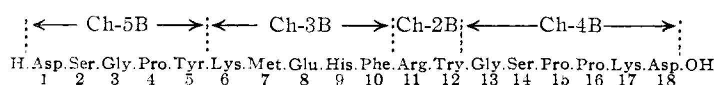
No other amino acids were detected even as trace contaminants. No amide-NH_3 was detected in the peptide, and the isoelectric point was found to be 7.0 as determined by zone electrophoresis on starch.[10] It may be noted that there is one additional residue of serine and one less residue of glutamic acid in the bovine MSH than in β-MSH.[3,4,11] Hence, as would be expected, the isoelectric point[4,5] (pH 5.8) of β-MSH is lower than that of the bovine hormone.

By the paper-strip modification[12] of the phenyl isothiocyanate method,[13] the amino acid sequence at the N-terminus was established as Asp.Ser.Gly.-Pro.Tyr.Lys.Met. A portion of this N-terminal sequence was confirmed by means of kinetic studies with leucine aminopeptidase.[14] Aspartic acid was identified as the C-terminal amino acid by reaction with carboxypeptidase. Digestion of the peptide hormone with chymotrypsin gave rise to four peptides in accordance with the specificity of the enzyme:



The separation and analysis of these peptide fragments in the chymotryptic hydrolysate were carried out as previously described.[4,15] The amino acid sequence in peptide Ch-3B was also elucidated by the action of leucine aminopeptidase. These data permit the formulation of the structure shown above for the bovine MSH.

It will be seen that this structure differs from that proposed for the porcine hormone[3,4] only at position 2, where a seryl residue replaces a glutamyl. As far as we are aware, the replacement of an uncharged residue (serine) by a charged one (glutamic acid) has not previously been encountered in connection with species variations among biologically active peptides.

(10) I. D. Raacke and C. H. Li, *J. Biol. Chem.*, **215**, 277 (1955).
(11) P. Roos, *Acta Chem. Scand.*, **10**, 1061 (1956).
(12) H. Fraenkel-Conrat, THIS JOURNAL, **76**, 3606 (1954).
(13) P. Edman, *Acta Chem. Scand.*, **4**, 283 (1950).
(14) D. H. Spackman, E. L. Smith and D. M. Brown, *J. Biol. Chem.*, **212**, 255 (1955). We are indebted to Dr. L. K. Ramachandran for a gift of this enzyme.
(15) I. I. Geschwind, C. H. Li and L. Barnafi, THIS JOURNAL, **79**, 620 (1957).
(16) This work was supported in part by grants from the U. S. Public Health Service (G-2907) and the Albert and Mary Lasker Foundation.

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FLUOROTELLURATES

Sir:

We have studied the behavior of tellurium hexafluoride and of selenium hexafluoride toward various inorganic fluorides. Neither of these hexafluorides showed evidence of strong interaction or of fluorine exchange with covalent fluorides; however, the tellurium compound did react with certain ionic fluorides to form the new fluorotellurate salts. Selenium did not form fluoroselenates; apparently, the requisite orbitals in selenium lie at too high an energy level.

The fluorotellurates, which have the limiting composition $2MF \cdot TeF_6$, were prepared by heating a metal fluoride with an excess (100%) of tellurium hexafluoride in a pressure vessel to 250° and cooling this reaction mixture over a period of 24 hours to room temperature. After the excess gas was released, the solid was recovered. In the case of cesium the solid product analyzed closely for $2CsF \cdot TeF_6$. The X-ray pattern of the cesium compound consisted of many weak lines which were not indexed; no crystalline cesium fluoride was present. Rubidium and potassium fluorides gave products that had the empirical compositions $2RbF \cdot 0.52TeF_6$ and $2KF \cdot 0.32TeF_6$. X-Ray diffraction patterns of these solids showed the presence of the alkali metal fluoride and a second crystalline phase. The fluorides of sodium, lithium, and barium were inactive under the experimental conditions. Under comparable conditions, selenium hexafluoride gave no sign of reaction with cesium and potassium fluorides. The F^{19} magnetic resonance spectra of binary systems of liquid tellurium (and selenium) hexafluoride with arsenic trifluoride and with boron trifluoride showed no evidence of compound formation or of a measurable fluorine exchange rate up to 60°.

