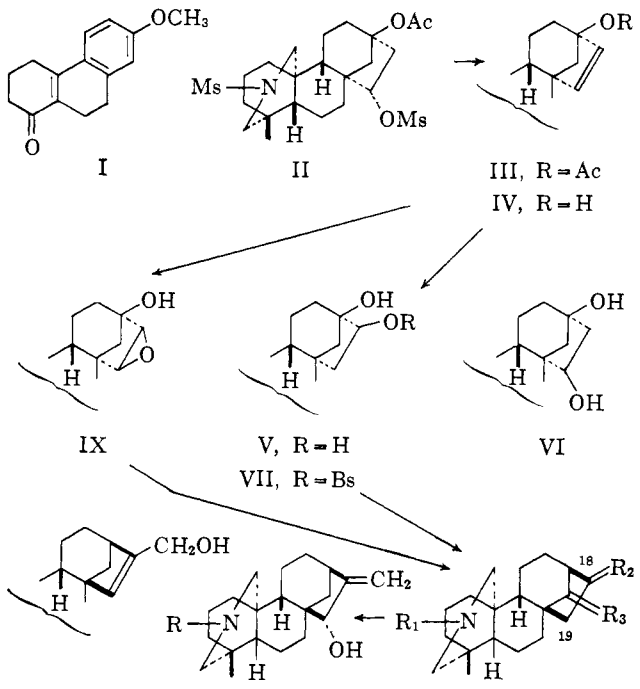


dl-garryine
and *dl*-veatchine

VIII, R_1 = Ms, R_2 = O, R_3 = H_2

X, R_1 = Ms, R_2 = O, R_3 = OH, H

XI, R_1 = Ms, R_2 = O, O, R_3 = OH, H

XII, R_1 = Ms, R_2 = O, O, R_3 = O

XIII, R_1 = Ms, R_2 = O, O, R_3 = H_2

XIV, R_1 = Ms, R_2 = CH_2, R_3 = H_2
XV, R_1 = H, R_2 = CH_2, R_3 = H_2
XVI, R_1 = $COOC_2H_5$, R_2 = CH_2
R_3 = H_2

XVII, m.p. 151–152°, together with the isomeric allylic alcohol XVIII, m.p. 129–130.5°. The over-all yields of XVII and XVIII from the N-mesyl olefin XIV were 9 and 10%, respectively (five steps). The hydrolytic elimination of the carbethoxy group was effected by refluxing[6] a solution of XVII in diethylene glycol with potassium hydroxide and a trace of hydrazine to afford the secondary base XIX, m.p. 182–182.5° ($\nu_{max}^{CHCl_3}$ 3606, 1661, 906 cm.$^{-1}$). Finally, the base XIX was alkylated with ethylene chlorohydrin, according to the process described in the literature,[7] to afford *dl*-dihydroveatchine (XX), m.p. 138–141° ($\nu_{max}^{CHCl_3}$ 3618, 3479, 1662, 905 cm.$^{-1}$), in 56% over-all yield (from XVII). Both bases XIX and XX were proved to be the racemic forms of the naturally derived dihydro pyrolysis base B[7-9] and dihydroveatchine[7,8] by the identity of infrared spectra ($CHCl_3$). Since transformation of dihydroveatchine to garryine[7] and further to veatchine[10] has already been accomplished in the natural series, this work constitutes a total synthesis of the racemic forms of these alkaloids. All the reactions employed in this synthesis, although not satisfactory in yield in a few of the steps, proceeded in a desired stereochemical sense.

Acknowledgment.—We wish to express our thanks to Professor emeritus E. Ochiai and Dr. K. Takeda for showing deep interest and encouraging us throughout this work.

(6) Cf. S. W. Pelletier and P. C. Parthasarathy, *Tetrahedron Letters*, **No. 4**, 205 (1963).

(7) K. Wiesner, W. I. Taylor, S. K. Figdor, M. F. Bartlett, J. R. Armstrong, and J. A. Edwards, *Chem. Ber.*, **86**, 800 (1953).

(8) We are very grateful to Prof. Z. Valenta for his courtesy of supplying valuable authentic samples of natural compounds.

(9) The authors wish to thank Prof. S. W. Pelletier for providing a sample of dihydro pyrolysis base B.

