THE SYNTHESIS OF 23,24-^{14}C-ECDYSONE

William B. Smith and George P. Newsoroff

Department of Chemistry

Texas Christian University

Fort Worth, Texas 76129

Received: 10/15/73

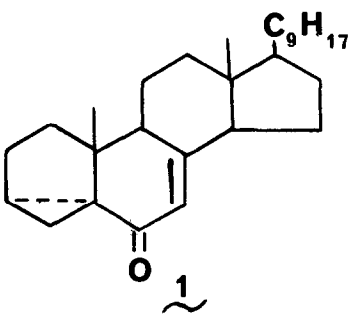
ABSTRACT

The synthesis of 23,24-^{14}C-ecdysone, using 3,4-^{14}C-2-methyl-3-butyn-2-yl tetrahydropyranyl ether, is described.

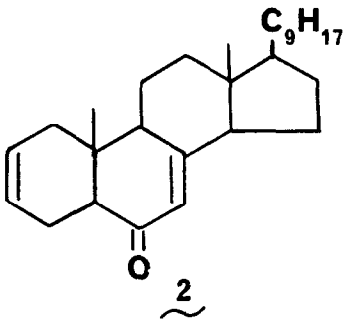
For certain contemplated biosynthesis studies it had appeared that carbon-14 labeled ecdysone (2β, 3β, 14α, 22R, 25-pentahydroxy-5β-cholest-7-en-6-one) would prove more utilitarian than the commercially available tritiated hormone. Towards this end, we have carried out a portion of a new synthesis of ecdysone (1) while concurrently investigating existing syntheses to accomplish our goal. We wish to report here some observations and improvements on two of these extant pathways.

A review of the literature (2) suggested that either the synthesis by Furlenmeier, et al. (3) or that by Barton, et al. (2b) could meet our purpose. Both start with readily available ergosterol, and both introduce the appropriate side chain late in the pathway with a small molecule convenient for tagging.

Initially and quite arbitrarily the Barton route was chosen. Ergosteryl tosylate was converted to 3α, 5α-cyclo-ergosta-7, 22-dien-6-one 1. It is reported (2b) that



1

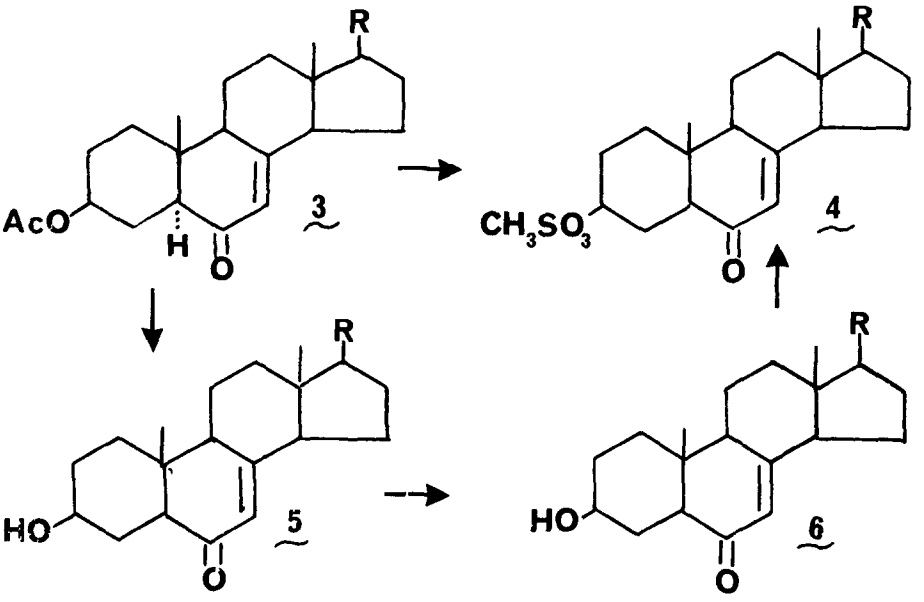


2

treatment of 1 with a small amount of p-toluenesulfonic acid in sulfolane at 160° gives a mixture of 14α and 14β-ergosta-2,7,22-trien-6-ones (2); while 1 treated with an equivalent of acid in refluxing benzene gives the appropriate 3β-tosylates which can be converted to an olefin mixture with lithium bromide in refluxing dimethyl formamide. In our hands, after repeated attempts, only extremely complex mixtures were formed by either ring opening procedure. The desired products were there in trivial amounts. Since NMR evidence suggested a much more deep seated rearrangement to be occurring, these reactions are under further investigation now.

