lack of orbital following, types of neighboring bonds), other than those included in the simple model, are important in determining the extent of the deviation from perfect pairing that occurs in a given system. Confirmation of these suggestions will have to await experimental measurements corresponding to those outlined here, as well as applications of the theory to more refined molecular wave functions.

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ORGANOSULFUR DERIVATIVES OF METAL CARBONYLS. I. THE ISOLATION OF TWO ISOMERIC PRODUCTS IN THE REACTION OF TRIIRON DODECACARBONYL WITH DIMETHYL DISULFIDE

Sir:

Compounds of the general formula $[Fe(CO)_3SR]_2$ have been isolated in the reaction of triiron dodecacarbonyl with dialkyl sulfides, alkyl mercaptans, and dialkyl disulfides.[1-3] I wish to report the separation of the methyl derivative into two isomeric compounds.

A mixture of 84 g. (0.167 mole) of triiron dodecacarbonyl, 150 ml. of dimethyl disulfide, and 1 liter of thiophene-free benzene was refluxed for 6 hr. under nitrogen with magnetic stirring. After cooling to room temperature, the reaction mixture was filtered giving a red filtrate and a black pyrophoric residue. Solvent was removed from the red filtrate at 30 mm. leaving about 28 g. (27% yield) of red crystals which may be purified either by recrystallization from pentane or by sublimation at 50° (0.1 mm.).

Samples of either recrystallized or resublimed $[Fe(CO)_3SCH_3]_2$ prepared in the above manner showed in the proton n.m.r. three methyl resonances at 2.13, 2.07 and 1.62 p.p.m.[4] of varying intensities suggesting that the product was a mixture of isomers. It was found possible to separate the product into two isomers by chromatography in pentane solution on a 2 × 110 cm. alumina column. This gave rise to two very distinct bands on the column, a large red band followed by a smaller orange band. Each band was eluted with pentane and the air-stable eluates were evaporated to dryness. From 3 g. of the original mixture about 2.4 g. of red crystals, m.p. 65–67.5°, hereafter designated as Isomer A, was isolated from the first red band and about 0.2 g. of orange crystals, m.p. 101.5–103.5°, hereafter designated as Isomer B, was isolated from the orange band.

Analyses showed Isomers A and B to have the same composition (Calcd. for $C_8H_6O_6S_2Fe_2$: C, 25.7; H, 1.6; S, 17.1; Fe, 29.9. Found on Isomer A: C, 25.4; H, 1.8; S, 17.2; Fe, 29.0. Found on Isomer B: C, 26.1; H, 1.9). The infrared spectra also were quite similar although not identical (Isomer A: 2085 (s), 2050 (vs), 2000 (vs), 1428 (sh), 1420 (w), 1310 (sh), 1303 (w), 1260 (vvw), 967 (w), 964 (m) and 710 (vw) cm.$^{-1}$; Isomer B: 2075 (s), 2040 (vs), 2000 (vs), 1995 (sh), 1430 (m), 1318 (m), 1260 (vw), 966 (w), 959 (w) and 703 (vw) cm.$^{-1}$). The proton magnetic resonance spectra provide information as to the nature of these isomers. Isomer A was found to exhibit two methyl peaks at 2.13 and 1.62 p.p.m.[4] in a 1:1 intensity ratio indicating each of the two methyl groups to be different. However, Isomer B was found to exhibit a single methyl peak at 2.07 p.p.m. indicating both methyl groups to be identical.

The absence of carbonyl bands in the 1700–1850 cm.$^{-1}$ region of the infrared spectrum indicates the absence of carbonyl bridging in each of the two isomers. In view of this and in view of the diamagnetism of each isomer as evidenced by the ability to obtain high resolution n.m.r. spectra, the most reasonable structure for each of the two isomers contains an iron–iron bond and two RS-bridges. The two isomers differ therefore only in the relative orientations of the methyl groups attached to the sulfur atoms. From the n.m.r. data it is apparent that the two methyl groups of Isomer A are in different positions but that the two methyl groups of Isomer B are in identical positions. However, on the basis of the presently available information on these compounds, the exact locations of the methyl groups and the nature of the iron-iron bonds in each of the isomers are still uncertain.

