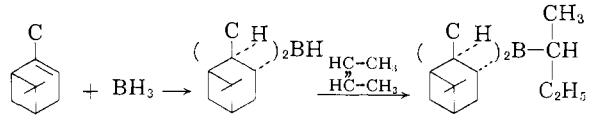
of varying structure.[1] Accordingly, it appeared possible that dialkylboranes derived from optically active terpenes or steroids might convert olefins into organoborane moieties capable of being transformed into optically active derivatives.

α-Pinene was hydroborated to form di-isopinocampheylborane, and the product utilized for the hydroboration of a number of representative olefins.



Oxidation of the resulting organoborane with alkaline hydrogen peroxide produced the corresponding alcohols with exceptionally high optical purities—in the range of 83–91%. Since the optical purity of the α-pinene ($[\alpha]^{20}D$ + 47.6°) is probably no better than 90%,[2] *it appears that this procedure achieves nearly complete asymmetric stereoselectivity.*

A representative procedure is given: α-Pinene, 27.2 g. (0.200 mole) was dissolved in 75 ml. of a 1.00 *M* solution of sodium borohydride in diglyme and the mixture, cooled to 0°, was treated with 14.2 g., (0.100 mole) of boron trifluoride etherate to form the di-isopinocampheylborane. To the reagent at 0° was added 6.1 g., 0.100 mole, of *cis*-2-butene and the reaction mixture maintained at 0° for four hours, then left overnight at room temperature. Oxidation at 30–50° with 31 ml. of 3 *N* sodium hydroxide followed by 31 ml. of 30% hydrogen peroxide produced 6.7 g. of 2-butanol, a yield of 90%: b.p. 98° at 744 mm.; $n^{20}D$ 1.3975; $[\alpha]^{20}D$ −11.8°, indicating an optical purity of 87%.[3]

Similarly, *cis*-3-hexene, readily synthesized via the hydroboration reaction from 3-hexyne,[4] was converted in 81% yield to 3-hexanol: b.p. 135–136° at 752 mm.; $n^{20}D$ 1.4148, $[\alpha]^{20}D$ −6.5°, indicating an optical purity of 91%.[5]

Application of the procedure to norbornene produced *exo*-norborneol in a yield of 62%. The product, m.p. 125–126°, exhibited the rotation $[\alpha]^{20}D$ −2.0°; acetate, $\alpha^{20}D$ + 7.9°, indicating an optical purity of 83%.[6]

The results clearly demonstrate that a boron atom at the asymmetric center, RR′C*HB<, is capable of maintaining asymmetry without significant racemization over periods of several hours. The ease with which organoboranes may be converted into other derivatives with retention of configuration and the unusually high optical purity achieved should make this approach to optically active derivatives a most valuable one for the synthetic chemist.

(1) H. C. Brown and G. Zweifel, *J. Am. Chem. Soc.*, **82**, 3222 (1960).

(2) F. H. Thurber and R. C. Thielke, *ibid.*, **53**, 1030 (1931), report $[\alpha]D$ +51.1° for α-pinene purified via the nitrosochloride.

(3) P. J. Leroux and H. J. Lucas, *ibid.*, **73**, 41 (1951), report for L(−)-2-butanol: b.p. 97.5–98° at 745 mm.; $n^{20}D$ 1.3970; $[\alpha]^{25}D$ −13.51°.

(4) H. C. Brown and G. Zweifel, *ibid.*, **81**, 1512 (1959).

(5) J. Kenyon and R. Poplett, *J. Chem. Soc.*, 273 (1945), report for 3-hexanol: b.p. 133–134°; $n^{20}D$ 1.4140; $[\alpha]^{18}D$ −7.13°.

(6) S. Winstein and D. Trifan, *J. Am. Chem. Soc.*, **74**, 1154 (1952), report for (−)-*exo*-norborneol: m.p. 126–126.8°; $[\alpha]^{24}D$ −2.41°; acetate, $\alpha^{25}D$ +10.39°.

Trans olefins and highly hindered olefins react only slowly with di-isopinocampheylborane. Consequently, we have undertaken both the development of less hindered reagents for the asymmetric hydroboration of such olefins and the investigation of the full scope of this new synthetic route to optically active derivatives.

We wish to acknowledge the generous gift of the α-pinene by Dr. R. A. Bankert of the Naval Stores Research Division of the Hercules Powder Company.

