forded N-carbobenzoxy-L-alanyl-L-leucyl-L-tyrosyl-L-leucyl-L-valyl-S-benzyl-L-cysteinylglycyl-γ-*t*-butyl-L-glutamyl-N$^{\omega}$-tosyl-L-arginylglycyl-L-phenylalanyl-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-N$^{\epsilon}$-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$^{\omega}$-tosyl-L-arginylglycyl-L-phenylalanyl-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-N$^{\epsilon}$-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.

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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).

tempts have been made[10] to prepare diphenylmethylcarbonium tetrafluoroborate by the reaction between 1,1-diphenylethyl chloride and silver tetrafluoroborate in ether solution. Although, as evidenced by silver chloride elimination, there appears to be a primary formation of the carbonium ion due to subsequent proton elimination, no stable complex could be isolated.

The phenyldimethylcarbonium ion is so far unreported in the literature.

It was possible now to obtain the diphenylcarbonium, diphenylmethylcarbonium, and phenyldimethylcarbonium ions in the form of their solvated hexafluoroantimonate salts in sulfur dioxide and sulfur dioxide–antimony pentafluoride solutions and to record their proton magnetic resonance spectra.

In previous work of this series[11] the isolation of the diphenylacetyl fluoride–antimony pentafluoride complex was reported, which, in contrast to many similar acyl fluoride–antimony pentafluoride complexes of oxocarbonium (acylium) salt nature, was found both in the solid state (based on infrared investigations) and in sulfur dioxide solution (based on n.m.r. investigations) to be only a polarized donor–acceptor complex.

$$(C_6H_5)_2CHCOF + SbF_5 \rightleftharpoons (C_6H_5)_2CHC(F)\!\!\nearrow\!\!O \rightarrow SbF_5$$

In continuation of this work a more detailed investigation of this complex in sulfur dioxide was carried out. It was observed that when a sample of the crystalline diphenylacetyl fluoride–antimony pentafluoride complex was dissolved in sulfur dioxide and left to stand for some hours at $-40°$, and the n.m.r. proton resonance spectrum then obtained at the same temperature, besides the previously reported donor–acceptor complex (I), a second, substantially more deshielded species (II) could also be resolved. Data are summarized in Table I.

TABLE I

NUCLEAR MAGNETIC PROTON RESONANCE[a] OF THE DIPHENYLACETYL FLUORIDE–ANTIMONY PENTAFLUORIDE SYSTEM IN SO_2 SOLUTION AT $-40°$

	Aromatic ring protons			CH
$(C_6H_5)_2CHCOF$ (neat)		−7.10		−4.97
$(C_6H_5)_2CHCOF$–SbF_5 (I)		−7.12		−5.35
Deshielded species (II)	−7.7	−8.2	−8.5	−9.7[b]

[a] 60 Mc., p.p.m. from TMS. [b] Peak area ratio 9.95:1.

Substantial deshielding is evident from the position of the ring protons and the aliphatic CH proton at −9.7 p.p.m. In preparative scale experiments, the evolution of carbon monoxide from the system could be established, thus indicating that the new deshielded species (II) could be diphenylcarbonium hexafluoroantimonate.

$$\underset{\text{I}}{(C_6H_5)_2CH\overset{\delta+}{C}(F)\!\!\nearrow\!\!O \rightarrow \overset{\delta-}{SbF_5}} \rightleftharpoons \underset{\text{II}}{CO + (C_6H_5)_2CH^+SbF_6^-}$$

A similar decarbonylation of the triphenylacetyl fluoride–antimony pentafluoride systems resulted in the quantitative formation of triphenylcarbonium hexafluoroantimonate, in this case isolable as the stable crystalline salt (m.p. 211°). Diphenylmethylacetyl fluoride–antimony pentafluoride and phenyldimethylacetyl fluoride–antimony pentafluoride in sulfur dioxide solution similarly yielded besides the acyl fluoride complexes *via* decarbonylation substantially deshielded species, in all probability the tertiary carbonium ions. The methyl protons were found in these species at −3.50 and −3.35 p.p.m., respectively; the aromatic ring protons also show deshielding.

