[CONTRIBUTION FROM THE RESEARCH LABORATORY, THE UPJOHN COMPANY]

ERGOSTENOL CHLOROACETATE

BY MERRILL C. HART AND FREDERICK W. HEYL

RECEIVED NOVEMBER 5, 1930 PUBLISHED APRIL 6, 1931

Windaus and Grosskopf[1] catalytically reduced ergosterol from yeast and obtained the tetrahydro reduction product, α-ergostenol, to which, however, they assigned the hexahydro, or completely reduced formula and corresponding name, *i. e.*, ergostanol. Reindel and Walter[2] made a number of quantitative reductions and demonstrated that the catalytic reduction of ergosterol acetate with Willstätter's platinum black gave the tetrahydro derivative, α-ergostenol. This substance, according to Windaus and Lüttringhaus,[3] melts at 131°, $[\alpha]_D$ +11.4°; and α-ergostenol acetate, m. p. 109–110°, $[\alpha]_D$ 0°. Hart, Speer and Heyl[4] reported α-ergostenol, m. p. 133°, $[\alpha]_D$ +10.5°; α-ergostenol acetate, m. p. 110–111°, $[\alpha]_D$ 0°.

Reindel and Walter stated further that the reduction of ergosterol acetate in ether with Adams' platinum oxide catalyst gave a hexahydro derivative, but this has since been shown to be incorrect,[5] the mixture actually containing dihydro-ergosterol.

Reindel, Walter and Rauch[6] state that chloroacetyl chloride reacts with α-ergostenol to form β-ergostenol chloroacetate, m. p. 167°. The β-form is characterized by easy reducibility to a hexahydro-ergosterol. This hexahydro-ergosterol, which is a well-known derivative, is known as allo-α-ergostanol. In a previous paper[4] we have prepared so-called β-ergostenol chloroacetate and reported that upon saponification a mixture of alcohols melting at 110–112° was obtained.

In the present paper we describe the fractional crystallization of the chloroacetate prepared from α-ergostenol. This work was undertaken for the purpose of preparing pure β-ergostenol, which should, of course, be readily prepared from the β-chloroacetate.

Although a careful fractionation of α-ergostenol acetate failed to reveal lack of uniformity in the product obtained by the catalytic reduction of ergosterol, we found that the fractionation of the chloroacetate yielded small amounts of allo-α-ergostanol. Furthermore, ergostenol chloroacetate was found to consist largely of the α-isomer and not exclusively of the β-form as Reindel states. The chloroacetates contain, in addition to allo-α-ergostanol, α-ergostenol chloroacetate, β-ergostenol chloroacetate and an addition product, $C_{31}H_{49}O_3Cl_3$.

[1] Windaus and Grosskopf, *Z. physiol. Chem.*, **124**, 8 (1923).
[2] Reindel and Walter, *Ann.*, **460**, 212 (1928).
[3] Windaus and Lüttringhaus, *ibid.*, **481**, 119 (1930).
[4] Hart, Speer and Heyl, THIS JOURNAL, **52**, 2016 (1930).
[5] Reindel and Detzel, *Ann.*, **475**, 80 (1929).
[6] Reindel, Walter and Rauch, *ibid.*, **452**, 34 (1927).

We have not been able to isolate pure β-ergostenol. That it is present is shown by the fact that upon catalytic reduction an increased amount of allo-α-ergostanol can be secured from the mixture of chloroacetates.

Attempts to separate a derivative of β-ergostenol from the mixture of the chloroacetates, by bromination or by oxidation with benzoyl hydroperoxide[7] failed to lead to any derivatives which might be characteristic for the β-form as contrasted with the α-form. One might expect, for example, to secure from an unsaturated sterol, which is readily reduced, either a dibromide or a peroxide.

Experimental

Fractionation of Ergostenol Chloroacetate.—Ergostenol chloroacetate was prepared from 25.6 g. of α-ergostenol (m. p. 132–133°) by gently refluxing with an excess of chloroacetyl chloride for twenty minutes. Hot acetic acid was added and the mixture stood aside to crystallize. The top fraction after five crystallizations from acetic acid weighed 16.1 g., m. p. 166–167°, and $[\alpha]_D^{22}$ +6.93.

Anal. Calcd. for $C_{29}H_{42}O_2Cl$: Cl, 7.66. Found: Cl, 7.71.

This material was systematically crystallized ten times from ethyl ether with the results shown in Table I.

