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Base-Catalyzed Autoxidation of 20-Hydroxyecdysone: Synthesis of Calonysterone and 9,20-Dihydroxyecdysone*

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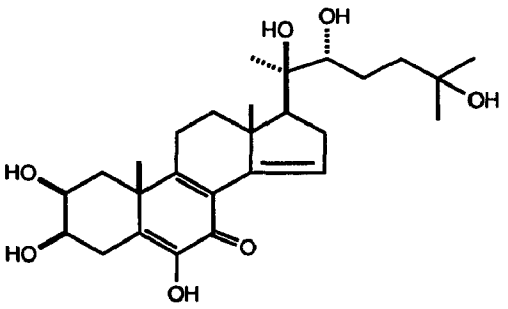
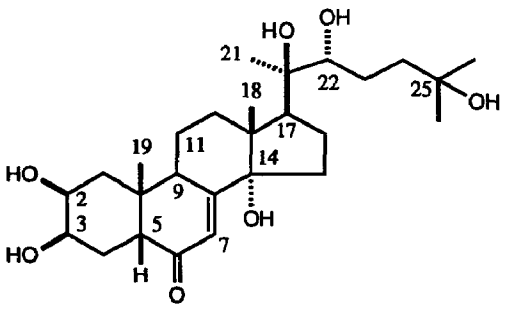
Abstract: Base-catalyzed autoxidation of 20-hydroxyecdysone (**2**) gave an unusually modified ecdysteroid calonysterone (**1**) and 9,20-dihydroxyecdysone (**4**).

In the course of an investigation of the minor ecdysteroids in *Vitex* plants, we isolated a small quantity (*ca* 1 mg) of calonysterone (**1**) from 4 kg of the bark of *Vitex glabrata*. This rare ecdysteroid was previously isolated as a minor constituent from *Ipomoea calonyction*[1] but there has been no report of its occurrence in other plant species since. In connection with structure-activity relationships, we wished to determine the moulting hormone activity of this unique, unusually modified ecdysteroid. However, we found that it was difficult to get sufficient **1** from either *V. glabrata* or other *Vitex* species. We therefore decided to study the possibility of synthesizing this compound from the readily available ecdysteroid, 20-hydroxyecdysone **2**.[2,3]



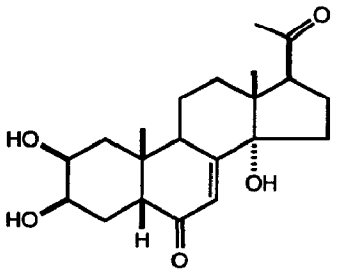
1



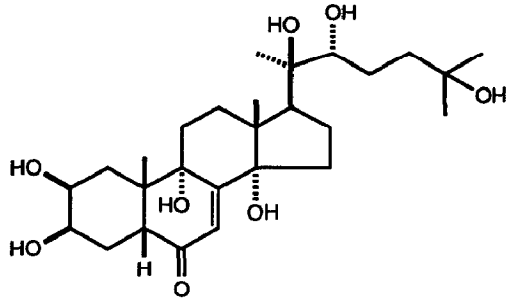
2

*Dedicated to Professor Jack R. Cannon for his unfailing efforts in the development of chemistry in developing countries.

In comparing the structure of **1** with that of **2**, it was obvious that introduction of an oxygen function in the B-ring of **2** followed by dehydration across C14-C15 should yield the compound **1**. As information concerning ring B oxidation of ecdysteroids was lacking, we investigated several possible methods. Preliminary results, however, indicated that under normal conditions common oxidizing agents (MnO_2, DDQ, SeO_2, $Pb(OAc)_4$, $KMnO_4$, CrO_3-pyridine, peracids, $(NH_4)_2Ce(NO_3)_6$, *etc.*) failed to yield the desired compound **1**. In most cases poststerone (**3**)[4] was identified as the product, indicating that oxidative cleavage at C20-C22 of the side chain was preferred.



