70 research staff members have been carrying on this specific work. Ten fellowships began operation during the year: alumina, cellulosic molding, chemical storage, coal products analysis, coke-plant physical technology, molasses technology, nickel, pencil technology, special lubricants and synthetic rubber hygiene. Four other new fellowships will start soon. During 1942–43 ten fellowships concluded their activities: Cotton Research Foundation, dielectrics, ethanol, Gartex, iodine, meat merchandising, oil cleaner, plate glass, powder metallurgy and special plastics. The Gartex project has been assigned temporarily to the mineral products fellowship; the iodine researches have been intermitted owing to the emergency; the fellowship on powder metallurgy and the multiple industrial fellowship on chain and welding technology have been consolidated with an increase in the personnel.

The investigations on cotton, 1937–43, have left a prominent record of achievement in the field and have opened the door to the future of an important American crop. The research findings have been incorporated in more than fifty publications, including patents. During the past year the multiple fellowship on plate glass technology finished an important study to determine the effect of bomb explosions on glass and other glazing materials. Much information of value in the design, use and protection of windows has been developed and made available to the services and the civilian defense organizations.

During the calendar year 1942, 2 books, 14 bulletins, 33 research papers and 43 other articles appeared from the institute. Twenty-nine United States patents and 17 foreign patents on fellowship inventions were issued. In 1942 investigational results were contributed to the literature by the following fellowships: chemical hygiene, chemical storage, cigaret technology, Cotton Research Foundation, ethanol, food varieties, gas purification, industrial hygiene, meat merchandising, meter, protected metals, Raolin, refractories, tar distillation and tar synthetics. The department of research in pure chemistry also has several papers to its credit during the past year. *Nutritional Observatory,* a quarterly periodical edited by the staff of the multiple fellowship on food varieties, has entered its fourth volume; this journal has a complimentary mailing list of 26,450.

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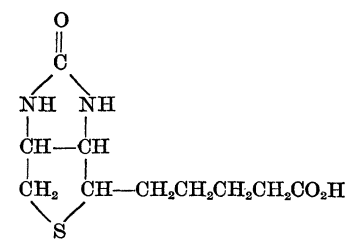
SPECIAL ARTICLES

SYNTHETIC BIOTIN

BIOTIN has been obtained by a total synthesis in this laboratory, and it has been found to be identical with the natural product. This verifies the structure assigned to biotin.

The isolation of biotin as the methyl ester from egg yolk[1] and vitamin H from liver[2] has been described, and the identity of vitamin H and coenzyme R with biotin has been established.[3]

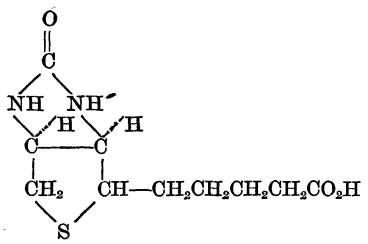
Results of chemical structure investigations in Europe[4] and in this country[5] gave evidence for a carboxylic acid containing a cyclic urea structure and sulfur in a thioether linkage. Further work in this country,[5, 6, 7] showed biotin to consist of a five-membered urea ring fused to a five-membered cyclic thioether having a normal valeric acid side chain. The essential evidence for the five-membered urea ring in biotin was obtained by hydrolysis to a diamine, which gave a dibenzoquinoxaline derivative and which was reconverted quantitatively to biotin by treatment with phosgene.[5] The essential evidence for the ring containing sulfur and having the side chain was furnished by oxidation of the diamine to adipic acid,[5] by the degradation of biotin to desthiobiotin by hydrogenolysis[6] and by degradation to thiophene valeric acid through a modified Hofmann reaction.[7] The structures of the last two compounds were proved by syntheses involving conventional reactions. Barring molecular rearrangements or other obscure reactions during the degradations, these results justified the conclusion drawn by du Vigneaud and collaborators[6, 7] that biotin has Structure I.



I

[1] Kögl and Tönnis, *Zeits. physiol. Chem.*, 242: 43, 1936.
[2] du Vigneaud, Hofmann, Melville and György, *Jour. Biol. Chem.*, 140: 643, 1941.
[3] György, Melville, Burk and du Vigneaud, SCIENCE, 91: 243, 1940; du Vigneaud, Melville, György and Rose, *ibid.*, 92: 62, 1940; György, Rose, Hofmann, Melville and du Vigneaud, *ibid.*, 92: 609, 1940.
[4] Kögl and Pons, *Zeits. physiol. Chem.*, 269: 61, 1941; Kögl and deMan, *ibid.*, 269: 81, 1941; Kögl, Erxleben and Verbeek, *ibid.*, 276: 63, 1942.
[5] Refer to review papers by du Vigneaud (SCIENCE, 96: 455, 1942), and Hofmann ("Advances in Enzymology," Vol. 3, p. 289, 1943. Interscience Publishers, Inc., New York).
[6] du Vigneaud, Melville, Folkers, Wolf, Mozingo, Keresztesy and Harris, *Jour. Biol. Chem.*, 146: 475, 1942.
[7] Melville, Moyer, Hofmann and du Vigneaud, *ibid.*, 146: 487, 1942.

