# STEROIDS. XV*

## Hydroxylation of dehydroepiandrosterone and dehydroepiandrosterone-3β-sulphonic acid sodium salt (Part 10)**

*(Preliminary Communication)*

By

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Hydroxylation of dehydroepiandrosterone and dehydroepiandrosterone-3β-sulphonic acid sodium salt has been examined in different „in vitro" systems. In Fenton-Cier and Udenfriend systems considerable, while on the effect of adrenal slices milder 16α-hydroxydehydroepiandrosterone formation could be observed. 7α-, or 7β-hydroxylation could not be detected.

A number of hydroxylation processes are involved in steroid metabolism catalysed by specific hydroxylases. The hydroxylation takes place in several organs, as proved by various in vitro experiments [1].

During the metabolism of dehydroepiandrosterone mainly the C-7 and C-16 atoms are hydroxylated. For instance, in mammalian liver 7α-hydroxylation [2], in placenta and liver 16α-hydroxylation and 16β-hydroxylation [3] and in human skin 7α-hydroxylation and 16α-hydroxylation [4] were detected. SULCOVA [5], studying homogenates of different organs, observed 7α-hydroxylation of dehydroepiandrosterone in liver, kidney, spleen, lung, heart, blood and in muscle, while STARKA [6] pointed out 7α-hydroxylation in adrenal glands, and COLAS [7] observed the 16α-hydroxylating ability of rat liver slices.

Very scarce data are, however, available about the in vitro hydroxylation of steroids under well defined conditions [8].

In our experiments we examined the effect of some non-specific hydroxylating systems as well as of adrenal slices on dehydroepiandrosterone and dehydroepiandrosterone-3β-sulphonic acid sodium salt.

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* Part XIV.: M. Marián, B. Matkovics, Sz. I. Varga: Acta Phys. et Chem. Szeged **17,** 85 (1971).

** Part 9.: B. Matkovics and Gy. Göndös: Biol. Közl. **15,** 23 (1967) (in Hungarian).

## *Experimental*

Hydroxylation systems were used as follows:

a) *Fenton system* [9]: 3 mmole $FeSO_4 \cdot 7H_2O$, 6 mmole $H_2O_2$, 3 mmole substrate and 100 ml 4*N* $H_2SO_4$. Reaction time: 30—60 minutes at room temperature or at 37°C.

b) *Modified Fenton system* [9]: 1 mmole $FeSO_4 \cdot 7H_2O$, 1 mmole EDTA.2Na, 2 mmole $H_2O_2$, 2 mmole substrate and 100 ml 0.1 M phosphate buffer (pH 7.2) Reaction time and temperature as above.

c) *Fenton—Cier system* [10]: 1 mmole $FeSO_4 \cdot 7H_2O$, 1 mmole EDTA.2Na, 0.5 mmole L-ascorbic acid, 2 mmole $H_2O_2$, 2 mmole substrate and 100 ml 0.1 M phosphate buffer (pH 7.2). Reaction conditions as before.

d) *Udenfriend system* [11]: 0.5 mmole $FeSO_4 \cdot H_2O$, 2.6 mmole EDTA.2Na, 5.6 mmole L-ascorbic acid, 2 mmole substrate and 100 ml 0.1 M phosphate buffer (pH 6.7). Aeration by shaking. Reaction time: 2—24 hours at room temperature or at 37°C.

e) *UV irradiation:* 1 mmole $FeCl_3$, 1 mmole EDTA.2Na, 1 mmole substrate, 100 ml distilled water, pH 7.0, 24 hours.

f) *Incubation with adrenals:* 10 g finely cut ox adrenals, $10^{-4}$ M substrate, $10^{-3}$ M potassium-fumarate, $10^{-3}$ M L-ascorbic acid, 0.001 g ascorbic acid oxidase, $2 \cdot 10^{-5}$ M NADH at 37°C. The solutions were shaken for five hours in oxygen stream.

Dehydroepiandrosterone was dissolved in propylene glycol (1,2-propanediol). The products were extracted with peroxide-free ether.

The TLC separation was carried out on Silica gel G (Merck) adsorbent, using $20 \times 20$ cm plates with 0.25 mm adsorbent thickness. For preparative purposes the mentioned adsorbent was used in 1 mm thickness [12].

Solvent systems: benzene-ethyl acetate (3:2) [4], cyclohexane-chloroform-acetic acid (7:2:1).

After separation, the plates were dried at 110°C for about 20 minutes, the spots were developed by iodine vapour or UV light, the plates sprayed with 25% sulphuric acid and dried.

In some experiments dehydroepiandrosterone sulphate ester was used as substrate.

The methods described below proved to be fairly useful in preparing different steroid sulphate esters [13—16].

### *Dehydroepiandrosterone*

Gift of G. RICHTER Pharmaceutical Works (Budapest), repeatedly recrystallized from methanol, m. p.: 152—153°C.

The preparative methods for both salts, treated in detail in [16], are only briefly mentioned here.

Dehydroepiandrosterone-3$\beta$-sulphate was prepared with chlorosulphonic acid in pyridine and the salt was precipitated with petroleum ether. M. p.: 208 °C. The substance dissolves well in water.

