OXIDATION OF ERGOSTEROL ACETATE WITH CHROMIC ANHYDRIDE

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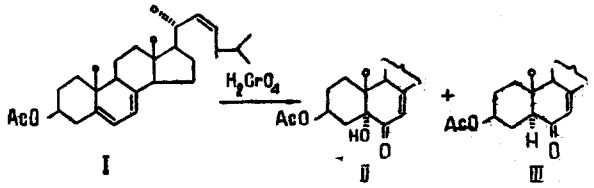
In 1937, Burawoy [1], and then Barton and Robinson [2], studied the oxidation of ergosterol acetate (I) with chromic acid and showed that this gave 3β-acetoxy-5α-hydroxyergosta-7,22-dien-6-one (II) (Burawoy's ketone), which served as the starting material for the synthesis of brassicasterol [2].



I II III

In recent years, this compound has become the object of numerous investigations on the synthesis of a new class of steroid hormones of insect ecdysis (ecdysterones) because its molecule already contains a number of the structural elements that are necessary for the appearance of hormonal activity. In particular, starting from the ketone (II) it has been possible to synthesize ecdysone and its derivatives [3]. However, in spite of the apparent ease of the synthesis of the ketone (II) in one stage the yield of this important intermediate compound is low. Thus, by Burawoy's method [1] (oxidation of ergosterol acetate with an excess of CrO_3 in 80% CH_3COOH at 80° for 30 min) it is 25%, and by Barton's method [2] (oxidation with CrO_3 in 80% CH_3COOH at room temperature for a day) it does not exceed 20%.

In view of this, it appeared to us to be of interest to study the conditions for the synthesis of Burawoy's ketone (II) more carefully and to perform the oxidation of ergosterol acetate with chromic anhydride under various conditions.

On repeating Barton's and Burawoy's experiments, we convinced ourselves that the oxidation products contained not only the ketone (II) but also a compound with a polarity similar to that of ergosterol acetate (I). Nevertheless, attempts to isolate the latter were unsuccessful, apparently because, during oxidation, under the influence of the acid partial isomerization takes place leading to the formation of B isomers of ergosterol.

In addition, it was found that the ketone (II) is unstable in an acid medium. When it was heated in glacial acetic acid (80-100° C, 5-10 min) and in dioxane (100° C, 5-10 min) in the presence of traces of sulfuric or chromic acids or of BF_3 in hexane, it underwent almost complete dehydration with the formation of a less polar product which, however, could not be isolated in the crystalline state. The UV spectrum of this compound purified by chromatography on silica gel showed a strong absorption maximum at 249 nm with an extinction ε = 19,100, which is characteristic for a cross-conjugated system. On this basis, we ascribe to the dehydration product the structure (IV).

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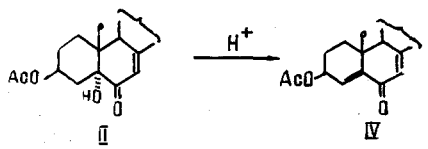


II IV

This induced us to study the oxidation of ergosterol acetate under milder conditions with a smaller amount of acetic acid and oxidizing agent.

On replacing a considerable part of the acetic acid by an equal volume of acetone (Table 1, experiment 1), we found a marked acceleration of the oxidation process. Thus, after only 1.5 h, and not after 24 h as in Burawoy's experiment, at room temperature the yield of Burawoy's ketone (II) was about 25-26%. However, an increase in the duration of the experiment did not lead to an increase in the yield of the desired compound and was accompanied by a considerable formation of a mixture of more polar products of further oxidation.

When the amount of acetic acid or oxidizing agent in the reaction mixture was reduced still further, the yield of Burawoy's ketone (see Table 1, experiment 2 and 5) reached 30-46%. We also observed some increase in the yield of the ketone (II).

In 1948, Fieser [4] observed for the first time that the oxidizing capacity of chromium trioxide rises with a reduction of the amount of water in the solvent. Similar results were also obtained later [5, 6], and it was found that the optimum concentration of water in the medium is 2-4%. Because of this, we performed an experiment on the oxidation of ergosterol acetate (I) in acetone with the complete absence of acetic acid and with a reduction in the water content in the reaction medium to 4%. Under these conditions (see Table 1, experiment 9), the yield of ketone (II) was 56-58%, and the time of oxidation shortened to 20-30 min.

