Pergamon

Tetrahedron Vol. 51, No. 38, pp. 10633-10650, 1995
Elsevier Science Ltd
Printed in Great Britain
0040-4020/95 $9.50+0.00

0040-4020(95)00635-4

Selective Acetylation of 20-Hydroxyecdysone Partial Synthesis of Some Minor Ecdysteroids and Analogues

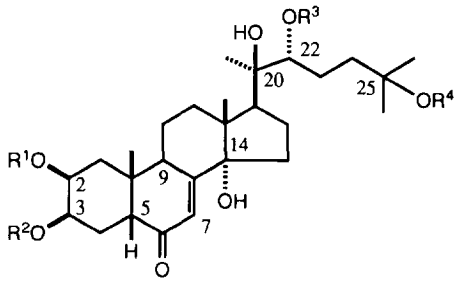
Apichart Suksamrarn* and Prasert Pattanaprateep

Department of Chemistry, Faculty of Science, Ramkhamhaeng University, Bangkok 10240, Thailand.

Abstract : Selective acetylation of the 2,3- and 20,22-acetonide and 2,3:20,22-diacetonide derivatives of 20-hydroxyecdysone and subsequent removal of the protecting groups led to partial synthesis of a number of mono-, di- and triacetate derivatives of 20-hydroxyecdysone. Some of these acetate derivatives are minor, naturally occurring ecdysteroids.

INTRODUCTION

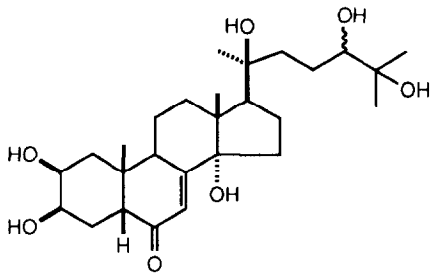
Ecdysteroids are polyhydroxysteroids found in most classes of invertebrates including insects and have also been isolated from many plants species. Their physiological functions in insects are to control moulting and metamorphosis processes and are involved in the control of reproduction. They also seem to be involved, to some extent, in the control of general physiological processes like metabolism and excretion.[1] The function of ecdysteroids in plants has not yet been clarified, but it has been proposed that they play a defensive role against non-adapted phytophagous insects.[2,3] 20-Hydroxyecdysone (20-ECD, **1**) is a widely distributed ecdysteroid found in many plant species and invertebrates.[4,5]



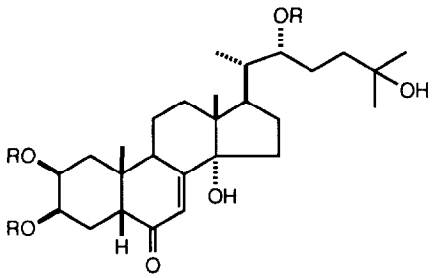
1 $R^1 = R^2 = R^3 = R^4 = H$
2 $R^1 = Ac, R^2 = R^3 = R^4 = H$
3 $R^1 = R^2 = R^3 = H, R^4 = Ac$
4 $R^1 = R^3 = Ac, R^2 = R^4 = H$
5 $R^1 = R^3 = R^4 = H, R^2 = Ac$
6 $R^1 = R^2 = R^4 = H, R^3 = Ac$
8 $R^1 = R^2 = R^3 = Ac, R^4 = H$
9 $R^1 = R^2 = R^3 = R^4 = Ac$

In recent years a number of acetate derivatives of ecdysteroids, especially those of 20-ECD (**1**), have been isolated from both plants and animals.[3,6] 20-ECD 2-acetate (**2**), 20-ECD 25-acetate (viticosterone E, **3**) and 20-ECD 2,22-diacetate (**4**) are examples of the acetate derivatives of **1** isolated from plants, while 20-ECD 3-acetate (**5**) and 20-ECD 22-acetate (**6**) have been isolated from both plants and invertebrates.[6] Information of the role of

these acetates in plants is still lacking. However discovery of some acetate derivatives of **1** in invertebrates led some groups of researchers to investigate the metabolic pathway of ingested **1** and it was concluded that the 3-acetate **5** in the migratory locust *Locusta migratoria*[7] and the 22-acetate **6** in the terrestrial snail *Cepaea nemoralis*[8] were derived from the ingested **1**. Conversion of ecdysteroids into their acetates is thought to be a mechanism to inactivate ecdysteroids present in the diet.[7] The low moulting hormone activity towards *Musca* bioassay of pinnatasterone (**7**)[9], an ecdysteroid lacking a C-22 hydroxyl group, could probably support the inactivation mechanism of **1** through C-22 acetylation. In view of structure-activity relationships, the moulting hormone activity of ecdysteroids depends on various factors including numbers and locations of the hydroxyl groups in the molecules presumably duing to variation in the ability of ecdysteroids to bind specifically to receptors.[2] It has been shown that the acetate **3**, the C-25 acetate derivative of **1**, is much less active than the parent compound **1** in the *Calliphora* bioassay for moulting hormone activity.[2,10] On the other hand, 20-ECD 2,3,22-triacetate (**8**) has been used more successfully to induce moulting in the American lobster *Homarus americanus* than **1**, though the former is less active than the latter.[11]



7



10 R = Ac

11 R = H

For those who are interested in biological activity of acetate derivatives of ecdysteroids, both naturally occurring and synthetic analogues and with varying numbers of acetyl groupings at different positions in the ecdysteroid skeleton, the availability of those acetates would make the study possible. Since most of the acetate derivatives of ecdysteroids occurred as minor constituents, it is therefore obvious that the need for obtaining them through synthesis is inevitable. Selective acetylation of the parent ecdysteroids is probably the most convenient synthesis of their acetate derivatives. It was known that acetylation of **1** at normal condition afforded 20-ECD 2,3,22-triacetate (**8**) and 20-ECD 2,3,22,25-tetraacetate (**9**).[12] Partial acetylation by controlling reaction time furnished, after a series of chromatographic separations, two monoacetates and two diacetates, including the recovered starting material **1**.[12] This method has currently been used by a number of investigators[13] and also with some modification.[14] An alternative preparation of acetate derivatives of **1** was partial deacetylation. In this case the triacetate **8** was hydrolysed with $KHCO_3$ to give a mixture of five acetates including the unreacted starting material.[12] This method has been modified for the partial deacetylation of ecdysone 2,3,22-triacetate (**10**) and a rather complex mixture of acetates was obtained.[15] In this paper we report partial synthesis of a number of acetate derivatives of 20-ECD (**1**). Selective acetylation by manipulation of protecting group(s), the target acetates could be obtained through only few and simple synthetic reactions.

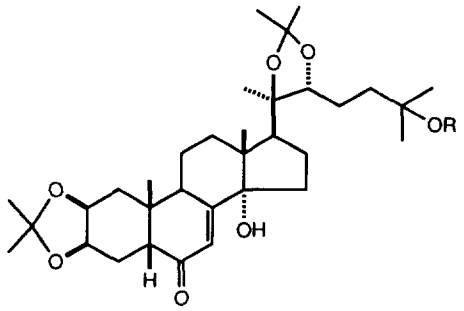
RESULTS AND DISCUSSION

Synthesis of monoacetate derivatives of 1

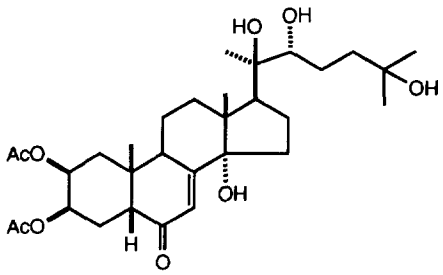
1) **Viticosterone E (3)**. The first naturally occurring ecdysteroid to be synthesized was 20-ECD 25-acetate (viticosterone E, **3**). It was isolated as a minor constituent from a number of plant species.[10,16-18] This ecdysteroid has in fact been synthesized previously.[16] However, there are some points in the synthesis of this compound worth mentioning and the compound **3** and its monoacetonide will be used in the synthesis of some di-and triacetate derivatives of **1**. Comparison of the structure of **3** with that of **1** it was clear that if the C-25 hydroxyl group was selectively acetylated, the required ecdysteroid **3** should be obtained. Studies of the reported[12] acetylation rate of **1** indicated that the reactivity of the secondary hydroxyl groups in **1** towards acetylation was 2 >> 22 > 3. The tertiary hydroxyl groups at C-25 was much less readily acetylated than the C-3 hydroxyl groups. The more hindered C-14 and C-20 tertiary hydroxyl groups are expected to be much less reactive. It was thus evident that if the secondary hydroxyl groups at C-2, C-3 and C-22 in **1** were protected with suitable protecting groups, the C-25 hydroxyl group would then be selectively acetylated.