The dehydroepiandrosterone-3$\beta$-sulphonic acid pyridinium salt, on the other hand, was converted to the sodium salt of the sulphate with sodium carbonate at a pH of about 7.5 in a saturated aqueous sodium sulphate solution. The salt was

finally precipitated similarly with petroleum ether. Mp.: 201°C. The salt is also well soluble in a phosphate buffer.

When the dehydroepiandrosterone sulphate salt was used for hydroxylation, the products were isolated in the following manner: The solution was saturated with sodium sulphate and then the sodium salt of the sulphates flocculated, which could be easily filtered. Then the solvolysis of sulphates [17] follows. For this purposes the sulphate salt was dissolved in 50 ml 2*N* sulphuric acid and extracted with ethyl acetate (about 60 ml) and about 100 ml ether previously saturated with water. The mixture was allowed to stand at room temperature for three days. Ethyl acetate-etheric solution was dried with anhydrous sodium sulphate, evaporated and chromatographed. The substances were identified by comparing their chromatographic properties with standards in different systems.

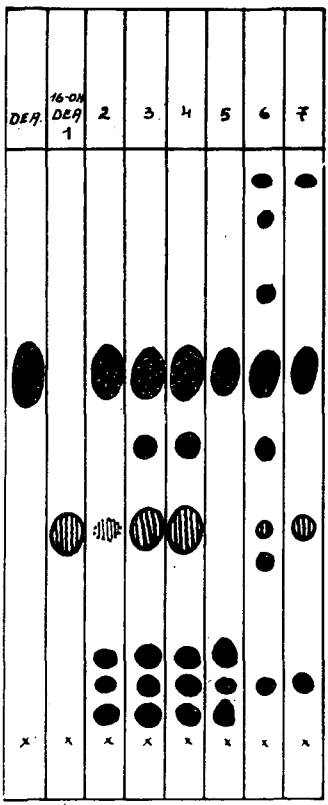


*Fig. 1*

## *Results*

Special attention was given to the formation of 16α-, 7α- or 17β-hydroxy derivatives. In this respect the following observations were made in different systems:

In the Fenton system no hydroxylation took place.

In the modified Fenton system 16α-hydroxydehydroepiandrosterone formed but only in a small amount (See Fig. 1 column 2).

In the Fenton-Cier and Udenfriend systems there was considerable 16α-hydroxylation (See Fig. 1 columns 3 and 4).

On the effect of UV light no 16α-hydroxylation took place (See Fig. 1 column 5).

With adrenal incubate there was a slight 16α-hydroxylation (See Fig. 1 column 7).

With adrenal incubate the dehydroepiandrosterone-3β-sulphonic acid sodium salt is also hydroxylated in the C-16 position (See Fig. 1 column 6). On the plate the chromatogram of the substances after solvolysis can be seen.

## *Discussion*

Up to this time merely the hydroxylation of a few corticosteroids has been studied in the mentioned chemical and in vitro systems [18, 19], however, the identification of the compounds was not quite convincing. Our experiments proved that the in vitro systems used by us are suitable for hydroxylation of certain steroids, which is unequivocally proved by 16α-hydroxylation of dehydroepiandrosterone.

Dehydroepiandrosterone-3$\beta$-sulphonic acid sodium salt was also hydroxylated the effect of adrenal slices.

Our experiments also proved that steroid sulphates, as water-soluble substrates can be easily prepared and are very useful substrates of different in vitro transformations. It is not surprising that more and more steroid sulphates have been detected in the last years and that they play a decisive role in the metabolism of steroids. Among others, a number of articles dealt with the biotransformation of dehydroepiandrosterone sulphate [20—22].

Data obtained so far are well supported by our experiments with adrenal slices, since we have proved the in vitro 16$\alpha$-hydroxylation of dehydroepiandrosterone. As supposed by several authors, L-ascorbic acid plays an important role in the steroid hydroxylations taking place in adrenals, but the role of the latter has not been completely clarified as yet. Our experiments were based on the concept that the electron transition between NADH and ascorbic acid is an important step of the formation of the hydroxylating agent. Really, the in vitro formation of NADH is accelerated by addition of ascorbic acid oxidase. We believe that our experiments described above furnish some additional data to the androgen biosynthesis depending on ascorbic acid in the adrenal gland.

The chemical systems mentioned above may also lead to the formation of several other derivatives which are not yet identified. It can also be supposed that the 16$\alpha$-hydroxylation of dehydroepiandrosterone is a consequence of a non-specific hydroxylation and it plays an important role in the inactivations of androgens. Further experiments are necessary to clarify these problems.

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# СТЕРОИДЫ XV.
# ГИДРОКСИЛИРОВАНИЕ НАТРИЕВОЙ СОЛИ ДЕГИДРОЭПИАНДРОСТЕРОНА И ДЕГИДРОЭПИАНДРОСТЕРОН-3$\beta$-СУЛФАТА (ЧАСТЬ 10.).

(Предварительное сообщение)

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Исследовалось гидроксилирование натриевой соли дегидроэпиандростерона и дегидроэпиандростерон-3$\beta$-сулфата в разных „in vitro" системах. Показали образование 16$\alpha$-гидроксидегидроэпиандросте-рона в системах Фентона-Сиэ и Уденфренда в значительном количестве, и образование незначинетельного количества 16$\alpha$-гидроксидегидроэпиандростерона под воздействием срезов надпочечника. 7$\alpha$- и 7$\beta$-гидрокси-производные не образовывались.