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STUDIES ON POLYPEPTIDES. XIII. THE SYNTHESIS OF A TRICOSAPEPTIDE POSSESSING ESSENTIALLY THE FULL BIOLOGICAL ACTIVITY OF NATURAL ACTH[1-3]

Sir:

As an outgrowth of our systematic studies[4] relating structure and function of peptides possessing melanophoretic and adrenocorticotropic activity, we have prepared seryltyrosylserylmethionylglutamylhistidylphenylalanylarginyltryptophylglycyllysylprolylvaline amide and seryltyrosylserylmethionylglutamylhistidylphenylalanylarginyltryptophylglycyllysylprolylvalylglycyllysyllysine amide and found these peptide derivatives which correspond to substantial portions of the N-terminal sequence of the corticotropins to possess, at best, a very low level of *in vivo* adrenocorticotropic activity (< 0.1 IU/mg.).[5] These results justify the conclusion that a sequence of more than 16 amino acid residues from the amino end of the corticotropin molecule is required for high adrenocorticotropic activity.

A recent communication by Li, *et al.*,[6] reporting *in vivo* adrenocorticotropic activity (29 IU/mg.) of a synthetic nonadecapeptide corresponding to the sequence of the 19 N-terminal amino acid residues of the corticotropins prompts us at this time to record further observations in this field.

We have prepared the tricosapeptide amide (I) and find that this compound possesses *in vivo* adrenocorticotropic activity. The most highly purified

		Formula	
I	R = H	R′ = OH	R″ = H
II	R = acetyl	R′ = NH_2	R″ = formyl

samples of the synthetic hormone derivative obtained to date exhibit 103 ± 10.4 IU/mg. of both ascorbic acid depleting and plasma corticosterone elevating

(1) Supported by grants from the U. S. Public Health Service, the National Science Foundation, the National Cancer Society and Armour and Company.

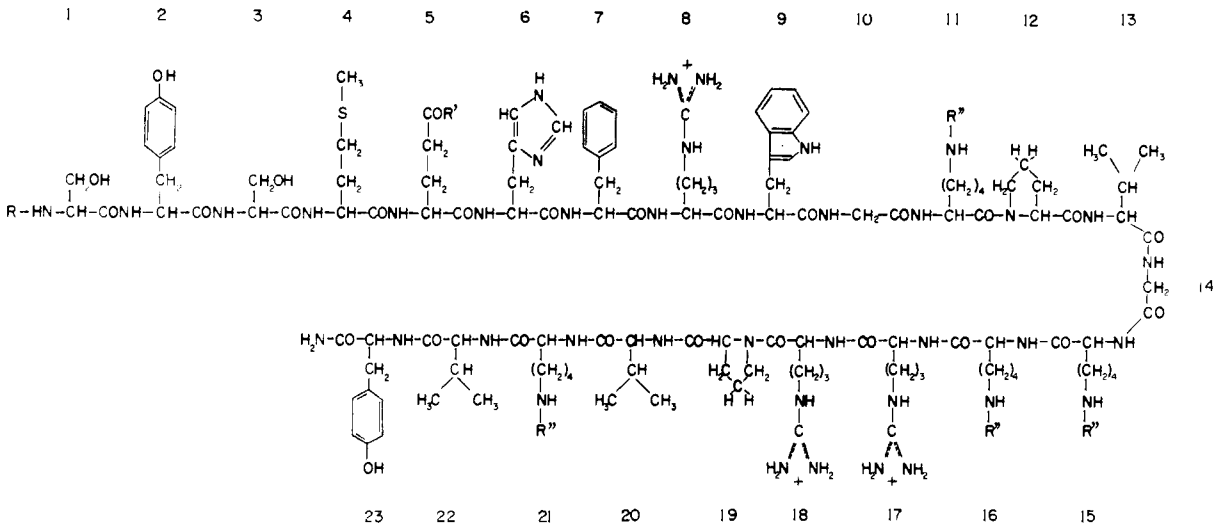
(2) The amino acid residues, except glycine, are of the L-configuration. In the interest of space conservation the customary L-designation of individual amino acid residues has been omitted.

(3) Amino acid analyses were carried out with a model 120 Beckman-Spinco amino acid analyzer. Unless noted otherwise, the R_f values refer to the Partridge system; S. M. Partridge, *Biochem. J.*, **42**, 238 (1948).

(4) See *J. Am. Chem. Soc.*, **82**, 3732 (1960), for Paper XVII in this series.

(5) K. Hofmann, "Brookhaven Symposia in Biology," Vol. 13, 184 (1960).