(10) S. W. Pelletier and K. Kawazu, *Chem. Ind.* (London), 1879 (1963).

SHIONOGI RESEARCH LABORATORY
SHIONOGI AND CO., LTD.
FUKUSHIMA-KU, OSAKA, JAPAN

WATARU NAGATA
MASAYUKI NARISADA
TOSHIO WAKABAYASHI
TSUTOMU SUGASAWA

RECEIVED DECEMBER 23, 1963

Insulin Peptides. X. The Synthesis of the B-Chain of Insulin and Its Combination with Natural or Synthetic A-Chain to Generate Insulin Activity[1]

Sir:

In a previous communication[2] we have reported the synthesis of the A-chain of sheep insulin, its isolation in the S-sulfonate form, and its combination with natural B-chain to generate insulin activity. We have now completed synthesis and isolation in the S-sulfonate form of a triacontapeptide with the amino acid sequence proposed by Sanger for the B-chain of insulin.[3] The proposed structure for sheep insulin is shown in Chart I. The synthetic B-chain upon combination with natural A-chain generated insulin activity equivalent to that produced when natural B-chain was recombined with natural A-chain. In addition, insulin activity was generated when the synthetic B-chain was combined with a synthetic preparation of A-chain. This last observation appears to represent the first chemical synthesis of a naturally occurring protein.

Im-Benzyl-L-histidine benzyl ester[4] was condensed with N-carbobenzoxy-L-glutamine *p*-nitrophenyl ester[5] to give N-carbobenzoxy-L-glutaminyl-Im-benzyl-L-histidine benzyl ester (I), m.p. 168°; $[\alpha]^{28}D$ − 25.7° (*c* 1.0, acetic acid) (*Anal.* Calcd. for $C_{33}H_{35}N_5O_6$: C, 66.3; H, 5.90; N, 11.8. Found: C, 65.8; H, 6.12; N, 11.5); R_f[6] (hydrobromide) 0.40. Decarbobenzoxylation of I with HBr in acetic acid and coupling of the ensuing product with N-carbobenzoxy-L-asparagine *p*-nitrophenyl ester[5] afforded N-carbobenzoxy-L-asparaginyl-L-glutaminyl-Im-benzyl-L-histidine benzyl ester (II), m.p. 184°; $[\alpha]^{28}D$ −20.9° (*c* 1.5, acetic acid) (*Anal.* Calcd. for $C_{37}H_{41}N_7O_8$: C, 62.4; H, 5.81; N, 13.8. Found: C, 62.1; H, 5.94; N, 13.8); R_f[6] (hydrobromide) 0.36. The tetrapeptide N-carbobenzoxy-L-valyl-L-asparaginyl-L-glutaminyl-Im-benzyl-L-histidine benzyl ester (III, m.p. 220°; $[\alpha]^{28}D$ −31.1° (*c* 0.9, acetic acid) (*Anal.* Calcd. for $C_{42}H_{50}N_8O_9$: C, 62.2; H, 6.21;

(1) Presented in part (P. G. K.) as the first Edwin J. Cohn Memorial Lecture at the 15th Annual Scientific Conference of Protein Foundation on November 25, 1963, Cambridge, Mass.; *Vox Sanguinis*, in press.

(2) P. G. Katsoyannis, A. Tometsko, and K. Fukuda, *J. Am. Chem. Soc.*, **85**, 2863 (1963).