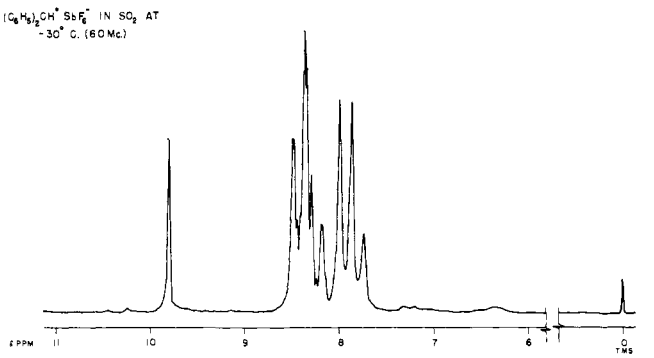

Fig. 1.—Spectrum (n.m.r.) of diphenylcarbonium ion.

In order to ascertain that the observed deshielded species in the phenylacetyl fluoride–antimony pentafluoride systems are indeed the decarbonylated phenylcarbonium ions, and to obtain these ions in pure form, the previously described[1] carbonium ion formation method using antimony pentafluoride as both Lewis acid and solvent was used. Diphenylchloromethane, 1,1-diphenyl-1-chloroethane, and 2-chloro-2-phenylpropane (α-chlorocumene) gave with excess antimony pentafluoride stable, solvated hexafluoroantimonate complexes of the diphenyl-, diphenylmethyl-, and phenyldimethylcarbonium ions. The nuclear magnetic proton resonance spectra of these ions could be obtained at $-30°$, using sulfur dioxide containing excess antimony pentafluoride as solvent. Table II summarizes the n.m.r. data compared with that of the known triphenylcarbonium ion. The spectrum of the diphenylcarbonium ion is shown in full in Fig. 1 as representative of the resolution obtained using a Varian Model A-60 spectrograph equipped with a low temperature probe.

TABLE II

NUCLEAR MAGNETIC PROTON RESONANCE[a] OF PHENYLCARBONIUM HEXAFLUOROANTIMONATE COMPLEXES IN SO_2–SbF_5 SOLUTION AT $-30°$

Compound	C^+–CH_3	Aromatic ring H—— *ortho*[b]	*meta*[b]	*para*	C^+H	Peak area ratio
$C_6H_5C^+(CH_3)_2$ SbF_6^-	−3.60	−7.95	−8.87	−8.56		6:5.04
$(C_6H_5)_2C^+CH_3$ SbF_6^-	−3.70	−7.53	−7.96	−8.12		3:9.95
$(C_6H_5)_2C^+H$ SbF_6^-		−7.92	−8.49	−8.37	−9.8	9.97:1
$(C_6H_5)_3C^+$ SbF_6^- (in neat SO_2)		−7.01	−7.51	−7.76		

[a] 60 Mc., p.p.m. from TMS. [b] See ref. 12.

The assignment of the deshielding sequence of the *ortho* and *meta* ring protons is tentative and arbitrary based only on the assumed analogy with the ring protons in the known triphenylcarbonium ion, where suc-

(10) D. W. A. Sharp, "Advances in Fluorine Chemistry," Vol. I, Butterworth and Co., London, 1960, p. 93.

(11) G. A. Olah, W. S. Tolgyesi, S. J. Kuhn, M. E. Moffatt, I. J. Bastien, and E. B. Baker, *J. Am. Chem. Soc.*, **85**, 1328 (1963).

cessive deuteration showed the *meta* protons more deshielded than the *ortho* protons.[12]

(12) One of the referees called attention to unpublished and independent work of Dr. D. G. Farnum on phenyl and phenylmethyl carbonium ions and suggested simultaneous publication, which was arranged by mutual agreement. Having learned of Dr. Farnum's data, one can say that there is excellent agreement of the observed chemical shifts of the aliphatic protons and the over-all ring proton shifts. However, based on Dr. Farnum's data, supported by calculation of theoretical spectra with the use of a computer program, the deshielding sequence of the *ortho* and *meta* protons is reversed in the diphenyl- and phenylmethylcarbonium ions, as compared with that of the triphenylcarbonium ion.

