kindly supplied by Dr. G. Slomp, The Upjohn Company, Kalamazoo, Mich., USA. After the elimination of the secondary β-hydroxyl in PGE the double bond migrates into the position indicated in (III).

The two isomeric compounds PGF, and PGF₂ must thus have structure IV and only differ in the steric position of the hydroxyl formed by reduction of the carbonyl. Compounds of this type do not seem to have been found in nature earlier. A cyclopentenone nucleus occurs in the "pyrethrins" present in Pyrethrum flowers, cf. pyrethrolone (V).

It does not appear unlikely that the prostaglandins are representatives of a group of hormonal compounds of general importance. Their high biological activity makes pharmacological exploration of the activity of similar compounds of interest.

A full report of this work will be published in this journal.

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Studies on Ester Sulphates

13. On the Enzymic Synthesis of Steroid Disulphates *

BO WENGER and HARRY BOSTRÖM

From the Department of Metabolic Research, Wenner-Gren Institute, University of Stockholm and the Pediatric Clinic, Karolinska Sjukhuset, Stockholm, Sweden

Formation of monosulphates of certain steroids in microsome-free liver extracts has been reported by several workers 1-4. Evidence of the occurrence in rat liver supernatant fluid of two different steroid sulphokinases was presented by Nose and Lipmann in 1958 5. One of these enzymes, dehydroepiandrosterone sulphokinase, was also shown to react with pregnenolone and androsterone, both of which have a hydroxyl group in the 3-position. The other enzyme was capable of sulphurylating the phenolic hydroxyl group of oestrone. On the other hand, no results with bearing on the enzymic in vitro formation of disulphates of steroids have so far been reported in the literature. In the present paper, an account is given of certain findings indicating the in vitro synthesis of steroid disulphates.

Androst-5-ene-3β-17β-diol **, in a final concentration ranging from 0.016 to 0.083 mM, was incubated for 120 min at 37.5°C in an open test tube, in a medium containing the following constituents: (1) 50 μl of a buffer solution containing equal parts of 0.3 M KH₂PO₄ (pH 6.8), 0.03 M K₂SO₄ and 0.005 M MgCl₂; (2) 20 μl of 0.02 M ATP disodium salt;***; (3) 50 μl of microsome-free supernatant fluid of female rat liver, homogenized in 2–3 volumes of 0.16 M KCl containing 0.001 M EDTA (pH 7.0), centrifuged at 105 000 g for 60 min, and diluted to contain 10 μg of protein per μl; (4) 0.1 mM of carrier-free ³⁵S-labelled sulphate †. Final volume of the incubation mixture: 120 μl. The steroid was added to the empty

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** Kindly placed at our disposal by Schering A.-G., Berlin, Germany.
*** Obtained from Sigma Chemical Company, St. Louis, U.S.A.
† Obtained from the Radiochemical Centre, Amersham, England.

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so far tested in our laboratory migrate to positions identical or close to the position of MS in paper chromatograms and electrophoretic strips. It is therefore assumed that MS constitutes a monosulphate of androstr-5-ene-3β,17β-diol. This assumption is further substantiated by the fact that the compound was desulphated by incubation with commercial steroid sulphatase * (37.5°C; pH 7.0; 120 min.).

With respect to the identity of DS, its electrophoretic migration at pH 5.5 suggested a conjugate of greater negative charge than MS. Furthermore, the chromatographic data indicated a higher polarity of DS than MS. On the basis of these facts, it appeared probable that DS was, in fact, a desulphated conjugate. Additional support for this view was obtained by the following findings. (1) When the labelled MS compound was incubated in the sulphurylating system in the absence of labelled inorganic sulphate, the radioactivity was almost quantitatively transformed into DS (Table I). (2) When the labelled MS compound was incubated as above in the sulphurylating system now containing labelled inorganic sulphate, the radioactivity of DS formed significantly exceeded that of DS in the former experiment. (3) Hydrolysis of DS in an acetate buffer (pH 5.5) for 4 h at 100°C split DS into a

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**Table 1. Transformation of steroid monosulphate into steroid disulphate.** Distribution of radioactivity between the steroid sulphate fractions MS and DS in the incubation medium, before and after incubation for 120' at 37.5°C in the sulphurylating system described in the text except that ³⁵S-labelled sulphate and androstr-5-ene-3β,17β-diol were replaced by partially purified ³⁵S-labelled MS.

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Before incubation</th>
<th>After incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>MS</td>
<td>DS</td>
</tr>
<tr>
<td>1</td>
<td>72</td>
<td>28</td>
</tr>
<tr>
<td>2</td>
<td>95</td>
<td>5</td>
</tr>
</tbody>
</table>

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* Obtained from Schering A.-G., Berlin, Germany.

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* Acta Chem. Scand. 16 (1962) No. 2
monosulphate and inorganic sulphate. (4) Desulphation of DS with commercial steroid sulphatase* at 37.5°C, pH 7.0, for 120 min could be demonstrated.

It was concluded from the aforementioned facts that DS is identical with a disulphate of androst-5-ene-3β,17β-diol. According to Bitman and Cohen, hydrolytic splitting of steroid sulphates at pH 5.5 under the conditions in question is limited to compounds containing β configuration of the sulphate, and a β,γ-unsaturation to this grouping. The monosulphate left in the hydrolysate, which contained approximately half the activity of DS added, is therefore assumed to be androst-5-ene-3β,17β-diol-17-sulphate. When the MS compound formed during incubation in the sulphurylating system was treated in the same way, almost all radioactivity was revealed as inorganic sulphate, indicating that this monosulphate was probably androst-5-ene-3β,17β-diol-3-sulphate.

Fig. 2 shows the formation in the sulphurylating system of MS and DS, respectively, as a function of androst-5-ene-3β,17β-diol concentration. Formation of DS was found to be optimal at a substrate concentration of approximately 0.035 mM. In low concentrations of the substrate, the only steroid conjugate detected was DS. This might indicate that all MS formed from androst-5-ene-3β,17β-diol at low substrate levels was transformed into DS. At higher concentrations, on the other hand, when DS formation had reached its maximum, greater amounts of MS were found (Fig. 2).

The foregoing hydrolysis data support the view that MS consisted mainly of the 3-sulphate of androst-5-ene-3β,17β-diol. This fact, as well as the shape of the substrate concentration curves, may imply that two different sulphokinases are operating in the sulphurylating system, one with the ability to transfer sulphate to the 3β-hydroxyl group, and the other to transfer it to the 17β-hydroxyl group. Of these enzymes, the latter seems to be the ratelimiting one for formation of the disulphate in the sulphurylating system used in the present study.

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