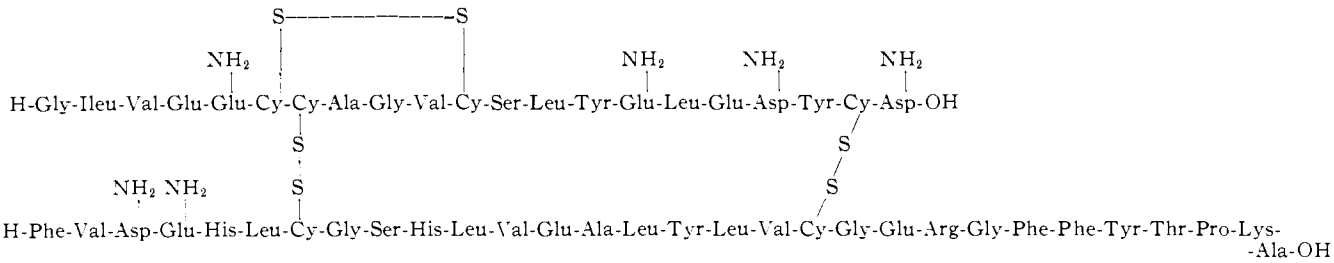
(3) F. Sanger and H. Tuppy, *Biochem. J.*, **49**, 463, 481 (1951); F. Sanger and E. O. L. Thompson, *ibid.*, **53**, 353, 366 (1953); H. Brown, F. Sanger, and R. Kitai, *ibid.*, **60**, 556 (1955); J. I. Harris, F. Sanger, and M. A. Naughton, *Arch. Biochem. Biophys.*, **65**, 427 (1956).

(4) D. Theodoropoulos and G. Fölsch, *Acta Chem. Scand.*, **12**, 1955 (1958).

(5) M. Bodanszky and V. du Vigneaud, *J. Am. Chem. Soc.*, **81**, 5688 (1959).

(6) The R_f refers to the Partridge system [S. M. Partridge, *Biochem. J.*, **42**, 238 (1948)].

CHART I



N, 13.8. Found: C, 62.3; H, 6.29; N, 14.0); R_f[6] (hydrobromide) 0.39) was prepared by the reaction of N-carbobenzoxy-L-valine *p*-nitrophenyl ester[7] with the product obtained by HBr in acetic acid decarbobenzoxylation of II. Removal of the carbobenzoxy group from III on exposure to HBr in acetic acid and coupling of the ensuing product with N-carbobenzoxy-L-phenylalanine *p*-nitrophenyl ester[8] yielded N-carbobenzoxy-L-phenylalanyl-L-valyl-L-asparaginyl-L-glutaminyl-Im-benzyl-L-histidine benzyl ester (IV), m.p. 232°; $[\alpha]^{28}D$ −27.3° (*c* 1.0, acetic acid) (*Anal.* Calcd. for $C_{51}H_{59}N_9O_{10}\cdot H_2O$: C, 62.7; H, 6.29; N, 12.9. Found: C, 62.7; H, 6.28; N, 13.2); R_f[6] (hydrobromide) 0.51. Saponification of IV with NaOH afforded N-carbobenzoxy-L-phenylalanyl-L-valyl-L-asparaginyl-L-glutaminyl-Im-benzyl-L-histidine (V), m.p. 222–224°; $[\alpha]^{29}D$ −3.4° (*c* 1.0, dimethyl sulfoxide) (*Anal.* Calcd. for $C_{44}H_{53}N_9O_{10}\cdot 0.5H_2O$: C, 60.3; H, 6.20; N, 14.4. Found: C, 60.0; H, 6.58; N, 13.9); for the hydrobromide: R_f[6] 0.42, R_f[9] 2.9 × his.

