7-Dehydrostigmasterol, α-Spinasterol, and Schottenol[1,2]

HENRY W. KIRCHER* AND FUMIKO U. ROSENSTEIN

Department of Agricultural Biochemistry, The University of Arizona, Tucson, Arizona 85721

Received October 10, 1972

The three title sterols are required for our studies on the growth, maturation, and reproduction of Sonoran Desert species of *Drosphilia*.[3] A number of preparations of 7-dehydrostigmasterol in 1–2% yield from stigmasteryl acetate have been reported.[4a–e] A more facile preparation of this sterol would readily lead to the other two compounds by selective hydrogenations.

Based on previous work[5–7] and more than 100 runs on the acetates of cholesterol, sitosterol, and stigmasterol in our laboratory, a method was developed that gives consistent, high yields of $\Delta^{5,7}$ acetates from the Δ^5 derivatives. The key steps involve the slow addition of solid *N*-bromosuccinimide (NBS) to a stirred, refluxing, illuminated solution of the Δ^5-steryl acetate in purified Skellysolve B and low-temperature removal of solvent from this mixture, followed by the dropwise addition of the 7-bromosteryl acetate in mesitylene to a refluxing, stirred solution of collidine in the same solvent.

α-Spinasterol has been isolated from many plants and has been prepared by hydrogenation of 7-dehydrostigmasteryl benzoate over Pt in ethyl acetate.[4d] The compound was readily obtained in our work by hydrogenation of 7-dehydrostigmasteryl acetate with tris(triphenylphosphine)chlororhodium in benzene–ethanol[8] or over Raney Ni in dioxane.[9]

Schottenol also occurs widely distributed among plants; its synthesis from α-spinasterol by hydrogenation over Pt in ether was described by Barton and Cox.[10] In our hands, hydrogenation of 7-dehydrostigmasterol or its acetate over Pt in ether or Raney Ni in several solvents always gave 8(14)-stigmasten-3β-ol as a substantial or principal reaction product when the reductions were run for an extended time to assure complete hydrogenation of the Δ^{22} bond.

The problem was solved by the addition of a small amount of triethylamine to the Ni-catalyzed reduction. Although this slowed the rate of reaction, the amine inhibited the $\Delta^7 \rightarrow \Delta^{8(14)}$ rearrangement and allowed a good yield of schottenol to be obtained from 7-dehydrostigmasterol. When the reduction was hastened by raising the temperature to 100°, 5α-8(14)-stigmasten-3β-ol and 5α-8(14)-stigmasten-3α-ol were the only products obtained. The latter compound has never been reported, although its isomer, 5β-8(14)-stigmasten-3α-ol, has been isolated from *Daemia extensa*.[11] The 5α,3α-ol was characterized by its chromatographic behavior, Liebermann–Burchard test, its inability to form an insoluble digitonide, and the analogies of the ir spectra[12] and melting points[13] of the 5α-8(14)-stigmasten-3α- and -3β-ols and their acetates with those of 5α-cholestan-3α-ol and 5α-cholestan-3β-ol.

The melting points of the products obtained in this study are given together with those from the literature in Table I.

Experimental Section

Melting points were taken *in vacuo* and are corrected. Glc on 5% OV-101, 260° (relative retention time) (cholesterol = 1.00): stigmasterol (1.38), 7-dehydrostigmasterol (1.48), α-spinasterol (1.52), schottenol (1.78), 5α-8(14)-stigmasten-3β-ol (1.51), 5α-8(14)-stigmasten-3α-ol (1.55); acetates comparable.

For tlc, 10% $AgNO_3$–silica gel plates were used: 1:1 $CHCl_3$–CCl_4 used for sterol acetates (R_f), 7-dehydrostigmasteryl acetate (0.26), 4,6,22-stigmastatrienyl acetate (0.46), stigmasteryl acetate (0.58); 95:5 $CHCl_3$–acetone used for free sterols (R_f), stigmasterol (0.39), 7-dehydrostigmasterol (0.20), α-spinasterol (0.39), schottenol (0.39), 8(14)-stigmasten-3β-ol (0.40), 8(14)-stigmasten-3α-ol (0.53).

