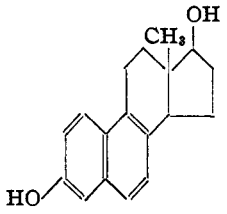
a possibility discussed in the first publication. Fractional crystallization yielded an apparently homogeneous product which did not change its melting point (226° corr.) on repeated recrystallization from various solvents. However, the analytical composition and specific rotation varied with different preparations ($[\alpha]_D$ +31 to +37°). The preponderant constituent of these mixtures appears to be a molecular compound consisting of two components. Only one component forms a picrate, and this property as well as its stronger acidic character enabled us to isolate this component in pure form.

The new compound melts at 215–217° and has the composition $C_{18}H_{20}O_2$ (calcd.: C, 80.55; H, 7.52; found: C, 80.53, 80.47; H, 7.61, 7.52); $[\alpha]^{25}D$ −4.7° (0.7% in dioxane). A di-*p*-nitrobenzoate (m. p. 250–252° corr.) and a monobenzoate (m. p. 203–205° corr.) have been prepared. The compound gives the color reactions originally attributed to the δ-follicular hormone.[1,3] Its absorption spectrum coincides with that of equilenin. Our measurements on equilenin reveal two new bands in addition to those found by Dirscherl and Hanusch,[4] namely, at 2310 Å., log ε 4.78, and at 2920 Å., log ε 3.58.

The monobenzoate was oxidized with chromic acid to a ketone which gave no depression of its melting point (223° corr.) when mixed with equilenin benzoate. The dihydroxy compound (m. p. 217°) is therefore dihydroequilenin.



Contrary to the rule, established for other oestrogenic compounds, that reduction of the C_{17} keto group to carbinol enhances the physiological activity, the dihydroequilenin isolated from urine possesses only about one-half of the potency of equilenin. On the other hand, David[5] found that the oily product which he obtained by reduction of equilenin with sodium was about three times as potent as equilenin. The possibility that our diol differs from the potent oestrogenic diols, obtained by reduction, in the configuration of the C_{17} hydroxyl group will be investigated. Whether the high potency originally reported for the δ-hormone resides in the other, as yet unidentified, component of the molecular compound (m. p. 226°) or in other diols present in the impure preparations, remains to be determined.

We wish to express our sincere thanks to Dr. A. Girard of Paris for sending us samples of equilenin and its benzoate for comparison.

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(3) Schwenk and Hildebrandt, *Biochem. Z.*, **259**, 240 (1933).
(4) Dirscherl and Hanusch, *Z. physiol. Chem.*, **233**, 13 (1935).
(5) David, *Acta brevia Neerl.*, **4**, 63 (1934).

Phenylmercuric Fluoride

BY GEORGE F. WRIGHT[1]

The recent discovery[2] of mercuric fluoride as a new fluorinating agent recalls the investigation in this Laboratory of phenylmercuric fluoride. This preparation was incidental in the study of the relative strengths of C–Hg and Hg–X bonds in organomercuric halides. According to our knowledge of organomercurials the tendency toward the dissociation reaction $2RHgX \rightleftarrows R_2Hg + HgX_2$ decreases in the order HgI > HgBr > HgCl. It was hoped that the introduction of fluorine would so stabilize the Hg–X bond as to favor the primary reaction $RHgX \longrightarrow R— + HgX$ which, it is considered, accounts for the interconversion possible with compounds like furyl and thienylmercuric halides containing more reactive, though unfortunately less stable, radicals.[3] The compound did not, however, fulfil these expectations. Instead of reacting with acetyl chloride to give acetophenone, acetyl fluoride was formed as easily as from mercuric fluoride.[2] Likewise when the compound was pyrolyzed at a temperature lower than that required to decompose diphenylmercury, only the latter substance, and no diphenyl, was produced.

In connection with the assertion of Henne and Midgley that pure mercuric fluoride cannot be prepared by treating mercuric oxide with aqueous hydrofluoric acid it may be significant that treatment of such a solution with phenyldiazonium fluoride, and subsequent treatment with copper, produced no trace of phenylmercuric fluoride.

(1) National Research Fellow in Chemistry.
(2) Henne and Midgley, THIS JOURNAL, **58**, 884 (1936).
(3) Steinkopf and Bauermeister, *Ann.*, **403**, 50 (1914); Gilman and Wright, THIS JOURNAL, **55**, 3302 (1933).

