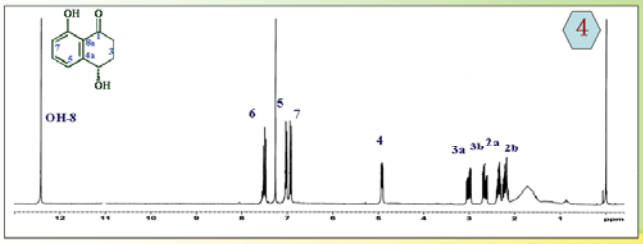
Xylaria cubensis PSU-MA34

Methodology: The flask culture of the fungus *X. cubensis* PSU-MA34 was filtered to separate into the filtrate and wet mycelia. The filtrate was extracted three times with an equal volume of EtOAc. The EtOAc layer was dried over anhydrous Na_2SO_4 and evaporated to dryness under reduced pressure to obtain a brown gum (2.0 g). The crude EtOAc extract was subjected to column chromatography over Sephadex LH20 to give five fractions (A-E). Fraction B was purified by column chromatography over silica gel to afford 1 (27.2 mg). Fraction C was separated by column chromatography over silica gel to give five fractions. The fourth fraction was further purified by column chromatography over reverse phase silica gel to furnish 2 (13.4 mg). Fraction D was separated by column chromatography over silica gel to give seven fractions. The first fraction was further separated by preparative thin layer chromatography to afford 3 (3.0 mg). 4 (4.1 mg) was obtained from the fifth fraction after purification by column chromatography over Sephadex LH20 and subsequent preparative thin layer chromatography.









Results, discussion and conclusion: Cytochalasin D (1), 2-hexylidene-3-methylsuccinic acid 4-methyl ester (2), 2-chloro-5-methoxy-3-methylcyclohexa-2,5-diene-1,4-dione (3) and isosclerone (4) were isolated from the fungus *X. cubensis* PSU-MA34. Their structures were assigned by spectroscopic methods and comparison of the ^{1}H and ^{13}C NMR data with those reported in the literature. Compound 3 exhibited weak antibacterial activity against SA and MRSA with equal MIC values of 128 μg/mL.

Keywords: mangrove-derived fungus, *Xylaria cubensis*, cytochalasin, succinic acid, quinone, tetralone

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Acknowledgements: The Royal Golden Jubilee Ph.D. Program of the Thailand Research Fund (Grant No. PHD/003/2552) and the Center for Innovation in Chemistry (PERCH-CIC) for a scholarship. The Commission on Higher Education and the Thailand Research Fund for the research grant No. RTA5180007 to Professor Dr. Vatcharin Rukachaisirikul. The Graduate School and Department of Chemistry, Faculty of Science, Prince of Songkla University for partial support.

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