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.

(6) P. L. Julian, E. W. Meyer, W. J. Karpel and I. R. Waller, THIS JOURNAL, **72**, 5145 (1950).

(7) T. Reichstein and H. G. Fuchs, *Helv. Chim. Acta*, **23**, 684 (1940); G. Rosenkranz, J. Pataki and C. Djerassi, *J. Org. Chem.*, **17**, 290 (1952).

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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.

(1) A. Neuberger, *Biochem. J.*, **32**, 1435 (1938).

(2) J. L. Oncley, M. Melin, D. A. Richert, J. W. Cameron and P. M. Gross, THIS JOURNAL, **71**, 541 (1949).

(3) R. J. Winzler, "Methods of Biochemical Analysis," edited by D. Glick, Vol. 2, 1955, p. 279.

(4) S. Moore and W. H. Stein, *J. Biol. Chem.*, **211**, 907 (1954).

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

Amino end group analysis by Sanger's method[6] gave DNP-aspartic acid from Glycopeptide 3. Glycopeptide 2, containing one additional glutamyl residue, yielded DNP-glutamic acid, and Glycopeptide 1, with two additional glutamyl residues, also gave DNP-glutamic acid. The only other DNP compound found in all three peptides in more than trace amounts was O-DNP-tyrosine. This suggests that Glycopeptide 1 has the structure: Glu.Glu.Asp.(Tyr, Glu, Asp, (CHO)), where the carbohydrate group (CHO) and the residues in parentheses are of undetermined sequence. Leucine aminopeptidase[7] released 2 residues of glutamic acid, 1 of asparagine and 0.8 of tyrosine from Glycopeptide 1. This is consonant with the above N-terminal sequence and indicates that tyrosine follows asparagine. The aminopeptidase liberated one residue each of aspartic acid, tyrosine and glutamic acid from Glycopeptide 3. This suggests the sequence Asp.Tyr.Glu; the only remaining residue is aspartic acid which must be at the C-terminal end of Glycopeptide 3 and bound to the carbohydrate.

The carbohydrate probably is attached to the β-carboxyl group of the aspartic acid by an amide or ester linkage. If it were attached by an α-amide bond, both leucine aminopeptidase and papain would hydrolyze such a bond. If it were attached by an α-ester bond, it would probably be split by papain. Incubation of Glycopeptides 1 and 3 with carboxypeptidase resulted in release of only traces of amino acids; this provides additional evidence that the carbohydrate is linked to the C-terminal residue.

(6) F. Sanger, *Biochem. J.*, **39**, 207 (1945).
(7) R. L. Hill and E. L. Smith, *J. Biol. Chem.*, **228**, 577 (1957).

TABLE I

COMPOSITION OF GLYCOPEPTIDES FROM A HUMAN γ-GLOBULIN[a]

Constituent	Glyco-peptide 1	Glyco-peptide 2	Glyco-peptide 3
Hexose	8.3	8.7	8.9
Glucosamine	6.0	6.0	3.1
Fucose	2.0	2.1	2.0
Sialic acid	1.0	0.6	0.2
Aspartic acid	2.1	1.8	1.8
Glutamic acid	3.3	2.4	0.90
Tyrosine[b]	0.95	1.0	1.15

[a] Residues are computed from a weighted average for the amino acids listed, on the assumption that each peptide contains one tyrosine residue. [b] Other amino acids were present in less than stoichiometric amount and varied in different preparations.

Present evidence indicates an over-all structure for Glycopeptide 1 as follows: Glu.Glu.Asp (NH_2).Tyr.Glu.Asp(CHO). Presumably the variations in the three glycopeptides are produced by the digestion procedure and the purification methods. The relatively good yield of the peptides indicates also that a single prosthetic group is present in this fraction of γ-globulin. Further studies are in progress to determine the structure of similar glycopeptides obtained from γ-globulins of other species and from specific antibodies.[8]

(8) Aided by grants from the National Institutes of Health, U. S. Public Health Service.
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BOOK REVIEWS

The 1,2,3- and 1,2,4-Triazines, Tetrazines and Pentazines. The Chemistry of Heterocyclic Compounds. Volume 10. A Series of Monographs. ARNOLD WEISSBERGER, Consulting Editor. By JOHN G. ERICKSON, Avochem, Inc., Minneapolis, Minnesota; PAUL F. WILEY, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana; and V. P. WYSTRACH, Stamford Research Laboratories, American Cyanamid Company, Stamford, Connecticut. Interscience Publishers, Inc., 250 Fifth Avenue, New York 1, New York. 1956. xi + 261 pp. 23.5 × 16 cm. Price, $10.50. Subscription price, $9.50.

In this latest volume of the Weissberger series on heterocyclic compounds, the 1,2,3-triazines, 1,2,4-triazines, 1,2,3,5-tetrazines and the pentazines are presented in chapters by Erickson; the 1,2,3,4-tetrazines by Wystrach; and the 1,2,4,5-tetrazines by Wiley. The compilation of this encyclopedic reference work is the result of a thorough and conscientious search of the literature through 1950. There is no question but that this is a very worthwhile contribution to the literature on heterocyclic compounds.

The compounds discussed in this review have received only sporadic attention in recent years, and the bulk of the work described was done thirty to fifty years ago. For anyone wishing to gain a background in these areas, the book will be a real help, for there is no other adequate review of the subject and independent searches of the literature of this vintage present some formidable obstacles. As is clearly pointed out in the text in each case, there has been no uniformity of nomenclature in the past and most of the compounds have been named in a variety of ways depending on the preferences of the individual authors. Secondly, our knowledge of organic chemistry has increased a good deal in the past thirty years and assignments of structure, which seemed sound at the time, no longer appear rational. A good deal of time is spent in reinterpreting the older work in the light of modern theory. In many instances no rational interpretation of the older experiments can be made and it is simply noted that additional experiments are needed. For anyone casting about for research problems, the book could be a veritable storehouse.

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