N-Carbobenzoxyglycyl-L-serine methyl ester[10] was decarbobenzoxylated on exposure to HBr in trifluoroacetic acid and the resulting product was condensed with N-carbobenzoxy-S-benzyl-L-cysteine *p*-nitrophenyl ester[5] to give N-carbobenzoxy-S-benzyl-L-cysteinylglycyl-L-serine methyl ester (VI), m.p. 151°; $[\alpha]^{29}D$ −29.2° (*c* 1.01, dimethylformamide) (*Anal.* Calcd. for $C_{24}H_{29}N_3O_7S_1$: C, 57.2; H, 5.80; N, 8.3. Found: C, 57.3; H, 5.70; N, 8.1); for the hydrobromide: R_f[6] 0.74, R_f[9] 3.28 × his. N-Carbobenzoxy-L-leucyl-S-benzyl-L-cysteinylglycyl-L-serine methyl ester (VII, m.p. 139°; $[\alpha]^{27}D$ −25.5° (*c* 1.1, dimethylformamide) (*Anal.* Calcd. for $C_{30}H_{40}N_4O_8S_1$: C, 58.4; H, 6.54; N, 9.1. Found: C, 58.6; H, 6.69; N, 9.0); for the hydrobromide: R_f[6] 0.85; R_f[9] 3.74 × his.) was prepared by the coupling of N-carbobenzoxy-L-leucine *p*-nitrophenyl ester[5] with the product obtained by HBr in trifluoroacetic acid decarbobenzoxylation of VI. Removal of the carbobenzoxy group from VII on exposure to HBr in trifluoroacetic acid and coupling of the ensuing product with V by the carbodiimide method[11] yielded N-carbobenzoxy-L-phenylalanyl-L-valyl-L-asparaginyl-L-glutaminyl-Im-benzyl-L-histidyl-L-leucyl-S-benzyl-L-cysteinylglycyl-L-serine methyl ester (VIII), m.p. 232–234°; $[\alpha]^{27}D$ −25.2° (*c* 1.05, diimethyl sulfoxide) (*Anal.* Calcd. for $C_{66}H_{85}N_{13}O_{15}S_1$: C, 59.5; H, 6.43; N, 13.7. Found: C, 59.1; H, 6.78; N, 13.5); for the hydrobromide: R_f[6] 0.81, R_f[9] 3.6 × his, R_f[12] 0.95; product completely digestible by leucine aminopeptidase (LAP) as judged by amino acid analysis of the digest; amino acid ratios in acid hydrolysate, $phe_{0.96}val_{1.04}asp_{1.07}glu_{1.09}leu_{0.93}ser_{0.64}gly_{0.91}$ (Im-benzylhistidine and S-benzylcysteine not determined); average amino acid recovery, 71%. Treatment of VIII with hydrazine afforded N-carbobenzoxy-L-phenylalanyl-L-valyl-L-asparaginyl-L-glutaminyl-Im-benzyl-L-histidyl-L-leucyl-S-benzyl-L-cysteinylglycyl-L-serine hydrazide (IX), m.p. 236–238°; $[\alpha]^{28}D$ −36.7° (*c* 1.3, acetic acid) (*Anal.* Calcd. for $C_{65}H_{85}H_{15}O_{14}S_1$: C, 58.6; H, 6.43; N, 15.8. Found: C, 58.1; H, 6.62; N, 15.6); R_f[6] 0.91, single spot (chlorine test[13]).

N^{α}-Carbobenzoxy-Im-benzyl-L-histidyl-L-leucyl-L-valyl-L-glutamic acid α-ethyl-γ-*t*-butyl ester (X, m.p. 168–172°; $[\alpha]^{28}D$ −20.3° (*c* 1.26, dimethylformamide) (*Anal.* Calcd. for $C_{43}H_{60}N_6O_9\cdot H_2O$: C, 62.8; H, 7.59; N, 10.2. Found: C, 62.8; H, 7.42; N, 10.5); R_f[6] 0.91 (chlorine test); after treatment with HBr in acetic acid, R_f[6] 0.73) was prepared from N^{α}-carbobenzoxy-Im-benzyl-L-histidyl-L-leucine[14] plus L-valyl-L-glutamic acid α-ethyl-γ-*t*-butyl ester[15] using the carbodiimide method. On exposure to alkali, X was converted to N^{α}-carbobenzoxy-Im-benzyl-L-histidyl-L-leucyl-L-valyl-γ-*t*-butyl-L-glutamic acid (XI), m.p. 178–182°; $[\alpha]^{29}D$ −16.5° (*c* 0.77, dimethylformamide) (*Anal.* Calcd. for $C_{41}H_{56}N_6O_9$: C, 63.4; H, 7.27; N, 10.8. Found: C, 63.5; H, 7.34; N, 10.9); after treatment with HBr in acetic acid: R_f[6] 0.67 (ninhydrin-positive and Pauly-negative spot). Reaction of XI with *p*-nitrophenol in the presence of N,N′-dicyclohexylcarbodiimide yielded N^{α}-carbobenzoxy-Im-benzyl-L-histidyl-L-leucyl-L-valyl-L-glutamic acid γ-*t*-butyl-α-*p*-nitrophenyl ester (XII), m.p. 113–116°; $[\alpha]^{27}D$ −14.5° (*c* 1, dimethylformamide) (*Anal.* Calcd. for $C_{47}H_{62}N_7O_{11}$: C, 62.7; H, 6.90; N, 10.7. Found: C, 62.2; H, 6.23; N, 10.5).

