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_1 and PGF_2 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 cyclopentenolone nucleus occurs in the "pyrethrins" present in *Pyrethrium* 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 *

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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, dehydro*epi*androsterone 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 open test tubes, in a medium containing the following constituents: (1) 50 µl of a buffer solution containing equal parts of 0.3 M KH_2PO_4 (pH 6.8), 0.03 M K_2SO_4 and 0.005 M $MgCl_2$; (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 0.15 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 mC of carrier-free ^{35}S-labelled sulphate †. Final volume of the incubation mixture: 120 µl. The steroid was added to the empty

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*** Obtained from Sigma Chemical Company, St. Louis, U.S.A.

† Obtained from the Radiochemical Centre, Amersham, England.

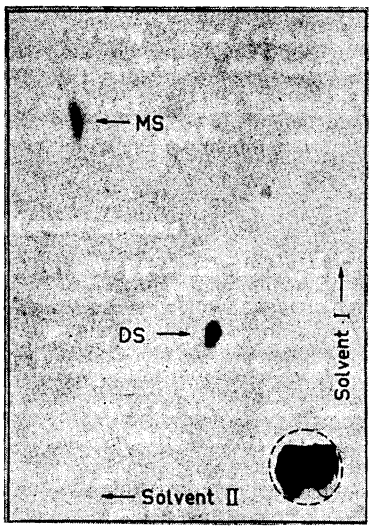

Fig. 1. Autoradiogram of a two-dimensional paper chromatogram, illustrating the separation of compounds MS and DS formed by incubation of androst-5-ene-3β,17β-diol in the sulphurylating system described in the text. The area indicated by the dotted lines contains inorganic and active sulphate. Solvent I: Phenol-water (400 g + 100 g); solvent II: Butanol-acetic acid-water (12/3/5 by vol.).

test tubes in ethanolic solution, the ethanol being evaporated *in vacuo* before addition of the other components.

The ester sulphates formed in the reaction mixture were separated by 2-dimensional ascending paper chromatography (solvent I: phenol-water, 400 g + 100 g; solution II: butanol-acetic acid-water, 12/3/5 by vol.), and by high-voltage electrophoresis, after which they were localized by autoradiography as previously described [6].

The radioactive areas indicated in Fig. 1 were eluted with water, and aliquots of the eluates were counted in a G.-M. counter after plating (infinite thinness) on frosted aluminium plates.

Incubation of androst-5-ene-3β,17β-diol in the sulphurylating system as described above gave rise to two different ^{35}S-labelled compounds (MS and DS), separable on paper chromatograms (Fig. 1) and electrophoretic strips. The sulphates of all sulphate-accepting monohydroxy steroids so far tested in our laboratory migrate to positions identical or close to the position of MS in paper chromatograms and electrophoretic strips [7]. It is therefore assumed that MS constitutes a monosulphate of androst-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 disulphated 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 1). (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

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 ^{35}S-labelled sulphate and androst-5-ene-3β,17β-diol were replaced by partially purified ^{35}S-labelled MS.

Expt. No.	Per cent of total steroid sulphate radioactivity			
	Before incubation		After incubation	
	MS	DS	MS	DS
1	72	28	11	89
2	95	5	7	93

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

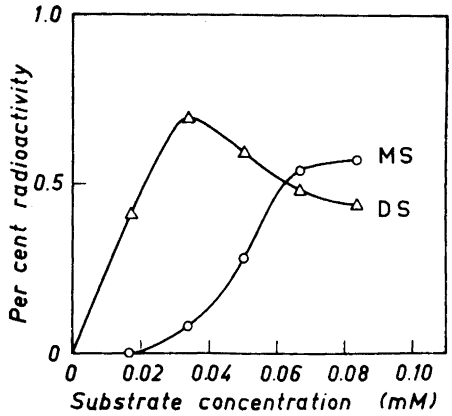

Fig. 2. Formation of MS and DS during incubation in the sulphurylating system described in the text, in relation to different androst-5-ene-3β,17β-diol concentrations of the medium. MS and DS are expressed in per cent of total radioactivity of the incubation mixture.

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 [8], 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 aforegoing 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 rate-limiting one for formation of the disulphate in the sulphurylating system used in the present study.

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