(6) C. H. Li, J. Meienhofer, E. Schnabel, D. Chung, T. Lo and J. Ramachandran, *J. Am. Chem. Soc.*, **82**, 5760 (1960).



activity.[7] The biological activity of this preparation is thus essentially of the same order of magnitude as that reported for homogeneous corticotropin preparations derived from natural sources.[8] The results demonstrate that a synthetic peptide of unequivocal structure which corresponds to the postulated arrangement of twenty-three amino acid residues from the amino end of the corticotropin sequence is endowed with full adrenocorticotropic activity.[9]

It is of considerable interest to note that the protected tricosapeptide amide (II), the immediate precursor of (I) in our method of synthesis, exhibits negligible *in vivo* adrenal ascorbic acid depleting and plasma corticosterone elevating activity.

The preparation of (11) $[[\alpha]^{27}D$ $-71.3°$ in 10% acetic acid, R_f 0.44, sharp, single ninhydrin negative, Pauly, Sakaguchi, methionine and Ehrlich positive spot; amino acid ratios in acid hydrolysate $ser_{2.15}tyr_{2.08}met_{0.89}glu_{1.00}his_{1.05}phe_{1.10}arg_{2.89}gly_{2.11}lys_{4.00}pro_{2.00}val_{2.87}$] involved coupling by the N,N'-dicyclohexylcarbodiimide procedure[10] of N-acetylseryltyrosylserylmethionylglutaminylhistidylphenylalanylarginyltryptophylglycine [diacetate tetrahydrate; *anal.* found: C, 51.6; H, 6.5; N, 1.50; $[\alpha]^{27}D$ $-30.6°$ in 10% acetic acid, R_f 0.68, single ninhydrin negative, Pauly, methionine, Sakaguchi and Ehrlich positive spot, amino acid ratios in acid hydrolysate $ser_{2.05}tyr_{0.99}met_{1.00}glu_{0.99}his_{1.00}phe_{1.04}arg_{1.00}gly_{1.00}$] (as the hydrochloride) with the dihydrochloride of N^{ϵ}-formyllysylprolylvalylglycyl-N^{ϵ}-formyllysyl-N^{ϵ}-formyllysylarginylarginylprolylvalyl-N^{ϵ}-formyllysylvalytyrosine amide [triacetate hydrate $[\alpha]^{28}D$ $-90.0°$ in 10% acetic acid; R_f 0.40, single ninhydrin, Pauly, and Sakaguchi positive spot, amino acid ratios in acid hydrolysate $lys_{3.89}pro_{2.04}val_{2.98}gly_{1.02}arg_{1.87}tyr_{0.93}$]. The latter compound, which contains the characteristic lysyllysylarginylarginine sequence of the corticotropins, resulted from hydrogenolysis of a carbobenzoxy derivative [diacetate tetrahydrate; *anal.* found: C, 53.0; H, 7.8; N, 17.5] obtained by coupling by the azide procedure of N^{α}-carbobenzoxy-N^{ϵ}-formyllysylprolylvalylglycyl-N^{ϵ}-formyllysyl-N^{ϵ}-formyllysine $[[\alpha]^{28}D$ $-56.5°$ in methanol] with arginylarginylprolylvalyl-N^{ϵ}-formyllysylvalyltyrosine amide [triacetate octahydrate; *anal.* found: C, 46.9; H, 7.6; N, 16.2; $[\alpha]^{29}D$ $-73.6°$ in 10% acetic acid, R_f 0.37, single ninhydrin, Sakaguchi and tyrosine positive spot, amino acid ratios in leucine aminopeptidase (LAP) digest $arg_{2.01}pro_{0.98}val_{2.01}$ N^{ϵ}-$formyls_{1.00}tyr_{0.97}$].

Chromatography on carboxymethylcellulose (CMC)[11] proved to be of outstanding value for purification of certain key intermediates and of the final product. Homogeneity of peptides was evaluated as described[12].

Exposure to 0.5 *N* hydrochloric acid at 100° for 60–80 minutes[13] converted the analytically pure biologically inactive protected tricosapeptide amide (II) into a mixture of products possessing adrenocorticotropic activity. Repeated assays[7] of various samples of this material gave values which ranged from 30 to 40 IU/mg. The highly potent hormone derivative (I) was isolated from acid treated (II) by CMC chromatography in a yield of 20–30% with excellent recovery of biological

(7) Ascorbic acid depleting activity was determined in 24 hour hypophysectomized rats according to the method of U. S. Pharmacopoeia XV against the USP reference standard. The plasma corticosterone levels were determined 15 minutes following administration, R. Guillemin, G. W. Clayton, J. D. Smith and H. S. Lipscomb, *Endocrinol.*, **63**, 349 (1958). The free steroid was separated chromatographically and assayed by a modification of the method of H. Kalant, *Biochem. J.*, **69**, 93 (1958). We are much indebted to Dr. Joseph D. Fisher of Armour Pharmaceutical Company, Kankakee, Illinois for all of the biological assays.