3



4

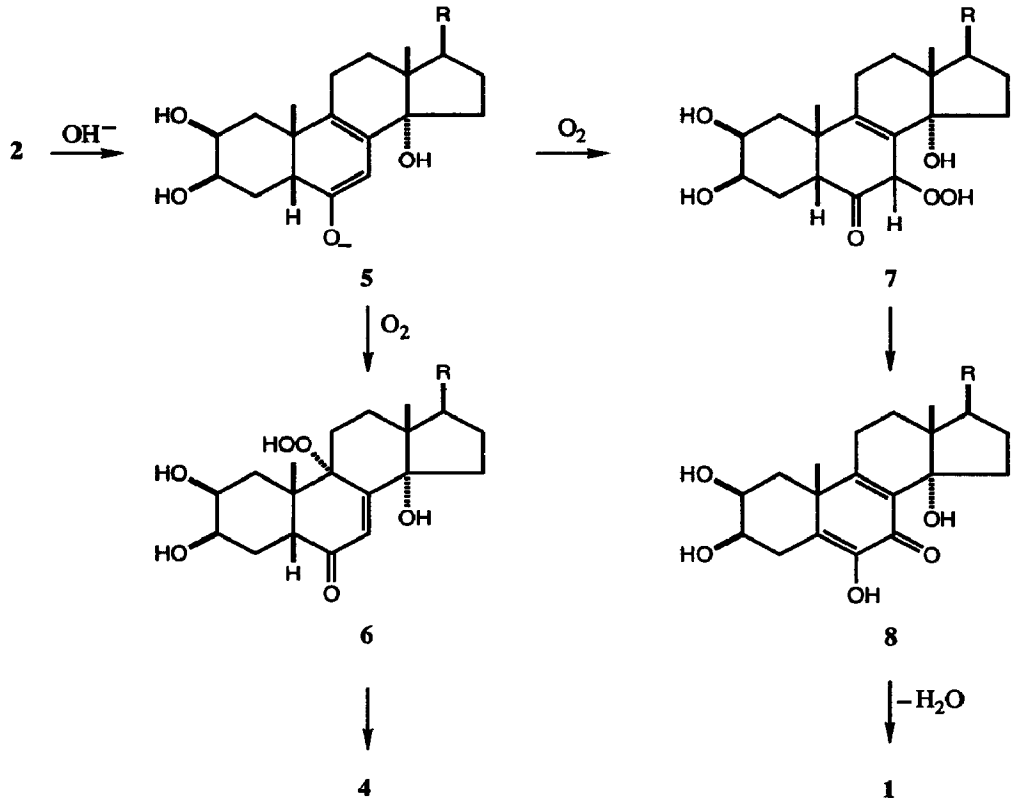
It is known that in certain cases molecular oxygen can oxidize unsaturated and / or allylic systems and the reactions can be catalyzed or accelerated by bases.[5] We then turned our interest to the base-catalyzed autoxidation of **2**. Thus a solution of **2** in 2% aqueous methanolic NaOH was stirred at ambient temperature (30-32 °C) for 2 h then the reaction mixture was worked up in the usual fashion. Column chromatography afforded **1** (35%) and 9,20-dihydroxyecdysone (**4**, 29%), based on the un-recovered starting material **2**. Prolonged reaction times increased the yields of **1** and **4**, but separation of the crude products became complicated duing to the presence of additional minor products. The rate of the reaction was also accelerated by bubbling O_2 into a basic solution of **2**, but, again, the products were accompanied by a number of minor compounds.

Spectroscopic (UV, IR, ^1H NMR and EIMS) data of compound **1** were identical with those reported for calonysterone.[1] The structure of **4** has been established as follows.[6] The IR absorption band of **4** at 1661 cm^{-1} indicated that an α,β-unsaturated keto function was still present. The quasi-molecular ion peak of **4** at m/z 497 in the ESMS spectrum indicated that base-catalyzed oxidation of **2** had resulted in the introduction of one hydroxyl group which should be located at position 9 from the ^1H NMR spectrum. Thus the C7 proton appeared as a sharp singlet, instead of a doublet (J = 2.5 Hz) in the case of **2**.[7] The absence of an H9 signal at *ca* 3.5 ppm and the relatively large downfield shifts of H3, H5 and 19-Me signals (*ca* 0.3, 0.6, and 0.5 ppm, respectively) as compared with those of **2** also supported structure **4**. The H17, 18-Me, 21-Me, 26-Me and 27-Me resonances were the same as those found in **2**, suggesting that the side chain and ring D of **4** remained intact.