7-Dehydrostigmasterol.—A 1-cm bore stopcock topped with a powder funnel was cemented (GE silicone RTV) to a 24/40 glass joint and placed in the center opening of a 1-l., three-necked flask equipped with a reflux condenser and nitrogen inlet. The flask was on a magnetic stirrer and was heated and illuminated by 2 GE DSB Photospot bulbs 5 cm away. Stigmasteryl acetate (68 g, 0.15 mol) was dissolved and brought to a reflux in 350 ml of purified Skellysolve B[5] (petroleum ether, bp 65–67°) in an N_2 stream with rapid stirring. NBS (45.5 g, 0.225 mol) was added in small portions during 30 min through the stopcock with the aid of 5–10 ml of solvent for each addition. Four minutes later the lights were removed and replaced with an ice bath. The cooled mixture was filtered through sintered glass into a 2-l. flask containing 35 ml of mesitylene and a magnetic stirring bar. The contents of the flask were evaporated *in vacuo* with an oil pump at 30–40° until a thick, honey-colored syrup remained. *Too high a temperature during this step results in low yields of product.*

The bromosteryl acetate was transferred to a dropping funnel with 150 ml of mesitylene and added over 35 min to a refluxing, stirred mixture of 75 ml of collidine and 350 ml of mesitylene under N_2. Five minutes later the mixture was cooled, low-boiling ether (400 ml) was added, and the mixture was filtered. After evaporation of the filtrate *in vacuo* at 50–60°, the brown semisolid residue was brought to a reflux under N_2 with 1 l. of acetone. After cooling to room temperature, 20 g of crude 7-dehydrostigmasteryl acetate was filtered off. An additional 2.5 g was obtained by work-up of the mother liquors.

The products from four such reactions were combined (88.7 g) and recrystallized from 1.3 l. of Skellysolve B under N_2 to yield 58.5 g of 7-dehydrostigmasteryl acetate, mp 175–177°. Further work-up of the mother liquors gave an additional 11.3 g of the product, mp 174–176°, for a total yield of 25.7% from stigmasteryl acetate. A pure sample, mp 178.3–179°, was obtained after an additional recrystallization from acetone. This was hydrolyzed to 7-dehydrostigmasterol and a benzoate was prepared (Table I).

(1) This work was supported by Grant GB28953X from the National Science Foundation. Contribution No. 1996 from the Arizona Agricultural Experiment Station.

(2) Trivial names for 5,7,22-stigmastatrien-3β-ol, 5α-7,22-stigmastadien-3β-ol, and 5α-7-stigmasten-3β-ol, respectively.

(3) H. W. Kircher and W. B. Heed, "Recent Advances in Phytochemistry," C. Steelink and V. C. Runeckles, Ed., Appleton-Century-Crofts, New York, N. Y., 1970.

(4) (a) O. Linsert, *Z. Physiol. Chem.*, **241**, 125 (1936); (b) O. Wintersteiner and W. L. Ruigh, *J. Amer. Chem. Soc.*, **64**, 1177 (1942); (c) G. A. D. Hazlewood, *Biochem. J.*, **33**, 454 (1939); (d) L. F. Fieser, M. Fieser, and R. N. Chakravarti, *J. Amer. Chem. Soc.*, **71**, 2226 (1949); (e) S. Agaki and K. Tsuda, *Chem. Pharm. Bull.*, **9**, 454 (1961); *Chem. Abstr.*, **55**, 27413 (1961).

(5) S. Bernstein, L. J. Binovi, L. Dorfman, K. J. Sax, and Y. Subbarow, *J. Org. Chem.*, **14**, 433 (1949).

(6) W. R. Nes, R. B. Kostic, and E. Mosettig, *J. Amer. Chem. Soc.*, **78**, 436 (1956).

(7) B. D. Kulkarni, G. Blondin, and W. R. Nes, *Steroids*, **1**, 21 (1963).

(8) A. J. Birch and K. A. M. Walker, *J. Chem. Soc. C*, 1894 (1966).

(9) L. F. Fieser and J. E. Herz, *J. Amer. Chem. Soc.*, **75**, 121 (1953).

(10) D. H. R. Barton and J. D. Cox, *J. Chem. Soc.*, 1354 (1948).