This structure possesses three different asymmetric carbon atoms which normally indicates eight optical isomers or four racemic modifications. However, it is believed from examination of models and reference to the literature[8,9] that two five-membered saturated heterocyclic nuclei fused in this manner can exist only in *cis* forms, as in Structure II.



II

trans Forms of the fused nuclei appear to involve strain which precludes their existence. Apparently, the synthesis of only one compound in which two five-membered rings fused in a *trans* manner through two adjacent atoms has been achieved, namely, *trans*-β-bicyclooctanone,[8] and this is carbocyclic. On this basis, only four isomers existing in two racemic modifications are indicated, and presumably biotin, which is optically active and optically stable,[10] is one of the four.

Any method of synthesis, therefore, has to take these factors into consideration. This we have done. Although the details of procuring best yields of desired intermediates, methods of resolution and other stereochemical problems, etc., are not completely worked out to our satisfaction for a detailed publication as a journal article, we wish to record at this time a comparison of our synthetic product with natural biotin. Synthetic biotin melts at 230–231°, which agrees with the recorded melting point,[11] and is identical with that of a specimen of natural biotin isolated by Dr. J. C. Keresztesy and kindly supplied by him. There is no depression of the melting point of a mixture. The rotation of the synthetic product, $(\alpha)^{25}_{D} + 90.7°$ (C = 2.0, N/10 sodium hydroxide solution) is in agreement also with that of the natural product, $(\alpha)^{25}_{D} + 91.4°$ determined here, and, $(\alpha)^{22}_{D} + 92°$, published[10] previously. The synthetic biotin crystallizes from water in long colorless needles and shows the same general solubility behavior as natural biotin. Its analysis follows: Calcd. for $C_{10}H_{16}N_2O_3S$: C, 49.16; H, 6.60; N, 11.46. Found: C, 49.12; H, 6.47; N, 11.23.

The authors wish to acknowledge the cooperation of Messrs. Glen E. Arth, Robert C. Anderson, Nelson R. Easton and Andrew N. Wilson, and Dr. Dorothea Heyl on the synthesis.

Results of assays on synthetic biotin, determined by Dr. J. L. Stokes, of the microbiology group, with *Lactobacillus arabinosus* No. 17–5 using a described medium,[12] modified to contain nicotinic acid but no biotin, shows that it has a biological activity equal to that of the natural biotin used as a standard.

Bioassays conducted by Dr. G. A. Emerson and Dr. W. H. Ott in the Merck Institute for Therapeutic Research with rats and chicks in which biotin deficiency had been induced by the feeding of egg-white-containing diets demonstrates the fact that the synthetic biotin produces a physiological response identical to that of natural biotin.

The results of the comparison of synthetic with natural biotin establishes their identity and, of course, proves unequivocally the assigned structure[6,7] of biotin.

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8 Linstead and Meade, *Jour. Chem. Soc.*, 935, 1934; Cook and Linstead, *ibid.*, 946, 1934; *ibid.*, 956, 1934.

9 Grigsby, Hind, Chanley and Westheimer, *Jour. Am. Chem. Soc.*, 64: 2606, 1942.

10 Melville, Hofmann and du Vigneaud, SCIENCE, 94: 308, 1941.

11 du Vigneaud, Hofmann, Melville and Rachele, *Jour. Biol. Chem.*, 140: 763, 1941.

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PERSISTENCE OF YELLOW FEVER VIRUS IN THE BRAINS OF MONKEYS IMMUNIZED BY CEREBRAL INOCULATION[1]

PERSISTENCE of virus in the body of the host after infection, despite a refractory state to reinfection from without, has been shown to occur in the case of a number of the viruses, and it has been suggested that lasting specific immunity following some virus diseases depends on this persistence. Psittacosis[2] and salivary gland virus infection of guinea pigs[3] are classic examples of diseases in which such conditions have been encountered. Recovery of virus from the

1 From the Laboratory of the Yellow Fever Research Service, Rio de Janeiro, Brazil. The studies reported in this paper were carried out in the Rio de Janeiro laboratory of the *Serviço de Estudos e Pesquisas sobre a Febre Amarela* (Yellow Fever Research Service) which is maintained jointly by the Ministry of Education and Health of Brazil and the International Health Division of The Rockefeller Foundation.

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