droxyl groups into the substrate has been observed.[1,2,5,6,12] However, dihydroxylated products have been formed to any appreciable extent only with progesterone[2,5,6,12] or 3-ketobisnor-4-cholen-22-al[1] as a substrate.

Since thus far no 17-hydroxylation by microbiological means has been reported, it is the purpose of this communication to describe the enzymatic oxygenation of the 17α-position of C_{21}-steroids by *Cephalothecium roseum* Cda (A.T.C.C. 8685). Upon incubation of 11-desoxysteroids with *C. roseum*, the 17α-hydroxyl group was introduced with or without concomitant hydroxylation of the 6β- or the 11α-position. When 11-keto or 11β-hydroxy-17-desoxysteroids were used as substrates only 17-hydroxylation took place.

When desoxycorticosterone was incubated with a 48-hour growth of *C. roseum* on a corn steep–glucose medium for 48 hours, extraction of the fermentation liquor with methylene dichloride and chromatography of the concentrates over Florisil[3] yielded two products: (a) 11α,17α,21-Trihydroxy-4-pregnene-3,20-dione[2,4,6,14] (11-epi F). The identity of this compound was established through these physical constants: m.p. 206–211°, m.p. in admixture with authentic 11-epi F, 208–212°; $[\alpha]_D +121°$ (methanol); ($[\alpha]_D +117°$ in methanol for authentic 11-epi F); the infrared spectrum of the diacetate (in chloroform solution) was identical to the spectrum of the authentic 11-epi F diacetate. (b) 6β,17α,-21-Trihydroxy-4-pregnene-3,20-dione[2,3,15] was also isolated; m.p. 234–236°, $[\alpha]_D +53°$; $\lambda_{max}^{alc.}$ 238 mμ, E 12,900. *Anal.* Calcd. for $C_{21}H_{30}O_5$: C, 69.58; H, 8.34. Found: C, 69.58; H, 8.38. The infrared spectrum of the diacetate, m.p. 190–193°, was identical to the spectrum of an authentic specimen.

Paper chromatography indicated that small amounts of Reichstein's compound S and epicorticosterone had been formed.

Fermentation of the following substrates with *C. roseum* gave the corresponding 17α-hydroxy derivatives:

Substrate	Conversion Product
Progesterone	11α,17α - Dihydroxyprogesterone
11-Dehydrocorticosterone	Kendall's Compound E
Corticosterone	Kendall's Compounds F and E

It is worthy of note that the enzyme systems of *Cephalothecium* can introduce a hydroxyl group at carbon atom 17 without any interference from a hydroxyl group already present at carbon atom 21 (as in desoxycorticosterone). This is in contrast to the performance of the mammalian adrenal which, at least under the *in vitro* conditions employed by the Worcester group,[16] cannot oxygenate the 17-position of desoxycorticosterone.

The authors are indebted to Misses Irene N.

(14) For chemical syntheses of this compound or its diacetate, *cf.* J. Romo, *et al.*, *Chem. and Ind.*, 783 (1952); H. L. Herzog, *et al.*, THIS JOURNAL, **74**, 4470 (1952); A. Lardon and T. Reichstein, *Pharm. Acta Helv.*, **27**, 287 (1952); J. Romo, *et al.*, THIS JOURNAL, **75**, 1277 (1953).

(15) For a chemical synthesis of this compound *cf.* K. Florey and M. Ehrenstein, *J. Org. Chem.*, in press.

(16) H. Levy, *et al.*, *J. Biol. Chem.*, **203**, 433 (1953).

Pratt, Jennie I. Mejeur and Henrietta Triemstra and Mr. J. R. Heald and G. Staffen for technical assistance, to Dr. J. L. Johnson and associates for the spectra and to Mr. W. A. Struck and associates for rotations and microanalyses.