I wish to thank Professor E. P. Kohler for suggestions offered during this investigation.

Experimental

Phenylmercuric Fluoride.—A solution of 40.6 g. (0.17 mole) of freshly precipitated silver oxide and 14 g. (0.35 mole) of 50% hydrofluoric acid in 400 cc. of water was shaken for five hours with 78 g. (0.25 mole) of phenylmercuric chloride, previously moistened with ethanol. The solid was filtered off and extracted with 400 cc. of boiling ethanol. After a small amount of precipitation by cooling, the solution was decanted and concentrated *in vacuo*. The residue was extracted with boiling ethanol and filtered. The cooled solution yielded 32 g. of phenylmercuric fluoride, m. p. 170°, or 43% of the theoretical. The chlorine-free compound was soluble in hot alcohol, hot xylene and hot chloroform and was crystallized from the latter solvent for analysis (m. p. 171°). It was insoluble in carbon tetrachloride, ethyl acetate, ether and hot acetone. The extracted silver chloride from the reaction mixture contained much unreacted phenylmercuric chloride; no doubt the yield can be increased by a longer period of reaction.

Anal. Calcd. for C_6H_5HgF: C, 24.27; H, 1.70. Found: C, 24.25; H, 1.99.

Reaction with Acetyl Chloride.—When 2.96 g. (0.01 mole) of phenylmercuric fluoride was refluxed with 1.57 g. (0.02 mole) of acetyl chloride, the acetyl fluoride was evolved immediately through the reflux condenser. After five hours the reaction was poured into iced sodium carbonate solution. No mercuric oxide precipitated. The solid was filtered off and crystallized from xylene to weigh 2.80 g. and melt at 257°. This 90% yield of phenylmercuric chloride was substantiated by mixed melting point.

Diphenylmercury.—When phenylmercuric fluoride was slowly destructively distilled at 200° under 10 mm. only diphenylmercury, and no diphenyl, could be found in the distillate.

CONVERSE MEMORIAL LABORATORY
HARVARD UNIVERSITY
CAMBRIDGE, MASS. RECEIVED SEPTEMBER 14, 1936

COMMUNICATIONS TO THE EDITOR

METAL ION ACTIVATION IN ENZYMATIC CATALYSIS. ARGINASE

Sir:

Recent investigations [Hellerman, Perkins, and Clark, *Proc. Nat. Acad. Sci.*, **19**, 855 (1933); Hellerman and Perkins, *J. Biol. Chem.*, **107**, 241 (1934); Bersin, *Z. physiol. Chem.*, **220**, 209 (1933)] support the idea that reversible chemical actions upon substituent thiol groups of certain enzymes (urease, papain, etc.) may account largely for their reversible inactivations by oxidation and by silver ion, phenylmercuric hydroxide, etc. However, the enzyme, arginase [Hellerman and Perkins, *J. Biol. Chem.*, **112**, 175 (1935)], was found to be little sensitive to phenylmercuric hydroxide, and most readily activated, not by reduction in the usual sense but rather by the use of reduced ions of the transition elements, manganese, cobalt or nickel, as well as ferrous ion, which had been associated previously with arginase activation. This, and other evidence cited, was considered to point strongly to metal coördination as a factor not merely in arginase activation but actually in the functioning of the enzyme itself. For example, dissociable, labile enzyme-substrate intermediates might be constructed by the "binding" to a metal ion of both enzyme and substrate through donor groupings of each. If so, an effective metal ion should alter the activity–*p*H curve of arginase in a characteristic way.

We have now studied the effect of such ions upon the arginase–arginine reaction in buffers of widely varying *p*H values but having approximately constant ionic strength ($\mu = 1$). Activity–*p*H curves constructed from the data clearly show characteristic differences. Liver-arginase action is enhanced greatly by nickelous and especially cobaltous ion from *p*H 5 to 7.7; activation by manganous ion is not significant below *p*H 6.7. The optima for cobaltous, nickelous and manganous ion are, respectively, *p*H 7.5 to 7.7, 6.7 to 7.7 and 10 as compared with the optimum for our arginase, without added metal, 7.7 to 9.0. The variations may be considered in relation to the corresponding stabilities of the coördination complexes of these ions with substituted ammonias. For example, we ascertained (by a potentiometric titration method) that the dissociation constants of the complex ions derived from *d*-arginine with cobaltous, nickelous