It was noteworthy that one should be careful in choosing any reagents to do reactions with ecdysteroids. It is known, for example, that under acidic condition of mineral acid like methanolic HCl, ecdysone (**11**) was converted into two dehydration products.[19] Under similar condition, 20-ECD (**1**) gave a mixture of at least eight components as seen from the TLC.[12] Under basic condition even aqueous methanolic $KHCO_3$ caused epimerization of C-5 hydrogen of acetate derivative of **11**.[15] We also found that upon treatment with aqueous methanolic NaOH caused autoxidation of **1** and two ecdysteroids calonysterone and 9,20-dihydroxyecdysone were obtained. Ammonium hydroxide, a milder base, also gave similar result.[20] The protecting group should therefore be introduced and removed by means not being harmful to the ecdysteroid itself. The most suitable protecting group used throughout our experiments was the acetonide. To protect the secondary hydroxyl groups in **1** the diacetonide **12** was prepared by the literature methods.[12] Thus treatment of **1** with dry acetone in the presence of phosphomolybdic acid as a catalyst gave the diacetonide **12**, which was acetylated with Ac_2O and pyridine to yield the corresponding acetate **13**. Acetylation of the hindered C-25 hydroxyl group of **12** took place slowly at ambient temperature but the yield was excellent. The acetylation rate was accelerated when the reaction mixture was brought up to 40-42 °C but the yield of **13** tended to decrease with increasing reaction temperature. In order to get the required compound **3**, the acetonide protecting groups had to be removed. It has been reported that hydrolysis of **12** and the 20,22-acetonide of **1** with *p*-TsOH or $HClO_4$ resulted in the removal of the protecting groups accompanied with dehydration product, the latter of which predominated.[21] However, we found that removal of the protecting groups in **13** was achieved by treatment of **13** with 70% aqueous AcOH. It should be noted that the rate of hydrolysis of the 2,3-acetonide protecting group was much faster than that of the corresponding 20,22-acetonide. Normally the former was removed within 1-2 h, while the latter required much longer period, ranging from 2 to 4 days. The rate of hydrolysis was much increased by performing reaction in the presence of $ZnCl_2$. Other Lewis acid like $AlCl_3$ could also be used, but the reaction tended to give more minor side-products. The hydrolysis was facilitated presumably by association of the Lewis acid with the acetonide oxygen function. Viticosterone E (**3**) obtained from this method was consistent (IR, ^1H NMR and MS) with the reported data.[10] Apart from the presence of an acetoxyl group in the ^1H NMR spectrum, the downfield shifts of the C-26 and 27 methyl protons (0.05 and 0.12 ppm), as compared to those of **1**, were observed. The overall

yield of **3** from the starting **1** was 21%. Compound **3** has also been synthesized by partial deacetylation of 20-ECD 2,3,22,25-tetraacetate (**9**).[16]

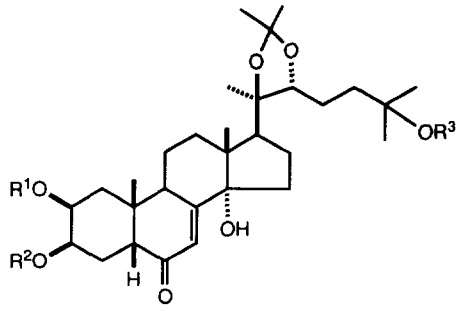


12 R = H
13 R =Ac



14

2) **20-Hydroxyecdysone 2-acetate** (**2**). The compound 20-ECD 2-acetate (**2**) was isolated from some plant species.[22,23] It was clear that selective acetylation at C-2 hydroxyl group of **1** would give this minor ecdysteroid. From the reported acetylation rate studies,[12] it might be possible to selectively acetylate **1** at position 2 to give **2**. However, previous result[12] also indicated that partial acetylation of **1** for a short period of time gave a mixture of acetate derivatives of **1**, i.e., the 2-acetate (**2**), 22-acetate (**6**), 2,3-diacetate (**14**), 2,22-diacetate (**4**), including a considerable amount of recovered **1**. Considering the mono- and diacetates arising from this acetylation condition[12] led to a conclusion that if the C-22 hydroxyl group was protected, the possible acetylated products would be the 2-acetate **2** and the 2,3-diacetate **14** only. Again the acetonide has been chosen to prevent the C-22 hydroxyl group from being acetylated and the appropriate acetonide would therefore be **15**, the 20,22-acetonide of **1**. It has been reported that treatment of **1** with acetone and perchloric acid, the monoacetonide **15** and the 2,3:20,22-diacetonide **12** were yielded, the latter being slightly predominated.[12] However, we found that by changing the acid catalyst to *p*-TsOH the required acetonide **15** was the major product. Acetylation of **15** gave a better result than that was expected as followed. Ac_2O was slowly added to a solution of **15** in a mixture of pyridine and $CHCl_3$ and the progress of the reaction was followed by TLC. The 2-acetate 20,22-acetonide **16** was obtained, accompanying by a small proportion (<10%) of the 2,3-diacetate 20,22-acetonide **17**. The product **16** could be prevented from being further acetylated to **17** if the reaction was terminated earlier, but approximately 5 to 10% of the starting material **15** was left. In both cases the required product was readily separable from **17** (or **15**) by conventional column chromatography. The yield of the product **16** thus obtained was 86% from **15**. That the acetylation took place at the most reactive C-2 hydroxyl group was evidenced by the presence of an AcO group and a large downfield shift (1.22 ppm) of H-2 signal in the ^1H NMR spectrum of **16** as compared to **15**. The minor product was shown to be 20-ECD 2,3-diacetate 20,22-acetonide (**17**) by spectroscopic methods (see Experimental). The main evidence was the presence of two AcO groups in the ^1H NMR spectrum and a large downfield shifts of H-2 and H-3 resonances in $CDCl_3$ (1.27 and 1.33 ppm, respectively) as compared to those of compound **15**.



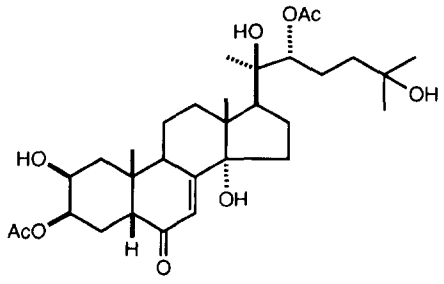
15 $R^1 = R^2 = R^3 = H$

16 $R^1 = Ac$, $R^2 = R^3 = H$

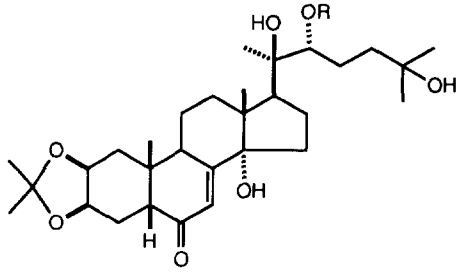
17 $R^1 = R^2 = Ac$, $R^3 = H$

18 $R^1 = R^3 = H$, $R^2 = Ac$

It should be noted that in some subsequent runs *ca* 5-10% (from ^1H NMR) of 20-ECD 3-acetate 20,22-acetonide (**18**) accompanied the corresponding major 2-acetate **16**. The acetates **16** and **18** co-migrated on TLC using a number of solvent systems. The result led us to investigate the reported partial acetylation of **1**[12] mentioned earlier and, with some modification, we found that apart from the reported acetate derivatives and a small quantity of the triacetate **8**, the 3-acetate **5** and the 3,22-diacetate **19** were also present as minor components of the 2-acetate **2** and 2,22-diacetate **4**, respectively. It was found that a mixture of 20-ECD 2-acetate (**2**) and 20-ECD 3-acetate (**5**) were inseparable by TLC.



19



20 R = H

21 R = Ac

In order to obtain the required 20-ECD 2-acetate (**2**) from **16**, the acetonide protecting group in **16** was removed by treatment with aqueous AcOH / $ZnCl_2$ and the product was obtained in 70% overall from **15**. However, ^1H NMR spectrum indicated that it was in fact a mixture of **2** and **5** in a ratio of *ca* 3:1. Acetonide deprotection of **16** with aqueous AcOH in the absence of $ZnCl_2$ gave similar result. A downfield (1.27 ppm) shift of H-3 and upfield (0.32 ppm) shift of H-5 in the ^1H NMR spectrum of **5**, as compared with those of **1**, indicated the presence of this compound in the mixture. The latter diamagnetic shift was also observed in all subsequent compounds possessing the 3-acetate group.

It worth mentioning that the presence of the 3-acetate **5** as a minor component of the corresponding 2-acetate **2** could arise from a number of possibilities. The first possibility might be due to direct acetylation at C-3 hydroxyl group to afford the minor acetate **5**, which accidentally gave the same R_f value as that of **2**. This possibility was less likely regarding the reported acetylation rate studies of **1**.[12] The second possibility involved migration of the acetyl group from C-2 to C-3. Acetyl migration has been observed in ecdysteroid derivatives.[14,15, 23,24] In our case this type of reaction could take place under acetylation condition or during the subsequent work up. The migration could also occur during the acetonide deprotection step or even during the chromatographic separation/purification.

In order to prepare pure 20-ECD 2-acetate (**2**), the reported[12,14] partial acetylation of **1** was re-investigated. Since acetylation rate of **1** was in the order 2>>22>3, it might be possible to obtain **2** as a major product and other products should be minimized. We found that by acetylating a pyridine solution of **1** at low temperature and, after column chromatography, the major product **2** fraction was obtained in 46 % yield. ^{1}H NMR spectrum indicated the presence of *ca* 5 % of the 3-acetate **5**. The 22-acetate **6** and 2,22-diacetate **4** were separated as minor components, together with the starting material **1**. Though the compounds **2** and **5** are inseparable on TLC, in practice the pure **2** could be obtained by dividing eluates corresponding to the 2-acetate portion into five or more subgroups and each subgroup was subjected to NMR investigation. Normally pure 20-ECD 2-acetate (**2**) was obtained, at least from one of these subgroups.