(11) M. M. Dhar, N. Anand, and M. L. Dhar, *J. Sci. Ind. Res.*, **18B**, 422 (1959).

(12) W. Neudert and H. Ropke, "Atlas of Steroid Spectra," Springer-Verlag, New York, N. Y., 1965, Compounds 816 and 817.

(13) L. F. Fieser and M. Fieser, "Steroids," Reinhold, New York, N. Y., 1959, p 28.

TABLE I
MELTING POINTS (°C) OF STEROLS AND THEIR DERIVATIVES

Registry no.	Sterol	This work, *in vacuo*, corrected: Free sterol	Acetate	Registry no.	Benzoate	Registry no.	Literature[a]: Free sterol	Acetate	Benzoate
481-19-6	7-Dehydrostigmasterol	158.5–159.3	178.3–179	39533-73-8	184.5–185.5	39533-21-8	152–154[b]	172–173[c]	178.5–180[d]
481-18-5	α-Spinasterol	172–173	188.5–189	4651-46-1	204–205	39599-22-9	172.5[e]	187[e]	201–202[f]
521-03-9	Schottenol	151–151.5	159.5–160	14473-77-9	184–184.5	39533-21-6	148–150[g]	161–163[h]	183.5[g]
14291-38-4	5α-8(14)-Stigmasten-3β-ol	116.5–117	117.8–118.5	14291-39-5	87.5–88.5	39533-23-8	114[i]	119[g]	89[i]
39533-72-7	5α-8(14)-Stigmasten-3α-ol	176–176.5	84.5–85	39533-24-9	99–100	39533-25-0			

[a] Highest melting point reported. [b] Reference 4a,e. [c] Reference 4a. [d] Reference 4b. [e] D. Larsen and F. W. Heyl, *J. Amer. Chem. Soc.*, **56**, 2663 (1934). [f] M. C. Hart and F. W. Heyl, *J. Biol. Chem.*, **95**, 311 (1932). [g] C. Djerassi, G. W. Krakower, A. J. Lemin, H. H. Liu, J. S. Mills, and R. Villotti, *J. Amer. Chem. Soc.*, **80**, 6284 (1958). [h] G. Biglino, *Farmaco, Ed. Sci.*, **14**, 673 (1959); *Chem. Abstr.*, **54**, 6812c (1960). [i] E. Fernholz and W. L. Ruigh, *J. Amer. Chem. Soc.*, **62**, 2341 (1940).

α-Spinasterol. A. Rh-Catalyzed Reaction.[8]—7-Dehydrostigmasteryl acetate (25 g) in 1200 ml of 3:1 benzene–ethanol was hydrogenated at room temperature and 1 atm pressure over 1.6 g of tris(triphenylphosphine)chlororhodium (Strem Chemical Co.) for 18 hr. Solvent was evaporated, the dry residue was extracted with petroleum ether and filtered to remove catalyst, and solvent was again evaporated. The residue was crystallized from 2:1 ethanol–benzene and then from acetone to give 15.1 g of α-spinasteryl acetate, mp 188.5–189°. An additional 7.7 g, mp 187.5°, was recovered by work-up of the mother liquors. A portion of the product was hydrolyzed to α-spinasterol, and a benzoate was prepared (Table I).

B. Raney Ni Catalyzed Reaction.[9]—7-Dehydrostigmasteryl acetate (11.3 g) in 450 ml of dioxane and 5 ml of Et_3N was hydrogenated over 10 ml of catalyst for 23 hr at room temperature and 1 atm pressure. Removal of catalyst and evaporation of solvent to 120 ml deposited 7.5 g of α-spinasteryl acetate, mp 186–186.5°. Further cooling of the filtrate yielded an additional 2.6 g of product, mp 183.5–184°.

Schottenol. A.—7-Dehydrostigmasterol (15 g) in 750 ml of ethyl acetate and 5 ml of Et_3N was hydrogenated for 4 days over 25 ml of Raney Ni at room temperature and 1 atm pressure. The reaction was followed by glc of samples periodically removed. Catalyst was then filtered off and the solvent was reduced in volume to 150 ml to precipitate 9.15 g of schottenol, mp 149.5–151.5°. Work-up of the mother liquors gave no more pure material.