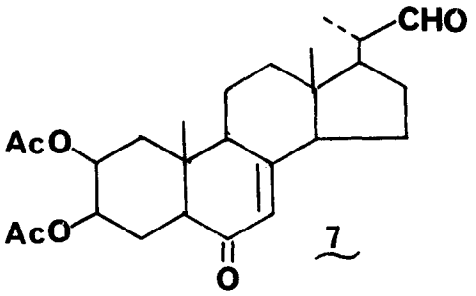
Turning to the Furlenmeier pathway (3), ergosteryl acetate was oxidized to the 5α-hydroxy-7-en-6-one structure. Reduction with zinc in acetic acid afforded a 3/1 mixture of the 5α and 5β isomers of 3β-acetoxyergosta-7,22-dien-6-one. While separable on column chromatography, the 5β isomer has not previously been reported as a product from this reduction.

Starting with the 5α isomer (3), Furlenmeier, et al. (3) converted this to the 3-mesylate (4) after a base hydrolysis. Their overall yield was 51%. In our experience the mild base hydrolysis caused a serious amount of deconjugation of the α,β-unsaturated ketone. Consequently, a new pathway from 3 to 4 was developed. Reduction of 3 with lithium aluminum hydride produced a diol 5. Oxidation of the allylic alcohol moiety with manganese dioxide gave 6 from which the mesylate



4 was formed. This route, while introducing more steps, avoided the deconjugation of the 7-en-6-one structure and increased the yield of 4 from 3 to 80%.

Cis-hydroxylation and ozonolysis of 4 gave the aldehyde 7 as per the literature results.



7

In both literature syntheses the side chain is derived from the tetrahydropyranyl (THP) ether of 2-methyl-3-butyn-2-ol. In the Barton synthesis (2b) the aldehyde 7 is hydroxylated in the 14α position then reacted with the lithium salt of the reagent. The presence of the 14α-hydroxyl is known to affect adversely the stereochemistry of the addition to the carbonyl group. Consequently, Furlenmeier, et al. (3) first added the Grignard reagent of the side chain moiety and subsequently carried out the 14α-hydroxylation. Unfortunately, the yield in their final reaction sequence to ecdysone is not given (3).

Carbon-14 labeled 2-methyl-3-butyn-2-ol was synthesized from acetone and labeled acetylene. Conversion to the Grignard

reagent of the tetrahydropyranyl (THP) derivative was readily accomplished in good yield.

The reaction of the Grignard reagent with 7 yields two isomeric alcohols in about equal amounts which are readily separated on an alumina column.

The hydrogenation of the 23-yne formed from 7 is complex due to incomplete reduction and hydrogenolysis of the THP protecting group. Separation of the products proved easier if the 14α-hydroxylation was carried on the crude reaction product first. In point of fact, it proved advantageous to proceed without isolating any products until arriving at ecdysone. Thus, the reaction sequence becomes reduction, 14α-hydroxylation, removal of the THP group, epimerization at the 5-position and hydrolysis of the 2,3-diacetate groups. Ecdysone was obtained in crude form in 18% yield from the 23-yn-22S-ol. Purification by crystallization reduced the yield to 8% of the labeled product.

Experimental

Infrared spectra were determined on a Perkin-Elmer model 237 spectrophotometer using the KBr pellet technique. NMR spectra were taken in deuterochloroform on a Varian A-60. All chemical shifts are in ppm from internal tetramethylsilane. Mass spectra were run on a Finnegan 1015 S/L mass spectrometer.

Melting points are uncorrected. Thin layer chromatography (TLC) utilized E. Merck silica gel H unless otherwise specified. Preparative scale thin layers (PLC) were made with E. Merck PF-254.

3β-Hydroxy-5α-ergosta-7,22-dien-6-one (6). Ergosteryl acetate was oxidized in 20 g batches by the method of Burawoy (4). Yields of 3β-acetoxy-5α-hydroxyergosta-7,22-dien-6-one averaged 25%. Reduction of this compound was carried out in 6 g batches using zinc dust from a freshly opened bottle following the procedure of Barton and Robinson (5). The reduction product was chromatographed over silica gel and eluted with benzene-ethyl acetate 85-15. From 41 g of crude product one obtains 23.7 g of the 5α reduction product (5) and 7.3 g of the 5β isomer (6). The melting point of the 5α-isomer was 187-189°, reported (6) m.p. 181-182°; while that for the 5β-isomer was 220-224°, reported (6) m.p. 218-219°. Infrared and NMR spectra for both isomers agreed closely with the reported (6) data.