N^{α}-Carbobenzoxy-N^{ω}-tosyl-L-arginylglycyl-L-phenylalanyl-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-N^{ϵ}-tosyl-L-lysyl-L-alanine methyl ester[16] was decarbobenzoxylated by catalytic hydrogenation and the ensuing product was condensed with N-carbobenzoxy-L-glutamic acid γ-*t*-butyl-α-*p*-nitrophenyl ester[17] to give N-carbobenzoxy-γ-*t*-butyl-L-glutamyl-N^{ω}-tosyl-L-arginylglycyl-L-phenylalanyl-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-N^{ϵ}-tosyl-L-lysyl-L-alanine methyl ester (XIII), m.p. 206–209°; $[\alpha]^{27}D$ −25° (*c* 1, dimethylformamide) (*Anal.* Calcd. for $C_{85}H_{110}N_{14}O_{21}S_2$: C, 59.1; H, 6.42; N, 11.4. Found: C, 59.5; H, 6.17; N, 11.1); after treatment with HBr in acetic acid: R_f[6] 0.90, R_f[9] 4.43 × his. Catalytic hydrogenolysis of XIII and coupling of the ensuing product with N-carbobenzoxy-L-alanyl-L-leucyl-L-tyrosyl-L-leucyl-L-valyl-S-benzyl-L-cysteinylglycine[18] by the N,N′-carbonyldiimidazole method[19] af-

(7) B. Iselin, W. R. Rittel, P. Sieber, and R. Schwyzer, *Helv. Chim. Acta*, **40**, 373 (1957).

(8) M. Bodanszky and V. du Vigneaud, *J. Am. Chem. Soc.*, **81**, 6072 (1959).

(9) The R_f refers to the system 1-butanol-pyridine–acetic acid–water 30:20:6:24 [S. G. Waley and G. Watson, *Biochem. J.*, **55**, 328 (1953)] and is expressed as a multiple of the distance traveled by a histidine marker.

(10) J. I. Harris and J. S. Fruton, *J. Biol. Chem.*, **191**, 143 (1951).

(11) J. C. Sheehan and G. P. Hess, *J. Am. Chem. Soc.*, **77**, 1067 (1955).

(12) The R_f refers to the 2-butanol–ammonia system [J. F. Roland, Jr., and A. M. Gross, *Anal. Chem.*, **26**, 502 (1954)].

(13) H. Zahn and E. Rexroth, *Z. Anal. Chem.*, **148**, 181 (1955).

(14) W. T. Huang, C. S. Yang, K. Z. Wang, and C. I. Niu, *Sci. Sinica* (Peking), **11**, 499 (1962).

(15) P. G. Katsoyannis, K. Fukuda, and A. Tometsko, *J. Am. Chem. Soc.*, **85**, 1681 (1963).

(16) P. G. Katsoyannis and (in part) K. Suzuki, *ibid.*, **85**, 2659 (1963).

(17) K. Hofmann, personal communication.

(18) P. G. Katsoyannis and M. Tilak, *J. Am. Chem. Soc.*, **85**, 4028 (1963).

(19) G. W. Anderson and R. Paul, *ibid.*, **80**, 4423 (1958); R. Paul and G. W. Anderson, *ibid.*, **82**, 4596 (1960).