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(2) W. Hieber and W. Beck, *Z. anorg. Chem.*, **305**, 265 (1960).

(3) S. F. A. Kettle and L. E. Orgel, *J. Chem. Soc.*, 3890 (1960).

(4) Proton chemical shifts are reported in p.p.m. downfield from tetramethylsilane.

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THE SYNTHESIS OF A HEPTADECAPEPTIDE POSSESSING ADRENOCORTICOTROPIC, MELANOTROPIC AND LIPOLYTIC ACTIVITIES

Sir:

Adrenocorticotropins (ACTH) isolated from pituitaries of various species[1,2,3,4] are polypeptides consisting of 39 amino acids. Since the first synthesis of a biologically active nonadecapeptide corresponding to the first 19 amino acid residues of the hormone was published,[5] two other laboratories[6,7,8] have described the synthesis of ACTH analogs with chain lengths of 19, 20, 23 and 24

(1) C. H. Li, I. I. Geschwind, A. L. Levy, J. I. Harris, J. S. Dixon, N. G. Pon and J. O. Porath, *Nature*, **173**, 251 (1954).

(2) P. H. Bell, *J. Am. Chem. Soc.*, **76**, 5565 (1954).

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(7) K. Hofmann, H. Yajima, N. Yanaihara, T. Y. Liu and S. Lande, *J. Am. Chem. Soc.*, **83**, 487 (1961).

(8) K. Hofmann, T. Y. Liu, H. Yajima, N. Yanaihara, C. Yanaihara and J. L. Humes, *ibid.*, **84**, 1054 (1962).

residues. It appears of considerable importance to ascertain the smallest peptide chain which, while still possessing *moderate* adrenal-stimulating activity, retains the *full* melanocyte-stimulating (MSH) and lipolytic potency of the natural product. In this communication, we wish to report the synthesis of a heptadecapeptide, L-seryl - L - tyrosyl - L - seryl - L - methionyl - L-glutamyl - L - histidyl - L - phenylalanyl - L - arginyl-L - tryptophyl - glycyl - L - lysyl - L - prolyl - L-valyl - glycyl - L - lysyl - L - lysyl - L - arginine, which corresponds to the first 17 amino acid residues of ACTH. By the *in vitro* steroidogenesis assay procedure,[9] the synthetic heptadecapeptide was shown to have an ACTH potency of 6 U.S.P. units per mg. It also was shown to be active by *in vivo* steroidogenesis assay after intravenous injection in man.[10] By both *in vitro*[11] and *in vivo*[12] frog assays, its MSH potency was found to be identical with that of the native hormone. In addition,[13] *in vitro* assays using rabbit perirenal adipose tissue indicated that the synthetic heptadecapeptide is as fully active a lipolytic agent as ACTH.

Carbobenzoxyglycine *t*-butyl ester[14] was hydrogenated and crystallized as the hydrochloric acid salt; this salt was converted into the free base and coupled with carbobenzoxy-L-tryptophan by the dicyclohexylcarbodiimide procedure.[15] Hydrogenation of the protected dipeptide ester yielded a crystalline product which was then allowed to react with crystalline N^{α}-carbobenzoxy-N^{G}-tosyl-L-arginine to obtain an amorphous protected tripeptide (I). After hydrogenation, the crystalline free base of I was condensed with carbobenzoxy-L-phenylalanine by Woodward's reagent[16] to produce the crystalline protected tetrapeptide (II). II was then hydrogenated and coupled with N^{α}-carbobenzoxy-L-histidine hydrazide[17] by the azide procedure. The protected pentapeptide (III) was obtained in crystalline form. III was next submitted to hydrogenation and the free base reacted with the crystalline *p*-nitrophenyl ester of N^{α}-*t*-butyloxycarbonyl-γ-benzylglutamic acid to obtain the crystalline protected hexapeptide (IV); m.p. 162–164°; $[\alpha]^{25}D$ + 22.5° (*c* 1, dimethylformamide); *Anal.* Calcd.: C, 60.5; H, 6.38; N, 13.7. Found: C, 60.2; H, 6.43; N, 13.7.

