was added slowly with stirring until the solution was no longer acid to congo red but still acid to litmus. After coupling was complete (two hours), the precipitate was isolated and washed until neutral. The nitro group was then reduced with a 10% solution of sodium sulfide crystals at 90° for three hours. After cooling to 30° the product was isolated and washed until neutral. It was then dissolved in 10% hydrochloric acid, separated by filtration from a small amount of insoluble impurities and reprecipitated at the boil with a 5% sodium hydroxide solution. The product, which separated as maroon-colored granules, was isolated, washed and dried in an oven at 65°; yield 82%. Recrystallized three times from water, the substance formed fine orange-colored rhomboids, m. p. 149°.

The compound is readily soluble in oxygen-containing solvents, slightly soluble in water and aromatic hydrocarbons, and very slightly soluble in aliphatic hydrocarbons. It forms solid solutions with cellulose esters and ethers. Its amino group is diazotizable and it yields an interesting series of insoluble compounds when coupled with phenols, naphthols and aromatic amines. The colors of these products range in general from various shades of blue to black.

Anal. Calcd. for $C_{17}H_{22}O_2N_4$: N, 17.82. Found: N, 17.60.

PFISTER CHEMICAL WORKS
RIDGEFIELD, NEW JERSEY GEORGE SHULMAN

RECEIVED SEPTEMBER 12, 1941

Dimethylneopentylacetic Acid (2,2,4,4-Tetramethylpentanoic Acid), its Methyl Ester, Amide and Acetanilide

1. Many attempts in the past had been unsuccessful in the preparation of the Grignard reagent of diisobutylene hydrochloride in the usual manner. It was found possible to force the formation of reagents of this type by the presence of ethylmagnesium bromide. Magnesium, 18 g., was placed in the conventional apparatus and a small amount of ethylmagnesium bromide was formed by dropping a 60-cc. aliquot of a solution of 33.3 g. of ethyl bromide in 150 cc. of anhydrous ether into the reaction flask. The remainder of the ethyl bromide solution was added to 180 cc. of anhydrous ether and 74 g. of diisobutylene hydrochloride. This solution was added to the magnesium with vigorous stirring at a rate of one drop per second. Titration indicated a yield of 52% of the Grignard of the tertiary chloride. The flask containing the Grignard reagent was cooled with a salt-ice bath and saturated with carbon dioxide. On decomposition in the usual manner, followed by distillation to remove propionic acid, a yield of 34% of dimethylneopentylacetic acid was obtained, m. p. 44–45°.

2. **Dimethylneopentylacetic acid,** 260 g., b. p. 126–130° at 16 mm, obtained from 2,4,4,6,6-pentamethyl-2-heptene by a large scale oxidation of triisobutylene,[1] was converted to the methyl ester by treating the acid with an excess of methanol saturated with hydrogen chloride. Repeated fractionation gave material b. p. 176.2° at 732 mm. (Cottrell), $n^{20}D$ 1.4222 (Valentine), d^{20} 0.879. The acid obtained by saponification of the ester, on fractionation gave b. p. 229.6° at 732 mm. (Cottrell), m. p. and mixed m. p. 45°.

Anal. Calcd. for $C_9H_{18}O_2$: C, 68.3; H, 11.5. Found: C, 68.7; H, 12.0.

3. **Dimethylneopentylacetamide** was prepared by treatment of an ether solution of the acid chloride with anhydrous ammonia. On recrystallization from petroleum ether the amide gave m. p. and mixed m. p. 71°.

4. **Dimethylneopentylacetanilide** was prepared by treating a benzene solution of the acid chloride with a benzene solution of aniline. On recrystallization from a mixture of benzene and petroleum ether the derivative gave m. p. and mixed m. p. 78°.

(1) Whitmore, *et al.*, THIS JOURNAL, **63**, 2036 (1941).

SCHOOL OF CHEMISTRY AND PHYSICS FRANK C. WHITMORE
PENNSYLVANIA STATE COLLEGE W. R. WHEELER
STATE COLLEGE, PA. J. D. SURMATIS

RECEIVED SEPTEMBER 2, 1941

COMMUNICATIONS TO THE EDITOR

THE FORMATION OF ADIPIC ACID BY THE OXIDATIVE DEGRADATION OF THE DIAMINOCARBOXYLIC ACID DERIVED FROM BIOTIN

Sir:

In recent communications[1,2] we have established the molecular formula as well as the functional groups of biotin ($C_{10}H_{16}O_3N_2S$). Biotin was found to be a monocarboxylic acid containing a cyclic urea structure and sulfur in a thio ether linkage. The basis for the cyclic urea structure was our obtaining a diaminocarboxylic acid ($C_9H_{18}O_2N_2S$) containing 2 primary amino groups by treatment of biotin at 140° with $Ba(OH)_2$. The urea structure was confirmed by the resynthesis of biotin from the diaminocarboxylic acid and phosgene.[3] The resynthesized biotin possessed identical chemical, physical, and biological properties with biotin isolated from natural sources. Evidence for the thio ether structure was based mainly on

(1) V. du Bigneaud, K. Hofmann, D. B. Melville and J. R. Rachele, *J. Biol. Chem.*, **140**, 763 (1941).