(8) For a recent review of this subject see C. H. Li in "Advances in Protein Chemistry, Volume XI, M. L. Anson, K. Bailey and J. T. Edsall, Editors, Academic Press, Inc., New York, N. Y., 1956, p. 101.

(9) Peptic digestion of the 39 amino acid residue β-corticotropin molecule affords, among other products, an N-terminal octacosapeptide fragment which is reported to retain the full ascorbic acid depleting activity of the intact hormone. From the results of partial hydrolysis with acid, it was inferred that the four C-terminal amino acids may be removed from this fragment with formation of a tetracosapeptide without altering significantly the biological potency, P. H. Bell, K. S. Howard, R. G. Shepherd, B. M. Finn, and J. H. Meisenhelder, *J. Am. Chem. Soc.*, **78**, 5059 (1956). The present findings are in accord with this deduction.

(10) J. C. Sheehan and G. P. Hess, THIS JOURNAL, **77**, 1067 (1955).

(11) E. A. Peterson and H. A. Sober, *J. Am. Chem. Soc.*, **78**, 751 (1956).

(12) K. Hofmann, *Ann. New York Acad. Sci.*, **88**, 689 (1960).

(13) Systematic studies with model peptides, corresponding to fragments of the corticotropin sequence, have shown these conditions of hydrolysis to remove the N-terminal acetyl group, the glutamine amide group and the Nε-formyl group from lysine residues.

activity. The peptide migrated at a rate somewhat slower than histidine in 24 hour Partridge paper chromatograms[3] and Stein–Moore analysis[14] of an acid hydrolysate gave the following amino acid ratios: $ser_{1.7}tyr_{1.8}met_{0.8}glu_{0.9}his_{0.8}phe_{0.9}arg_{3.1}gly_{2.0}lys_{4.3}pro_{2.3}val_{3.4}$. The average recovery of individual amino acids was 95%.

This first synthesis of a peptide which possesses essentially the full *in vivo* ascorbic acid depleting and plasma corticosterone elevating activity of the natural corticotropins opens the way to systematic investigations relating peptide structure to this important physiological activity.

(14) S. Moore, D. H. Spackman and W. H. Stein, *Anal. Chem.*, **30**, 1185 (1958).

(15) The skillful technical assistance of Mrs. Chizuko Yanaihara and Mr. John L. Humes is gratefully acknowledged.

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RECEIVED DECEMBER 1, 1960

STRUCTURE OF $(OC_3)Fe(C_8H_8)Fe(CO)_3$

Sir:

The cycloöctatetraene (COT) ring has the tub form in COT itself[1,2] and in the $AgC_8H_8^+$ ion.[3] Electron transfer to the ring to form[4] $(COT)^-$ and $(COT)^{-2}$ changes the geometry of the original molecule to a new form, suggested[4] to be the planar form. The many suggestions[5,6,7,8,9,10] concerning the geometry of the $(OC)_3Fe(COT)Fe(CO)_3$ molecule include the tub and planar forms of the (COT) ring, with mention[6] of a ring of D_4 symmetry. The puckered ring of D_{4d} symmetry, which has similar molecular orbital degeneracies to those of the planar ring, and less strain, but poorer π-π overlap, has not been included in these discussions.

We have completed a determination of the structure of a single crystal of $(OC)_3Fe(COT)Fe(CO)_3$, which shows a geometry of the COT residue different from any of the above proposals. The ring (Fig. 1) is oval, but with little residual bonding expected across the closest "non-bonded" C. . .C distance of 2.85 Å. The form is approximately an eight membered "chair" form, neither tub nor crown. Each $Fe(CO)_3$ is associated with four CH groups, with each set of 4C forming a planar or nearly planar group, somewhat suggestive of butadiene. Assuming very approximate sp^2 hybrids in the various C-C-C planes, the $\pi \ldots \pi$ overlap integral is 0.25 between C_2-C_3, but only 0.11 between C_1-C_2 or C_3-C_4. All of these π orbitals are fairly well

