Synthesis of β-Sitosteryl Acetate [(24*R*)-24-Ethyl-3β-acetoxycholest-5-ene] and Its 24*S* Epimer[1]

R. IKAN,* A. MARKUS, AND E. D. BERGMANN

Department of Organic Chemistry, Hebrew University, Jerusalem, Israel

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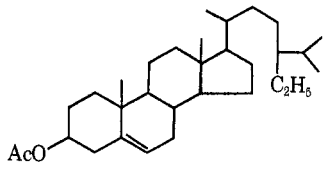
A semitotal synthesis of β-sitosteryl acetate and its 24*S* epimer (clionasterol) has been carried out, starting from the optically active 3-ethyl-4-methylpentylmagnesium bromides and pregnenolone acetate.

In a previous communication[2a] we have described the synthesis of campesteryl acetate and its 24*S* epimer. We then reported that the two epimers are differently utilized by the larvae of *Dermestes maculatus*. In order to clarify further the correlation between the stereochemical arrangement of C-24 alkyl groups and the biological activity of the sterols, we have synthesized β-sitosteryl acetate **1** and its 24*S* epimer (clionasteryl acetate) **2**.[2b]

β-Sitosteryl acetate **1** was synthesized as follows. Dextrarotatory 4-ethyl-5-methylhexanoic acid (**3**) was converted to 3-ethyl-4-methylpentyl bromide (**4**) by the Hunsdieker reaction. Grignard reaction of pregnenolone acetate with 3-ethyl-4-methylpentylmagnesium bromide yielded (24*R*)-24-ethyl-3β-acetoxycholesta-5,20(22)-diene (**5**) which was selectively reduced to (24*R*)-24-ethyl-3β-acetoxycholest-5-ene (β-sitosteryl acetate, **1**). The parallel synthesis using the optical antipode of **3** yielded the 24*S* epimer **2** of **1**, clionasteryl acetate.



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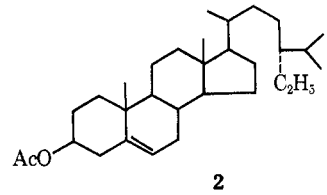

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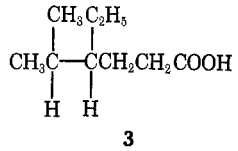

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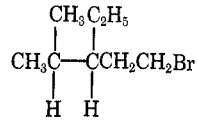

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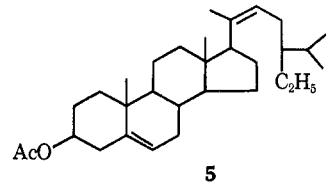

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In agreement with our previous experience, only cinchonidine brought about the resolution of 4-ethyl-5-methylhexanoic acid into its optical isomers, whereas brucine, quinine, and 3-*p*-nitrophenyl-2-aminopropane-1,3-diol failed to do so.

Experimental Section[3]

Diethyl ethylmalonate was prepared according to Vogel.[4]

2-Ethyl-3-methylbutyric acid was prepared according to Ikan, *et al.*,[2a] starting from diethyl ethylmalonate, bp 115° (30 mm), yield 70%.

Anal. Calcd for $C_7H_{14}O_2$: C, 64.6; H, 10.9. Found: C, 64.6; H, 10.7.

Methyl 2-ethyl-3-methylbutyrate was prepared according to Ikan, *et al.*,[2a] bp 123–126°, yield 90%.

Anal. Calcd for $C_8H_{16}O_2$: C, 66.7; H, 11.1. Found: C, 66.4; H, 11.3.

2-Ethyl-3-methylbutanol.—To a slurry of 23 g of lithium aluminum hydride in 385 ml of dry ether, 100 g of the preceding ester in 240 ml of the same solvent was added with vigorous agitation. The mixture was refluxed for 6 hr, cooled (the excess of lithium aluminum hydride was destroyed with methanol), and acidified with dilute hydrochloric acid. The product was thoroughly extracted with ether (after the aqueous layer was saturated with sodium chloride) and dried, bp 145°, yield 68 g (77%).

Anal. Calcd for $C_7H_{16}O$: C, 72.4; H, 13.8. Found: C, 72.2; H, 13.9.

2-Ethyl-3-methylbutyl Bromide.—To a cooled solution (0°) of 2-ethyl-3-methylbutanol (35 g), 30 g of phosphorus tribromide was added dropwise. After 15 hr, ice was added and the organic layer separated. It was washed twice with 1-ml portions of concentrated sulfuric acid, twice with water, and with a dilute solution of sodium bicarbonate (10%). After drying over magnesium sulfate, the product was distilled, bp 65° (30 mm), yield 27.5 g (50%).