The 3β-acetoxy-5α-ergosta-7,22-dien-6-one (3) prepared above was reduced in 5 g batches in dry tetrahydrofuran (50 ml) at 0° under nitrogen. Lithium aluminum hydride (0.5 g) was added in portions. The mixture was stirred at room temperature for one hour, a small amount of methanol was added with cooling, and the mixture shaken with 0.1 N cold hydrochloric acid (150 ml). The precipitate was collected and air dried. Besides a ν_{max} 3300 cm^{-1}, the 3,6-diol (5) was not farther characterized as it was extremely insoluble in most organic solvents. The yield of crude 5 was quantitative.

Crude 5 (5 g) was suspended in 100 ml of chloroform and added to azeotropically dried activated manganese dioxide (100 g) (7) in benzene (300 ml). This mix was shaken for 20 minutes.

Three batches of oxidation product (14 g) were combined and chromatographed on 300 g of silica gel. Applied initially in benzene-ethyl acetate 95-5; the desired 3β-alcohol 6 was eluted with benzene-ethyl acetate 70-30. The yield was 80%. A small portion was crystallized from methanol as white needles, m.p. 198-201°, δ 0.625 (CH_3-18), 0.875 (CH_3-19), 3.60 (br. m, H-3), 5.25 (m, H-22,23), and 5.75 (t, H-7, J 2Hz). Reported (6) m.p. 199-200°, δ 0.615 (s, CH_3-18), 0.870 (s, CH_3-19), ca. 3.58 (m, H-3), 5.20 (m, H-22,23), and 5.75 (t, H-7).

3β-Methanesulfonyloxy-5α-ergosta-7,22-dien-6-one (4). The ketoalcohol 6 (12 g) was taken up in 170 ml of dry pyridine, cooled in an ice bath, flushed with nitrogen and reacted with 7 ml of methanesulfonyl chloride added dropwise. After 2 hrs. at room temperature the mixture was poured into ice water with stirring. and the crystals of the desired 3β-mesylate 4 were collected by filtration followed by washing

with water. The dried cake represented a quantitative yield of ester (14.2 g). A small portion was crystallized from acetonitrile yielding white needles, m.p. 176-177°, ν_{max} 1655, 1605, 1170 cm^{-1}; δ 0.625 (CH_3-18), 0.895 (CH_3-19) 3.03 (s, $\underline{CH_3}SO_2O$), ca. 4.60 (br. m, H-3), 5.22 (m, H-22,23) and 5.75 (m, H-7). Reported (6) m.p. 176-177°, δ 0.625 (CH_3-18), 0.90 (CH_3-19), 3.04 (s, $\underline{CH_3}SO_2O$), ca. 4.65 (m, H-3), 5.22 (m, H-22,23) and 5.75 (t, H-7).

Furlenmeier, et al. (3) reported the preparation of the mesylate 4 (m.p. 162°) in 51% from the acetate 3. Subsequently, Scherrer (6) reported a melting point identical with ours.

2β,3β-Diacetoxy-5α-ergosta-7,22-dien-6-one. The mesylate ester (13.3 g) was reacted with lithium carbonate in dimethylacetamide under the conditions ultiized by Furlenmeier, et al. (3) to give the crude 2-ene in 80% yield.

The crude 2-ene (10.6 g) was reacted with 9.8 g of silver acetate, 7.4 g of iodine, and 1.3 ml of water in 500 ml of glacial acetic acid via the conditions of Barton, et al. (2b). The product (13 g) was isolated as a gum which was acetylated by being allowed to stand for 44 hrs in 200 ml of pyridine and 100 ml of acetic anhydride. The reaction mixture was partitioned between benzene-hexane (3:1) (200 ml) and an equal volume of water. Each layer was backwashed with fresh portions of the other. The combined organic solvent extracts were washed with dilute cold hydrochloric acid, dilute potassium carbonate, dried and the solvent evaporated to give 14.5 g of a yellow gum.