B.—The reaction as in A was repeated in a Parr stirred pressure vessel at 14 atm H_2 pressure. After 50 hr the product was worked up as before to yield 8.9 g of schottenol, mp 150–151.5°.

The products from several runs (24.5 g) were combined in 500 ml of hot ethyl acetate and cooled to room temperature to yield 18.5 g of schottenol, mp 151–151.5°. An acetate and a benzoate were prepared (Table I).

5α-8(14)-Stigmasten-3α- and -3β-ol.—7-Dehydrostigmasterol (17 g) in 700 ml of ethyl acetate and 5 ml of Et_3N was hydrogenated over 20 ml of Raney Ni at room temperature for 10 min at 14 atm and for 2 hr at 100° and 14 atm. The reaction was followed by glc. An additional 22 hr at 14 atm and 100° and 24 hr at 140 atm and 100° with fresh catalyst produced no changes in the glc pattern.

The autoclave was cooled, catalyst was removed, and the product (one peak on glc, two spots on tlc) was chromatographed on 1 kg of 2:1 10% $AgNO_3$–silica gel–Celite with 10% ether in petroleum ether. The two compounds were cleanly separated (tlc) to yield 4 g of the higher R_f material and 8 g of the lower R_f material.

The former was crystallized from methanol–benzene to yield 5α-8(14)-stigmasten-3α-ol, mp 176–176.5°, acetate, mp 84.5–85° (from methanol–benzene), and benzoate, mp 99–100° (from ethanol). The compound gave a fast blue color with the Liebermann–Burchard reagent, no precipitate with digitonin, and had an ir spectrum similar to that of 5α-cholestan-3α-ol.[12] The material last to emerge from the column was recrystallized from methanol–benzene and identified as 5α-8(14)-stigmasten-3β-ol, mp 116.5–117.0° (from ethanol), acetate mp 117.8–118.5° (from ethanol), and benzoate mp 87.5–88.5° (from acetone). It corresponded to the lower R_f spot on tlc, had the same retention time on glc as the 3α-ol, gave a precipitate with digitonin and a fast blue Liebermann-Burchard test, and had an ir spectrum similar to that of 5α-cholestan-3β-ol.[12]

Registry No.—NBS, 128-08-5.

Percyclophane-4

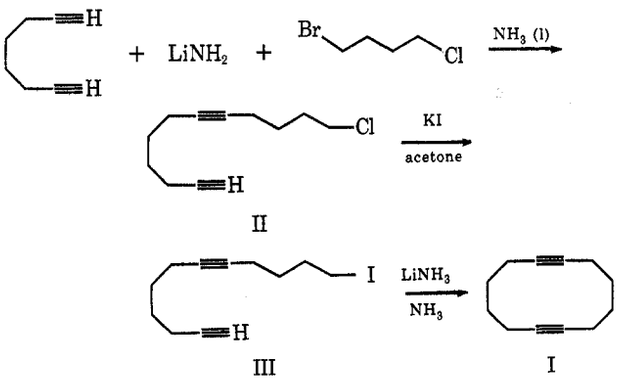
ROBERT D. STEPHENS

Department of Chemistry, Washington State University, Pullman, Washington 99163

Received December 12, 1972

We wish to report the synthesis of an unusual new cage aromatic compound, percyclophane-4[1] (Figure 1). Percyclophane-4 was synthesized by the cyclic trimerization of cyclododecadiyne-1,7, catalyzed by organochromium and organocobalt compounds.

Cyclododecadiyne-1,7 (I) was synthesized from commercially available octadiyne-1,7 in the manner described below.



Overall yield of this synthesis was approximately 20%. This is a new synthesis of a previously reported compound.[2] Cyclododecadiyne-1,7 is a colorless, crystalline compound, mp 23 ± 2°, which on exposure

(1) "Percyclophane" is a name coined by the author to describe this cage molecule. The name is derived from the word paracyclophane, which refers to molecules with a configuration of two benzene rings linked together in the para positions.

(2) (a) D. J. Cram, N. L. Allinger, and H. Steinberg, *J. Amer. Chem. Soc.*, **76**, 726 (1959); (b) D. J. Cram and N. L. Allinger, *ibid.*, 6132 (1954); (c) *ibid.*, **78**, 2518 (1956).