4-Ethyl-5-methylhexanoic Acid (3).—To a solution of 12 g of sodium in 250 ml of anhydrous ethanol, 79 g of diethyl malonate was added and the temperature was raised to 60°. Then 90 g of 2-ethyl-3-methylbutyl bromide was added dropwise during 45 min and the mixture refluxed with stirring for 25 hr. The precipitated sodium bromide was filtered off and the filtrate refluxed for 2 hr with 60 g of potassium hydroxide in 80 ml of ethyl alcohol, until neutral. Then 50 ml of water was added, the alcohol distilled off, and the residue acidified with concentrated hydrochloric acid and boiled for 10 hr. The product was extracted with methylene chloride, washed with a solution of sodium bicarbonate (10%) and water, and dried over anhydrous magnesium sulfate, bp 145° (25 mm), yield 45 g (58%).

Anal. Calcd for $C_8H_{18}O_2$: C, 68.3; H, 11.5. Found: C, 68.2; H, 11.6.

Resolution of 4-Ethyl-5-methylhexanoic Acid.—The acid and cinchonidine (1 mol each) were dissolved in acetone; the mixture was heated until the solution became clear and then allowed to crystallize at room temperature. The crystals of the salt were filtered on a Büchner funnel as soon as they formed. After five recrystallizations from acetone the salt was treated with dilute hydrochloric acid and the acid was extracted with methylene chloride and distilled under reduced pressure. The optical rotation of the product was $[\alpha]_D$ −8°.

(1) This work was supported in part by U. S. Department of Agriculture, Grant No. FG-Is-268.

(2) (a) R. Ikan, A. Markus, and E. D. Bergmann, *Steroids*, **16**, 517 (1970). (b) Preliminary tests have indicated that clionasterol is partially utilized by *Dermestes maculatus*.

(3) Melting points were determined on a Thomas-Hoover apparatus. Optical rotations were measured in chloroform. Nmr spectra were recorded for deuteriochloroform solutions using a Varian Hz-100 spectrometer. Infrared spectra were recorded on a Perkin-Elmer 137 spectrophotometer, using Nujol oil.

(4) A. I. Vogel, "Practical Organic Chemistry," Longmans, Green and Co., New York, N. Y., 1962, p 1002.

(−)-3-Ethyl-4-methylpentyl Bromide (4).—The levorotatory acid (4.5 g) was dissolved in 15 ml of carbon tetrachloride in a three-necked flask (protected from light with aluminum foil). Then 6.6 g of mercuric oxide was added followed, after a short heating period, by 4.8 g of bromine in 15 ml of carbon tetrachloride which was added dropwise. The solution was refluxed for 1 hr, the mercuric bromide filtered off, and the filtrate washed with a solution of sodium hydroxide (5%) and water. The product was fractionally distilled, bp 80° (20 mm), yield 2.1 g (40%), $[\alpha]_D$ −3°.

(24*S*)-24-Ethyl-3β-acetoxycholesta-5,20(22)-diene.—To the Grignard reagent prepared from 0.6 g of magnesium and 2.5 g of (−)-3-ethyl-4-methylpentyl bromide in 50 ml of ether, 1.8 g of 3β-acetoxypregn-5-en-20-one (pregnenolone acetate) in 50 ml of dry benzene was added, and the mixture was refluxed for 4 hr and allowed to stand overnight at room temperature. Hydrochloric acid (5%) was added and the product was extracted with benzene. Distillation of the benzene left an oily residue which was treated with 10 ml each of acetic anhydride and dry pyridine and left overnight at room temperature. Then 20 ml each of methanol and benzene was added, and the solution was concentrated *in vacuo*. This operation was repeated several times in order to remove the last traces of pyridine and acetic anhydride. The oily residue was chromatographed on a Florisil (60 g) column. The products were eluted with 100 ml each of solutions of benzene in hexane with the following concentrations, 5, 10, 20, 50% (v/v), followed by solutions of chloroform in benzene, 5 and 50% (v/v), and finally with chloroform. The product was recrystallized from methanol and melted at 135°, yield 0.5 g (30%), $[\alpha]_D$ −73.5°. The molecular ion in the mass spectrum was 394 (calcd, 394); ν_{max}^{Nujol} 1730 (CH_3COO^-), 975, 1640 cm^{-1} (C=C); nmr δ 5.2 ppm (>C=CH). Obviously, the tertiary alcohol formed in the Grignard reaction had undergone spontaneous dehydration.