γ - Benzyl - L - glutamyl - L - histidyl - L - phenylalanyl - N^{G} - tosyl - L - arginyl - L - tryptophylglycine ditrifluoroacetate (V) was obtained in crystalline form by treatment of IV with trifluoroacetic acid and then the crystalline product was coupled with carbobenzoxy - L - seryl - L - tyrosyl-L - seryl - L - methioninehydrazide[5,18] by the azide procedure. The protected decapeptide acid was crystallized from aqueous dimethylformamide with m.p. 214–216°; $[\alpha]^{25}D$ −22.3° (*c* 1, dimethylformamide): *Anal.* Calcd.: C, 56.8; H, 5.88; N, 13.1. Found: C, 56.9; H, 6.13; N, 12.7.

The synthesis of N^{α}-*t*-butyloxycarbonyl-N^{ϵ}-tosyl - L - lysyl - L - prolyl - L - valylglycyl - N^{ϵ}-tosyl - L - lysyl - N^{ϵ} - tosyl - L - lysyl - N^{G} - tosyl-L - arginine benzyl ester [VI, m.p. 120–125°; $[\alpha]^{25}D$ −33.8° (*c* 1, methanol); Calcd.: C, 56.4; H, 6.67; N, 11.3; S, 7.93. Found: C, 56.0; H, 6.68; N, 11.2; S, 8.01] was achieved by coupling N^{α} - *t* - butyloxycarbonyl - N^{ϵ} - tosyl - L - lysyl-L - prolyl - L - valyl - glycine (VII) with N^{ϵ} - tosyl-L - lysyl - N^{ϵ} - tosyl - L - lysyl - N^{G} - tosyl - L - arginine benzyl ester (VIII) using the Woodward reagent.[16] The preparation of VII was the same as previously described[5] except that N^{α}-*t*-butyloxycarbonyl-N^{ϵ}-tosyl-lysine *p*-nitrophenyl ester was employed in the last step; VII was crystallized from ethyl acetate: m.p. 104–106°; $[\alpha]^{25}D$ −68.3° (*c* 1, methanol); *Anal.* Calcd.: C, 55.1; H, 7.25; N, 10.7; S, 4.91. Found: C, 54.9; H, 7.21; N, 10.6; S, 4.72.

Peptide VIII [m.p. 82–86°; $[\alpha]^{25}D$, −4° (*c* 1, dimethylformamide); $[\alpha]^{25}D$ −4.3° (*c* 2, methanol); Calcd.: C, 56.2; H, 6.36; N, 11.4. Found: C, 56.5; H, 6.42; N, 11.5] was achieved by treatment with hydrogen bromide in glacial acetic acid of N^{α}-carbobenzoxy-N^{ϵ}-tosyl-L-lysyl-N^{ϵ}-tosyl-L-lysyl-N^{G}-tosyl-L-arginine benzyl ester which was obtained by the reaction of crystalline N^{α}-carbobenzoxy-N^{ϵ}-tosyl-L-lysyl-N^{ϵ}-tosyl-L-lysine hydrazide [m.p. 145–146°; $[\alpha]^{25}D$ −7.2° (*c* 2, acetic acid); Calcd.: C, 55.9; H, 6.34; N, 11.5. Found: C, 56.2; H, 6.39; N, 11.8], with crystalline N^{G}-tosyl-L-arginine benzyl ester [m.p. 74–75°; $[\alpha]^{25}D$, + 3.3° (*c* 2, dimethylformamide); Calcd.: C, 57.4; H, 6.26; N, 13.4; S, 7.66. Found: C, 57.2; H, 6.44; N, 13.4; S, 7.52] *via* the azide procedure.