3) **20-ECD 22-acetate** (**6**). This ecdysteroid has been isolated from snails[8], pycnogonids[25] and insects,[26] and also from some plant species.[23,27,28] Logically, the synthesis of **6** should proceed *via* the 2,3-acetonide **20** by conventional acetylation. If this key intermediate is available, not only the C-22 hydroxyl group in **1** and many other ecdysteroids could be functionalized selectively but also modifications of the side chain of **1** would easily be manipulated. Indeed there are quite a number of naturally occurring C-22 ester and C-22,25 diester derivatives of **1**.[4,6] If C-2 and C-3 hydroxyl groups have not been suitably protected, efficient synthesis of these esters would not be possible. Moreover the acetonide **20** itself has been reported as a minor constituent of a few plant species.[29-31] As a potential starting material with a suitable protecting group, we therefore would like to synthesize this compound. We reconsidered the formation of the diacetonide **12** and 20,22-acetonide **15**. As mentioned earlier, utilization of phosphomolybdic acid as a catalyst the diacetonide **12** was obtained, presumably through the 20,22-acetonide **15**. On the other hand, when *p*-TsOH was chosen for a catalyst the 20,22-acetonide **15** was resulted, together with the diacetonide **12**. We believed that **12** might also be derived from the 2,3-acetonide **20**, which might be more rapidly converted to **12** than that of the corresponding acetonide **15**. If our assumption was the case, it might then be possible to isolate the acetonide **20** by careful selection of a milder acid catalyst. Eventually we found that pyridinium *p*-toluenesulphonate[32] served this purpose; the 2,3-acetonide **20** was obtained together with the 20,22-acetonide **15** in a ratio between 1:3 to 3:5. These two compounds were readily separable by column chromatography. The spectroscopic (IR, ^{1}H NMR) data of the acetonide **20** was consistent with the naturally occurring ecdysteroid, 20-ECD 2,3-acetonide.[29] Acetylation of **20** smoothly converted to **21**, which was subsequently treated with aqueous AcOH to give the required ecdysteroid, 20-ECD 22-acetate (**6**). The presence of a C-22 acetate grouping in **6** was evident by a large downfield shift (1.66 ppm) of the H-22 signal in the ^{1}H NMR spectrum as compared with that of **1**. The structure of **6** was further confirmed by the presence of a fragment ion at m/z 159, corresponding to a side chain ion arising from cleavage between C-20 and C-22. The overall yield of **6** from the acetonide **20** was 85%.

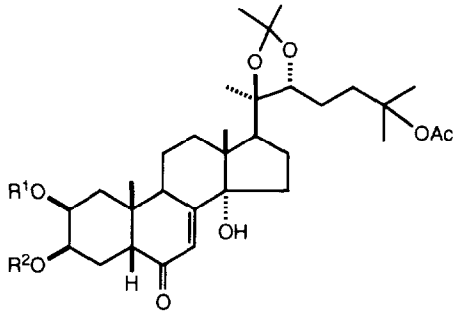
Synthesis of diacetate derivatives of 1

1) **20-ECD 2,22-diacetate** (**4**). This diacetate of **1** has recently been isolated as a minor component from *Serratula tinctoria*.[23] Synthesis of **4** has been accomplished as followed. Selective acetylation of 20-ECD 22-acetate (**6**) synthesized previously, using similar condition to that employed for the synthesis of **16** from **15**, the required 2,22-diacetate **4** together with a small quantity of 20-ECD 2,3,22-triacetate (**8**), were obtained in 80 and 1%, respectively. The ^{1}H NMR spectral data of the diacetate **4** was consistent with the structure **4**.[23] A large downfield shift of H-2 clearly indicated that the second AcO group was located at position 2. In this case the presence of 20-ECD 3,22-diacetate (**19**)[23] was detected from the ^{1}H NMR spectrum of some fractions of

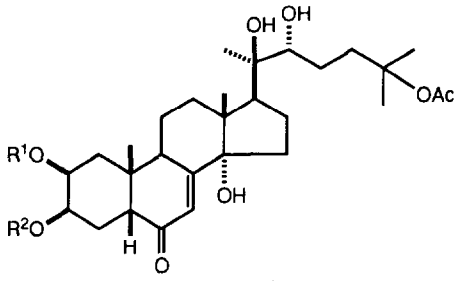
eluates of **4**. The proton signals corresponding to compound **19** was first misunderstood to be those of impurity. However, it was later concluded to be those of **19** after the spectrum being compared with that of a 10:3 mixture of **4** and **19** obtained from partial acetylation of **1** (method 1, see Experimental). Again, we believed that the co-occurrence of these two compounds resulted from C-2 to C-3 acetyl migration.

2) **20-ECD 2,3-diacetate (14)**. The diacetate **14** was synthesized by acetylating the 20,22-acetonide **15** under normal acetylation condition and the resulting diacetate acetonide **17** was subjected to hydrolysis to remove the protecting group by the method similar to that employed for the synthesis of **2**. The spectroscopic (^{1}H NMR) data of **14** were consistent with the reported data.[12]

3) **20-ECD 2,25-diacetate (23) and 20-ECD 3,25-diacetate (24)**. Taking the advantage of a large difference in the hydrolysis rate of the 2,3- and 20,22-acetonides, partial deprotection of 20-ECD 25-acetate 2,3:20,22-diacetonide (**13**) by treatment with aqueous AcOH for 2 h afforded the corresponding monoacetonide **22** as a single product in high yield. Selective acetylation under similar condition as employed for the synthesis of **16** from **15**, a *ca* 4:1 mixture of the 2,25-diacetate acetonide **25** and 3,25-diacetate acetonide **26** was obtained in 82% together with 5% of the corresponding 2,3,25-triacetate acetonide **27**. In this case the less polar, minor diacetate acetonide **26** was supposed to be derived from the corresponding 2,25-diacetate acetonide **25** through the acetyl migration. Subsequent hydrolysis of the protecting group in **25** and **26** mixture afforded a mixture of 20-ECD 2,25-diacetate (**23**) and 20-ECD 3,25-diacetate (**24**) in 82% yield. The diacetates **25** and **26** could be separated by column chromatography and the two isomers could easily be distinguished by ^{1}H NMR data (see Table).



22 $R^1 = R^2 = H$
25 $R^1 = Ac$, $R^2 = H$
26 $R^1 = H$, $R^2 = Ac$
27 $R^1 = R^2 = Ac$

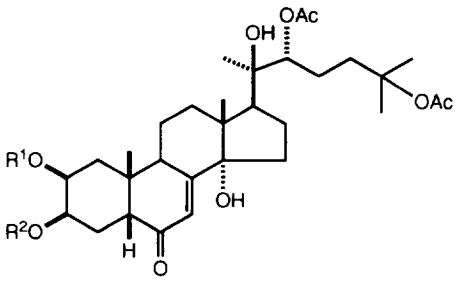


23 $R^1 = Ac$, $R^2 = H$
24 $R^1 = H$, $R^2 = Ac$
30 $R^1 = R^2 = Ac$

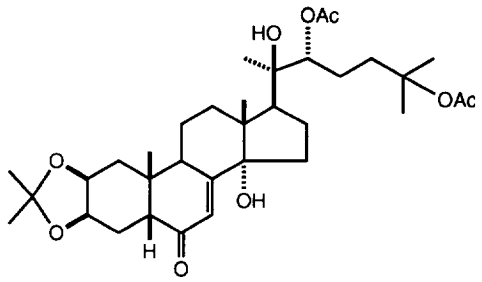
4) **20-ECD 22,25-diacetate (28)**. The procedure for the synthesis of 20-ECD 22-acetate (**6**) was applied for the synthesis of **28** from **20**, but the acetylation time of **20** was prolonged so that the more hindered C-25 hydroxyl group was also acetylated and finally the 22,25-diacetate 2,3-acetonide **29** was resulted. Again the rate of acetylation of this tertiary hydroxyl group could be accelerated by warming up the reaction mixture. The 22,25-diacetate **28** was finally obtained in 77% overall from **20** after usual hydrolysis of the protecting group of **29**. The ^{1}H NMR spectrum of **28** exhibited a large (1.60 ppm) downfield shift of H-22 as compared to that of **1**. Acetylation shifts were also observed for the C-26 and C-27 methyl protons.

Synthesis of triacetate derivatives of 1

1) **20-ECD 2,3,25-triacetate (30).** As mentioned earlier, 20-ECD 2,3,22-triacetate (**8**) has been synthesized by acetylating **1** directly.[12] However, synthesis of the triacetate **30** from **1** had to go through a suitable protecting group and the 20,22-acetonide **15** seemed to serve the purpose. Thus prolonged acetylation of **15** and subsequent hydrolysis of the protecting group of the resulting 2,3,25-triacetate 20,22-acetonide **27** afforded the required triacetate **30** in 38% overall from **15**. The spectroscopic data were consistent with the structure **30**. It should be mentioned that compound **27** was also obtained as a minor product from the synthesis of 20-ECD 2,25-diacetate (**23**) but the strategies for the synthesis of **23** and **30** were different.



28 $R^1 = R^2 = H$
31 $R^1 = Ac$, $R^2 = H$
32 $R^1 = H$, $R^2 = Ac$



29

2) **20-ECD 2,22,25-triacetate (31) and 20-ECD 3,22,25-triacetate (32).** Partial acetylation of 20-ECD 22,25-diacetate (**28**) synthesized previously under the same condition as employed for the synthesis of **4** from **6**, the triacetate **31** was resulted together with the isomeric 3,22,25-triacetate **32** in a ratio of 5:1. A small proportion of a faster moving component could be seen on the TLC and it was identified as the known 2,3,22,25-tetraacetate **9**. The structures of **31** and **32** were established mainly from ^1H NMR data. Again, acetylation shift of the carbinol proton at appropriate position was the reliable evidence in confirming the structure of the acetylated products.