forded N-carbobenzoxy-L-alanyl-L-leucyl-L-tyrosyl-L-leucyl-L-valyl-S-benzyl-L-cysteinylglycyl-γ-*t*-butyl-L-glutamyl-N^{ω}-tosyl-L-arginylglycyl-L-phenylalanyl-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-N^{ϵ}-tosyl-L-lysyl-L-alanine methyl ester (XIV), m.p. 256–259°; $[\alpha]^{27}D$ −33.6° (*c* 1, dimethylformamide) (*Anal.* Calcd. for $C_{125}H_{169}N_{21}O_{29}S \cdot H_2O$: C, 59.0; H, 6.77; N, 11.5. Found: C, 58.7; H, 6.90; N, 11.5.); after treatment with HBr in acetic acid: R_f^6 0.93, R_f^9 6.58 × his; amino acid ratios in acid hydrolysate, $lys_{1.13}arg_{1.00}$S-benzylcysteine$_{0.94}thr_{0.88}glu_{0.97}gly_{2.09}ala_{1.88}val_{1.16}leu_{2.19}pro_{0.97}tyr_{1.97}phe_{1.84}$; average amino acid recovery, 91%. Exposure of XIV to HBr in acetic acid and reaction of the resulting product with XII yielded the protected heneicosapeptide; this, in turn, on exposure to alkali and then to HBr in acetic acid afforded the C-terminal segment of the B-chain Im-benzyl-L-histidyl-L-leucyl-L-valyl-L-glutamyl-L-alanyl-L-leucyl-L-tyrosyl-L-leucyl-L-valyl-S-benzyl-L-cysteinylglycyl-L-glutamyl-N^{ω}-tosyl-L-arginylglycyl-L-phenylalanyl-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-N^{ϵ}-tosyl-L-lysyl-L-alanine hydrobromide (XV), $[\alpha]^{27}D$ −24.4° (*c* 0.9, dimethylformamide) (in none of the usual paper chromatographic systems employed in these studies did this peptide move from the origin, hence, no paper chromatographic criteria could be obtained); amino acid ratios in acid hydrolysate, $lys_{0.85}arg_{0.95}thr_{1.10}glu_{1.95}pro_{1.15}gly_{2.40}ala_{1.95}val_{1.80}leu_{2.70}tyr_{1.90}phe_{2.40}$ (S-benzylcysteine and Im-benzylhistidine not determined); average amino acid recovery, 87%.

Conversion of IX to the corresponding azide and coupling of the later product with XV yielded the protected sulfhydryl form of the B-chain of insulin. The protecting groups were removed by treatment with sodium in liquid ammonia and the deblocked product was converted to the S-sulfonate form and purified by ion-exchange chromatography. The S-sulfonate of the B-chain obtained exhibited on ion-exchange chromatography a pattern identical with that of the S-sulfonate of natural B-chain[20] and on paper chromatography exhibited a single Pauly-positive spot with the same R_f[21] (0.96) as natural B-chain. Amino acid analysis of the synthetic material after acid hydrolysis gave a composition in molar ratios which corresponds to that of the natural B-chain, $lys_{0.80}arg_{1.00}his_{1.8}asp_{1.10}thr_{0.84}ser_{0.85}glu_{3.3}pro_{0.85}gly_{3.34}ala_{1.64}val_{3.14}leu_{4.34}tyr_{1.84}phe_{3.3}$ (cystine was not determined).

Combination experiments[22] between the synthetic material and the A-chain, natural or synthetic, provided further proof that the synthetic product is indeed the B-chain of insulin. As judged by biological assays, using the mouse-diaphragm method, insulin activity was generated when the synthetic B-chain was combined with natural A-chain. Furthermore the amount of activity generated was quantitatively identical with the activity produced when an equal amount of natural B-chain was used for the combination experiments.[23] Considerable insulin activity was also obtained when synthetic B-chain was combined with a partially purified preparation of synthetic A-chain. The quantitative results of the recombination experiments and the biological assays will be reported in a later communication.[22]

The data on the synthesis of the B-chain presented in this report in conjunction with our previous communication regarding the synthesis of the A-chain[2] strongly suggest that the structure proposed by Sanger for the insulin chains is correct. This constitutes a unique case where a proposed primary structure for a protein has been confirmed by chemical synthesis. Du, *et al.*,[24] have reported the isolation of crystalline insulin, identical with the natural hormone, by recombination of natural A- and B-chains. On this basis, work is now in progress in our laboratory on the synthesis of amounts of the two chains adequate to permit the isolation of synthetic insulin by combination of synthetic A- and B-chains. It is expected that the material thus obtained also will be identical with the natural protein.

(20) Natural B-chain was prepared in our laboratory from crystalline zinc-insulin by a modification of the method of Meienhofer and Brinkhof [J. Meienhofer and O. Brinkhof, *Nature*, **199**, 1096 (1963)].

