PREPARATION OF 3-EPI-ECDYSONE AND 3-EPI-20-HYDROXYECDYSONE

Laurence Dinan and Huw H. Rees

Department of Biochemistry, University of Liverpool,

P.O. Box 147, Liverpool, L69 3BX, U.K.

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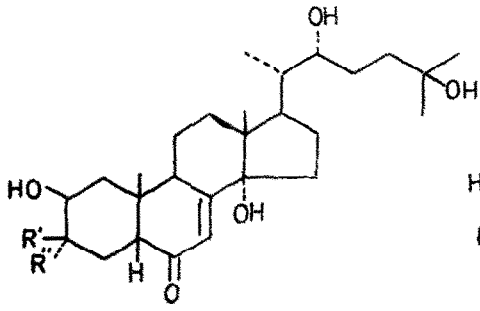
ABSTRACT

3-Dehydro-ecdysone and 3-dehydro-20-hydroxyecdysone were prepared and characterized. Reduction of these compounds with $NaBH_4$ gave 3-epi-ecdysone and 3-epi-20-hydroxyecdysone, which were characterized fully by mass and p.m.r. spectrometry as well as by derivative formation.

INTRODUCTION

Investigation of the hormonal control of insect development frequently necessitates the availability of reasonable quantities of some of the nine ecdysteroids which have been either isolated from, or detected as metabolic products in insects (1). Both enzymic and chemical methods (2-4) have been reported for synthesis of 3-dehydro-ecdysone (I) and 3-dehydro-20-hydroxyecdysone (II). We now report the preparation of 3-epi-ecdysone (III) and 3-epi-20-hydroxyecdysone (IV). The latter compound was originally isolated from meconium of Manduca sexta (5), whereas the former was characterized originally as a metabolic product of ecdysone in an in vitro system from midgut of this species (6).

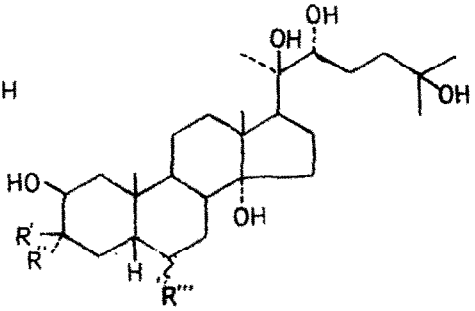
In the present work, ecdysone (V) and 20-hydroxyecdysone (VI) were oxidized using the method of Spindler et al. (4) to their 3-dehydro-derivatives, which were then reduced with $NaBH_4$. There are eight plausible products (VIII - XV) of this reduction reaction for each 3-dehydroecdysteroid. The reduction was carried out initially on a small



I $R',R''= =O$

III $R'=H, R''=OH$

V $R'=OH, R''=H$

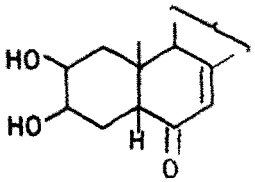


II $R',R''= =O, R'''= =O$

IV $R'=H, R''=OH, R'''= =O$

VI $R'=OH, R''=H, R'''= =O$

VII $R'=OH, R''=H, R'''=$ 



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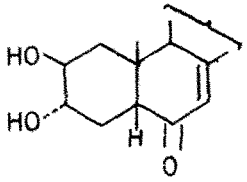

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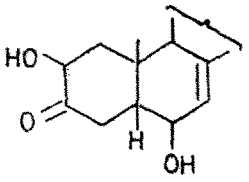

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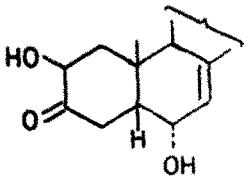

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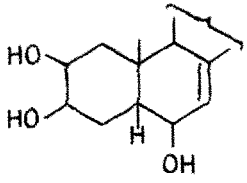