The synthesis of 20-ECD 2,3,22-triacetate (**8**) and 20-ECD 2,3,22,25-tetraacetate (**9**) have not been mentioned, since these two acetate derivatives of **1** have been synthesized by direct acetylation of **1**.[12]

CONCLUSIONS

1. The following mono-, di- and triacetate derivatives of 20-ECD (**1**) have been partially synthesized from **1**: the 2-acetate (**2**), 22-acetate (**6**), 25-acetate (viticosterone E, **3**), 2,22-diacetate (**4**), 2,3-diacetate (**14**), a mixture of 2,25-diacetate (**23**) and 3,25-diacetate (**24**), 22,25-diacetate (**28**), 2,3,25-triacetate (**30**), 2,22,25-triacetate (**31**) and 3,22,25-triacetate (**32**). The first four compounds are naturally occurring ecdysteroids.

2. The known C-2 to C-3 acetyl migration[14,15,23,24] has also been observed in our synthesis. We found that all 2-acetate derivatives of **1** with free C-3 hydroxyl groups suffered from this type of reaction under the reaction conditions and/or purification procedures employed and the corresponding C-3 acetates were obtained as minor components. The two isomeric acetates could be isolated from each other by conventional column

Table ^{1}H NMR data of ecdysteroids

H	1	2	3	4	5[†]	6	8	9	12	
	C_5D_5N	C_5D_5N	C_5D_5N	C_5D_5N	C_5D_5N	C_5D_5N	$CDCl_3$	$CDCl_3$	$CDCl_3$	C_5D_5N
2	4.15 (m)[≠]	5.24 (m)	4.16 (br d, 10.5)	5.23 (m)	4.28 (m)[¶]	4.19 (m)	5.07 (m)	5.07 (ddd, 12, 5, 3)	4.23 (m)	4.16 (m)
3	4.20 (br s)[≠]	4.28 (br s)	4.23 (br s)	4.28 (br s)	5.47 (br s)	4.23 (br s)	5.37 (br s)	5.35 (br s)	4.26 (br s)	4.09 (br s)
5	2.97 (m)[§]	3.01 (dd, 13.5, 3.6)	3.02 (dd, 13.2, 3.5)	3.02 (dd, 13.4, 4.3)	2.65 (dd, 13.4, 3.9)	3.03 (m)[≠]	2.40 (m)[≠]	2.39 (m)[≠]	2.36 (dd, 12.5, 4.7)	2.54 (dd, 12.5, 4.7)
7	6.22 (d, 2.1)	6.23 (d, 2.3)	6.24 (d, 2.2)	6.21 (d, 2.1)	6.21 (d, 2.3)	6.24 (d, 2)	5.88 (d, 2.2)	5.88 (d, 2.2)	5.83 (d, 2.2)	6.18 (d, 2.2)
9	3.55 (m)	3.63 (m)	3.58 (m)	3.62 (m)	3.55 (m)	3.59 (m)	3.11 (m)	3.10 (m)	2.80 (m)	3.15 (m)
17	2.96 (m)[§]	2.96 (t, 9)	2.98 (t, 9.1)	2.94 (m)	2.96 (t, 9)	2.97 (m)[≠]	2.40 (m)[≠]	2.36 (m)[≠]	2.22 (t, 8)	2.75 (t, 8.3)
22	3.84 (br d, 8.9)	3.85 (br d, 9.1)	3.83 (br d, 10)	5.48 (br d, 9)	3.85 (br d, 9.1)	5.50 (br d, 9.5)	4.86 (br d, 8)	4.80 (dd, 9, 1.5)	3.65 (dd, 9.5, 2.5)	3.93 (dd, 9.5, 2.5)
18-Me	1.18 (s)	1.18 (s)	1.21 (s)	1.14 (s)	1.18 (s)	1.16 (s)	0.85 (s)	0.85 (s)	0.78 (s)	0.98 (s)[a]
19-Me	1.02 (s)	1.06 (s)	1.05 (s)	1.05 (s)	1.06 (s)	1.05 (s)	1.03 (s)	1.03 (s)	0.98 (s)	0.97 (s)[a]
21-Me	1.56 (s)	1.55 (s)	1.60 (s)	1.59 (s)	1.55 (s)	1.62 (s)	1.27 (s)	1.25 (s)	1.16 (s)	1.54 (s)[b]
26-Me	1.34 (s)	1.37 (s)	1.39 (s)	1.34 (s)	1.34 (s)	1.32 (s)	1.22 (s)	1.41 (s)	1.24 (s)	1.32 (s)[c]
27-Me	1.34 (s)	1.37 (s)	1.46 (s)	1.35 (s)	1.34 (s)	1.33 (s)	1.24 (s)	1.44 (s)	1.25 (s)	1.32 (s)[c]
$>C(Me)_2$	-	-	-	-	-	-	-	-	1.33, 1.41; 1.33, 1.49 (each s)	1.34,[c] 1.53[b] 1.30,[c] 1.44 (each s)
Ac	-	1.93 (s)	-	1.91, 2.01 (each s)	1.96 (s)	2.01 (s)	2.01, 2.11, 2.12 (each s)	1.99, 2.01, 2.11, 2.12 (each s)	-	-

[†] Signals were assigned from a mixture of compounds **2** and **5**.

[≠, §] Signals within the same column denote partially overlapping signals. [¶] Obscured by other signals.

[a-c] Assignments may be reversed for signals with the same superscript.

Table ^{1}H NMR data of ecdysteroids (continued)

H	13	14	15		16	17	19	20	21	22
	$CDCl_3$	C_5D_5N	$CDCl_3$	C_5D_5N	$CDCl_3$	$CDCl_3$	C_5D_5N	C_5D_5N	C_5D_5N	C_5D_5N
2	4.21 (m)	5.33 (ddd, 11.9, 5, 3)	3.80 (m)	4.15 (m)	5.02 (m)	5.07 (ddd, 11.6, 5, 3)	4.29 (m)	4.17 (m)	4.18 (m)	4.14 (m)
3	4.27 (br s)	5.46 (br s)	4.03 (br s)	4.21 (br s)	4.12 (br s)	5.36 (br s)	5.42 (br s)	4.08 (br s)	4.07 (br s)	4.22 (br s)
5	2.36 (dd, 12.6, 4.7)	2.63 (dd, 13.1, 3.8)	2.39 (m)[≠]	2.98 (dd, 13.1, 3.3)	2.55 (dd, 13, 4)	2.40 (dd, 13, 4.2)	2.64 (dd, 13.5, 4)	2.55 (m)[¶]	2.54 (m)[¶]	3.01 (dd, 12.9, 3.6)
7	5.83 (d, 2.1)	6.22 (d, 2)	5.84 (d, 2.1)	6.24 (d, 2.1)	5.89 (d, 2)	5.88 (d, 2.3)	6.19 (d, 2.1)	6.20 (d, 2)	6.17 (d, 1.8)	6.25 (d, 2.1)
9	2.80 (m)	3.56 (m)	2.97 (m)	3.54 (m)	3.08 (m)	3.10 (m)	3.53 (m)	3.15 (m)	3.18 (m)	3.55 (m)
17	2.22 (m)	2.97 (t, 8.8)	2.39 (m)[≠]	2.74 (t, 8.6)	2.24 (m)	2.24 (m)	2.94 (m)	3.00 (t, 8.9)	2.98 (t, 8.6)	2.74 (t, 8)
22	3.60 (dd, 9.3, 3.1)	3.86 (br d, 8.9)	3.60 (m)	3.93 (dd, 9.7, 2.1)	3.68 (br d, 9)	3.66 (br d, 8)	5.48 (br d, 9)	3.87 (br d, 8.1)	5.50 (br d, 8)	3.89 (dd, 9.3, 2.5)
18-Me	0.79 (s)	1.18 (s)	0.78 (s)	1.02 (s)[d]	0.80 (s)	0.79 (s)	1.14 (s)	1.19 (s)	1.14 (s)	1.03 (s)
19-Me	0.98 (s)	1.03 (s)	0.96 (s)	0.99 (s)[d]	1.00 (s)	1.03 (s)	1.05 (s)	0.99 (s)	0.98 (s)	1.00 (s)
21-Me	1.15 (s)	1.56 (s)	1.15 (s)	1.52 (s)	1.16 (s)	1.16 (s)	1.59 (s)	1.60 (s)	1.63 (s)	1.52 (s)
26-Me	1.43 (s)	1.38 (s)	1.20 (s)	1.33 (s)	1.24 (s)	1.24 (s)	1.31 (s)	1.35 (s)	1.31 (s)[e]	1.43 (s)[f]
27-Me	1.47 (s)	1.38 (s)	1.29 (s)	1.34 (s)	1.25 (s)	1.25 (s)	1.32 (s)	1.35 (s)	1.31 (s)[e]	1.43 (s)[f]
$>C(Me)_2$	1.31, 1.40; 1.33, 1.49 (each s)	-	1.32, 1.36 (each s)	1.31, 1.43 (each s)	1.33, 1.41 (each s)	1.33, 1.41 (each s)	-	1.31, 1.55 (each s)	1.33,[e] 1.54 (each s)	1.34, 1.42[f] (each s)
Ac	1.98 (s)	1.99, 2.03 (each s)	-	-	2.10 (s)	2.01, 2.11 (each s)	1.96, 2.00 (each s)	-	2.01 (s)	1.90 (s)

[≠] Signals within the same column denote partially overlapping signals. [¶] Obscured by other signals.
[d-f] Assignments may be reversed for signals with the same superscript.