The protected heptapeptide ester VI was treated with trifluoroacetic acid to remove the *t*-butyloxycarbonyl group and the benzyl ester of heptapeptide base [m.p. 118–122°; $[\alpha]^{25}D$ −28.9° (*c* 1, methanol); Calcd.: C, 57.4; H, 6.12; N, 12.8. Found: C, 56.9; H, 5.98; N, 12.4] was then coupled with the protected decapeptide acid by either the Woodward method[16] or the dicyclohexylcarbodiimide[15] procedure. The protected heptadecapeptide formed was purified by countercurrent distribution in the two solvent systems (CCl_4 and toluene).[5] The purified protected heptadecapeptide [Calcd.: C, 57.4; H, 6.12; N, 12.8. Found: C, 56.9; H, 5.98; N, 12.4; $[\alpha]^{25}D$ −17.4 (*c* 1, dimethylformamide)] was treated with sodium–liquid ammonia[19]; the crude heptadecapeptide was desalted and then purified chromatographically in a carboxylmethylcellulose[20] column. The final product behaved as a homogeneous substance

(9) M. Saffran and A. V. Schally, *Endocrinol.*, **56**, 523 (1955).

(10) We are indebted to Dr. P. H. Forsham and his colleagues of this University for the clinical tests of our synthetic product.

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(12) L. T. Hogben and D. Slome, *Proc. Roy. Soc.* (London), **B108**, 10 (1931).

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(19) V. du Vigneaud and O. K. Behrens, *J. Biol. Chem.*, **117**, 27 (1937).

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in paper electrophoresis and ion-exchange chromatography. Analysis of the acid hydrolysate of the synthetic heptadecapeptide by both microbiological means[21] and the chromatographic method of Spackman, *et al.*,[22] disclosed an amino acid composition consistent with the theoretically calculated values (microbiological: $Lys_{2.8}His_{0.9}Arg_{1.7}Ser_{2.3}Glu_{1.0}Gly_{2.0}Val_{1.1}Met_{0.9}Tyr_{0.9}Phe_{1.0}Pro_{1.0}$; chromatographic: $Lys_{3.2}His_{1.0}Arg_{2.1}Ser_{1.9}Glu_{1.1}Gly_{2.1}Val_{1.2}Met_{0.9}Tyr_{1.0}Phe_{1.0}Pro_{0.9}$). The intact heptadecapeptide was found to contain tyrosine and tryptophan in a molar ratio of one to one, as determined spectrophotometrically.[23]

(21) The microbiological assay was carried out by the Shankman Laboratories, Los Angeles.

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(23) T. W. Goodwin and R. A. Morton, *Biochem. J.*, **40**, 628 (1946).

(24) We wish to acknowledge the generous support of the National Institutes of Health of the United States Public Health Service (G 2907) and the Albert and Mary Lasker Foundation.

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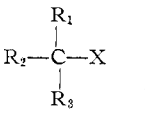
REACTIONS OF TRICYCLOPROPYLCARBINOL DERIVATIVES[1,2]

Sir:

Some time ago we reported[3] that a second cyclopropyl group was nearly as effective as the first in accelerating the solvolysis of cyclopropylcarbinyl derivatives. Thus, the ratios of the solvolysis rates $I_0:I_1:I_2$ (X = *p*-nitrobenzoate) in 80% aqueous dioxane at 60° were 1:246:23,500. At the time, we were unsuccessful in attempts to



I_0[4]: $R_1 = R_2 = R_3$ = isopropyl
I_1: $R_1 = R_2$ = isopropyl; R_3 = cyclopropyl
I_2: R_1 = isopropyl; $R_2 = R_3$ = cyclopropyl
I_3: $R_1 = R_2 = R_3$ = cyclopropyl

prepare the *p*-nitrobenzoate of tricyclopropylcarbinol (I_3, X = PNB) but we have now prepared a solvolyzable derivative, the unsubstituted benzoate (II), which solvolyzes with alkyl–oxygen fission. The ester was obtained in quantitative yield[5] from the reaction of the potassium salt of the alcohol with benzoyl chloride in pentane at 0°.[6] The n.m.r. spectrum of the benzoate showed only the five aromatic protons (ortho, 2.18 τ; meta and para, 2.60 τ) and fifteen cyclopropane protons (methine, 8.6 τ; methylene, 9.2–9.7 τ).