(21) The R_f refers to a descending paper chromatography in the system 2-butanol–acetic acid–8 *M* urea 12.5:1:11.5 [Y.-C. Du, Y.-S. Zhang, Z.-X. Lu, and C.-L. Tsou, *Sci. Sinica* (Peking), **10**, 84 (1961)].

(22) We are most indebted to Dr. G. H. Dixon of the Department of Biochemistry, University of British Columbia, who carried out the combination experiments, and to Dr. J. K. Davidson of the Best Institute, University of Toronto, who assayed the combination reaction mixtures for insulin activity.

(23) A crude preparation of B-chain which generated only slight insulin activity upon combination with the A-chain was obtained by the condensation of the N-terminal tridecapeptide with the C-terminal heptadecapeptide. This work was presented (P. G. K.) at the Brook Lodge Conference on Proteins and Polypeptides on October 7–9, 1963, Kalamazoo, Mich.

(24) See reference in footnote 21.

(25) This work was supported by a Research Career Development Award (GM-K3-15151) from the Public Health Service and a grant (A-3067) from the National Institute of Arthritis and Metabolic Diseases, Public Health Service, for which we wish to express our appreciation.

(26) The authors wish to express their appreciation to Mrs. Jemele Hudson for the enzymatic analyses and amino acid analyses and Mrs. Gudrun Hjorth for technical assistance.

BIOCHEMISTRY DEPARTMENT
UNIVERSITY OF PITTSBURGH
SCHOOL OF MEDICINE
PITTSBURGH, PENNSYLVANIA

PANAYOTIS G. KATSOYANNIS[25,26]
KOUHEI FUKUDA
ANDREW TOMETSKO
KENJI SUZUKI
MANOHAR TILAK

RECEIVED JANUARY 28, 1964

Stable Carbonium Ions. VI.[1] Nuclear Magnetic Resonance Investigation of the Diphenylcarbonium, Diphenylmethylcarbonium, and Phenyldimethylcarbonium Ions

Sir:

The triphenylcarbonium ion is the best investigated carbonium ion. Its structure has been established by ultraviolet,[2] infrared,[3] and n.m.r. investigations.[4]

In contrast, the diphenylcarbonium ion (benzhydryl cation) is much less known and has not yet been directly characterized with the exception of ultraviolet investigations. Gold[5] and Deno and his co-workers[6] reported the ultraviolet absorption of diphenylmethanol in concentrated sulfuric acid. Hafner[7] and Holmes and Pettit[8] possibly obtained salts of $(C_6H_5)_2CH^+$, but the structure of these was not more closely characterized, possibly due to their instability in solution.

The diphenylmethylcarbonium ion was investigated by Gold[5] through the ultraviolet spectra of 1,1-diphenylethylene and 1,1-diphenylethanol in sulfuric acid solution, as well as by O'Reilly and Leftin[9] through the n.m.r. proton spectrum of 1,1-diphenylethanol in sulfuric acid solution. Although the position of the main aromatic proton peak and the methyl group could be established, due to fast hydrogen exchange in sulfuric acid solution, no fine structure was observed. At-

(1) Part V: *J. Am. Chem. Soc.*, in press.

(2) For a review see, L. N. Ferguson, *Chem. Rev.*, **43**, 385 (1948).

(3) D. W. A. Sharp and N. Sheppard, *J. Chem. Soc.*, 674 (1957).

(4) R. B. Moodie, T. M. Connor, and R. Stewart, *Can. J. Chem.*, **37**, 1402 (1959); R. Dehl, W. R. Waughan, and R. S. Berry, *J. Org. Chem.*, **24**, 1616 (1959); R. S. Berry, R. Dehl, and W. R. Waughan, *J. Chem. Phys.*, **34**, 1460 (1961).

(5) V. Gold and F. L. Tye, *J. Chem. Soc.*, 2172 (1952).

(6) N. C. Deno, J. J. Jaruzelski, and A. Schriesheim, *J. Am. Chem. Soc.*, **77**, 3044 (1955).

(7) K. Hafner and H. Pelster, *Angew. Chem.*, **73**, 342 (1961).

(8) J. Holmes and R. Pettit, *J. Org. Chem.*, **28**, 1965 (1963).

(9) D. E. O'Reilly and H. P. Leftin, *J. Phys. Chem.*, **64**, 1555 (1960).