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Chemical Shifts and Long Range Shielding Effects in the Nuclear Magnetic Resonance Spectra of Phenyldimethyl, Diphenyl, Triphenyl, and Related Carbonium Ions

Sir:

Analyses of the n.m.r. spectra of triphenylcarbonium and methyldiphenylcarbonium ions have been reported in the literature.[1-3] We wish to describe results of an analysis of the n.m.r. spectra of a number of phenylcarbonium ions which (1) suggest that shielding of *ortho* protons by neighboring phenyl rings in di- and triphenylcarbonium ions is more important than has been recognized in earlier papers, and (2) permit a qualitative correlation of the extent of phenyl delocalization of the positive charge with the position of the *para* proton resonance.

TABLE I
RING PROTON CHEMICAL SHIFTS FOR SOME PHENYLCARBONIUM IONS[4]

Case	Carbonium ion	*para*, τ-units	*ortho*, τ-units	*meta*, τ-units	*ortho*–*para*, p.p.m.	*meta*–*para*, p.p.m.
1	$C_6H_5CH_2CH_2C^+(OH)_2$[a]	2.80	2.80	2.80	0	0
2	$C_6H_5C^+(OH)_2$	1.94	1.73	2.24	−0.21	+0.30
3	$(C_6H_5)_2C^+OH$	1.85	1.88	2.17	+0.03	+0.32
4	$C_6H_5C^+(OH)CH_3$[b]	1.84	1.57	2.22	−0.30	+0.38
5	$(C_6H_5)_3C^+$	1.76	2.31	2.13	+0.55	+0.37
6	$(C_6H_5)_2C^+CH_3$[c]	1.72	1.97	2.12	+0.25	+0.40
7	$(C_6H_5)_2C^+H$[d]	1.62	1.54	2.02	−0.08	+0.40
8	$C_6H_5C^+(CH_3)_2$[e]	1.45	1.20	2.03	−0.25	+0.58

[a] Since sulfonation in chlorosulfonic acid was very rapid, the n.m.r. spectrum of this substance was determined in concentrated sulfuric acid. [b] CH_3 resonance at τ 6.69. [c] CH_3 resonance at τ 6.30. [d] >CH resonance at τ 0.19. [e] CH_3 resonance at τ 6.43.

The *ortho*, *meta*, and *para* ring proton chemical shifts for a number of phenylcarbonium ions are given in Table I. Values were determined by comparing n.m.r. spectra of chlorosulfonic acid solutions of the appropriate carbinol or carbonyl compound[4] with calculated spectra obtained using the computer program of Bothner-By and Naar-Colin.[6,7] The calculated and de-