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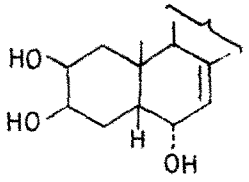

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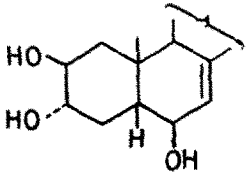

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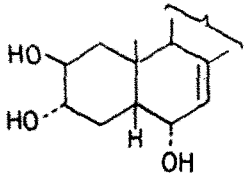

XV

scale on 3-dehydro-20-hydroxyecdysone (II) and the progress of the reaction was monitored by t.l.c. After 5 min. the reaction was essentially complete with no detectable starting material remaining. Two close u.v.-absorbing bands of greater polarity than the starting compound were present, the lower of which co-chromatographed with 20-hydroxyecdysone. Based on their u.v. absorption and position on t.l.c. (5), these two products are probably 20-hydroxyecdysone (VI), and 3-epi-20-hydroxyecdysone (IV), which are the only u.v.-absorbing compounds of the eight possible reaction products. When the t.l.c. plate was sprayed with berberine sulphate and viewed under u.v. light, there were no other perceptible non-u.v. absorbing compounds on the plate. However, such non-u.v. absorbing materials could be present at the same R_f as 20-hydroxyecdysone (VI) and/or 3-epi-20-hydroxyecdysone (IV) and be masked by the u.v. absorption of these compounds.

Any possible 6-hydroxy-ecdysteroid contaminants in the products of the reduction reaction were removed by reoxidation of the allylic 6-hydroxy group to a 6-oxo function using dichlorodicyanobenzoquinone (DDQ). As a model system, the 6-oxo group of 20-hydroxyecdysone (VI) was first reduced with $NaBH_4$ to give the 6-hydroxy derivative, which was used to ascertain the conditions necessary for oxidation of the 6-hydroxy group using DDQ.

EXPERIMENTAL

Analytical methods - P.m.r. spectra were determined at 220 MHz by the Physico-Chemical Measurements Unit (P.C.M.U.), Harwell, Berks, U.K. with a Varian HR220 spectrometer. Low resolution mass spectra were recorded on an AEI MS12 spectrometer whereas accurate mass measurements were carried out on an AEI MS902 spectrometer.

Thin-layer chromatography - Preparative t.l.c. was carried out on 0.5mm thick Kieselgel GF_{254} (E. Merck A.-G., Darmstadt, Germany) plates,

which had been pre-washed by developing once in methanol and then dried overnight at room temperature before use. Chromatograms were developed as specified in the text, after which the ecdysteroids were visualized under u.v. light and eluted well with methanol/dichloromethane (1/1, v/v).

Acetate and acetonide derivatives - Acetonide and 2-acetoxy derivatives of ecdysteroids were prepared by the methods of Galbraith and Horn (7).

5β-Cholest-7-en-2β,3β,6ξ,14α,20R,22R,25-heptol (VII) - 20-Hydroxyecdysone (5.5mg) was dissolved in dry redistilled ethanol/tetrahydrofuran (1/1, v/v) and after the addition of $NaBH_4$ (1 mg) the mixture was refluxed for 1-3/4 hr. The mixture was allowed to cool, the reaction stopped by the addition of one drop of glacial acetic acid and subjected directly to t.l.c. with chloroform/methanol (7/3, v/v) for development. There were no bands visible under u.v. light, but on spraying with berberine sulphate in acetone/methanol (1/1, v/v) and viewing under u.v. light a major band was perceptible. This band (R_f 0.32; cf. 20-hydroxyecdysone (VI) R_f 0.43, was eluted to give 4.5mg of (VII); m/e 464 (M^+-H_2O, 2%), 446 (3%), 431 (3%), 428 (10%), 413 (6%), 410 (5%), 395 (3%), 365 (7%), 348 (18%), 347 (70%), 346 (29%), 331 (28%), 330 (81%), 329 (89%), 313 (20%), 312 (48%), 311 (51%), 287 (22%), 161 (15%), 143 (39%), 99 (100%), 81 (67%).