The fluorotellurates dissociated at elevated temperatures to yield the starting materials. Crude measurements of the dissociation pressure of cesium fluorotellurate yielded an average value for the heat of dissociation of 0.4 kcal. over the temperature range 200–250°. Addition of the fluorotellurates to water resulted in evolution of tellurium hexafluoride and dissolution of the alkali metal fluoride. The fluorotellurates displayed no stability outside the solid state, and the stability of the lattice appeared to be inversely related to the polarizing power of the cation. The lattice could either be composed of M^+ and $TeF_8^=$ or of M^+, F^-, and TeF_7^- aggregates. A unique solution of the crystal structure is being sought in analyses of the X-ray diffraction pattern and the F^{19} magnetic resonance spectrum of the cesium compound.

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FORMATION OF 6β-HYDROXY AND 6-KETO DERIVATIVES OF ESTRADIOL-16-C[14] BY MOUSE LIVER MICROSOMES

Sir:

Further study of the protein-binding of estradiol by fortified liver preparations[1] has revealed that mouse liver microsomes in the presence of reduced triphosphopyridine nucleotide and oxygen convert estradiol-16-C^{14} to at least five new metabolites[2] extractable into an acetone-benzene mixture (3:4). Preliminary chromatographic analysis on

(1) I. L. Riegel and G. C. Mueller, *J. Biol. Chem.*, **210**, 249 (1954).
(2) G. Rumney, *Fed. Proc.*, **15**, 343 (1956).

Whatman No. 1 paper[3] demonstrated that two of the radioactive metabolites migrated identically with 6β-hydroxyestradiol (I) and 6-ketoestradiol (II),[4] respectively.

For absolute identification of these metabolites twenty-four reaction flasks, each containing 20 μg. of estradiol (7,500 c.p.m./μg.) in 1.0 ml. of 0.05 M potassium phosphate buffer pH 7.4, 0.3 ml. of TPN H (2.5 mg.), 0.15 ml. of 1.0 M nicotinamide, 0.35 ml. of 1.0 M KCl, 0.1 ml. of 1.0 M $MgSO_4$ and 0.4 ml. of mouse liver microsome suspension (1.0 ml. = microsomes from 1.25 g. of liver) were incubated for 15 minutes at 37°. The reactions were stopped with 0.3 ml. 1 N HCl, 25 mg. each of (I) and (II) were added, and extraction with acetone–benzene and then with chloroform–ethylene dichloride (1:1) carried out. The residue from the organic phase was chromatographed on a 100 × 1.8 cm. column of Florisil with increasing concentrations of methanol in benzene (Fig. 1). Radioactive peaks T and X_2 contained the carrier (I) and (II), respectively.

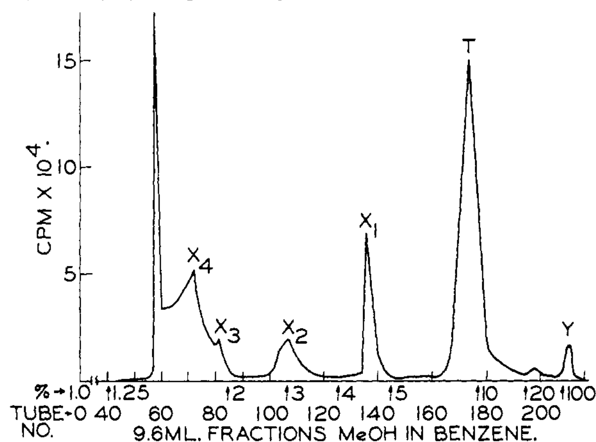

Fig. 1.