This gum was chromatographed over 200 g of silica gel made up in benzene. Elution with benzene-ethyl acetate (95-5) was followed by TLC, and a 7.5 g fraction was isolated which formed a gum on evaporation of the solvent. Trituration with and crystallization from methanol gave the desired 2β,3β-diacetate as white needles (4.3 g, 28%), m.p. 192-194°, ν_{max} 1748, 1675, 1620, 1250 cm^{-1}; δ 0.62 (CH_3-18), 1.00 (CH_3-19), 2.01 and 2.04 (singlets, OAc), ca. 4.85 (br. m, H-3), 5.22 (m, H-22,23), ca. 5.30 (m, H-2), 5.74 (m, H-7). Reported (3) m.p. 195-196°, ν_{max} 1746, 1668, 1620 cm^{-1}, δ 0.61 (CH_3-18), 1.00 (CH_3-19), 2.01 and 2.07 (singlets, OAc), 4.83 (m, H-3), 5.19 (m, H-22,23), 5.31 (m, H-2), 5.74 (m, H-7).

Our sample was homogeneous upon high pressure liquid chromatography (LC) on Corasil II using pentane-ethyl ether (4-1).

(20 S)-2β,3β-Diacetoxy-20-formyl-5α-pregn-7-en-6-one (7). The 2β,3β-diacetate (2 g) was ozonized by the procedure of Furlenmeier, et al. (3) to give a white solid (1.6 g) which was shown to be mainly the aldehyde (7) by TLC (hexane-acetone 3-1) and NMR. A small portion of the crude 7 was crystallized from ether-methylene dichloride, m.p. 210-213°; reported (3) 211-212°.

(22S)-2β,3β-Diacetoxy-22-hydroxy-25-(tetrahydropyran-2-yloxy)-5α-cholest-7-en-23-yn-6-one. The procedure of Furlenmeier, et al. (3) was used to add the side chain to 1.6 g of the aldehyde 7. The addition product was decomposed with saturated cold ammonium chloride as described. The crude product was chromatographed over neutral alumina (200 g, Woelm activity III) made up in hexane-acetone 9-1. Unreacted 2-methyl-3-butyn-2-ol tetrahydropyranyl ether was eluted first. Elution with hexane-acetone 3-1 gave first the (22S)-22-hydroxy-23-yne then the (22R)-22-hydroxy-23-yne. The former (1.0 g, 43%) was recrystallized from isopropyl ether as white needles, m.p. 197-200°, $[\alpha]_D$ + 35° (C 0.084, chloroform), δ 0.625 (CH_3-18), 1.00 (CH_3-19), 1.07 (d, CH_3-21), 1.48 and 1.52 (singlets, CH_3-26,27), 2.00 and 2.07 (singlets, -OAc), 3.50 and 3.90 (multiplets, -O-CH_2-), 4.48 (br. d, H-22, J 2.5 H_3), 4.80 (m, H-3), 5.05 (m, -O-CH-O-), 5.27 (m, H-2), and 5.73 (m, H-7). Reported (3) m.p. 188°, $[\alpha]_D$ = +36°, δ 0.63 (CH_3-18), 1.00 (CH_3-19), 1.08 (d, CH_3-21), 1.49 and 1.53 (singlets, CH_3-26,27), 2.00 and 2.06 (singlets, -OAc), 3.47 and 3.96 (multiplets, -O-CH_2-), 4.48 (d, H-22), 4.82 (m, H-3), 5.04 (m, -O-CH-O-), 5.27 (m, H-2), and 5.74 (m, H-7).

Ecdysone. The (22S)-22-hydroxy-23-yne (300 mg) was hydrogenated at room temperature and pressure in ethyl acetate (45 ml) containing 0.5 ml of triethylamine with 100 mg of 5% palladium on charcoal. The calculated amount of hydrogen was taken up in 4 hr:

Removal of the catalyst and solvent gave 305 mg of gum which was taken up in 15 ml of purified dioxane and stirred at room temperature for 15 hrs with 0.6 g of resublimed selenium dioxide. The reaction mixture was filtered through celite and diluted with an additional 200 ml of chloroform. The mixture was washed with water, dried over sodium sulfate, and the solvents evaporated to give 320 mg of reddish residue which was chromatographed over 50 g of neutral alumina (Merck, activity III) made up in hexane-acetone 4-1. Washing with this solvent mix was followed by elution with hexane-acetone 7-3 yielding the major polar product. This was taken up in 10 ml of 0.05 N methanolic hydrochloric acid for 10 minutes. The solution was poured into dilute potassium bicarbonate, extracted with chloroform and the solvent evaporated to give 210 mg of crude 5α-analog of ecdysone as the 2β,3β-diacetate.