Table ^{1}H NMR data of ecdysteroids (continued)

H	23	24†	25	26	27	28	29	30	31	32
	C_5D_5N	C_5D_5N	$CDCl_3$	$CDCl_3$	$CDCl_3$	C_5D_5N	C_5D_5N	C_5D_5N	$CDCl_3$	$CDCl_3$
2	5.22 (m)	4.28 (m)¶	5.00 (m)	4.00 (m)	5.08 (m)	4.17 (m)	4.14 (m)	5.30 (m)	5.02 (m)	4.03 (ddd, 11.5, 4, 3)
3	4.30 (br s)	5.48 (br s)	4.10 (br s)	5.19 (br s)	5.36 br s)	4.24 (br s)	4.09 (br s)	5.48 (br s)	4.13 (br s)	5.21 (br s)
5	3.04 (dd, 13, 4)	2.63 (dd, 13.5, 4)	2.49 (dd, 13.4, 4)	2.30 (dd, 13.4, 4)	2.40 (dd, 13.2, 4.3)	3.02 (dd, 13, 3.8)	2.56 (m)¶	2.64 (dd, 13.2, 3.5)	2.52 (dd, 13.6, 4.1)	2.33 (dd, 13, 4)
7	6.24 (d, 2)	6.22 (d, 2)	5.84 (d, 2.5)	5.85 (d, 2.4)	5.88 (d, 2.3)	6.22 (d, 2)	6.17 (d, 2)	6.22 (d, 1.9)	5.87 (d, 2.3)	5.87 (d, 2.2)
9	3.65 (m)	3.57 (m)	3.07 (m)	3.00 (m)	3.11 (m)	3.58 (m)	3.18 (m)	3.58 (m)	3.10 (m)	3.03 (m)
17	2.94 (t, 8.8)	2.94 (t, 8.8)	2.20 (m)	2.20 (m)	2.23 (m)	2.94 (t, 8.5)	2.94 (t, 8.5)	2.94 (t, 8.9)	2.35 (t, 9)	2.34 (t, 9)
22	3.83 (br d, 10)	3.83 (br d, 10)	3.59 (dd, 9.5, 3.1)	3.59 (dd, 9.5, 3.1)	3.61 (dd, 9.3, 3)	5.44 (br d, 10)	5.45 (br d, 10)	3.83 (br d, 9.6)	4.81 (dd, 9.5, 2)	4.81 (dd, 9.7, 2)
18-Me	1.20 (s)	1.20 (s)	0.77 (s)	0.78 (s)	0.80 (s)	1.16 (s)	1.14 (s)	1.19 (s)	0.85 (s)	0.86 (s)
19-Me	1.06 (s)	1.06 (s)	0.97 (s)	1.00 (s)	1.03 (s)	1.05 (s)	0.93 (s)	1.04 (s)	1.00 (s)	1.02 (s)
21-Me	1.59 (s)	1.59 (s)	1.13 (s)	1.13 (s)	1.15 (s)	1.63 (s)	1.65 (s)	1.59 (s)	1.26 (s)	1.26 (s)
26-Me	1.43 (s)	1.39 (s)	1.38 (s)	1.38 (s)	1.41 (s)	1.39 (s)	1.38 (s)	1.43 (s)	1.41 (s)	1.40 (s)
27-Me	1.49 (s)	1.46 (s)	1.42 (s)	1.42 (s)	1.45 (s)	1.40 (s)	1.40 (s)	1.49 (s)	1.44 (s)	1.43 (s)
$>C(Me)_2$	-	-	1.29, 1.43 (each s)	1.29, 1.45 (each s)	1.32,1.48 (each s)	-	1.31, 1.55 (each s)	-	-	-
Ac	1.93, 1.94 (each s)	-	1.96, 2.07 (each s)	1.96, 2.11 (each s)	1.99, 2.01, 2.12 (each s)	1.94, 2.05 (each s)	1.94, 2.05 (each s)	1.93, 1.99, 2.03 (each s)	1.98, 2.10, 2.12 (each s)	1.98, 2.12, 2.14 (each s)

† Signals were assigned from a mixture of compounds **23** and **24**. ¶ Obscured by other signals.

chromatographic techniques, except for the compound **2** that separation from the mixture of **2** and **5** has been achieved only partially and separation of **23** and **24** from the mixture of these two compounds has not been attempted. Acetyl migration resulted in an additional separation step of the isomeric acetates, it was nevertheless a useful preparation method of 3-acetate derivatives of ecdysteroids. It is obvious that in order to synthesize a 3-acetate derivative of an ecdysteroid, the C-2 hydroxyl group has to be suitably protected. To our knowledge, there was no report for the preparation of such protecting group.

3. Selective acetylation *via* protecting group(s) is useful especially in the partial synthesis of a particular acetate derivative of 20-ECD (**1**). In case a number of acetate derivatives are needed and purification of a complex mixture of the products is not a problem, the reported partial acetylation and/or deacetylation of **1** and/or acetate derivatives of **1**[12] may then be a better choice. However, the latter two methods have some limitations and only some certain acetates are obtained.

4. 20-ECD 2,3-acetonide (**20**) is a versatile intermediate in the partial synthesis of the 22-acetate (**6**) and 22,25-diacetate (**28**), including other ester derivatives of **1** and the acetonide protecting group which survives under basic condition can easily be removed under mild acidic condition. The 2,3-acetonide protecting group in **1** and other ecdysteroids allows modifications of the side chains to be possible. The protecting group in its isomeric 20,22-acetonide **15**, is, on the other hand, more difficult to be hydrolyzed. Lewis acids (e.g. $ZnCl_2$) could be used to facilitate the 20,22-acetonide deprotection, but care must be exercised for an ecdysteroid bearing a 25-acetoxyl group since minor side products frequently accompanied the required product and this led to a decrease in the yield of the latter.

EXPERIMENTAL

Melting points were determined with an Electrothermal melting point apparatus and were uncorrected. IR spectra were recorded in KBr on a Jasco IR-700 spectrophotometer. ^1H NMR spectra were recorded on a Bruker AM300 spectrometer operating at 300 and 75.5 MHz, respectively. EIMS were measured on a Hewlett Packard 5896 instrument operating at 70 eV. The microanalyses were performed by the Elemental Analysis Unit, Department of Chemistry, Faculty of Science, Silpakorn University and the Department of Chemistry, Faculty of Science, Mahidol University. Column chromatography and TLC were carried out using Merck's silica gel 60 (>230 mesh) and precoated silica gel 60 F_{254} plates, respectively. Spots on TLC were visualized under UV light and by spraying with anisaldehyde-H_2SO_4 reagent followed by heating. 20-Hydroxyecdysone (**1**) used throughout the experiments was isolated from the bark of *Vitex glabrata*.[33]

20-ECD 2,3:20,22-diacetonide (12).Compound **1** (80 mg, 0.167 mmol) was dissolved in MeOH (1 ml) and dry acetone (15 ml, excess) added. Phosphomolybdic acid (8 mg, *ca* 0.004 mmol) was added and the reaction mixture stirred for 3 h. The mixture was neutralized with 1% $NaHCO_3$ and the product extracted with $CHCl_3$. The organic phase was washed with H_2O, dried over anhydrous Na_2SO_4, evaporated under reduced pressure and chromatographed ($CHCl_3$-MeOH = 99:1) to give **12** (61 mg, 65%), mp 233-234 °C from acetone-hexane (lit.[12] 234-236 °C); IR : ν_{max} 3404, 2966, 1640, 1444, 1378, 1315, 1243, 1224, 1151, 1062, 951, 753 cm^{-1}; ^1H NMR data is given in Table; EIMS : m/z (% rel. intensity) 545 $[M-CH_3]^+$ (4), 527(3), 509 (2), 467(7), 403(7), 143(20), 125(28). Anal. Calcd for $C_{33}H_{52}O_7 \cdot 1/2H_2O$: C, 69.59; H, 9.31. Found: C, 69.42; H, 9.26.

20-ECD 20,22-acetonide (15). Acetone (1 ml, excess) and *p*-TsOH (monohydrate, 52 mg, 0.273 mmol) were added to a solution of **1** (960 mg, 2 mmol) in MeOH (8 ml) and the mixture stirred for 1 h, during

which time the progress of the reaction was monitored by TLC. The reaction mixture was worked up in the same manner as described for the preparation of **12**, except that EtOAc has been used in place of $CHCl_3$ and the crude products chromatographed using $CHCl_3$ and $CHCl_3$-MeOH as eluting solvents, with a gradual increase in the concn of more polar component to yield **12** (20 mg, 2%) and **15** (962 mg, 93%).

15 : mp 222-224 °C (lit.[12] 222.5-223.5 °C); IR : ν_{max} 3420, 2974, 1649, 1454, 1377, 1216, 1170, 1103, 1057, 1001, 877 cm^{-1}; ^1H NMR data is given in Table; EIMS : m/z (% rel. intensity) 487 $[M-CH_3-H_2O]^+$ (0.4), 469(0.5), 363(5), 143(17), 125(25). Anal. Calcd for $C_{30}H_{48}O_7$: C, 69.23; H, 9.23. Found : C, 69.42; H, 9.26.

20-ECD 2,3-acetonide (20). Acetone (10 ml, excess) and pyridinium *p*-toluenesulphonate (8 mg, 0.032 mmol) were added to a solution of **1** (90 mg, 0.187 mmol) in MeOH (1 ml) and the mixture stirred for 3 h. The reaction mixture was worked up as described for the preparation of **15** and the crude products separated by column chromatography, using $CHCl_3$-MeOH (99:1 to 93:7) as eluting solvent to afford **12** (<1mg), **20** (22 mg, 23%) and **15** (37 mg, 38%).

