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DETECTION OF ESTERASE ACTIVITY CONVERTING RENIERAMYCIN M TO JORUNNAMYCIN A IN THE CRUDE ENZYME EXTRACTS OF *JORUNNA FUNEBRIS*

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ดาลัด วโรภาสตระกูล : การตรวจสอบกิจกรรมของเอสเทอเรสซึ่งเปลี่ยนสารเรเนียรามัยชินเอ็ม เป็นโจรันนามัยชินเอในสารสกัดเอนไซม์จาก *Jorunna funebris*. (DETECTION OF ESTERASE ACTIVITY CONVERTING RENIERAMYCIN M TO JORUNNAMYCIN A IN THE CRUDE ENZYME EXTRACTS OF *JORUNNA FUNEBRIS*) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ภญ. ดร.ทักษิณา ชวนอาษา, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: อ. ภก. ดร.คณิต สุวรรณบริรักษ์, รศ. ดร.วันชัย ดีเอกนามกูล, 85 หน้า.

โจรันนามัยชินเอ เป็นสารแอลคาลอยด์ในกลุ่มบิสเตตราไฮโดรไอโซควิโนลินควิโนน ซึ่งถูกนำมาใช้เป็นสารตัวกลางเพื่อสังเคราะห์สารอนุพันธ์ 22-*O*-acyl ของสารเรเนียรามัยชินเอ็ม ในการเตรียมสารโจรันนามัยชินเอทำได้โดยกระบวนการทางเคมีเพื่อตัดหมู่ 22-*O*-angeloyl ของสารเรเนียรามัยชินเอ็ม นอกจากนี้ยังสามารถแยกสารโจรันนามัยชินเอ ได้จากทากเปลือย *Jorunna funebris*

ได้ศึกษาคุณสมบัติในการเปลี่ยนสารเรเนียรามัยชินเอ็มเป็นสารโจรันนามัยชินเอ ของสารสกัดหยาบเอนไซม์ที่เตรียมได้จากเนื้อเยื่ออวัยวะภายในของ *J. funebris* ทำให้เชื่อว่ามีเอนไซม์เอสเทอเรส ที่สามารถเร่งปฏิกิริยาไฮโดรลิซิสในการตัดพันธะเอสเทอร์ หมู่ 22-*O*-angeloyl ของสารเรเนียรามัยชินเอ็มได้ และพบว่าเอนไซม์นี้ตกตะกอนด้วยแอมโมเนียมซัลเฟตในช่วงความเข้มข้นร้อยละ ๕๕ - ๗๕ จากการศึกษาครั้งนี้พบว่าสารสกัดเอนไซม์สามารถทำงานได้ดีที่สุดเมื่อใช้สารละลายบัฟเฟอร์ Tricine ที่อุณหภูมิ ๒๕ องศาเซลเซียส ภายใต้สภาวะด่างที่พีเอช ๘ รวมทั้งพบว่าสารสกัดหยาบเอนไซม์มีความจำเพาะต่อโครงสร้างของสารกลุ่มเรเนียรามัยชิน ดังนี้ ๑) คาร์บอนตำแหน่งที่ ๑๕ และ ๑๘ ต้องเป็นหมู่ควิโนน ๒) คาร์บอนตำแหน่งที่ ๑๔ ไม่ควรมีหมู่แทนที่ออกซิเจน ๓) พันธะคู่ที่คาร์บอนตำแหน่งที่ ๒๕ เป็นแบบทรานส์ และ ๔) หมู่ 22-*O*-aliphatic acyl ที่มีจำนวนมากกว่า ๕ คาร์บอนมีความจำเพาะน้อยกว่าหมู่ที่มีจำนวนคาร์บอนน้อย

ผลการศึกษานี้เป็นการรายงานครั้งแรกเพื่อเป็นข้อมูลในการนำเอนไซม์ที่ได้จากทากเปลือย *J. funebris* ซึ่งเป็นเอนไซม์ที่มีความจำเพาะต่อสารเรเนียรามัยชินเอ็ม สามารถนำมาพัฒนาใช้ในการเตรียมสารโจรันนามัยชินเอ แทนกระบวนการสังเคราะห์ทางเคมี

ภาควิชา เภสัชเวทและเภสัชพฤกษศาสตร์ ลายมือชื่อนิสิต .....

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DALAD WAROPASTRAKUL: DETECTION OF ESTERASE ACTIVITY CONVERTING RENIERAMYCIN M TO JORUNNAMYCIN A IN THE CRUDE ENZYME EXTRACTS OF *JORUNNA FUNEBRIS*. ADVISOR: ASST. PROF. TAKSINA CHUANASA, Ph.D., CO-ADVISOR: KHANIT SUWANBORIRUX, Ph.D., ASSOC. PROF. WANCHAI DE-EKNAMKUL, Ph.D., 85 pp.

JorunnamycinA, a member of the *bis*-tetrahydroisoquinolinequinone alkaloids, has been recently used as an important starting material to prepare various 22-*O*-acyl derivatives of renieramycin M. It was synthesized by chemical reactions to remove the 22-*O*-angeloyl of renieramycin M. Besides, jorunnamycin A was recently isolated from a marine nudibranch *Jorunna funebris*.

Our preliminary result suggested that the crude enzyme extract prepared from visceral organs of *J. funebris* possibly contained the esterase enzyme. The enzyme extract was able to convert renieramycin M to jorunnamycin A by exhibiting the catalytic activity for ester bond hydrolysis of the 22-*O*-angeoyl moiety of renieramycin M. The enzyme was suitably precipitated by ammonium sulfate at the concentrations of 55-75%. According to our study, the optimal condition for the enzyme activity was performed with Tricine buffer at 45°C and pH 8. Additionally, the crude enzyme showed specificity to the renieramycin structures as follows: i) C-15 and C-18 must be a quinone moiety, ii) C-14 should not contain an oxygenated substituent, iii) the *trans* double bond geometry at C-25 is more preferable than the *cis*, and iv) the 22-*O*-aliphatic acyl group containing more than 5 carbons is less favorable than the shorter chain.

This is the first study to report the information of the esterase enzyme from the nudibranch *J. funebris* which is highly specific to the substrate renieramycin M. The enzyme will be useful as an alternative approach replacing the chemical reactions to prepare jorunnamycin A.

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