20-Hydroxyecdysone (VI) - 5β-Cholest-7-en-2β,3β,6ξ,14α,20R,22R,25-heptol (VII; 4mg) dissolved in dry dioxan (0.3 ml) was treated with DDQ at room temperature and the progress of the reaction followed periodically by t.l.c. The reaction was essentially complete after 16 hr. and the mixture was subjected directly to t.l.c. with chloroform/methanol (7/3, v/v) for development. A single product (u.v. absorbing), which co-chromatographed with 20-hydroxyecdysone (VI) was obtained (3.9 mg); m/e 480 (<1%), 462 (<1%), 444 (1%), 429 (3%), 426 (12%), 411 (2%), 408 (3%), 393 (1%), 363 (7%), 346 (11%), 345 (30%), 344 (26%), 328 (17%), 327 (19%), 300 (13%), 145 (8%), 143 (8%), 99 (100%), 81 (27%).

3-Dehydro-20-hydroxyecdysone (II) - Platinum IV oxide (145 mg) in glacial acetic acid (5 ml) was reduced with hydrogen and the reduced platinum formed was washed exhaustively with distilled water. 20-Hydroxyecdysone (VI; 115mg) was dissolved in warm distilled water (100 ml), the solution cooled to room temperature, and the reduced platinum was added. A gentle stream of oxygen was bubbled continuously through the reaction mixture, which was stirred at room temperature. The progress of the reaction was monitored periodically by t.l.c.; the best yield of 3-dehydro-20-hydroxyecdysone was obtained after a variable time period, usually in the region 3-6 hr (4). The reaction was stopped by addition of methanol (300 ml), the mixture centrifuged, and after removal of the supernatant the catalyst pellet was washed twice with methanol/dichloromethane (1/1, v/v). The combined supernatants were then evaporated to dryness under vacuum. The crude reaction products were dissolved in a minimum volume of methanol/dichloromethane, (1/1, v/v) and separated by t.l.c. with methanol/dichloromethane (1/4, v/v) for development. An u.v. absorbing band (R_f 0.44) was eluted to give

77.1mg of crude product, which was then recrystallized from acetone-light petroleum to give 3-dehydro-20-hydroxyecdysone (II; 53.5mg); u.v. λ_{max} in ethanol 242 nm; i.r. ν max (KBr) 1640 (α,β unsaturated C=0) and 1700 cm^{-1} (C=0); m/e 478 (< 1%), 460 (< 1%), 442 (1%), 427 (5%), 424 (11%), 409 (2%), 406 (3%), 361 (14%), 345 (24%), 343 (73%), 342 (27%), 327 (16%), 326 (27%), 325 (27%), 283 (20%), 269 (22%), 189 (18%), 161 (10%), 143 (24%), 99 (100%), 81 (73%); p.m.r. C_5D_5N) δ, 1.24 (s, C-18 methyl), 1.06 (s, C-19 methyl), 1.62 (s, C-21 methyl), and 1.38 (s, C-26/27 methyls) p.p.m.

The 3-dehydro-20-hydroxyecdysone (II) was further characterized by derivative formation as follows:

3-Dehydro-20-hydroxyecdysone 20,22-acetonide - A portion of the 3-dehydro-20-hydroxyecdysone (II) prepared above was transformed (7) into 3-dehydro-20-hydroxyecdysone 20,22-acetonide and the product purified by t.l.c. with chloroform/ethanol (9/1, v/v) for development; m/e 518 (< 1%), 503 (< 1%), 500 (1%), 485 (< 1%), 467 (< 1%), 361 (6%), 345 (7%), 343 (8%), 327 (4%), 325 (2%), 298 (11%), 201 (5%), 102 (59%), 99 (49%), 85 (30%), 81 (16%), 69 (22%), 59 (100%).