We also studied the oxidation of ergosterol acetate in heterogeneous media, using for this purpose such solvents as cyclohexane, benzene and ether.

However, the oxidation of the acetate (I) in cyclohexane or benzene (see Table 1, experiments 10 and 11) did not lead to definite results, and although the oxidation of the ergosterol acetate took place completely, the oxidation product contained, according to thin-layer chromatography, at least four compounds, two of which were isolated in the crystalline state. The ketone (II) was obtained with a yield of only about 8% together with a small amount of less polar compound.

As the IR spectrum shows, compound (III) does not contain hydroxyl groups (absence of absorption band in the 3300-3600 cm^{-1} region) but it does have an ester grouping (1743 and 1253 cm^{-1}) and also an α, β-unsaturated keto group (1695 cm^{-1}). The latter was also confirmed by its IR spectrum: λ_{max} (ethanol) 246 nm (ε 14,500) and a shoulder at 305 nm (ε 130). This is characteristic for compounds of the type of desoxyviperidone (3β-hydroxycholest-7-en-6-one) acetate [7] and 3β-hydroxyergosta-7-22-dien-6-one [2]. The latter compound, which we prepared by Barton's method [2] of the reduction of the ketone (II) with

TABLE 1

Experiment No.	Oxidation temperature, °C	Reaction time, h	Amount*				Yield of ketone (II), %
			solvent, ml	CrO_3, g	AcOH, ml	H_2O, ml	
1	20	1,3	Acetone 300	0,5	80	20	25—26
2	20	4		0,5	80	20	20—25
3	20, acc. to Barton	24	—	0,6	300	5	22
4	80, acc. to Burawoy	3	—	0,6	300	5	24
5	20	1,5	Acetone 300	0,5	25	6	45—46
6	20	1,5		0,3	25	6	40
7	20	1,3	Acetone 200	0,5	3	0,6	30
8	4	4		0,5	25	6	37
9	20	0,3—0,5		0,5	—	0,6	56—58
10	80	10	Benzene 100	0,5	—	25	8
11	80	10—12	Cyclohexane 100	0,5	—	25	10—12
12	100	1	Dioxane 100	0,5	—	10	39
13	35	1	Ether 100	0,6	—	15—20	70—75
14	20	Two weeks	Pyridine 100	0,5	—	—	Traces
15	20, acc. to Fieser	24	Benzene 28	1,2 g $Na_2Cr_2O_7 \cdot 2H_2O$	28	3	28

*All the figures are calculated to 1 g of ergoesterol acetate.

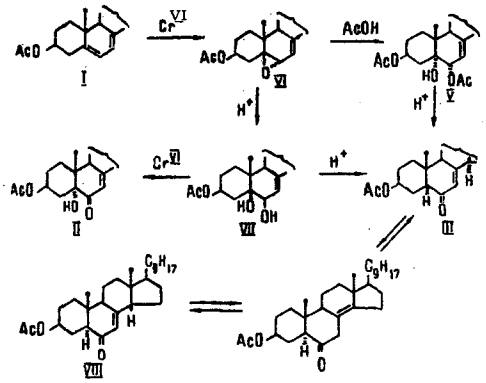
zinc in acetic acid, proved to be identical in all respects with compound (III). Thus, on slow heterogeneous oxidation a compound with a lower degree of oxidation is formed in addition to Burawoy's ketone.

The appearance of a similar compound (according to TLC) was also observed in the slow oxidation of ergosterol by Sarret's method (CrO_3 + C_5H_5N, experiment 14). Unfortunately, the yield of intermediate product was very low.

Oxidation in ether [a solvent capable, on the one hand, of dissolving a small amount of water - (up to 3%) - and, on the other hand, of forming an onium compound with the oxidizing agent and thereby serving as a carrier of the $HCrO_4^-$ ion] sharply raised the yield of Burawoy's ketone to 70-75% (with a reaction time of 45-60 min), making it an extremely accessible compound.

In 1953, Fieser et al. [8] showed that in the oxidation of ergosterol acetate (I) in a mixture of acetic acid and benzene in addition to the ketone (II) ergosta-7,22-diene-3β,5α,6α-triol 3,6-diacetate (V) was also formed. They suggested that the triol (V) is a product of the acidolysis of the 5,6-oxide (VI) formed as an intermediate.