20 : mp 269-271 °C (lit.[29] 232-233 °C); IR : ν_{max} 3458, 2966, 1641, 1449, 1379, 1243, 1224, 1150, 1057 cm^{-1}; ^1H NMR data is given in Table; EIMS : m/z (% rel. intensity) 466 $[M-3H_2O]^+$ (2), 403(16), 386(21), 385(25), 269(17), 161(14), 117(5), 99(100). Anal. Calcd for $C_{30}H_{48}O_7 \cdot 3/2H_2O$: C, 65.81; H, 9.32. Found: C, 66.13; H, 9.61.

Viticosterone E 2,3:20,22-diacetonide (13). A mixture of the diacetonide **12** (300 mg, 0.535 mmol), pyridine (2.5 ml) and Ac_2O (2 ml, 21.176 mmol) was stirred for 3 weeks. H_2O (200 ml) was added to the mixture and the product extracted with $CHCl_3$ (2x100 ml); the organic phase washed with H_2O, dried, evaporated and chromatographed ($CHCl_3$-MeOH = 99:1) to afford **13** (321 mg, quantitative), mp 199-201 °C (from $CHCl_3$-hexane); IR : ν_{max} 3474, 2976, 1735, 1662, 1452, 1370, 1246, 1215, 1167, 1108, 1057 cm^{-1}; ^1H NMR data is given in Table; EIMS : m/z (% rel. intensity) 527 $[M-CH_3-AcOH]^+$ (0.4), 484 (0.5), 404(1), 403 (4), 385(0.4), 368(2), 201(1), 159(2). Anal. Calcd for $C_{35}H_{54}O_8$: C, 69.73; H, 9.02. Found : C, 69.35; H, 9.05.

The acetylation rate could be accelerated by keeping the reaction temperature at 40-42 °C for 4-5 days, but the yield tended to decrease considerably.

Viticosterone E (3). **13** (45 mg, 0.074 mmol) in 70% AcOH (1 ml) was stirred for 3/2 h. $ZnCl_2$ (15 mg, 0.109 mmol) was then added and the mixture stirred for another 7 h. H_2O (150 ml) was added to the mixture; the product extracted with *n*-butanol (3x20 ml), the organic phase washed with H_2O and the solvent evaporated by co-distillation with H_2O under reduced pressure. Purification by column chromatography ($CHCl_3$-MeOH = 19 :1) gave **3** (13 mg, 33%); IR : ν_{max} 3428, 2950, 1725(sh), 1709, 1648, 1444, 1370, 1277, 1207, 1127, 1056, 1022, 950, 876 cm^{-1}; ^1H NMR data is given in Table; EIMS : m/z (% rel. intensity) 426[M-AcOH-$2H_2O$]$^+$(0.7), 363(27), 345(88), 327(36), 309(13), 203(2), 185(6), 159(8), 143(14), 141(2), 125(16), 117(3), 99(36), 81(39).

20-ECD 2-acetate 20,22-acetonide (16) and 20-ECD 2,3-diacetate 20,22-acetonide (17). Ac_2O (0.5 ml, 5.294 mmol) was slowly added to a solution of the acetonide **15** (327 mg, 0.628 mmol) in a mixture of pyridine (1 ml) and $CHCl_3$ (0.3 ml). The progress of the reaction was followed by TLC while the mixture was kept stirring for 4 h. The reaction was worked up and the products separated in the usual manner to afford **16** (303 mg, 86%) and **17** (26 mg, 7%).

16 : mp 233-235 °C (lit.[14] 206-210 °C); IR ν_{max} 3446, 2970, 1735(sh), 1710, 1649, 1445, 1375, 1253, 1205, 1154, 1106, 1046, 1000, 954 cm^{-1}; ^1H NMR data is given in Table; EIMS : m/z (% rel. intensity) 469 [M-CH_3-AcOH-H_2O]$^+$(1), 451(1), 405(10), 387(9), 345(15), 327(13), 159(4), 158(5), 157(4), 143(28). Anal. Calcd for $C_{32}H_{50}O_8 \cdot H_2O$: C, 66.20; H, 8.96. Found : C, 66.21; H, 9.15.

17 : mp 243-244 °C (form EtOAc-hexane); IR : ν_{max} 3448, 2964, 1735, 1659, 1449, 1372, 1317, 1247, 1170, 1145, 1104, 1083, 1043, 1000, 952, 927, 882 cm^{-1}; ^1H NMR data is given in Table; EIMS : m/z (% rel. intensity) 511 [M-CH_3-AcOH-H_2O]$^+$ (0.4), 477(4), 429(3), 387(1), 345(5), 327(5), 158(3). Anal. Calcd for $C_{34}H_{52}O_9 \cdot 1/2H_2O$: C, 66.55; H,8.64. Found : C, 66.43; H, 8.17.

A mixture of 20-ECD 2-acetate (2) and 20-ECD 3-acetate (5). A mixture of **16** (12 mg, 0.021 mmol), $ZnCl_2$ (10 mg, 0.073 mmol) in 70% AcOH (1 ml) was stirred for 5 h and the reaction mixture worked up and purified in the usual way to give a mixture of **2** and **5** (9 mg, 81%); IR : ν_{max} 3432, 2964, 1719, 1653, 1446, 1379, 1258, 1150, 1048 cm^{-1}; ^1H NMR data indicated a *ca* 3:1 mixture of **2** and 20-ECD 3-acetate (**5**). ^1H NMR data of these compounds are given in Table; EIMS : m/z (% rel. intensity) 486 [M-$2H_2O$]$^+$ (1), 471(1), 468(3), 450(1), 405(22), 388 (47), 387(65), 370(18), 369(4), 345(31), 327(66), 309(19).

Acetonide deprotection was repeated, but without using the Lewis acid, and similar result was obtained.

20-ECD 22-acetate 2,3-acetonide (21). A mixture of the acetonide **20** (120 mg, 0.230 mmol), pyridine (3 ml) and Ac_2O (1 ml, 10.588 mmol) was stirred for 6 h. Working up and purification of the product were carried out in similar manner as described in the above experiments to yield **21** (118 mg, 91%), mp 220-222 °C (from $CHCl_3$-hexane); IR : ν_{max} 3444, 2964, 1716, 1657, 1443, 1377, 1243, 1055 cm^{-1}; ^1H NMR data is given in Table; EIMS : m/z (% rel. intensity) 484 [M-AcOH-H_2O]$^+$ (0.8), 469(0.8), 466(2), 403(10), 385(9), 159(6). Anal. Calcd for $C_{32}H_{50}O_8$: C, 68.32; H,8.90. Found : C, 68.15; H, 9.35.

20-ECD 22-acetate (6). **21** (110 mg, 0.195 mmol) in 70% AcOH (5 ml) was stirred for 2 h and the product subjected to column chromatography ($CHCl_3$-MeOH = 91:9) to give **6** (95 mg, 93%), mp 146-148 °C (from EtOAc-hexane) (lit.[12] 145-147 °C); IR : ν_{max} 3428, 2962, 1714, 1651, 1444, 1377, 1253, 1052 cm^{-1}; ^1H NMR data is given in Table; EIMS : m/z (% rel. intensity) 486 [M-$2H_2O$]$^+$(0.4), 468(2), 426(13), 408(3), 363 (15), 345(15), 327(14), 309(4), 159(8).

20-ECD 2,22-diacetate (4). Compound **6** (60 mg, 0.115 mmol) was partially acetylated and worked up in similar manner as employed for the preparation of **16** and the products were separated by column chromatography ($CHCl_3$-MeOH = 24:1) to yield **4** (52 mg, 80%), together with 20-ECD 2,3,22-triacetate (**8**) (1 mg, 1%). Compound **8** was identified by direct comparison (TLC and IR) with authentic sample.[33]

4 : mp 160-162 °C (from EtOAc-hexane); IR : ν_{max} 3428, 2968, 1717, 1657, 1445, 1377, 1317, 1250, 1150, 1121, 1049, 948 cm^{-1}; EIMS : m/z (% rel. intensity) 486 [M-AcOH-H_2O]$^+$ (0.5), 471(1), 468(2), 453(3), 405(16), 388(10), 387(8), 345(25), 327(22), 309(8), 292(29), 291(11), 159(9). Anal. Calcd for $C_{31}H_{48}O_9 \cdot 2H_2O$: C, 62.00; H, 8.66. Found : C, 61.97; H, 8.29.

20-ECD 2,3-diacetate 20,22-acetonide (17). The reaction and working up were carried out in the same manner as employed for the preparation of **21** to give **17** (29 mg, 83%) from 30 mg (0.057 mmol) of **15**. This compound was identical (TLC, IR, ^1H NMR and EIMS) to the minor product **17** obtained from the foregoing synthesis.

20-ECD 2,3-diacetate (14). Compound **17** (20 mg, 0.033 mmol) was subjected to acetonide deprotection as described for the preparation of **2**. The product was purified by column chromatography to afford **14** (14 mg, 75%), mp 128-130 °C (lit.[12] 131-133 °C); IR : ν_{max} 3436, 2964, 1742, 1659, 1447, 1369, 1253,

1145, 1045 cm^{-1}; ^1H NMR data is given in Table; EIMS : m/z (% rel. intensity) 510 $[M-3H_2O]^+$ (2), 447(15), 430(44), 429(43), 412(14), 387(20), 345(15), 327(61), 309(19).

