STEROIDS. XCIII.[1] INTRODUCTION OF THE CORTICAL HORMONE SIDE-CHAIN

Sir:

Introduction of the important 21-hydroxyl function into Δ^4-3-keto steroids with the pregnane (C-20 ketone) side chain has been accomplished microbiologically[2] or by a chemical reaction sequence[3] involving selective oxalate condensation at C-21 followed by iodination, hydrolysis and iodide displacement.

We wish to report a simple procedure for the direct preparation of 21-iodo steroids and thus of the cortical hormone side-chain. Iodination of 17α-hydroxyprogesterone in a mixture of tetrahydrofuran–methanol with an excess of iodine in the presence of a base such as solid calcium oxide or aqueous sodium hydroxide gave mainly the 21-iodo steroid which without purification was converted directly to Reichstein's substance "S" acetate[4] by treatment with potassium acetate in acetone followed by brief reaction with aqueous-methanolic bisulfite to remove residual iodine. Over-all yields of 60% have been consistently realized in this reaction. This thus constitutes a facile three stage[5] conversion of 16α,17α-oxidopregnenolone,[6] readily accessible from diosgenin, to substance "S" acetate (Δ^4-pregnene-17α,21-diol-3,20-dione acetate) and one additional microbiological step to hydrocortisone.[2] Similarly, progesterone has been converted to desoxycorticosterone acetate in 40% to 45% yield making this by far the simplest method for the large scale synthesis of this hormone. Furthermore, 11β-hydroxyprogesterone[7] was converted to corticosterone acetate by the same reaction sequence.

Versatility of this reaction was further demonstrated by the preparation of Δ^1-dehydro substance "S" acetate[8] from $\Delta^{1,4}$-pregnadiene-17α-ol-3,20-dione[8] and by the conversion of 11-ketoprogesterone and pregnan-3α-ol-11,20-dione to the corresponding 21-acetoxy compounds, Δ^4-pregnen-21-ol-3,11,20-trione acetate[9] (dehydrocorticosterone acetate) and pregnane-3α,21-diol-11,20-dione[10] acetate.

The 21-acetoxy introduction could be carried out in one step but with inferior yield by treatment of the corresponding steroid with excess iodine, potassium acetate and potassium bicarbonate in aqueous dimethylformamide solution.

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(4) Compounds were characterized by mixture melting point, rotation, ultraviolet and infrared spectral determination.

(5) A. Ercoli and P. de Ruggeri, *Gazz. chim. ital.*, **84**, 479 (1954), recorded the conversion of 16α,17α-oxido-progesterone, derived from Oppenauer oxidation of 16α,17α-oxido-pregnenolone, to 17α-hydroxyprogesterone through Raney nickel reduction of the 16,17-iodohydrin.

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STRUCTURE OF GLYCOPEPTIDES FROM A HUMAN γ-GLOBULIN

Sir:

The presence of carbohydrate in many proteins has long been recognized. Neuberger[1] was able to isolate a polysaccharide which accounted for the carbohydrate content of ovalbumin. We wish to report the isolation and characterization of three glycopeptides from human γ-globulin, in which the carbohydrate is covalently linked to an aspartyl residue of the peptide. This represents the first case in which the nature of protein binding to carbohydrate has been ascertained.

The glycopeptides were obtained from human γ-globulin, Fraction II-1,2[2] by digestion with papain at *p*H 6.5, removal of amino acids and small peptides on Dowex 50 × 8 (hydrogen cycle, 20–50 mesh), ethanol precipitation of the glycopeptides, and zone electrophoresis on a starch column at *p*H 8.5. The isolation was followed by an orcinol–sulfuric acid method[3] and a ninhydrin method.[4] Analysis of effluent fractions from the starch column permitted separation of three glycopeptides. The hexose in the glycopeptides before electrophoresis accounted for about 60% of the hexoses in the γ-globulin.

The similarity of the three glycopeptides (Table I) suggests that they are derived from the same structure. The largest, Glycopeptide 1, probably represents the prosthetic group of the intact γ-globulin. Glycopeptides 2 and 3 appear to be partially degraded; they contain less than 1 residue of sialic acid and Glycopeptide 3 has only half the glucosamine of the others. The peptide portions differ only in the number of glutamyl residues. Some variation in peptide chain length can be expected since enzymic hydrolysis at one site would inhibit splitting at adjacent sites. Traces of other amino acids were present in less than stoichiometric amount as judged by the finding that the approximate molecular weight was less than 5,000 for a mixture of the three glycopeptides.[5]

Analyses for amino acids and glucosamine were performed on ion-exchange columns and for carbohydrate components by suitable colorimetric methods. All components were also identified by paper chromatography. Galactose and mannose were present in a ratio of about 3 to 5. Other hexoses, pentoses, hexonic acids, hexuronic acids and hexosamines could not be detected.

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(5) We are indebted to Dr. H. K. Schachman for a sedimentation determination on this material.