rium is involved between a carbonyl of cobalt (possibly dicobalt octacarbonyl or the hydrocarbonyl), carbon monoxide and the olefin, the latter being able to substitute for some of the CO in the carbonyl. One possible scheme for the reaction is

$$\mathrm{[Co(CO)_4]_2 + C_6H_{10} \rightleftharpoons \underset{(A)}{[Co_2(CO)_7C_6H_{10}]} + CO} \quad (1)$$

$$\mathrm{2Co(CO)_7C_6H_{10} + 2H_2 \longrightarrow 2C_6H_{11}CHO + [Co(CO)_3]_4} \quad (2)$$

$$\mathrm{[Co(CO)_3]_4 + 4CO \rightleftharpoons 2[Co(CO)_4]_2} \quad (3)$$

It is possible that the postulated intermediate (A) is formed from the decomposition of the complex $\mathrm{[Co(CO)_4{\cdot}olefin]^+[Co(CO)_4]^-}$ recently suggested by Orchin[3] but further work is required, especially since the above tentative scheme does not assign a role to the hydrocarbonyl.

[3] M. Orchin, in "Advances in Catalysis," Vol. V, Academic Press, Inc., New York, 1953, p. 407.

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RECEIVED MAY 3, 1954

POLYATOMIC SPECIES IN SILVER VAPOR[1]

Sir:

Calculation of the molecular weight of silver vapor from the measured vapor velocity[2] and success of the famous Stern–Gerlach experiment in yielding in an inhomogeneous field the splitting predicted for a beam of silver atoms[3] apparently established beyond doubt that silver vapor is monoatomic at low pressures. We have found, however, a high concentration of polyatomic species in silver vapor at $\sim 10^{-6}$ to 10^{-4} atm. pressure.

By use of a method developed by Mayer[4] and Volmer,[5] we have measured the apparent molecular weight of silver vapor in a graphite box containing two small holes.

Pressure inside the box can be calculated from the hole areas, rate of weight loss due to escaping vapor, temperature and the molecular weight of the vapor by use of Knudsen's equation.[6] The pressure can also be calculated from the angle ϕ through which the force of the escaping vapor causes the box to rotate about a wire of torsion constant D. The equation used is $P = 2D\phi/(q_1a_1 + q_2a_2)$, where a_1 and a_2 are the areas of the holes and q_1 and q_2 are distances from the axes of the holes to the wire. Simultaneous solution of the equations yields the molecular weight M of the vapor.

Fifteen determinations at 1310 to 1420°K. gave $M = 278$ (2.56 times the atomic weight) with an estimated maximum uncertainty of ± 90. This high average molecular weight cannot arise from systematic error because concurrent determinations of the molecular weights of tin[7] and germanium[8] vapors yielded values within estimated error of the atomic weights.

The existence of polyatomic ionic species in the vapor of silver metal was recently established through direct observation of the ions in a 60° single direction focusing mass spectrometer of very high sensitivity. In one run at various temperatures above the melting point and below 1500° silver vapor was found to contain ionic species (presumably formed by thermal ionization) corresponding to the compositions $\mathrm{Ag^+}$, $\mathrm{Ag_2^+}$, $\mathrm{Ag_3^+}$ and $\mathrm{Ag_4^+}$ in approximate relative intensities 15:7:30:1, respectively.

Identification of these species was made on the basis of the mass numbers observed, which were known from magnetic field calibration with a proton resonance fluxmeter, and on the basis of a characteristic mass spectrum for each species which results from the isotopic composition of silver. It has not been possible as yet to search for higher polymers which may also exist.

There is, of course, considerable uncertainty associated with the extension of these data to the relative abundances of neutral polyatomic species in the vapor state, but it may be noted that the above relative intensities would give an average molecular weight for silver vapor in good agreement with the above molecular weight determination.

[1] Supported in part by the Office of Naval Research and in part by the Atomic Energy Commission.

[2] O. Stern, *Z. Physik*, **3**, 417 (1920).