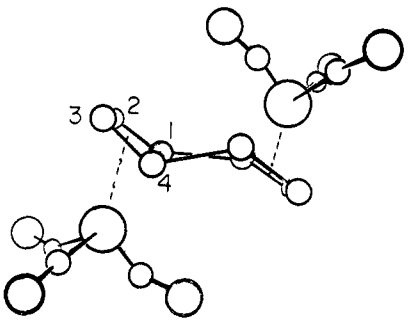

Fig. 1.—The $(OC)_3Fe(COT)Fe(CO)_3$ molecule: bond distances average to 1.39 Å. for the two distances at the ends of the COT residue, 1.49 Å. for the two central bonds, and 1.44 Å. for the other four C—C bonds, all ±0.03 Å. Fe. . . C distances are 2.06 Å. to the end carbon atoms (bonded together) and 2.15 Å. to the central four carbon atoms, to be compared with 2.05 Å. for Fe. . . .C in ferrocene.[16] The average Fe—C and C≡O distances are 1.76 Å. and 1.15 Å., respectively, in the Fe—C≡O groups.

directed toward the corresponding Fe atoms, which are actually closer to the bond centers than to the C atoms themselves. A comparison of distances in a butadiene type of complex[11,12,13] is not yet available, but would be of interest. Little $\pi \ldots \pi$ interaction between the two halves of the COT complex is probable, because of the 96° to 101° angle between π orbitals as inferred from the assumption of sp^2 hybrids in planes defined by C-C-C. Also, the average C-C distance of 1.49 Å. joining the two nearly planar C_4 groups seems reasonable for little more than a single bond in view of the immediate strong-bonding coördination numbers,[14,15] but we do not eliminate a very small $\pi \ldots \pi$ interaction between the C_4 halves. The distortion of sp^2 bond angles of C_8H_8 in this complex occurs almost entirely in the central four C-C-C angles, which average to 130°. As usual, the most naive way of counting electrons around Fe is unbelievably satisfactory. Each C_4 group can be thought of as donating four electrons to its Fe, which already has eight, and receives six more from its three C≡O groups. Octahedral coördination is apparent around the Fe, as the three C—C bonds are staggered with respect to the three C≡O groups around each corresponding Fe. However, a more detailed description of the bonding and back coördination will be deferred until completion of our study of the $(COT)Fe(CO)_3$ structure, presently in progress.

The equivalence of the H^1 nuclear magnetic resonances[5,6] is suggestive of a dynamical effect, of negligible relative chemical shifts of the C-H groups, or of different geometries in the solid and solution. A study of the n.m.r. spectrum of $(OC)_3Fe(COT)Fe(CO)_3$ as a function of temperature may

(1) H. S. Kaufman, H. Mark and I. Fankuchen, *Nature*, **161**, 165 (1948).

(2) J. Bregman, private communication.

(3) F. S. Mathews and W. N. Lipscomb, *J. Phys. Chem.*, **63**, 845 (1959); also *J. Am. Chem. Soc.*, **80**, 4745 (1958).

(4) T. J. Katz and H. L. Strauss, *J. Chem. Phys.*, **32**, 1873 (1960).

(5) T. A. Manuel and F. G. A. Stone, *Proc. Chem. Soc.*, 90 (1959).

(6) T. A. Manuel and F. G. A. Stone, *J. Am. Chem. Soc.*, **82**, 336 (1960).

(7) A. Nakamura and N. Hagihara, *Bull. Chem. Soc. Japan*, **32**, 880 (1959).

(8) D. A. Brown, *J. Inorg. and Nuclear Chem.*, **10**, 39 (1959); **10**, 49 (1959).

(9) F. A. Cotton, *J. Chem. Soc. (London)*, 400 (1960).

(10) P. L. Pauson, *Proc. Chem. Soc. (London)*, 297 (1960).

(11) H. Rheilin, A. Gruhl, G. Hessling and O. Pfrangle, *Annalen*, **482**, 161 (1930).

(12) B. F. Hallam and P. L. Pauson, *J. Chem. Soc. (London)*, 646 (1958).

(13) R. B. King, T. A. Manuel and F. G. A. Stone, *J. Inorg. and Nucl. Chem.*, in press.

(14) C. C. Costain and B. P. Stoicheff, *J. Chem. Phys.*, **30**, 777 (1959).

(15) Values of 1.50 Å. (C. A. Coulson, "V. Henri Memorial Lectures," Desoer, Liege, 1948, p. 25) and 1.48 Å. (D. W. J. Cruickshank and R. A. Sparks, *Proc. Roy. Soc., London*, **A258**, 270 (1960)) have been suggested for a single bond between two sp^2 carbon atoms.