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THE STEREOCHEMISTRY OF METAL CHELATES OF A POLYDENTATE LIGAND

Sir:

Metal complexes with sexadentate ligands were first described by Dwyer and Lions.[1] Diehl and co-workers[2] seemingly prepared some similar compounds with triethylenetetramine and salicylaldehyde (also substituted salicylaldehydes) by the reaction of cobalt salts during their investigation on the oxygen-carrying synthetic chelates. They, however, did not isolate the pure compounds or study their properties. A Schiff base (I) from triethylene-

$$HOC_6H_4CHN(CH_2)_2NCH_2CH_2N(CH_2)_2N=CHC_6H_4OH$$

$$\begin{array}{c} CH \\ | \\ C_6H_4OH \end{array} \quad I$$
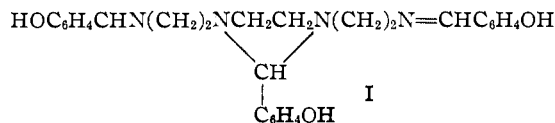
tetramine (II) and salicylaldehyde (III) has been reported by Mukherjee,[3] which gives a brown hygroscopic substance with $[Co(py)_4Cl_2]Cl$. This is soluble in water, insoluble in alcohol, and decomposes at 110°. The same substance is also said to be obtained by the reaction of (III) on [Co trien Cl_2]Cl.

The nitrogen analog of Dwyer's schiff base (IV)

$$HOC_6H_4CH=N(CH_2)_2NH(CH_2)_2NH(CH_2)_2N=CHC_6H_4OH$$

could not be prepared from (II) and (III), only (I) being obtained. We have, however, been able to prepare a large number of compounds of the sexadentate ligand (IV) by the action of (II) on bis-salicylaldehyde compounds of cobalt, copper and other bivalent metals in about 80–85% yield. These can be prepared also by adding a mixture of (II) and (III) in four equivalents of alkali (KOH) in a cold methanol solution to a solution of the metal salt concerned. (I) also gives a poor yield (10–20%) of these compounds by careful manipulation. A compound of the composition [Co trien CHOC₆-$H_4O]^{++}$ is obtained as the main product from [Co trien Cl_2]Cl and (III).

When base (I) reacts with metal salts, one molecule of salicylaldehyde is hydrolyzed from it, and derivatives of base (IV) result. For bivalent metal ions such as Fe^{II} and Pd^{II} these have the composition intermediate between MTS_2 and MTS_3, where TS_2 and TS_3 represent the bi-negative ions of the bases (IV) and (I), respectively.

The complexes of (IV) will be asymmetric

(1) F. P. Dwyer and F. Lions, THIS JOURNAL, **69**, 2917 (1947).
(2) H. Diehl and co-workers, *Iowa State Coll. J. Sci.*, **21**, 109 (1947).
(3) A. K. Mukherjee, *Science and Culture*, **19**, 107 (1953).

whether the base behaves as a tetradentate or hexadentate ligand. When (IV) behaves as a tetradentate ligand (as in the copper and palladium compounds obtained by the first two methods of preparation), the metal complexes have an asymmetric center in the metal ion, due to the two aromatic rings lying in different planes, irrespective of whether the arrangement be planar or tetrahedral. There is of course a possible meso-form.

Some of the compounds prepared are tabulated:

Compound	Color and crystal form	Solubility Water EtOH		Magnetic moment	Melting point, °C.
$[Co^{II}TS_2] \cdot 0.5H_2O$	Brownish yellow powder	insol.	sol.	4.37	dec. 226
$[Co^{III}TS_2]Cl \cdot 2.5H_2O$	Dark black-brown hexagonal plates	sol.	sol.	0	240
$[Fe^{III}TS_2]I \cdot 1.5H_2O$	Dark purple rectangular prisms	sol.	sol.	1.83	117–118
$[Cu^{II}TS_2]$	Bluish green prismatic needles, green when anhydrous	sol.	sol.	2.01	78
$[Al^{III}TS_2]I \cdot 0.5H_2O$	Colorless, hexagonal	sol.	sol.	...	<285

The cobalt, iron and aluminum complexes have been resolved through their d-antimonyl tartrates and d-bromocamphor sulfonates (in aqueous solution for the first and methanol–water solutions for the others), $[\alpha]^{30}$D being 300, 357 and 68°, respectively.