II solvolyzes at an extraordinarily rapid rate, as seen from the data in the table.[7]

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(2) Paper X in a series on Cyclopropane Chemistry. For the previous paper see *J. Am. Chem. Soc.*, **82**, 6362 (1960).

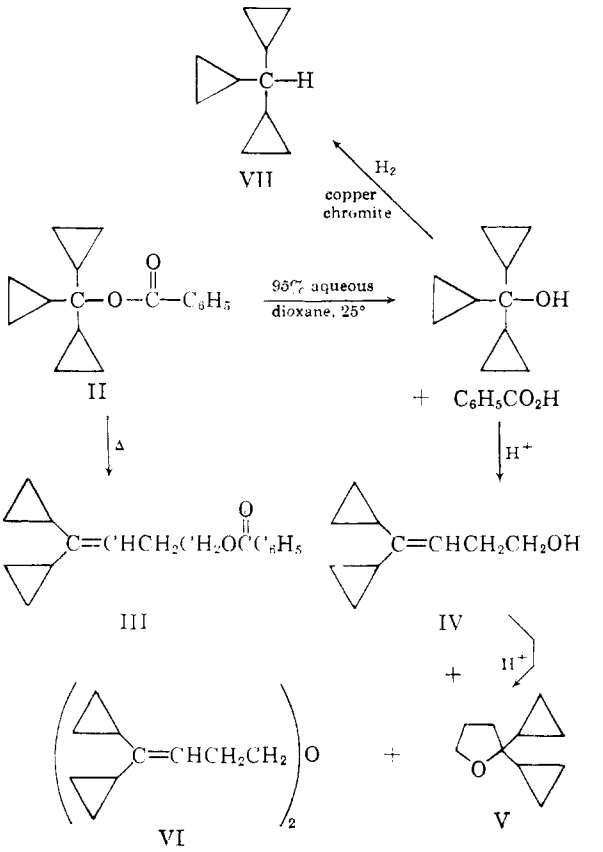
(3) H. Hart and J. M. Sandri, *ibid.*, **81**, 320 (1959).

(4) The subscript indicates the number of cyclopropyl groups.

(5) All compounds reported gave satisfactory analyses.

(6) A similar procedure, and many variations of it, were totally unsuccessful with *p*-nitrobenzoyl chloride.

(7) Rates were followed by quenching samples in anhydrous acetone at −10°, then rapid titration in a nitrogen atmosphere with dilute base in aqueous dioxane at −5 to 0°. Values in the table are mean values for at least three separate determinations, often on different samples of ester, and are accurate to ± 10%.



The acceleration for replacement of isopropyl (I_2) by a third cyclopropyl is about 1080-fold in 95% dioxane at 25°; the comparison is perhaps made more vivid by noting the half-lives (II 9.4 minutes, I_2 (X-benzoate) = 169 hours). This rate enhancement for the third cyclopropyl group, comparable (or greater) in magnitude than for the first or second, implies that each cyclopropyl group is involved in stabilizing the charge on the tricyclopropylcarbonium ion; the truly unusual solvolysis rate for a benzoate ester implies unusual stability for this carbonium ion. The exclusive hydrolysis product was tricyclopropylcarbinol (no rearranged alcohol, no olefin).[8] In accord with each ring being involved in positive charge stabilization, the n.m.r. spectrum of tricyclopropylcarbinol in 98% sulfuric acid shows a single peak[9] at 7.79 τ_m.[10]

TABLE I

SOLVOLYSIS RATES

Ester	% Aqueous dioxane	t, °C.	$k_1 \times 10^4$, sec.$^{-1}$
II	95	25.0	12.3
		15.5	4.37
		7.9	2.52
	90	7.9	22.9
I_2 (X-Benzoate)	95	25.0	0.0114

(8) Methanolysis gives the corresponding methyl ether.

(9) See communication by N. C. Deno, H. G. Richey, Jr., J. S. Liu, H. D. Hodge, J. D. Houser and M. J. Wisotsky, *J. Am. Chem. Soc.*, **84**, 2016 (1962).