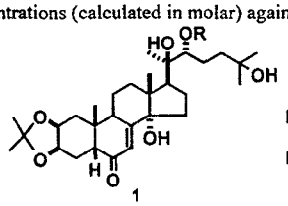
MOULTING ACTIVITY OF 22-*O*-ALKYL ETHER AND ACYL ESTER DERIVATIVES OF ECDYSTEROIDS: ALTERNATIVE STRUCTURAL REQUIREMENTS FOR HIGH MOULTING ACTIVITY

Tanud Tanachatchairatana[1], Prasert Pattanaprateep[2], Woraphot Haritakul[1], Boon-ek Yingyongnarongkul[1], Nitirat Chimnoi[3] and Apichart Suksamrarn[1]*
[1]Department of Chemistry, Faculty of Science, Ramkhamhaeng University, Bangkok 10240, Thailand; [2]Department of Chemistry, Faculty of Science, Srinakharinwirot University, Bangkok 10110, Thailand; [3]Chulabhorn Research Institute, Vipavadee-Rangsit Highway, Bangkok 10210, Thailand; e-mail address: tanud2000@hotmail.com

Abstract: 22-*O*-alkyl ethers and acyl esters of 20-hydroxyecdysone have been prepared and their moulting activity determined, using the *Musca* assay. It was found that a free 22-hydroxyl group is not an essential structural requirement for an ecdysteroid to exhibit high moulting activity. The activity of a 22-*O*-substituted ecdysteroid may even be higher than that of the parent compound, providing that the substituents constituted a functional group that can enhance biological activity. The moulting activity is not sensitive to steric factor at the 22-position. Ecdysteroid without a 22-hydroxyl group may exhibit high moulting activity, if a functional group that can enhance activity is present at an appropriate position.

Methodology: Starting from 20-hydroxyecdysone 2,3-acetonide (**1**), which in turn was prepared from 20-hydroxyecdysone (**2**), the 22-*O*-methyl ether **3** and 22-O-ethyl ether **4** were prepared by methylation and ethylation with appropriate alkyl iodide and silver oxide in dimethylformamide and the resulting products were subjected to deacetonation with 70% acetic acid. 20-Hydroxyecdysone 22-acetate (**5**) was prepared according to the published method [1]. 20-Hydroxyecdysone 22-*O*-palmitate (**6**) was prepared by reacting compound **1** with palmitoyl chloride in pyridine and benzene , followed by deacetonation. 20-Hydroxyecdysone 22-*O*-(pyrrole-2-carboxylate) (**7**) was prepared by reacting compound **1** with pyrrole-2-carboxylic acid anhydride and 4-dimethylaminopyridine (DMAP) in triethylamine-dioxane under reflux, followed by deacetonation. The 22-*O*-(furan-2-carboxylate) **8**, 22-*O*-(thiophene-2-carboxylate) **9**, 22-*O*-glycolate **10**, 22-*O*-acetylglycolate **11**, and 22-*O*-chloroacetate **12** were prepared in the same manner as that of compound **6**. The *Musca domestica* assays were scored according to Othaki et al [2] and EC_{50}s were determined by plotting concentrations (calculated in molar) against % effectiveness of puparium formation.



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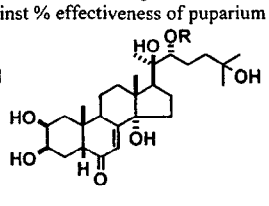
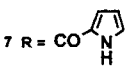
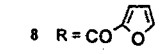


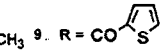
2 R = H
3 R = CH_3
4 R = CH_2CH_3
5 R = Ac
6 R = $CO(CH_2)_{14}CH_3$



7 R = CO



8 R = CO



9 R = CO

10 R = $COCH_2OH$
11 R = $COCH_2OCOCH_3$
12 R = $COCH_2Cl$

Results, Discussion and Conclusion: Inactivation by acylation at the 22-hydroxyl group could possibly be due to the bulkiness of the acyl group and/or lack of a free hydroxyl group at the 22-position to bind with the ecdysteroid receptor. To find out which factor is operating, the 22-*O*-methyl ether **3** and 22-*O*-ethyl ether **4** were prepared and subjected to biological evaluation. The *in vivo Musca* assay has been used throughout the present study. It was found that compound **3** and **4** did show some decrease in moulting activity as compared with the parent ecdysteroid **2**. Substituent at the 22-position was changed to the acyl group. We found the 22-*O*-acetate **5** was slightly less active than the parent ecdysteroid **2**. We therefore decided to study moulting activity of higher acyl ester derivative and the 22-*O*-palmitate **6** was prepared and tested for activity. Despite a very large acyl group, compound **6** was only slightly less active than the acetate **5**. In order to have information about the influence of the nitrogen heterocyclic functional group, it was planned to place the pyrrole-2-carboxylate moiety at the 22-position of compound **2** and compound **7** has therefore been prepared. To our surprise, compound **7** was twice as active as the parent ecdysteroid **2**. The furan-2-carboxylate **8** and thiophene-2-carboxylate **9** have been chosen as the oxygen and sulphur analogues. As expected, compound **8** and **9** were much less active than compound **7**. In order to see whether the hydroxyl group located at approximately the same distance as that of the NH group in **7** would bind to the receptors as efficiently as the NH group, 20-hydroxyecdysone 22-glycolate (**10**) was prepared. Compound **10** exhibited very high moulting activity in the *Musca* assay; it was about 5 times more active than the parent ecdysteroid **2** and almost 2.5 times more active than the 22-pyrrole-(2-carboxylate) ester **7**. In order to see whether the hydroxyl function of the glycolate moiety was responsible for such high activity, the corresponding acetate derivative **11** was prepared and moulting activity determined. The acetate derivative **11** was less active than its parent compound **10**, it was nevertheless more active than the ecdysteroid **2**. In order to have more information of the glycolate group, it was planned to replace the hydroxyl function with other group that could not form hydrogen bond or act as a ligand as that of compound **10** and the chloroacetate **12** was prepared. Again, the activity of compound **12** was less than that of compound **11**, thus confirming the role of a free hydroxyl group in enhancing activity.

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References: (1) Suksamrarn, A. and Patanaprateep, P. (1995) *Tetrahedron* **51**, 10633-10650.
(2) Ohtaki, T., Milkman, R.D. and Williams, C.M. (1967) *Proc.Natl. Acad. Sci.* **58**, 981-984.

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