(2) K. Hofmann, D. B. Melville and V. du Vigneaud, *ibid.*, **141**, 207 (1941).

(3) D. B. Melville, K. Hofmann and V. du Vigneaud, *Science*, **94** 308 (1941).

the formation of a sulfone. Taking into account the absence of an ethylenic linkage, as well as the nature of the functional groups and the ratio of hydrogen to carbon, it is evident that biotin must contain a bicyclic ring system. In two papers recently come to hand, Kögl and co-workers[4,5] arrived at similar conclusions and they present evidence that the sulfur is part of a ring.

We should now like to report the isolation of adipic acid ($C_6H_{10}O_4$) from the degradation products obtained by treatment of the diaminocarboxylic acid with nitric acid or alkaline permanganate. The adipic acid was purified by crystallization from ether and sublimation *in vacuo*. The melting point of the purest samples was 152–153°.[6] A mixture of the isolated material with an authentic sample of adipic acid showed no depression in melting point. The neutral equivalent determined by Dr. Julian Rachele of this Laboratory was found to be 73 in agreement with the theory. To characterize the acid further we have prepared the diamide, m. p. 223–226°, as well as the di-β-naphthylamide, m. p. 266–267°, and both of these compounds proved to be identical with the analogous derivatives prepared from the authentic sample of adipic acid.

It is quite obvious that the finding of a 6-carbon straight chain moiety in the biotin molecule greatly reduces the number of possible structures that might be ascribed to biotin. We prefer to reserve, however, a discussion of structure until experiments underway conclusively demonstrate whether one of the carboxyl groups of the adipic acid is the original carboxyl group of biotin.

(4) F. Kögl and L. Pons, *Z. physiol. Chem.*, **269**, 61 (1941).
(5) F. Kögl and Th. J. de Man, *ibid.*, **269**, 81 (1941).
(6) All the melting points given are micro melting points.

DEPARTMENT OF BIOCHEMISTRY KLAUS HOFMANN
CORNELL UNIVERSITY MEDICAL COLLEGE
NEW YORK, N. Y. DONALD B. MELVILLE
VINCENT DU VIGNEAUD

RECEIVED OCTOBER 23, 1941

THE TETRAACETYL-β-GLUCOSIDE OF DESOXYCORTICOSTERONE

Sir:

A number of considerations are consistent with the suggestion that the true hormone of the adrenal cortex may be a substance resembling a glycoside retaining the corticosterone type of structure as the aglycone. Some light may be thrown on this speculation by a study of the physiological action of synthetic compounds containing the corticosteroid nucleus linked to a sugar residue. The formation of the glucoside linkage in desoxycorticosterone therefore has been investigated. This work was in progress at the time of the announcement by Zwemer, Lowenstein and Pines [*Endocrinology*, **27**, 945 (1940)] of their intention to pursue similar lines of research.

Since the work described below was completed, British Patent 525,307 has come to my attention [*Chem. Abst.*, **35**, 6599 (1941)]. The claims involve the preparation of the tetraacetylglucoside of desoxycorticosterone by the conventional Helferich silver oxide method. The melting point, 175–176°, is in agreement, but no other physical constants, analysis or yields are recorded.

Because of the general inaccessibility of desoxycorticosterone it seemed necessary to investigate a technique for the small-scale preparation of glucosides of steroids. Cholestanol was chosen for this work since both the α- and β-glucoside tetraacetates are easily isolated and have been well characterized [Linstead, THIS JOURNAL, **62**, 1766 (1940)]. In the present work the Zemplén and the Helferich methods were adapted to the semi-micro preparation of the α- and β-isomers, respectively. From 30-mg. samples of sterol the tetraacetyl-α-glucoside was obtained in 35–40% yields; in the case of the β-isomer the yields were higher, 52–54%. A report of this work will appear in detail at a later date.

The Helferich silver oxide method on 25.7-mg. samples of desoxycorticosterone gave the tetraacetyl-β-glucoside in yields of 10–14%. These low yields may be attributed to difficulty in isolation of the reaction product, doubtless explained by its soluble properties in comparison with the cholestanol derivatives. The success of the preparation seemed to depend principally upon the maintenance of anhydrous conditions. A mixture of desoxycorticosterone (25.7 mg.), acetobromoglucose (63.6 mg.), silver oxide (40 mg.) and drierite (about 20–30 mg.) in chloroform or benzene (0.2 cc.) was sealed in a small soft glass tube and allowed to shake for twenty-four hours. The filtrate and washings were evaporated to dryness in a stream of air, and the residue treated with ether (1.7 cc.). On standing several days colorless crystals of the tetraacetyl-β-glucoside separated, yield 5–7 mg., m. p. 170–173° (cor.). Crystallization from 50% alcohol gave glistening clusters of colorless needles, m. p. 176–176.5° cor.

Anal. (Microanalysis by Arlington Laboratories). Calcd. for $C_{35}H_{48}O_{12}$: C, 63.62; H, 7.32.