Epimerization and hydrolysis was carried by treating 200 mg of the 5α-compound in 27 ml of methanol plus 2.7 ml of 0.3% aqueous potassium carbonate by refluxing under nitrogen for 3 hrs. Upon cooling the mixture was poured into saturated brine (50 ml) and extracted with five 30 ml portions of ethyl acetate. The organic layer was washed (brine), dried, and evaporated to give 160 mg of white solid. TLC of this material (E. Merck, alumina T, development chloroform-methanol 9-1) showed two spots the more polar of which corresponded with an authentic sample of ecdysone.

The reaction product was chromatographed over 60 g of neutral alumina (Merck activity III) made up in chloroform. The less polar material (85 mg) was eluted with chloroform-methanol 95-5. Increasing amounts of methanol were utilized until at 80-20 the more polar substance (57 mg) was eluted. Crystallization from acetone gave 25 mg of pure ecdysone, m.p. 234-237°; reported (3) m.p. 241°. The infrared spectrum and mass spectrum of this material were identical with authentic samples of ecdysone.

2,3-^{14}C-2-Methyl-3-butyn-2-ol tetrahydropyranyl ether. This material was prepared by adapting the method Kriz, et al. (8). The reactions were carried out using a conventional vacuum line with gases being moved by displacement with mercury.

An approximately 1 M solution of sodium methylsulfinyl carbanion (9) was prepared in dimethyl sulfoxide. Two mls of this reagent was introduced into a round bottom flask on the line, and 1 m Ci of ^{14}C-acetylene (2 m Ci/m M) was allowed to react. Regular acetylene (45 ml) was then added to the system and allowed to react for 0.5 hr. Acetone (0.22 ml) was added dropwise to the cloudy reaction mixture. The resulting homogeneous yellow solution was stirred at room temperature for 2 hr. Ammonium chloride (0.2 g) was added, and the mixture stirred for 0.5 hr. Following the addition of water (5 ml), the mixture was extracted with five 15 ml portions of ether. Little or no radioactivity was found in the aqueous portion. The ether layer was dried over sodium sulfate then molecular sieves (Linde 4A) and evaporated to give 0.3 g of yellow oil. This oil was treated overnight with 0.5 ml of dihydropyran and a few small crystal of p-toluenesulfonic acid. The mixture was shaken with a small amount of anhydrous potassium carbonate and then filtered through 30 g. of neutral alumina (Woelm, activity II) with pentane-ether 9-1 to give 3,4-^{14}C-2-methyl-3-butyn-2-ol tetrahydropyranyl ether (0.14 g) identified by infrared and NMR spectroscopy and comparison with authentic samples.

Conversion of the 0.14 g of labeled reagent in the Grignard reagent was accomplished using the established conditions (3). This was then allowed to react with 0.311 g of the aldehyde 7. The addition product was treated as above to give 0.021 g of crude labeled ecdysone which was crystallized to give 0.011 g of pure 23,24-^{14}C-ecdysone.

ACKNOWLEDGMENTS

We wish to express our appreciation to the Robert A. Welch Foundation for their generous support of this work which is a contribution from the Chemistry of Behavior program at Texas Christian University. We also wish to thank Drs. D. H. S. Horn and K. Nakanishi for samples of authentic ecdysone.

REFERENCES

1. Smith, W. B., Newsoroff, G. P., and Wu, N. Y., STEROIDS, 21, 609 (1973).

2. (a) For a review of existing syntheses see Horn, D. H. S., in NATURALLY OCCURRING INSECTICIDES, Editors, M. Jacobson and D. G. Crosby, M. Dekker, Inc., New York, 1971. (b) Barton, D. H. R., Feakins, P. G., Poyser, J. P., and Sammes, P. G., J. CHEM. SOC. (C), 1584 (1970).

3. Furlenmeier, A., Furst, A., Langeman, A., Waldvogel, G., Hocks, P., Kerb, U. and Wiechert, R., HELV. CHIM. ACTA, 50 2387 (1967).

4. Burawoy, A., J. CHEM. SOC., 409 (1937).

5. Barton, D. H. R. and Robinson, C. H., ibid., 3045 (1954).

6. Scherrer, H., HELV. CHIM. ACTA, 52, 2428 (1969).

7. (a) Pratt, E. F. and Van de Castle, J. F., J. ORG. CHEM., 26, 2973 (1961); (b) Goldman, I. M., ibid., 34, 1979 (1969).

8. Kriz, J., Benes, M. J., and Peska, J., COLL. CZECH. CHEM. COMM., 32, 398 (1967).

9. Corey, E. J. and Chaykovsky, J. AMER. CHEM. SOC., 87, 1345 (1965).