Since in the slow oxidation of the acetate (I) in benzene we were still able to isolate the ketone (III), we have suggested the following sequence of the multistage oxidation of compound (I):



The conversion of the diacetate (V) into the α,β-unsaturated ketone (III) can apparently be explained by the pinacolone rearrangement of substance (V) or (VII), which takes place readily in an acid medium.

In order to confirm this scheme, Fieser's conditions for the oxidation of ergosterol acetate (I) were reproduced, and the diacetate (V) was isolated. As we have shown, when it is heated for a short time (10 min) with p-toluenesulfonic acid in benzene (without the azeotropic distillation of water) or under the conditions of the oxidation, keto acetate (III) is actually formed, which supports the scheme given above. More prolonged boiling of the reaction product (30 min and above) leads to a mixture of the keto acetate (III) and a more polar product which is apparently the 14β isomer of (III) – (VIII). The ease of such an isomerization under the influence of acids has been established recently [9].

EXPERIMENTAL

Typical Experiment on the Oxidation of the Acetate (I) in Acetone. A solution of CrO_3 in the amount given in the table in 80% acetic acid or water was added (one portion) to a solution of 1 g of ergosterol acetate (I) in 300 ml of acetone (previously treated with solid CrO_3 at room temperature for 2 days and then distilled). The reaction mixture was left at room temperature or was heated (under Burawoy's conditions, see experiment 4) at 80° C for the time shown. After the end of the reaction, it was poured into water. The product was extracted three times with chloroform, and the extract was washed with aqueous $NaHCO_3$ solution and with water, dried over calcined $MgSO_4$, and evaporated. The resulting oil, which partially crystallized, was chromatographed on a column containing 20 g of silica gel. Benzene and benzene-chloroform (1 : 1) eluted compounds close in polarity to ergosterol acetate, while elution with pure chloroform gave the acetate of the ketone (II) with mp 262-263° C (from ethyl acetate), $[\alpha]_D^{20}$ – 3.1° (c 1; $CHCl_3$). Literature data: mp 264° C [1]. UV spectrum: λ_{max} (in ethanol) 252 nm (ε 15,800) and a shoulder at 308 nm (ε 123). IR spectrum (KBr tablet, cm^{-1}): 3428 (OH group), 1737 and 1259 (CH_3COO group), and 1683 (α,β-unsaturated

CO group). In experiments 1 and 5 (see Table 1), the formation of a product of the oxidation of the acetate (I) more polar than substance (II) was found; its structure is being studied.

Oxidation of the Acetate (I) in Benzene. A solution of 0.5 g of CrO_3 in 25 ml of water was added to a solution of 1 g of ergosterol acetate in 100 ml of benzene, and the mixture was boiled with stirring for 10 h. After cooling, the organic layer was separated off and the aqueous layer was twice extracted with benzene. The combined extract was washed with aqueous sodium bicarbonate solution and with water and was dried with calcined Na_2SO_4 and evaporated. The residue was chromatographed on a column containing 20 g of silica gel. Benzene eluted 0.36 g of an oil which was treated with cold methanol. The crystals that deposited were filtered off and recrystallized from methanol. This gave 150 mg of the keto acetate (III) with mp 183-184°, $[\alpha]_D^{20} - 15°$ (c 1.5; $CHCl_3$). UV spectrum (in ethanol): λ_{max} 246 nm (ε 14,500) and a shoulder at 305 nm (ε 130); UV spectrum (mull in paraffin oil, cm^{-1}): 1743 and 1253 (CH_3COO group) and 1695 (α,β-unsaturated CO group).

The subsequent elution of the column with chloroform yielded 20 mg of a mixture of ketones and 80 mg of the ketone acetate (II) with mp 261-263° (see Table 1, experiment 10).

The ketol acetate (III) prepared by Barton's method [2], with mp 183-184.5° C (from methanol), proved to be identical in all respects with the compound obtained above.