Rechromatography of the X_2 peak on Florisil with increasing concentration of methanol in ethylene dichloride (1–4%) showed that the elution of carrier mass coincided with elution of radioactivity. Crystallization to constant specific activity gave 4,860 c.p.m./mg. Conversion to the 2,4-dinitrophenylhydrazone[5] and chromatography on aluminum oxide (Activity = Brockmann II, elution with ethanol–$CHCl_3$ mixtures) gave a single peak in which the specific activity of the two halves of peak were 3040 and 3130 c.p.m./mg. (Calculated specific activity for a mono phenylhydrazone derivative = 2990 c.p.m./mg.) Therefore the identity of X_2 is established as 6-ketoestradiol (yield 3.4%).

The "T" peak was rechromatographed on Florisil using gradually increasing concentrations of methanol (1.25–4%) in ethylene dichloride. The elution of radioactivity coincided with the elution of the carrier I; the two halves of the peak had specific activities of 39,070 and 39,570 c.p.m./mg., respectively. Partition chromatography on Celite with ethylene dichloride-methanol[6] yielded a similar result; the two halves of the peak gave a specific activity of 36,300 and 35,890 c.p.m./mg., respectively. Conversion of the carrier to the triacetate yielded a derivative (m.p. 140–143°) with specific activity of 24,000 c.p.m./mg. Expected specific activity = 25,080 c.p.m./mg. Thus the identity of "T" is established as 6β-hydroxyestradiol (yield = 25%).

Preliminary results on the reduction X_1 to (I) and the oxidation of both X_1 and (II) to the same new product, presumed to be the 6-ketoestrone, indicated that the identity of X_1 is 6β-hydroxyestrone. More complete evidence for this conclusion will be reported later.

Accordingly it has been demonstrated that mouse liver microsomes in the presence of $TPN{\cdot}H^+$ hydroxylate estradiol primarily to form 6β-hydroxyestradiol which is converted[2] to 6-ketoestradiol and 6β-hydroxyestrone. This family of compounds appears to constitute a major metabolic pathway for estradiol in mouse and rat liver preparations.[7,8]

(3) Chromatograms were developed by descension of benzene in a paper lined jar saturated from a mixture of 500 ml. of methanol and 250 ml. of water placed in bottom of jar: G. Rumney, Ph.D. Thesis, Hebrew Univ., Jerusalem, 1954.

(4) We are extremely grateful to Dr. O. Wintersteiner for supplying us with authentic samples of I and II. Additional II has been prepared in this laboratory according to B. Longwell and O. Wintersteiner: *J. Biol. Chem.*, **133**, 219 (1940), and reduction of this product to I with $NaBH_4$.

(5) H. Reich, K. F. Crane and S. J. Sanfilippo, *J. Org. Chem.*, **18**, 822 (1953).

(6) W. S. Bauld, *Biochem. J.*, **59**, 294 (1955).

(7) This work was supported by a grant from the Alexander and Margaret Stewart Trust Fund, grant No. C-1897 from the United States Public Health Service and an Institutional Grant from the American Cancer Society.

(8) Authors wish to thank Miss Elba I. Porrato for valuable technical assistance.

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CONFIGURATIONAL EFFECTS IN THE PROTON MAGNETIC RESONANCE SPECTRA OF ACETYLATED CARBOHYDRATES[1]

Sir:

We have observed configurational effects in the proton magnetic resonance (NMR) spectra[2] of acetylated carbohydrates which promise to be of value in configurational and conformational analyses and which raise rather important questions regarding the interpretation of chemical shifts in general.

(a) There is a shift of 5 to 10 cycles per second (c.p.s.) between the signals for the methyl hydrogens of equatorial and axial acetoxy groups.

(b) There is a shift of approximately 8 c.p.s. between the signals for axial and equatorial hydrogens.

(c) The carbon-1 hydrogens of anomeric acetylated aldopyranoses produce signals which are separated by 10 to 26 c.p.s. when these hydrogens are axial in one of the anomeric forms and equato-

(1) Presented in part at the 130th Meeting of the American Chemical Society, Atlantic City, N. J., Sept. 16–21, 1956, Abstract of Papers, p. 10-D. N.R.C. Contribution No. 4257.

(2) The spectra were determined in chloroform solution at room temperature with a Varian High Resolution Spectrometer at 40 megacycles per second. The positions of the signals were measured in cycles per second from the chloroform signal.