

STEROIDS. XVIII*

Effect of Ultraviolet Radiation on Aqueous Solution of Sodium 17 α -Hydroxy-11-desoxycorticosterone-21-sulfate

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Sodium 17 α -hydroxy-11-desoxycorticosterone-21-sulfate was irradiated with ultraviolet light in aqueous medium. During the reaction the 21-sulfate ester is hydrolyzed. The main products of the irradiation are androst-4-en-3,17-dione and the hydrolysis product Reichstein S.

A number of studies have dealt with the transformations of steroids, both in the solid state and in solution, as a result of irradiation with γ , β and X-rays [1—3]. The effect of the radiation is strongly dependent on the nature of the solvent.

The reactions in aqueous media in the presence of O₂ differ considerably from those under anaerobic conditions. In general, reduction processes occur in anaerobic aqueous solution (dehydroxylation, hydrogenation of double bonds), while in the presence of O₂ the main reaction path involves oxidation and hydroxylation, and the formation of the keto group and unstable peroxides. The breakdown of the steroid skeleton may result, as also the incorporation of active groups from the solvent (*e.g.* acetylation in a glacial acetic acid medium).

The compound used in the present experiments was an ester of a corticosteroid sulfate, which was well soluble in water. This afforded the possibility of an extensive study of the reaction induced by ultraviolet radiation, as well as comparison with the nonconjugated steroid reaction. The effects of ultraviolet light (UV light) on aqueous solutions of steroids had previously not been investigated in any great detail, such work having been concentrated mainly on the aromatic compounds [4].

Experimental

Sodium 17 α -hydroxy-11-desoxycorticosterone-21-sulfate (Reichstein S-21-sulfate) was prepared by a modification [6] of the procedure described by KORNEL *et al.* [5] for 17-hydroxycorticosterone.

The effects of UV light were studied in solutions of various pH containing certain additives. Each solution contained 10⁻² M sodium Reichstein S-21-sulfate in distilled water, the other characteristics being as follows (see: Table 1):

A: pH = 3.8; B: pH = 7.0; C: 10⁻² M FeCl₃, pH = 2.0;

D: 2 \times 10⁻³ M H₂O₂, pH = 7.0.

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Irradiation was carried out from a distance of 10 cm with a quartz lamp Type AKO2 (Budapest, Hungary). The surface of the solution was in free contact with the air. The temperature of the solution during the irradiation was 40°C, and the duration of the irradiation was 1—72 hours.

At the end of the reaction the free steroids were extracted with methylene chloride (3 times with an equal volume), the extracts were dried over Na₂SO₄, and the solvent was evaporated.

The method of KORNEL [7] was applied for the extraction of the steroid sulfate and for the hydrolysis of the sulfate group.

A 10⁻² M solution of Reichstein S in water was prepared: dissolution was induced with a little propylene glycol; the pH of the final solution was adjusted to 7.0. After irradiation the product was extracted 3 times with an equal volume of methylene chloride.

The products were separated by thin-layer chromatography on Silicagel HF₂₅₄, with chloroform-ethanol (9:1), methylene chloride-acetone (8:2), benzene-ethanol (95:5) as solvents. For qualitative separation and identification a 250 mμ layer-thickness was used, whereas for preparative separation the thickness of the absorbents was 1 mm. Elution from the layer was achieved with methanol or a methanol-methylene chloride (1:1) mixture.

Development: spraid with 25% H₂SO₄ solution, dried at 100°C; UV fluorescence; tetrazolium chloride reaction; alkaline fluorescent reagent [8].

The compounds were identified on the basis of their infrared spectra [9] and melting points [10].

Quantitative determinations were carried out after methanol elution, by measurement of the absorption at 240 mμ and comparison with a standard curve.

Results

The following free steroids were found in the solutions of sodium Reichstein S-21-sulfate after irradiation with ultraviolet light. Two main products are formed: androst-4-en-3,17-dione (androstendione, AD) and 17α-hydroxy-11-desoxycorticosterone (Reichstein S). A further characteristic product consisted of two substances (one of them marked by X in the Table) with almost the same chromatographic properties as those of cortisol; the structures have not been specifically determined till now, but the infrared spectra (almost identical) indicate the presence of the unchanged 4-en-3-keto group, while a carboxyl group has probably been formed, too (most likely in position 20).

The "other" compounds include chromatographically less well-defined substances, formed in amounts of about 1—5%. During the irradiation of the solution the extinction at 240 mμ showed a slow, but constant decrease. This change could not be caused by the main products; it was due to the hydrogenation of the olefinic bond in position 4, resulting in the conjugation and reduction of the characteristic absorption at 240 mμ. The extinction was reduced by about 1—5%, in agreement with the above.