This 3-dehydro-20-hydroxyecdysone 20,22-acetonide sample was then transformed (7) into 2β-acetoxy-3-dehydro-20-hydroxyecdysone 20,22-acetonide; m/e 560.3344 (M^+, < 1%, $C_{32}H_{48}O_8$ requires m/e 560.3349), 545 (< 1%), 542.3238 (M^+-H_2O, < 1%, $C_{32}H_{46}O_7$ requires m/e 542.3244), 527 (< 1%), 509 (1%), 467 (9%), 449 (8%), 403.2120 ($M^+-[C_{22}-C_{27}]$, 7%, $C_{23}H_{31}O_6$ requires m/e 403.2121), 393 (10%), 386 (12%), 385 (17%), 369 (9%), 343 (13%), 325 (13%), 201.1490 (9%, $C_{11}H_{21}O_3$ requires m/e 201.1491), 143 (27%), 125 (24%), 102 (76%), 99 (86%), 81 (22%), 59 (100%); p.m.r. ($CDCl_3$) δ, 0.79 (s, C-18 methyl) 1.10 (s, C-19 methyl), 1.19 (s, C-21 methyl), 1.25 (s, C-26/27 methyls), 1.37 (d, J=19Hz, acetonide methyls), 2.16 (s, AcO), 3.64 (m, C-22H), and 5.90 (C-7H).

A separate sample of 2β-acetoxy-3-dehydro-20-hydroxyecdysone 20,22-acetonide was prepared by oxidation of 2β-acetoxy-20-hydroxyecdysone 20,22-acetonide, which was synthesized as described previously (8). 2β-Acetoxy-20-hydroxyecdysone 20,22-acetonide (18mg) was dissolved in acetic anhydride (250ul) and dimethyl sulphoxide (500ul) and left for 18 hr at room temperature. The reaction mixture was then subjected directly to t.l.c. with chloroform/ethanol (9/1, v/v) for development and the 2β-acetoxy-3-dehydro-20-hydroxyecdysone 20,22-acetonide was eluted. This sample of 2β-acetoxy-3-dehydro-20-hydroxyecdysone 20,22-acetonide and the material prepared from 3-dehydro-20-hydroxyecdysone were indistinguishable on the basis of their thin-layer chromatographic, mass spectral and p.m.r. properties.

3-Dehydro-ecdysone (I) - Oxidation of ecdysone (V; 120mg) by the method used to prepare 3-dehydro-20-hydroxyecdysone (II) gave 58.8mg of 3-dehydro-ecdysone (I) after recrystallization from acetone-light petroleum; u.v. λ_{max} in ethanol 242 nm; i.r. ν max (KBr) 1660 (α,β unsaturated C=0) and 1720 cm^{-1} (C=0); m/e 462 (<1%), 444 (3%), 426 (7%), 411 (4%), 346 (3%), 328 (18%), 298 (10%), 248 (18%), 126 (20%), 117 (12%), 99 (100%),

81 (71%); p.m.r. (C_5D_5N) δ, 0.74 (s, C-18 methyl), 1.06 (s, C-19 methyl), 1.32 (d, J = 6.5 Hz, C-21 methyl) and 1.40 (s, C-26/27 methyls) p.p.m.