20-ECD 25-acetate 20,22-acetonide (22). 70% AcOH (2 ml) was added to a solution of viticosterone E 2,3:20,22-diacetonide (**13**) (139 mg, 0.231 mmol) in MeOH (0.5 ml) and the mixture stirred for 2 h during which time the progress of the reaction has been followed by TLC. The reaction mixture was worked up in the usual way to give **22** (121 mg, 93%), mp 206-208 °C (from EtOAc-hexane); IR : ν_{max} 3428, 2970, 1732, 1712, 1655, 1450, 1369, 1253, 1212, 1108, 1105 cm^{-1}; ^1H NMR data is given in Table; EIMS : m/z (% rel. intensity) 445 $[M+H-CH_3COCH_3]^+$ (1), 444(1), 427(1), 426(0.6), 409(2), 363(7), 201(2), 200 (3), 159(3), 101(10). Anal. Calcd for $C_{32}H_{50}O_8 \cdot H_2O$: C, 66.20; H,8.96. Found : C, 66.16; H, 8.84.

20-ECD 2,25-diacetate 20,22-acetonide (25) and **20-ECD 3,25-diacetate 20,22-acetonide (26).** **22** (82 mg, 0.146 mmol) was selectively acetylated in the same manner as described for the preparation of **16**. After the work up the crude products were subjected to column chromatography ($CHCl_3$-MeOH = 49 : 1) to afford 20-ECD 2,3,25-triacetate 20,22-acetonide (**27**) (5 mg, 3%) and a *ca* 4:1 mixture of **25** and **26** (73 mg, 83%). The two compounds gave almost completely overlapping spots on TLC.

A portion (47 mg) of the above mixture was subjected to column chromatography and the compound **26** (5 mg) was eluted first, followed by a mixture of **26** and **25** (9 mg) and finally the compound **25** (20 mg).

25 : IR : ν_{max} 3428, 2970, 1720, 1652, 1448, 1370, 1249, 1213, 1154, 1103, 1045, 1001, 874, 754 cm^{-1}; ^1H NMR data is given in Table; (EIMS : m/z (% rel.intensity) 469 $[M-2AcOH-CH_3]^+$(2), 405(5), 345(7), 343(13), 200(6), 183(10), 126(41), 125(60), 97(61), 82(100). Anal. Calcd for $C_{34}H_{52}O_9 \cdot H_2O$: C, 65.59; H, 8.68. Found : C, 65.27; H, 8.87).

26 : IR : ν_{max} 3428, 2924, 1741, 1640, 1452, 1370, 1246, 1211, 1026, 883 cm^{-1}; ^1H NMR data is given in Table; EIMS : m/z (% rel. intensity) 405 $[M+H-C_{11}H_{20}O_3]^+$ (1), 343(8), 185(10), 126(26), 125(52), 97(61), 82(100).

27: This minor product was identical to ECD 2,3,25-triacetate 20,22-acetonide (**27**) synthesized by a different method (see under the preparation of compound **27**).

A mixture of 20-ECD 2,25-diacetate (23) and 20-ECD 3,25-diacetate (24). The acetonide protecting groups in **25** and **26** mixture were removed in the same manner as employed for the preparation of **2** to yield a *ca* 7:3 mixture of the diacetates **23** and **24** (56 mg, 82%) from 61 mg of **25** and **26** (*ca* 4:1 mixture). IR : ν_{max} 3428, 1712-1725(br), 1654, 1448, 1369, 1250, 1127, 1046, 945, 876 cm^{-1}; ^1H NMR data is given in Table; EIMS : m/z (% rel. intensity) 405 $[M-C_8H_{15}O_3]^+$ (9), 388(16), 387(54), 345(16), 328(18), 327(78), 309 (20), 292 (20), 291(6), 285(20), 284(32), 270(16), 269(73), 203(4), 191(59), 187(11), 185(11), 161(18), 159 (12), 143(16), 126(54), 125(26).

20-ECD 22,25-diacetate 2,3-acetonide (29). A mixture of **20** (83 mg, 0.159 mmol), pyridine (2 ml) and Ac_2O (2 ml, 21.176 mmol) was stirred for 3 weeks. After working up the product was purified by column chromatography ($CHCl_3$-MeOH = 49 : 1) to give **29** (83 mg, 86%), mp 211-213 °C (from EtOAc-$CHCl_3$); IR : ν_{max} 3436, 2932, 1737, 1656, 1444, 1369, 1243, 1211, 1057 cm^{-1}; ^1H NMR data is given in Table; EIMS : m/z (% rel. intensity) 484 $[M-2AcOH]^+$ (0.4), 466(1), 448(1), 403(4), 385(6), 201(2), 159(3). Anal. Calcd for $C_{34}H_{52}O_9 \cdot 1/2H_2O$: C, 66.55; H, 8.64. Found : C, 66.92; H, 8.32.

An alternative preparation of **29** was to perform acetylation of **20** at 30-33 °C for 2 days followed by heating at 40-43 °C for another 2 days. The yield in this case decreased slightly.

20-ECD 22,25-diacetate (28). **29** (63 mg, 0.104 mmol) was subjected to acetonide deprotection and the product purified as described for the preparation of **6** to give **28** (53 mg, 90%); IR : ν_{max} 3428, 2938, 1717, 1652, 1445, 1371, 1253, 1213, 1145, 1052, 1023, 949, 877 cm^{-1}; ^1H NMR data is given in Table; EIMS : m/z (% rel. intensity) 486 [M-AcOH-H_2O]$^+$ (0.2), 468(1), 450(2), 408(6), 393(3), 363(13), 345(24), 327(18) , 309 (4), 201(3), 159(6). Anal. Calcd for $C_{31}H_{48}O_9 \cdot 3/2\ H_2O$: C, 62.94; H, 8.62. Found : C, 62.82; H, 8.24.

20-ECD 2,3,25-triacetate 20,22-acetonide (27). A mixture of **15** (20 mg, 0.038 mmol), pyridine (2 ml) and Ac_2O (1 ml, 10.588 mmol) was stirred at 30-32 °C for 20 h and at 48-52 °C for another 2 days. The reaction mixture was worked up and the product chromatographed ($CHCl_3$-MeOH = 99:1) to yield **27** (15 mg, 60%); IR : ν_{max} 3396, 2956, 1731(br), 1666, 1449, 1373, 1246(br), 1143, 1104, 1046, 929, 883 cm^{-1}; ^1H NMR data is given in Table; EIMS : m/z (% rel. intensity) 528 [M-AcOH-CH_3COCH_3]$^+$ (<1), 511(0.2), 447(1), 387(1), 327(2), 309 (1), 201(1), 159(1). Anal. Calcd for $C_{36}H_{54}O_{10}$: C, 66.87; H, 8.35. Found : C, 67.26; H, 8.01.

20-ECD 2,3,25-triacetate (30). 70% AcOH (3 ml) was added to a solution of **27** (10 mg, 0.015 mmol) in dioxane (0.5 ml) and the mixture stirred at 38-42 °C for 2 days. The reaction mixture was worked up and the product purified in normal fashion to give **30** (6 mg, 64%); IR : ν_{max} 3428, 2964, 1739, 1659, 1446, 1369, 1262, 1143, 1044, 947, 878 cm^{-1}; ^1H NMR data is given in Table; EIMS : m/z (% rel. intensity) 528 [M-AcOH-H_2O]$^+$ (0.6), 510(2), 492 (0.7), 447(19), 429(53), 412(8), 387(15), 369(3), 327(58), 159(5) , 99(40).

20-ECD 2,22,25-triacetate (31) and 20-ECD 3,22,25-triacetate (32). Compound **28** (98 mg, 0.174 mmol) was partially acetylated in similar manner to that employed for the preparation of **16** from **15**, except that the reaction was performed at 5-10 °C. The products were separated by column chromatography ($CHCl_3$-MeOH from 99:1 to 99:3) to afford 20-ECD 2,3,22,25-tetraacetate **9** (8 mg, 7%), 20-ECD 3,22,25-triacetate (**32**) (10 mg, 10%) and 20-ECD 2,22,25-triacetate **31** (50 mg, 48%).

31 : IR : ν_{max} 3474, 2964, 1732, 1659, 1446, 1369, 1250, 1149, 1046, 944, 876 cm^{-1}; ^1H NMR data is given in Table; EIMS : m/z (% rel. intensity) 468 [M-2AcOH-H_2O]$^+$ (1), 405(8), 387(11), 345(12), 327(27), 309(7), 292(54), 291(16), 201(7), 159(9). Anal. Calcd for $C_{33}H_{50}O_{10} \cdot 1/2H_2O$: C, 64.39; H,8.29 Found : C, 64.53; H, 7.90.

32 : IR : ν_{max} 3452, 2962, 1726, 1656, 1452, 1370, 1249, 1127, 1047, 1023, 947, 879 cm^{-1}; ^1H NMR data is given in Table; EIMS : m/z (% rel. intensity) 468 [M-2AcOH-H_2O]$^+$ (6), 450(5), 435(4), 426(1), 408(3), 390(1), 372(1), 343 (10), 201(17), 159(25).

9 : ^1H NMR data (Table) were identical to those of the reported 20-ECD 2,3,22,25-tetraacetate. [34]

Partial acetylation of 1. *Method 1*. Compound **1** was partially acetylated according to the literature procedure, [12] with some modification. To a solution of **1** (380 mg, 0.791 mmol) in pyridine (6 ml) and $CHCl_3$ (8 ml) was added Ac_2O (2 ml, 21.176 mmol) and the solution stirred for 25 min. More pyridine (2 ml), $CHCl_3$ (10 ml) and Ac_2O (1.6 ml,16.941 mmol) were successively added and stirring continued for 5 h. H_2O (200 ml) was added and the mixture extracted with EtOAc (100 ml) and then with *n*-BuOH (2x50 ml). The combined organic phases were washed with H_2O and evaporated to dryness to give a mixture of acetate derivatives of **1** (372 mg), which was chromatographed and eluted with $CHCl_3$ containing increasing amounts of MeOH to afford a number of acetylated 20-ECD, which were isolated and purified as follows.