The Co(III) compound is quite stable even in aqueous solution, while the other two racemize in solution—more quickly in water than in methanol. The half-life for the aluminum compound is 1.5 hours in 75% ethanol and 2.5 hours in 95% methanol.

The cupric compound, when prepared by the action of (II), (III) and alkali in methanol on an aqueous solution of the d-tartrate complex of copper, comes out as an active compound, with $[\alpha]_D$ −65°. (Tartaric acid, tartrates and Cu d-tartrates are all dextrorotatory.)

The details of the work together with that on other metal compounds will be shortly communicated.

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RECEIVED JUNE 30, 1954

THE INFLUENCE OF PH ON ANTIGEN-ANTIBODY EQUILIBRIA

Sir:

Continuing our studies of soluble complexes of antigen (Ag) and antibody (Ab),[1,2] we have investigated the effect of pH on solutions containing crystalline bovine serum albumin (BSA) as antigen and rabbit antibodies to BSA. A solution of complexes was preapred[1] containing 21 mg. of protein/ml., consisting of 63.0% total Ag and 37.0% total Ab by weight.[3] Aliquots of this solution were dia-

(1) S. J. Singer and D. H. Campbell, THIS JOURNAL, 74, 1794 (1952).

(2) S. J. Singer, and D. H. Campbell, ibid., 75, 5577 (1953).

(3) Analyses for total Ag and total Ab were performed by electrophoresis in glycine–HCl buffer, pH 2.4, ionic strength 0.1, using known mixtures of BSA and normal γ-globin for calibration. This method will be described in detail elsewhere.

lyzed against buffers of different pH, all at 0.1 ionic strength, and were then ultracentrifuged at about 25°. Between pH 7.5 and 4.6, the ultracentrifuge diagrams were essentially unchanged, closely resembling those of Fig. 2c of reference (1). From pH 4.6 to 3.1 progressively larger amounts of a component with sedimentation rate corresponding to free antibody γ-globulin appeared in the diagrams, while the peaks due to complexes diminished in area. At pH 2.4, the diagram was that of a corresponding mixture of BSA and normal γ-globulin. A solution at pH 3.1 dialyzed back to pH 7.5 exhibited an ultracentrifuge pattern indistinguishable from that of a solution kept at pH 7.5, indicating that the acid dissociation of the complexes was completely reversible under these conditions.

The apparent and corrected[4] relative areas of free Ag, free Ab, and of the slowest-sedimenting complex peak (a-complex) only, are given in Table I. At pH 7.5, the a-complex peak was shown[1] to be due largely to the $(Ag)_2Ab$ complex. At acid pH values, however, where considerable amounts of free Ab appear in the ultracentrifuge diagrams, we expect appreciable amounts of the AgAb complex to be present as well. We infer that the sedimentation rates of $(Ag)_2Ab$ and AgAb are similar enough so that the two complexes are not resolved in these experiments, and that both together constitute the a-complex area at acid pH values. That fraction of the a-complex area attributable to AgAb may be calculated, as a good first approximation as follows. If all Ag reactive sites have equal affinity for Ab sites, and vice versa, regardless of the size or shape of the complex in which these sites are bound, it must follow[5,6,2] that $c_{(Ag)_2Ab} = c^2{}_{AgAb}/4c_{Ab}$, where c represents molar concentration. This permits evaluation of the quasi-experimental relative areas of AgAb which are given in column 9 of Table I.

With these data we may evaluate apparent equilibrium constants, K, which are almost entirely experimental, for the reaction $Ag \times Ab \rightleftarrows AgAb$, as a function of pH. K and log K are listed in the last two columns of Table I. In view of the approximations made, the K values may be uncertain to ±25%, but this introduces an uncertainty of only ±0.1 unit in log K. We conclude therefore that in this pH range log K is a linear function of pH

(4) The empirical corrections are made to take account of the anomaly described by J. P. Johnston and A. G. Ogston, Trans. Faraday Soc., 42, 789 (1946).

(5) L. Pauling, D. Pressman, D. H. Campbell and C. Ikeda, THIS JOURNAL, 64, 3003 (1942).

(6) R. J. Goldberg, THIS JOURNAL, 74, 5715 (1952).