3-Epi-20-hydroxyecdysone (IV) - 3-Dehydro-20-hydroxyecdysone (II; 30mg) in 2 ml dry ethanol/tetrahydrofuran (1/1, v/v) was treated with $NaBH_4$ (10mg) and the mixture left at room temperature for 10 min. The reaction was then stopped by addition of one drop of glacial acetic acid and the mixture was subjected directly to t.l.c. with chloroform/methanol (4/1, v/v) for development. This system does not separate 20-hydroxyecdysone (VI) from the corresponding 3-epimer (IV), but does separate these reaction products from 3-dehydro-20-hydroxyecdysone (II). The combined region corresponding to compounds IV and VI was then eluted and the solvent was removed. To the residue (28.1mg) dissolved in dry dioxan (0.5 ml), DDQ (10mg) was added and the mixture left at room temperature for 16 hr. The reaction mixture was then applied directly to t.l.c. plates, which were developed by multiple elution (4 times) in chloroform/methanol (90/15, v/v). Two close u.v.-absorbing bands were discernible (R_f 0.25 and 0.33) the most polar co-chromatographing with authentic 20-hydroxyecdysone (VI). The two bands were eluted separately and cross contamination was shown to be less than 5% by t.l.c. of a small portion of each band.

The upper band (15.5mg) had properties expected (5) for 3-epi-20-hydroxyecdysone (IV); 462 (M^+-H_2O, < 1%), 444 (1%), 426.2789 (M^+-3H_2O, 16%, $C_{27}H_{38}O_5$ requires m/e 426.2770), 411 (10%), 408 (6%), 393 (4%), 375 (2%), 363 (4%), 352 (10%), 345 (45%), 344 (17%), 329 (17%), 328 (38%), 327 (48%), 313 (11%), 311 (10%), 310 (14%), 309 (17%), 301 (20%), 300 (33%), 285 (31%), 269 (21%), 267 (21%), 173 (26%), 99 (100%), 81 (62%); p.m.r. (C_5D_5N) δ, 1.06 (s, C-19 methyl), 1.22 (s, C-18 methyl), 1.57 (s, C-21 methyl), 1.39 (s, C-26/27 methyls).

When a portion of the 3-epi-20-hydroxyecdysone (IV) was treated under conditions (7) which result in the transformation of 20-hydroxyecdysone (VI) into the corresponding diacetonide derivative, only the 20,22-monoacetonide derivative was detectable. This was purified by t.l.c. using chloroform/methanol (90/15, v/v) for development (R_f 0.12; cf. 20-hydroxyecdysone 20,22-acetonide, R_f 0.09); m/e 505 (M^+-CH_3; < 1%), 502 (2%), 487 (1%), 484 (1%), 469 (1%), 462 (< 1%), 444 (1%), 426 (7%), 363 (22%), 347 (6%), 345 (29%), 329 (8%), 327 (7%), 301 (20%), 300 (69%), 201 (7%), 125 (16%), 99 (100%), 81 (23%), 59 (100%), 43 (100%).

The lower band (7 mg) had properties expected for 20-hydroxyecdysone (VI); m/e 480 (< 1%), 464 (< 1%), 444 (3%), 429 (4%), 426 (14%), 411 (6%), 408 (4%), 363 (35%), 347 (10%) 346 (35%), 345 (77%), 344 (20%), 329 (14%), 328 (29%), 327 (36%), 310 (10%), 301 (14%), 300 (16%), 285 (13%), 143 (23%), 99 (100%), 81 (64%). This compound was transformed into 20-hydroxyecdysone 2,3-20,22-diacetonide (R_f 0.74 on t.l.c. with chloroform/methanol (90/15, v/v) for development); m/e 560 (1%), 545 (2%), 527 (2%), 484 (1%), 403 (20%), 385 (10%), 341 (12%), 340 (5%), 201 (5%), 149 (26%), 125 (15%), 102 (23%), 99 (30%), 85 (23%), 83 (25%), 81 (25%), 71 (60%), 69 (45%), 59 (53%), 43 (100%).

3-Epi-ecdysone (III) - Reduction of 3-dehydro-ecdysone (I; 58mg) with $NaBH_4$ was carried out in an analogous manner to the reduction of 3-dehydro-20-hydroxyecdysone (II), and yielded 36.3 mg of a mixture of putative 3-epi-ecdysone (III) and ecdysone (V). This mixture was then subjected to the DDQ treatment as before and the ecdysone and 3-epi-ecdysone were separated by t.l.c.