Although the compound **4** has not yet been found in either a plant or animal, some ecdysteroids have been reported to possess C9 hydroxyl groups.[8] Base-catalyzed autoxidation could also prove useful for partial syntheses of these compounds.

A possible mechanism for the transformations 2→1 and 2→4 is proposed in Scheme. The initial step would involve abstraction of the C9 proton of **2** by the base to generate the anion of **2**, or its corresponding dienolate anion **5**. Reaction of **5** with O_2 would give rise to the hydroperoxide **6**, which would then be cleaved[5] to 9,20-dihydroxyecdysone (**4**). Alternatively, the anion **5** would attack O_2 across the 7-position to give the hydroperoxide **7**, which would then be transformed to the intermediate **8**. Subsequent dehydration of **8** would then give rise to calonysterone (**1**). The dehydration step could alternatively occur at an earlier stage.

Scheme



We then turned our attention to the reported extraction procedure of **1**.[1] The solvent system used in this case was $CHCl_3$-MeOH-NH_3 (9:0.9:0.1). We repeated our oxidation by replacing NaOH with excess ammonium hydroxide and the compounds **1** and **4** were obtained in 3 and 65% yields respectively, based on the un-recovered starting material **2**. It should be noted that by decreasing the strength of the base, a decrease in the yield of **1** resulted. This was nevertheless compensated for by an increase in the yield of **4**. However, this experiment did not provide the evidence that **1** was an artifact arising from **2** during the extraction.[1] If **1** were derived from **2**, the compound **4** should be isolated as well. The compound **1** could also be one of the secondary metabolites of *I. calonyction* as well as *V. glabrata*, or an air-oxidation product of **2** before the plant

materials were subjected to extraction. Our finding indicates that extraction of ecdysteroids under basic condition should be avoided.

9,20-Dihydroxyecdysone (4) showed extremely low moulting hormone activity in the *Musca domestica* bioassay, based on the activity of the ecdysteroid 2. The result suggested that the C9 hydroxyl substituent prevented the ecdysteroid 4 from binding efficiently to the receptor.[9] Moreover, calonysterone (1) exhibited no activity in this bioassay. Clearly the negative bioassay result for 1 was due to lack of a 7-en-6-one system in ring B, an A/B *cis*-ring junction and a 14α-hydroxyl group which are essential for an ecdysteroid to exhibit moulting hormone activity.[9]

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6. The compound **4** was obtained crystalline, mp 269-272 °C (MeOH-$CHCl_3$); UV λ_{max}^{MeOH} nm (log ε) 228 (3.87); IR ν_{max}^{KBr} cm^{-1}: 3460, 3380(OH), 1661 (conj.C=O); ^1H NMR (d_5-Pyridine): δ 1.20 (s, 3H, 18-Me), 1.39 (s, 2x3H, 26-Me and 27-Me), 1.56 and 1.58 (each s, 2x3H, 19-Me, 21-Me), 2.96 (t, J = 8.5 Hz, 1H, H17), 3.63 (dd, J = 11.7, 4.0 Hz, 1H, H5), 3.88 (brd, J = 8.3 Hz, 1H, H22), 4.01(m, 1H, H2), 4.52 (m, 1H, H3), 6.18 (s, 1H, H7); ESMS m/z (%rel. intensity): 497 $[M+H]^+$(12), 479 $[M+H-H_2O]^+$ (37), 443 $[M+H-3H_2O]^+$(61), 425 $[M+H-4H_2O]^+$(7). Anal. Calcd For $C_{27}H_{44}O_8$: C, 65.30 ; H, 8.93. Found : C, 65.09; H, 8.95.
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