[3] W. Gerlach and O. Stern, *ibid.*, **9**, 349 (1922).

[4] H. Mayer, *ibid.*, **67**. 240 (1931).

[5] M. Volmer, *ibid.*, *Chem.*, *Bodenstein Festband*, 863 (1931).

[6] M. Knudsen, *Ann. Physik*, [4] **28**, 999 (1909).

[7] A. W. Searcy and R. D. Freeman, THIS JOURNAL, in press.

[8] A. W. Searcy and R. D. Freeman, unpublished data.

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RECEIVED JULY 8, 1954

MICROBIOLOGICAL TRANSFORMATIONS OF STEROIDS. XII.[1] 17α-HYDROXYLATION

Sir:

Preceding publications from our own as well as other laboratories have been concerned with the microbiological oxygenation of the steroid molecule at various positions of the skeleton. The introduction of a hydroxyl group into positions 6β,[2,3] 7β,[2,4] 8ξ,[2] 11α,[1,2,3,5,6] 11β,[2,7,8,9] 14α,[2,10] 15,[11] and 16α,[12,13] by the action of various genera of the filamentous fungi has been described. In some instances a simultaneous introduction of two hy-

[1] Paper XI of this series, THIS JOURNAL, in press.

[2] H. C. Murray and D. H. Peterson, U. S. Patent 2,602,769 (July 8, 1952).

[3] Papers III, IV, V, IX, and X of this series, THIS JOURNAL, **75**, 408, 412, 416, 5768 (1953); **76**, 3174 (1954).

[4] F. W. Kahnt, *et al.*, *Experientia*, **8**, 422 (1952).

[5] See, besides ref. 3, also papers I, II, VI and VII of this series, THIS JOURNAL, **74**, 5933 (1952); **75**, 55, 419, 421 (1953).

[6] J. Fried, *et al.*, *ibid.*, **74**, 3962 (1952).

[7] D. R. Colingsworth, *et al.*, *ibid.*, **74**, 2381 (1952); *J. Biol. Chem.*, **203**, 807 (1953).

[8] F. R. Hanson, *et al.*, THIS JOURNAL, **75**, 5369 (1953).

[9] G. M. Shull, D. A. Kita and J. W. Davisson, U. S. Patent 2,658,023 (Nov. 3, 1953).

[10] P. D. Meister, *et al.*, Abstracts of the 123rd Meeting of the American Chemical Society, Los Angeles, Calif., March 15–19 (1953), page 5C.

[11] (a) Personal communication from Dr. J. Fried, The Squibb Institute for Medical Research, New Brunswick, N. J.; (b) unpublished results, these laboratories.

[12] D. Perlman, E. Titus and J. Fried, THIS JOURNAL, **74**, 2126 (1952).

[13] E. Vischer, J. Schmidlin and A. Wettstein, *Helv. Chim. Acta*, **37**, 321 (1954).

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°; $\lambda^{alc.}_{max.}$ 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.

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[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).

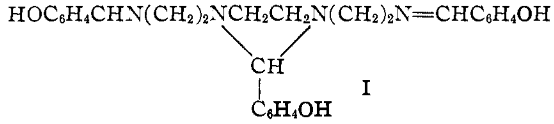
[15] For a chemical synthesis of this compound *cf.* K. Florey and M. Ehrenstein, *J. Org. Chem.*, in press.

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

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 triethylenetetramine (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 $[Co\ trien\ Cl_2]Cl$.



I

$$HOC_6H_4CH\underset{\underset{C_6H_4OH}{|}}{N(CH_2)_2N\!-\!CH\!-\!CH_2CH_2}N(CH_2)_2N{=}CHC_6H_4OH$$

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 $[Co\ trien\ CHOC_6H_4O]^{++}$ 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

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[1] F. P. Dwyer and F. Lions, THIS JOURNAL, **69**, 2917 (1947).

[2] H. Diehl and co-workers, *Iowa State Coll. J. Sci.*, **91**, 109 (1947).

[3] A. K. Mukherjee, *Science and Culture*, **19**, 107 (1953).