The less polar band (R_f 0.41; cf. ecdysone, R_f 0.34) had properties expected (6) for 3-epi-ecdysone (III); m/e 446.3042 (M^+-H_2O, 1% $C_{27}H_{42}O_5$ requires m/e 446.3032), 431 (3%), 428 (16%), 413 (8%), 410 (4%), 395 (4%), 348 (14%), 330 (30%), 315 (14%), 301 (14%), 300 (30%), 285 (13%), 279 (26%), 250 (29%), 249 (18%), 173 (13%), 161 (13%), 145 (10%), 126 (16%), 121 (14%), 117 (14%), 109 (17%), 107 (10%), 105 (13%), 99 (100%), 81 (77%); p.m.r. (C_5D_5N) δ, 0.72 (s, C-18 methyl), 1.06 (s, C-19 methyl), 1.31 (d, J = 6Hz, C-21 methyl), and 1.39 (s, C-26/27 methyls) p.p.m. The 3-epi-ecdysone (III) did not form an acetonide derivative.

The lower band (9.8 mg) from t.l.c., co-chromatographing with ecdysone (V), had properties expected for that compound; m/e 446 (2%), 431 (2%), 428 (9%), 413 (2%), 410 (2%), 348 (8%), 330 (10%), 300 (24%), 250 (11%), 249 (10%) 99 (100%), 81 (70%). This compound was transformed into ecdysone 2,3-acetonide; m/e 504 (4%), 489 (5%), 486 (6%), 471 (7%), 468 (8%), 453 (3%), 410 (10%), 388 (20%), 370 (32%), 341 (16%), 340 (22%), 319 (12%), 290 (12%), 173 (18%), 161 (12%), 99 (100%), 81 (100%).

RESULTS AND DISCUSSION

Oxidation of ecdysone and 20-hydroxyecdysone with platinum as catalyst by the method of Spindler et al (4) gave the corresponding 3-oxo compounds. The small downfield shift (0.03 p.p.m.) in the p.m.r. signal of the C-19 methyl group in both oxo-compounds as compared to the corresponding 3β-hydroxy-ecdysteroids indicates that the extra oxo group is located at C-3 (3). This was corroborated by conversion of the 3-dehydro-20-hydroxyecdysone via 3-dehydro-20-hydroxyecdysone 20,22-acetonide into 2-acetoxy-3-dehydro-20-hydroxyecdysone 20,22-acetonide, which had indistinguishable t.l.c., mass spectral and p.m.r. properties from another sample of the latter compound prepared by the sequence : 20-hydroxyecdysone ⟶ 2-acetoxy-20-hydroxyecdysone ⟶ 2-acetoxy-20-hydroxyecdysone 20,22-acetonide ⟶ 2-acetoxy-3-dehydro-20-hydroxy -

ecdysone 20,22-acetonide.

Reduction of the α,β-unsaturated 6-oxo group of 20-hydroxyecdysone with $NaBH_4$ in ethanol/tetrahydrofuran (1/1, v/v) did not occur at room temperature even after a period of 3 days. This implies that very little, if any, 6-hydroxy contaminants would be produced during the reduction of 3-dehydro-ecdysteroids at room temperature for 10 min; refluxing for about 1 hr was necessary to reduce the 6-oxo group. The present observation that selective reduction occurs at C-3 during treatment of 3-dehydro-ecdysteroids in ethanol/tetrahydrofuran with $NaBH_4$ at room temperature is in general agreement with previous observations on the reduction of other steroids containing two oxo-groups, one of which is conjugated to a double bond (9, 10). In the case of 3-dehydro-ecdysteroids, steric considerations also probably markedly hinder reduction of the 6-oxo group.