TABLE I

FRACTIONATION OF ERGOSTENOL CHLOROACETATE

Fraction no.	Weight, g.	$[\alpha]_D$	M. p., °C.
1	0.909	+ 5.3	190–192
2	0.715	+ 5.5	169–170
3	1.187	+ 6.24	169–170
4	2.216	+ 4.86	168–169
5	3.650	+ 6.24	167–168
6	3.452	+ 6.94	165–166
7	2.490	+15.56	162–164
8	0.848	+20.82	153–156

Fraction 1 is practically pure allo-α-ergostenol; recrystallized from ether, m. p. 199–200°; $[\alpha]_D$ +2.3°. Reindel gives 200–201°. Upon saponification and subsequent acetylation pure allo-α-ergostanol acetate, m. p. 149–149.5°, was obtained.

Anal. Calcd. for $C_{27}H_{47}OCOCH_3$: C, 80.9; H, 11.7. Found: C, 81.0; H, 11.5.

The alcohol itself melted at 144°. Reindel gives 144–145°. The isolation of allo-α-ergostanol chloroacetate from the most insoluble fraction proves that the reduction of ergosterol acetate in acetic acid solution with platinum oxide (Adams') as a catalyst results in the formation of small amounts of the hexahydro derivative, allo-α-ergostanol.

Fractions 2 to 8 of the chloroacetate crystallization were separately saponified and the fractions showed melting points that varied from 118–120° to 129–131°, while the rotations varied from +11.8 to +17.3°. All these rotations are considerably higher than that required for α-ergostenol, and those fractions showing a rotation considerably higher than that reported by Reindel for β-ergostenol (+15.9°) were obviously mixtures. It may be predicted safely that β-ergostenol, when finally isolated, will show a higher positive specific rotation than +17.3°.

[7] Westphalen, *Ber.*, **48**, 1064 (1915).

In these fractions the α-isomer so largely predominated that it was possible to isolate it in comparatively pure form as the characteristic plates of the acetate. Fractions in which the α-form was present in much smaller amounts yielded acetates crystallizing in needles. The elementary composition of the fractions agreed with that required for $C_{29}H_{48}O_2$.

Isolation of an Addition Product of α-Ergostenol Chloroacetate and Chloroacetyl Chloride.—During the main crystallizations of ergostenol chloroacetate, in addition to the top fraction weighing 16.1 g. and melting at 166–167°, four other fractions of ergostenol chloroacetate were obtained. These fractions melted at 157–159°, 144–147°, 107–110° and 110–114°. The first three of these fractions were exhaustively fractionated from ethyl acetate, acetone and ether and various fractions melting from 110–160° were obtained.

The lowest fraction of ergostenol chloroacetate crystallization (m. p. 110–114°) was treated with hot alcohol. The material insoluble in the hot alcohol was crystallized twice from acetone and once from acetic acid; 0.3 g. of irregular plates melting at 129–130° were obtained, $[\alpha]_D$ +8.7°.

Anal. Calcd. for $C_{31}H_{49}O_3Cl_3$: Cl, 18.5. Found: Cl, 17.0, 17.3.

This substance on saponification from 3% methyl alcoholic and crystallization from alcoholic solution gave needles of α-ergostenol melting at 132–133°. The acetates prepared from acetic anhydride crystallized in plates melting at 110–111°. Mixed melting points with α-ergostenol and α-ergostenol acetate, respectively, were not depressed.

Catalytic Reduction of Ergostenol Chloroacetate.—Six grams of ergostenol chloroacetate, m. p. 166–167°, was reduced in acetic acid solution with Adams' platinum oxide as catalyst. The reduced chloroacetate after several crystallizations from ether, acetic anhydride and acetic acid, melted at 184–186°, and on saponification with 5% methyl alcoholic potash and after four crystallizations from alcohol and ether gave crystalline plates of allo-α-ergostanol melting at 144–145°, yield 1.1 g. The acetate crystallized in plates melting at 149–149.5°, $[\alpha]_D$ +4.7°. The benzoate prepared by means of benzoyl chloride and pyridine melted at 163–164°.