1) **20-ECD 2,3,22-triacetate (8)**, eluted with $CHCl_3$-MeOH (97:3) was rechromatographed to give **8** (4 mg, 0.8 %). TLC and NMR data were identical to the triacetate **8** prepared by normal acetylation of **1**.[12,33]

2) **20-ECD 2,3-diacetate (14)**, eluted with $CHCl_3$-MeOH (24:1) and rechromatographed using the same eluent of similar proportion to afford **14** (21 mg, 5%). TLC, IR and ^1H NMR confirmed the identity of this compound with **14**, prepared previously by acetylation of ECD 20,22-acetonide (**15**) and subsequent hydrolysis of the protecting group.

3) **A mixture of 20-ECD 2,22-diacetate (4) and 20-ECD 3,22-diacetate (19)**, eluted with $CHCl_3$-MeOH (24:1) was rechromatographed to give 30 mg (7 %) of the product. TLC examination indicated that the isolated fraction gave a single, homogeneous spot. However, comparison of the ^1H NMR spectra of the product with that obtained from selective acetylation method previously described revealed that it was a *ca* 10:3 mixture of 20-ECD 2,22-diacetate (**4**) and 20-ECD 3,22-diacetate (**19**).

4) **A mixture of 20-ECD 2-acetate (2) and 20-ECD 3-acetate (5)**, eluted with $CHCl_3$-MeOH (24:1 to 19:1) was rechromatographed twice to give 116 mg (28%) of the product which was homogeneous on TLC. However, ^1H NMR indicated that it was a *ca* 3:1 mixture of 20-ECD 2-acetate (**2**) and 20-ECD 3-acetate (**5**).

5) **20-ECD 22-acetate (6)** was eluted with $CHCl_3$-MeOH (47:3 to 23:2) and was rechromatographed twice to give **6** (26 mg, 6%). TLC, IR and ^1H NMR revealed the identity of this compound with 20-ECD 22-acetate (**6**) prepared by the foregoing selective acetylation method.

6) **Recovered 20-ECD (1)** (14 mg) was finally eluted with $CHCl_3$-MeOH (9:1 to 8:1). The identity of the eluted compound was made by TLC and IR comparison with the starting material **1**.

Method 2. A solution of **1** (60 mg, 0.125 mmol) in pyridine (3 ml) was kept in an ice bath and a portion of Ac_2O (1 ml, 10.588 mmol) added. The mixture was kept stirring and the progress of the reaction monitored by TLC. After 1 h more Ac_2O (1 ml, 10.588 mmol) was added and stirring continued for another 1 h. Cold water (150 ml) was added to the mixture and the solution extracted with EtOAc (2x50 ml), then with *n*-butanol (2x30 ml). The organic phases were combined, washed with H_2O, dried over anhydrous Na_2SO_4 and the evaporated residue chromatographed ($CHCl_3$-MeOH from 99:1 to 9:1). Fractions corresponding to 20-ECD 2,3-diacetate (**14**) (1 mg), 20-ECD 2,22-diacetate (**4**) (4 mg), 20-ECD 22-acetate (**6**) (3 mg) and 20-ECD (**1**) (10 mg) were not purified further. Those corresponding to 20-ECD 2-acetate (**2**) and 20-ECD 3-acetate (**5**) (30 mg) were subjected to column chromatography ($CHCl_3$-MeOH = 93:7) and the eluates collected into 8 subgroups. The second to seventh subgroups which corresponded to **2** and **5** (total weight 19 mg) were separately subjected to ^1H NMR investigations.

Acknowledgements. This work was supported by the International Foundation for Science (IFS). We are indebted to Dr. L. T. Byrne, University of Western Australia for recording the NMR spectra. Partial support from the Network for the Chemistry of Biologically Important Natural Products is gratefully acknowledged.

REFERENCES

1. Lafont, R. *Insect Biochem.* **1991**, *21*, 697-721, and references cited therein.
2. Bergamasco, R.; Horn, D. H. S. The Biological Activities of Ecdysteroids and Analogues. In *Progress in Ecdysone Research*; Hoffmann, J. A. Ed.; Elsevier / North-Holland Biomedical Press : Amsterdam, **1980**; pp 299-324.

3. Lafont, R.; Horn, D. H. S. Phytoecdysteroids : Structures and Occurrence. In *Ecdysone-From Chemistry to Mode of Action* ; Koolman, J. Ed.; Georg Thieme : Stuttgart, **1989**; pp 39-64.
4. Camps, F. Plant Ecdysteroids and Their Interaction with Insects. In *Ecological Chemistry and Biochemistry of Plant Terpenoids* ; Harborne, J. B.; Tomas-Barberan, F. A. Eds.; Claredon Press: Oxford, **1991**; pp 331-376.
5. Rees, H. H. Zooecdysteroids : Structures and Occurrence. In *Ecdysone-From Chemistry to Mode of Action* ; Koolman, J. Ed.; Georg Thieme: Stuttgart, **1989**; pp 28-38.
6. Lafont, R.; Wilson, I. D. *The Ecdysone Handbook*; The Chromatographic Society : Nottingham. **1992.**
7. Modde, J.-F.; Lafont, R.; Hoffmann, J. A. *Int. J. Invert. Reprod. Devel.* **1984**, *7*, 161-183.
8. Garcia, M.; Girault, J.-P.; Lafont, R. *Int. J. Invert. Reprod. Devel.* **1986**, *9*, 43-58.
9. Suksamrarn, A.; Sommechai, C. *Phytochemistry* **1993**, *32*, 303-306.
10. Rimpler, H. *Tetrahedron Lett.* **1969**, 329-333.
11. Gilgan, M. W.; Burns, B. G. *Comp. Biochem. Physiol.* **1979**, *64A*, 125-131.
12. Galbraith, M. N.; Horn, D. H. S. *Aust. J. Chem.* **1969**, *22*, 1045-1057.
13. Davies, T. G.; Lockley, W. J. S.; Boid, R.; Rees, H. H.; Goodwin, T. W. *Biochem. J.* **1980**, *190*, 537-544.
14. Lloyd-Jones, J. G.; Rees, H. H.; Goodwin, T. W. *Phytochemistry* **1973**, *12*, 569-572.
15. Greenwood, D. R.; Dinan, L. N.; Rees, H. H. *Biochem. J.* **1984**, *217*, 783-789.
16. Zatsny, I. L.; Gorovits, M. B.; Abubakirov, N. K. *Khim. Prir. Soedin.* **1973**, 175-178.
17. Novosel'skaya, I. L.; Gorovits, M. B.; Abubakirov, N. K. *Khim. Prir. Soedin.* **1981,** 668-669.
18. Saatov, Z., Gorovits, M.B.; Abubakirov, N. K. *Khim. Prir. Soedin.* **1987**, 852-855.
19. Karlson, P.; Hoffmeister, H.; Hummel, H.; Hocks, P. ; Spiteller, G. *Chem. Ber.* **1965**, *98*, 2394-2402.
20. Suksamrarn, A.; Ganpinyo, P.; Sommechai, C. *Tetrahedron Lett.* **1994**, *35*, 4445-4448.
21. Tomas, J.; Camps, F.; Coll, J.; Mele, E.; Pascual, N. *Tetrahedron* **1992**, *48*, 9809-9818.
22. Nien, S.-L.; Hsu, H.-Y.; Ho, M.; Yo, Y. C. *Acta Chimica Sinica* **1978**, *36*, 137-141.
23. Rudel, D.; Bathori, M.; Gharbi, J.; Girault, J.-P.; Racz, I.; Melis, K.; Szendrei, K.; Lafont, R. *Planta Med.* **1992**, *58*, 358-364.
24. Isaac, R. E.; Rees, H. H.; Goodwin, T. W. *J. Chem. Soc., Chem. Commun.* **1981**, 594-595.
25. Buckmann, D.; Starnecker, G.; Tomaschko, K.-H.; Wilhelm, E.; Lafont, R.; Girault, J.-P. *J. Comp. Physiol.* **1986**, *B156*, 759-765.
26. Maroy, P.; Kaufmann, G.; Dubendorfer, A. *J. Insect Physiol.* **1988**, *34*, 633-637.
27. Girault, J.-P.; Bathori, M.; Varga, E.; Szendrei, K.; Lafont, R. *J. Nat. Prod.* **1990**, *53*, 279-293.
28. Saatov, Z.; Umarova, R. U.; Gorovits, M. B. ; Abubakirov, N. K. *Khim. Prir. Soedin.* **1990**, 480-483.
29. Baltaev, U. A.; Abubakirov, N. K. *Khim. Prir. Soedin.* **1987**, 681-684.
30. Saatov, Z.; Abdullaev, N. D. ; Gorovits, M. B. ; Abubakirov, N. K. *Khim. Prir. Soedin.* **1990**, 363-366.
31. Pis, J.; Budesinsky, M.; Vokac, K.; Laudova, V.; Harmatha, J. *Phytochemistry* **1994**, *37*, 707-711.
32. Miyashita, M.; Yoshikoshi, A. Grieco, P. A. *J. Org. Chem.* **1977**, *42*, 3772-3774.
33. Werawattanametin, K.; Podimuang, V.; Suksamrarn, A. *J. Nat. Prod.* **1986**, *49*, 365-366.
34. Bandara, B. M. R.; Jayasinghe, L.; Karunaratne, V.; Wannigama, G. P.; Bokel, M.; Kraus, W.; Sotheeswaran, S. *Phytochemistry* **1989**, *28*, 1073-1075.

(*Received in UK* 15 *June* 1995; *revised* 27 *July* 1995; *accepted* 4 *August* 1995)