Since 6,20-dihydroxyecdysone (VII) could be reoxidized quantitatively to 20-hydroxyecdysone upon treatment with DDQ for 16 hr at room temperature, these conditions were used for removing any conceivable 6-hydroxy contaminants from the mixed 3β- and 3α-hydroxy ecdysteroid preparation. After this treatment, there was no detectable 3-dehydro-ecdysone (I) or 3-dehydro-20-hydroxyecdysone (II), indicating that no selective reduction of the 6-oxo group had occurred.

Reduction of 3-dehydro-ecdysone (I) with $NaBH_4$ gave a mixture of ecdysone (V) and 3-epi-ecdysone (III). Similarly, reduction of 3-dehydro-20-hydroxyecdysone (II) gave a mixture of 20-hydroxyecdysone (VI) and 3-epi-20-hydroxyecdysone (IV). In agreement with published properties (5,6), the 3-epi-ecdysteroids were slightly less polar than the corresponding 3β-hydroxy compounds on t.l.c. (silica gel). Also

as expected, the 3-epi-ecdysone (III) and 3-epi-20-hydroxyecdysone (IV) had similar mass spectra and also practically the same methyl resonances in their p.m.r. spectra as the corresponding 3β-hydroxy-ecdysteroids (5,6).

Ecdysone (V) and 20-hydroxyecdysone (VI) produced by reduction of the corresponding 3-dehydro-ecdysteroids formed 2,3-monoacetonide and 2,3-20,22-diacetonide derivatives, respectively, upon treatment with acetone in the presence of phosphomolybdic acid. An ion at m/e 340 (M^+-side chain $-H_2O$) in the mass spectra of these acetonides indicates the presence of an acetonide group on the ecdysteroid nucleus. Furthermore, an ion at m/e 201 ($C_{11}H_{21}O_3$) is characteristic of a 20-hydroxyecdysone side chain bearing a 20,22-acetonide group. 3-Epi-ecdysone (III) did not form an acetonide derivative under the conditions used to prepare ecdysone 2,3-acetonide. However, the 3-epi-20-hydroxy-ecdysone formed a 20,22-monoacetonide derivative, which had slightly greater mobility than 20-hydroxyecdysone 20,22-acetonide on t.l.c. (silica gel). These t.l.c. properties would be expected by analogy with the chromatographic behaviour of 20-hydroxyecdysone (VI) and its 3-epi-derivative (IV). The occurrence of an ion at m/e 201 and the absence of an ion at m/e 340 in the mass spectrum of the monoacetonide derivative of 3-epi-20-hydroxyecdysone is consistent with the location of the acetonide grouping on the side chain.

In summary, 3-epi-ecdysone and 3-epi-20-hydroxyecdysone have been prepared from the corresponding 3-dehydroecdysteroids in 33% and 52% yield, respectively. During this reduction procedure, 6-hydroxy-ecdysteroids are probably not produced, so that treatment of crude products of the reaction with DDQ might not be essential. However, this

oxidation procedure removes any possible traces of 6-hydroxy-compounds.

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TRIVIAL AND I.U.P.A.C. NOMENCLATURE

Ecdysone = 2β,3β,14α 22R,25-pentahydroxy-5β-cholest-7-en-6-one
20-Hydroxyecdysone = 2β,3β, 14α,20R,22R,25-hexahydroxy-5β-cholest-7-en-6-one
3-Dehydro-ecdysone = 2β, 14α,22R,25-tetrahydroxy-5β-cholest-7-en-3, 6-dione
3-Dehydro-20-hydroxyecdysone = 2β, 14α,20R,22R,25-pentahydroxy-5β-cholest-7-en-3,6-dione
3-Epi-ecdysone = 2β,3α,14α,22R,25-pentahydroxy-5β-cholest-7-en-6-one
3-Epi-20-hydroxyecdysone = 2β,3α,14α,20R,22R,25-hexahydroxy-5β-cholest-7-en-6-one
6,20-Dihydroxyecdysone = 5β-cholest-7-en-2β,3β,6β,14α,20R,22R,25-heptol