Oxidation of the Acetate (I) in Ether. A solution of 1 g of ergosterol acetate in 100 ml of ether was boiled with a solution of 0.6 g of CrO_3 in 20 ml of water with stirring for 1 h and was then diluted with 200 ml of water. The product was extracted three times with ether and was chromatographed on silica gel as described above. This gave 812 mg of the hydroxy ketone acetate (III) with mp 262-263° C (from ethyl acetate). Yield 72%.

Oxidation of the Acetate (I) with Sodium Dichromate. A solution of 1.2 g of $Na_2CrO_7 \cdot 2H_2O$ in 3 ml of water was added (in one portion) to a solution of 1.0 g of ergosterol acetate in 28 ml of benzene and 28 ml of glacial acetic acid. The mixture was left at room temperature for a day. The product obtained after the usual working up was washed with cold ethyl acetate, and 0.15 g of the ketone acetate (II) with mp 261.5-263° C was filtered off. The mother solution was evaporated and the residue was chromatographed on silica gel. Benzene eluted 0.16 g of ergosterol acetate, benzene–chloroform (2:1.5) eluted 0.143 g of the diacetate (V), and chloroform eluted 80 mg of the ketol acetate (II).

The diacetate (V), after crystallization from 80% methanol, had mp 180-181.5° C, $[\alpha]_D^{20} + 40°$ C (c 1.4; $CHCl_3$). Literature data: mp 180.5-181° C [10]. IR Spectrum (mull in paraffin oil), cm^{-1}: 3480 (OH group), 1728, 1735 and 1258, 1247 (two CH_3COO groups).

Dehydration of the Ketone (II). A. A solution of 0.33 g of the ketone (II) in 20 ml of glacial AcOH containing two drops of conc. sulfuric acid was heated at 80-100° C for 5-10 min and was then poured into water. The product was extracted with ether, and the extract was washed with water, aqueous sodium carbonate solution, and water again, dried over calcined sodium sulfate, and evaporated. This gave 0.23 g of an oil which was percolated through a column filled with silica gel, and chloroform eluted 0.16 g of the yellow oily ketone (IV). UV spectrum (ethanol): λ_{max} 249 nm (ε 19,100); IR spectrum (paraffin oil), cm^{-1}: 1728 and 1249 (CH_3COO group), 1690 (α,β-unsaturated CO group), 973 (trans olefin).

B. A mixture of 0.1 g of the ketone (II) and 0.5 ml of BF_3 etherate was boiled in 20 ml of hexane for 1 h, cooled to room temperature, and poured into water. The product was extracted with ether, and after working up similar to the preceeding case, 78 mg of ketone (IV) identical with the sample described above was obtained.

C. A solution of 80 mg of the ketone (II) in 5 ml of dioxane heated to the boil was treated with 0.5 ml of 5% aqueous sulfuric acid, and the mixture was boiled for 10 min and poured into water. Ether extraction and working up as described above gave 60 mg of the ketone (IV).

Rearrangement of the Diacetate (V). A. A mixture of 80 mg of the diacetate (V) and 15 mg of p-toluenesulfonic acid in 25 ml of benzene was boiled for 10 min. The solution was cooled and neutralized with sodium carbonate. The benzene layer was dried and evaporated. This gave an oil, which was chromatographed on silica gel. Benzene–chloroform (1:1) eluted 20 mg of the ketone acetate (III), with mp 181-182° C (from methanol), giving no depression of the melting point with the sample described above.

The product obtained by the more prolonged heating of the diacetate (V) with p-toluenesulfonic acid (1.5 h) was shown by thin-layer chromatography to contain a compound somewhat more polar than the acetate (III).

B. A mixture of 50 mg of the diacetate (V) in 5 ml of benzene and 15 mg of CrO_3 in two drops of water was boiled for 1 h. According to thin-layer chromatography [chloroform–ethyl acetate (5 : 1) system] in a fixed layer of silica gel, the reaction product contained both the acetate (III) and the product of its isomerization.

SUMMARY

1. The conditions for the oxidation of ergosterol acetate with chromic anhydride have been studied, and it has been shown that the yield of the 3β-acetoxy-5α-hydroxyergosta-7,22-dien-6-one (Burawoy's ketone) so obtained is a maximum when oxidation is performed in ether.

2. On the basis of the results obtained, a multistage mechanism for the oxidation of ergosterol acetate is suggested.

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