Attempt to Isolate a Bromide from the Isomerization Mixture.—When ergostenol chloroacetate (5.95 g.) in 450 cc. of ether was treated with 24 cc. of 9% bromine solution, no insoluble bromide separated. The yellow ether solution was washed with 10% sodium thiosulfate solution and water and then dried over anhydrous sodium sulfate. The product was free from bromine. It was saponified with methyl alcoholic potash and an extremely small yield of crystalline plates that melted at 144–145° was obtained. This in no way represented any characteristic derivative which should be useful in working with this mixture. Upon analysis it showed the composition of an oxidation product, $C_{27}H_{43}OH$. The acetate formed plates melting at 145–146°.

Anal. Calcd. for $C_{29}H_{46}O_2$: C, 81.6; H, 10.9. Found: C, 81.7; H, 11.0.

It gave α-ergostenol acetate upon catalytic reduction. This is undoubtedly a dehydro-ergostenol but it shows a rather wide divergence from the compound isolated by Windaus and Lüttringhaus[3] by the action of bromine or benzoyl hydroperoxide on α-ergostenol acetate. They found 135–136° for the melting point of the acetate of their dehydro compound and 141° for that of the alcohol. We attempted to establish the identity of our product by preparing the dehydro derivative by the action of benzoyl hydroperoxide[8] on α-ergostenol acetate. Similarly a slight yield of a dehydro derivative, the acetate of which melted at 145–146°, was obtained (C, 81.8; H, 10.9). When this acetate was mixed with the acetate obtained by the action of bromine on the chloroacetate as described above there was no depression of the mixed melting point.

[8] Baeyer and Villiger, *Ber.*, **33**, 1569 (1900).

Summary

1. α-Ergostenol, prepared by the catalytic reduction of ergosterol acetate in acetic acid solution with platinum oxide (Adams') as a catalyst, contains small amounts of allo-α-ergostanol.

2. Ergostenol chloroacetate was found to be very largely α-ergostenol chloroacetate with smaller amounts of the chloroacetates of β-ergostenol and of allo-α-ergostanol.

3. Ergostenol reacts with chloroacetyl chloride to form, in small amount, an addition product, $C_{31}H_{49}O_3Cl_3$.

4. Ergostenol chloroacetate forms neither a bromide nor a peroxide nor does it react with potassium permanganate. Upon reduction evidence of some degree of isomerization is found in the increased yield of the reduction product, allo-α-ergostanol.

KALAMAZOO, MICHIGAN

[A COMMUNICATION FROM THE LABORATORY OF ORGANIC CHEMISTRY OF THE UNIVERSITY OF WISCONSIN]

THE PREPARATION OF DIMETHYLACETOACETIC ESTER AND OF Δ^3,2,2-DIMETHYLBUTENOL-1

BY KARL FOLKERS AND HOMER ADKINS

RECEIVED NOVEMBER 6, 1930 PUBLISHED APRIL 6, 1931

A considerable quantity of Δ^3,2,2-dimethylbutenol-1 was desired in order to study its behavior over oxide catalysts.[1] It appeared that this compound could be most easily prepared from acetoacetic ester through the following series of compounds: (1) monomethylacetoacetic ester, (2) dimethylacetoacetic ester, (3) 3-hydroxy-2,2-dimethylbutyric ester, (4) Δ^3,2,2-dimethylbutyric ester, (5) Δ^3,2,2-dimethylbutenol-1.

There does not seem to be available in the literature adequate directions for the methylation of acetoacetic ester, although the two derivatives have been prepared in a more or less pure state by a number of investigators.[2] The method of preparation described below has been developed.

Ethyl 2-Methylacetoacetate.—Thirty-nine and eight-tenths grams (1.73 moles) of sodium was very finely powdered in xylene so that it was like sand. After decanting the xylene, the sodium was washed with two 75-ml. portions of dry toluene (Note 1). The sodium was then covered with one liter of dry toluene, and the flask fitted with a reflux condenser, immersed in an ice-bath, and allowed to cool for fifteen minutes, after which 218.3 g. (1.68 moles) of ethyl acetoacetate (Note 2) was added down the condenser during the course of ten to fifteen minutes (Note 3) while the flask was vigorously shaken and kept in the ice-bath. The top of the condenser was now fitted with a calcium chloride tube and, after an additional ten minutes' cooling, was allowed to stand at room

[1] Adkins and Folkers, THIS JOURNAL, **53**, 1095 (1931).

[2] Frankland and Duppa, *Ann.*, **138**, 328 (1860); Conrad and Limpach, *ibid.*, **192**, 153 (1878); Michael, *Ber.*, **38**, 2083 (1905); Clark, THIS JOURNAL, **33**, 527 (1911).