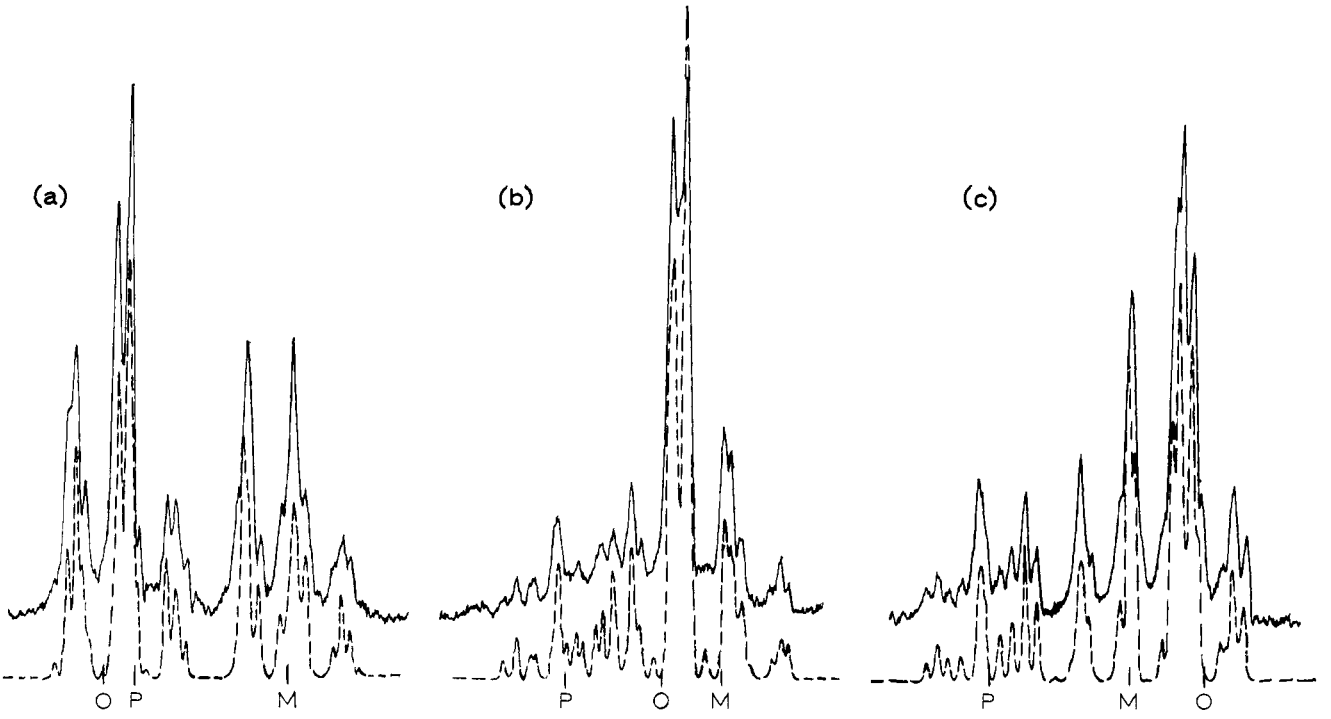

Fig. 1.—Calculated (dashed line) and experimental (solid line) n.m.r. spectra of some phenylcarbonium ions. Chemical shift differences (δ) and coupling constants (J) for the *ortho* (O), *meta* (M), and *para* (P) protons used for the calculation are given in c.p.s. Changes of 0.5 c.p.s. in chemical shifts or the large coupling constants gave noticeably poorer agreement. The small coupling constants are probably within 1 c.p.s. of the correct value. (a) Diphenylcarbonium ion: δ(O–P) −5, δ(O–M) +24; J(O, M) 8.2, (O, P) 1.2, (O, M′) 0.5, (O, O′) 1.2, (M, P) 7.5, (M, M′) 1.7. (b) Methyldiphenylcarbonium ion: δ(O–P) +15, δ(M, P) +24.5; J(O, M) 8.2, (O, P) 1.2, (O, M′) 0.5, (O, O′) 1.2, (M, P) 7.5, (M, M′) 1.7. (c) Triphenylcarbonium ion: δ(O–P) +33.5, δ(M–P) +22; J(O, M) 7.7, (O, P) 1.2, (O, M′) 0.5, (O, O′) 1.2, (M, P) 7.5, (M, M′) 1.0.

(1) R. Dehl, W. R. Vaughan, and R. S. Berry, *J. Org. Chem.*, **24**, 1616 (1959); *J. Chem. Phys.*, **34**, 1460 (1961).

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

(3) R. B. Moodie, T. M. Connor, and R. Stewart, *Can. J. Chem.*, **37**, 1402 (1959).

(4) Solutions were made up to approximately 10% concentration by dropwise addition of a 1:1 solution of the compound in thionyl chloride to vigorously stirred chlorosulfonic acid at −20° (−40° for phenyldimethylcarbonium ion). The n.m.r. spectra were determined on a Varian A-60 spectrometer at room temperature (−40° for phenyldimethylcarbonium ion). Chemical shifts are reported on the τ-scale with tetramethylammonium fluoroborate (τ 6.87) as an internal standard.[5]

(5) D. G. Farnum, M. A. T. Heybey, and B. Webster, *Tetrahedron Letters*, **No. 5**, 307 (1963).

(6) A. A. Bothner-By and C. Naar-Colin, *J. Am. Chem. Soc.*, **83**, 231