When the steroid-sulfate remaining in the solution after the methylene chloride extraction is extracted and then hydrolyzed, only Reichstein S is obtained, showing that no product containing the sulfate ester group is formed.

Among the products of the irradiation of nonconjugated Reichstein S, only androstendione is found in significant amounts; the other products are not characteristic, and are difficult to separate by chromatography.

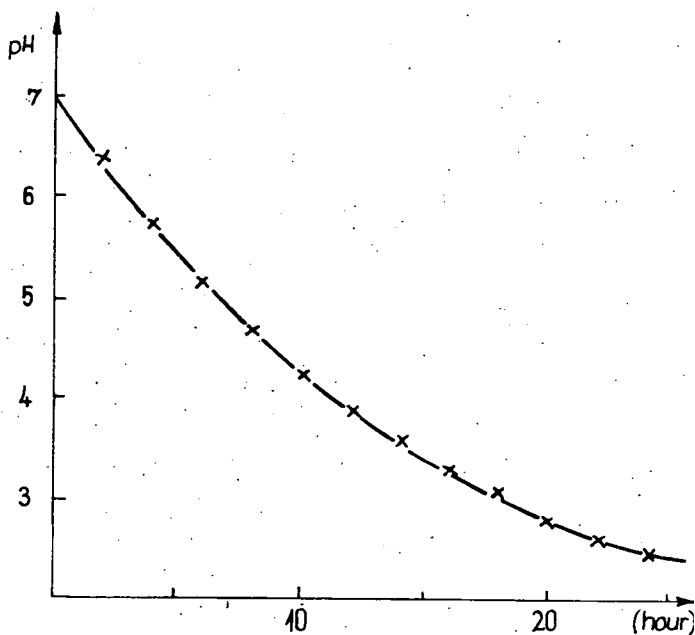


Fig. 1

The results given in Table I refer to a 24 hours irradiation. The amounts of the products increase proportionally to the time of the treatment. (The relative ratios gradually shift towards the increase of the amount of androstendione.)

Table I

Mixture Product %	Na Reichstein S-21- sulfate				Reichstein S compound
	A	B	C	D	E
AD -	19	14	11	10	1
Reichstein S	12	9	7	8	—
X	5	4	3	1	—
Other	5	3	2	15	1
Total	41	30	23	34	2

It can be seen that the reaction proceeds to the greatest extent in the acidic aqueous medium.

The change in pH during the reaction was measured (Fig. 1), a constant shift to lower pH values being apparent (similarly in solutions of different composition), in agreement with the hydrolysis of the sulfate ester.

Control experiments (40°C, light-free) showed that no changes took place in the above systems.

Discussion

Work similar to the above has been performed by ALLINSON *et al.* [2]. In their experiments aqueous solutions of cortisone and desoxycorticosterone were irradiated with X-rays. The transformations found in the presence of air were: decomposition of the 4-ene-3-keto system, formation of a very small amount of the 17-keto group by side-chain splitting, and the formation of a product which was presumed to be of a hydroperoxide type.

Our experiments indicate that a considerable proportion of the corticosteroid-21-sulfate is converted by side-chain splitting to androstenedione. The decomposition of the 4-ene-3-keto system (by hydrogenation) occurs only to a small extent. The presence of the original steroid in considerable amount as a result of simple hydrolysis makes it probable that the process is primarily hydrolysis, this being followed by the oxidative decomposition of the side-chain. The formation of the sulfate ester means that the steroid is soluble and its reactivity is thereby considerably increased.

This hydrolytic process is of interest, because in aqueous medium no hydrolysis of steroid sulfates is found, even if the medium is acidic or heated to 100°C. The hydrolysis is therefore carried out in an organic medium. At the same time, however, a trace of water is always necessary in the case of solvolysis in the organic phase. The rate of the process is controlled by the number of available lone electrons on the ethers [11–13].

It is to be expected that the steroid will be attacked by the radicals formed on the action of the ultraviolet light during the hydrolysis process.

It can be established from our results, as well as from the literature that certain preferred positions are attacked in the reactions due to the irradiation. The main target of attack in the system studied was the side-chain of the steroid.

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ВЛИЯНИЕ УЛЬТРАФИОЛЕТОВОГО ОБЛУЧЕНИЯ НА ВОДНЫЙ
РАСТВОР НАТРИЕВОЙ СОЛИ 17-ГИДРОКСИ-ДЕЗОКСИКОРТИКОСТЕРОН-
21-СУЛФАТА

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17 α -гидрокси-11-дезоксикортикостерон-21-сульфат-соль (Рейштейн-S-сульфат-Na-соль) в водной среде подвергалась ультрафиолетовому облучению. Во время облучения сульфат эфир гидролизуется. Вторым главным продуктом облучения является андрост-4-ен-3,17-дион и небольшое количество другого неидентифицированного вещества.