(24*S*)-24-Ethyl-3β-acetoxycholest-5-ene (Clionasteryl Acetate) (2).—(24*S*)-24-Ethyl-3β-acetoxycholesta-5,20(22)-diene (30 mg) was dissolved in 10 ml of ethyl acetate and reduced catalytically in a microhydrogenator[5] in the presence of Pd/C (10%). The hydrogenation was stopped after saturation of the 20,22 double bond. The residue was recrystallized from methanol, yielding 28 mg of crystals melting at 139° (lit.[6] mp 143–144°), $[\alpha]_D$ −44.9° (lit.[6] $[\alpha]_D$ −45.3°). The molecular ion in the mass spectrum was 396 (calcd, 396).

Synthesis of (24*R*)-24-Ethyl-3β-acetoxycholesta-5,20(22)-diene (5). (+)-4-Ethyl-5-methylhexanoic Acid.—The mother liquors of the last two fractional crystallizations of cinchonidine (−)-4-ethyl-5-methylhexanoate were concentrated and the residue was treated with dilute hydrochloric acid. The acid was extracted with methylene chloride and distilled under reduced pressure, bp 145° (25 mm), yield 20%, $[\alpha]_D$ +8°.

(5) N. Clauson-Kaas and F. Limborg, *Acta Chem. Scand.*, **1**, 884 (1949).
(6) W. Bergmann and E. M. Low, *J. Org. Chem.*, **12**, 67 (1947).

(+)-3-Ethyl-4-methylpentyl bromide was prepared analogously to the (−) isomer, bp 80° (25 mm), yield 1.8 g (40%), $[\alpha]_D$ +3°.

(24*R*)-24-Ethyl-3-acetoxycholesta-5,20(22)-diene was prepared analogously to the 24*S*-ethyl isomer: mp 129°; yield 28%; $[\alpha]_D$ −84.3°; molecular ion in the mass spectrum 394 (calcd, 394); ν_{max}^{Nujol} 1730 cm^{-1} (CH_3COO^-); nmr δ 5.2 ppm.

(24*R*)-24-Ethyl-3β-acetoxycholest-5-ene (β-Sitosteryl Acetate) (1).—The catalytic hydrogenation was carried out analogously to the 24*S* epimer. The product melted at 121–122° after recrystallization from methanol: mp 124–125°; $[\alpha]_D$ −43° (lit.[7] mp 127°; $[\alpha]_D$ −41°); molecular ion 396 (calcd, 396); ν_{max}^{Nujol} 1730 cm^{-1} (CH_3COO^-).

Biological Tests.—The dietary components of the semisynthetic diet[8] were thoroughly extracted with ether, in order to remove traces of sterols. Sterol additions (0.1%) were made to the diet. Each sterol was tested on at least 20 larvae of *Dermestes maculatus* in 2–4 replications. The results of the tests are summarized in Table I.

TABLE I

Sterol	Av wt of larva (mg) after 25 days	% larvae pupating	Mortality of larvae
Sterol free (control)		None	Complete
Cholesteryl acetate	38	100	None
Campesteryl acetate[a]	33	95	1 in 20
β-Sitosteryl acetate[a]	2	None	Complete
Clionasteryl acetate[a]	3	None	Complete

[a] Synthetic; infrared and mass spectra of the synthetic sterols were identical with those of the natural sterols. No depression of melting points was observed on admixture of the synthetic and the natural compounds.

Registry No.—**1**, 915-05-9; **2**, 4651-54-1; (−)-**3**, 32444-27-2; (+)-**3**, 32444-28-3; (−)-**4**, 32444-29-4; (+)-**4**, 32444-30-7; (24*R*)-**5**, 32444-31-8; (24*S*)-**5**, 32444-36-3; 2-ethyl-3-methylbutyric acid, 32444-32-9; methyl 2-ethyl-3-methylbutyrate, 32444-33-0; 2-ethyl-3-methylbutanol, 32444-34-1; 2-ethyl-3-methylbutyl bromide, 32444-35-2.

Acknowledgment.—The authors wish to thank Mr. G. Grossman for the biological tests.

(7) E. S. Wallis, *ibid.*, **2**, 341 (1937).
(8) Z. H. Levinson, Y. Barelkovsky, and A. Bar-Ilan, *J. Stored Prod. Res.*, **3**, 345 (1967).