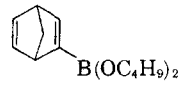
saturated with sodium chloride at −70 to −40°, extraction with 1-butanol and the standard work-up procedure[4] (except that the boronic ester solution was made neutral by washing with saturated aqueous sodium chloride, not sodium bicarbonate) yielded 52 g. (57%) of dibutyl acetyleneboronate, b.p. 30–32° (0.3 mm.), $n^{26}D$ 1.4180, C≡C absorption at 2070 cm.$^{-1}$, ≡C-H at 3230 cm.$^{-1}$ (in CCl_4). Calcd. for $C_{10}H_{19}BO_2$: C, 65.96; H, 10.52; B, 5.94. Found[5]: C, 66.09; H, 10.73; B, 5.94. The acetylenic group is hydrolyzed from the boron atom with extreme ease by aqueous bases, even sodium bicarbonate being sufficient to cause rapid evolution of acetylene (confirmed with Ag^+). The carbon–boron bond is not noticeably attacked by pure hydroxylic solvents or dilute acids. General applicability of the method of synthesis is indicated by the conversion of 1-hexynylmagnesium bromide to dibutyl 1-hexyne-1-boronate in 40% yield, b.p. 85–90° (0.1 mm.), C≡C absorption 2180 cm.$^{-1}$ (in CCl_4). Calcd. for $C_{14}H_{27}BO_2$: C, 70.60; H, 11.43; B, 4.54. Found: C, 70.40; H, 11.53; B, 4.76.

Dibutyl acetyleneboronate is a moderately active dienophile. A solution of 1.82 g. of the boronic ester in 7.5 ml. of chlorobenzene refluxed (130°) vigorously with 3 ml. of cyclopentadiene for 15 hr. yielded 0.65 g. (25%)[6] of dibutyl bicyclo-[2.2.1]hepta-2,5-diene-2-boronate, b.p. 74–75 (0.1



mm.), twin C=C absorption bands at 1580 and 1545 cm.$^{-1}$ (in CCl_4). Calcd. for $C_{15}H_{25}BO_2$: C, 72.59; H, 10.15; B, 4.36. Found: C, 72.72; H, 10.12; B, 4.46. The compound was further characterized by treatment with hydrogen peroxide and 2,4-dinitrophenylhydrazine[4] to yield 35% of the 2,4-dinitrophenylhydrazone of bicyclo-[2.2.1]hept-5-ene-2-one, m.p. (one recrystallization) 169–172°, reported,[7] 174–175° The acetylenic boronic ester is a less active dienophile than dibutyl ethyleneboronate,[4] which forms an adduct with cyclopentadiene in 54% yield in 3 hr. at 90–95°, b.p. 75–76°(0.1 mm.),[8] to be reported in detail later.

Dibutyl acetyleneboronate reacts at the triple bond with free radicals to form adducts of the expected types. With an equimolar quantity of 1-hexanethiol and 5 g. of azobisisobutyronitrile per mole at 80–85° for 3 hr., a 72% yield of the 1:1 adduct, $C_6H_{13}SCH{=}CHB(OC_4H_9)_2$,[8] was obtained, b.p. 120°(0.1 mm.), C=C absorption strong and broad, 1550 cm.$^{-1}$ (pure liquid). Degradation of the adduct with solid potassium hydroxide at 140–160° yielded acetylene (70%). In the presence of ultraviolet light at −70° or if excess mercaptan was present, two moles of mercaptan added to the triple bond, but the product decomposed during distillation at 150° (0.1 mm.). With 7.5 ml. of bromotrichloromethane and 0.06 g. of azobisisobutyronitrile, 1.8 g. of dibutyl acetyleneboronate formed the adduct $CCl_3CH{=}CBrB(OC_4H_9)_2$[8] in 90% yield, b.p. 102° (0.1 mm.), C=C absorption 1635 cm.$^{-1}$ (pure liquid). Light (incandescent lamp) is required to initiate the addition of bromine to dibutyl acetyleneboronate in methylene chloride at 25–35°; the 1:1 adduct $BrCH{=}CBrB(OC_4H_9)_2$[8] is formed in 88% yield, b.p. 73° (0.1 mm.), C=C absorption 1590 cm.$^{-1}$. The acetylenic compound again is less reactive than dibutyl ethyleneboronate, which requires no apparent catalyst to form $BrCH_2CHBrB(OC_4H_9)_2$[8] very rapidly at −70° in methylene chloride, 89% yield, b.p. 94–95°(0.1 mm.).

The Diels–Alder reactions and the positions of the infrared bands described above provide further qualitative support for the magnitudes of the parameters chosen for boron in previous molecular orbital calculations.[4]

(4) D. S. Matteson, THIS JOURNAL, **81**, 5004 (1959); **82**, 4228 (1960).

(5) Galbraith Laboratories, Knoxville, Tenn.

(6) About 1.0 g. of dibutyl acetyleneboronate was recovered. The conversion increased to 49% in refluxing cumene (150°) but some decomposition occurred.

(7) J. D. Roberts, E. R. Trumbull, Jr., W. Bennett and R. Armstrong, THIS JOURNAL, **72**, 3116 (1950).

(8) Correct analytical values were obtained for all elements (omitting O).

(9) National Defense Education Act Fellow, 1959–.

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THE SYNTHESIS OF A NONADECAPEPTIDE POSSESSING ADRENOCORTICOTROPIC AND MELANOTROPIC ACTIVITIES

Sir:

We wish to report herein the synthesis of a nonadecapeptide, 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-arginyl-L-arginyl-L-proline (V), which has an amino acid sequence identical with the first nineteen residues from the NH_2-terminus of ovine,[1] porcine,[2] and bovine[3] adrenocorticotropins (ACTH) and which possesses both adrenocorticotropic and melanocyte-stimulating (MSH) activities.

The protected tetrapeptide, carbobenzoxy-(Z)-Ser-Tyr-Ser-Met-$NHNH_2$ (I), was synthesized from Z-Ser-Tyr-$NHNH_2$ and H-Ser-Met-OCH_3 by the azide procedure; the resulting ester[4] was converted to the crystalline hydrazide, m.p. 244–245° (dec.); $[\alpha]^{25}D$ −15° (*c* 1, acetic acid).

Anal. Calcd.: C, 52.98; H, 6.03; N, 13.24; Found: C, 53.21; H, 6.22; N, 13.03.

For the synthesis of the protected hexapeptide, OBzBz Tos Tos

Z-Glu-His-Phe-Arg-Try-Gly-OH (II), Z-Arg-Try-Gly-OCH_3[5] was catalytically hydrogenated, and the product was condensed by the *p*-nitrophenyl

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(3) C. H. Li, J. S. Dixon and D. Chung, *ibid.*, **80**, 2587 (1958).

(4) K. Hofmann, A. Jöhl, A. E. Furlenmeier and H. Kappeler, *ibid.*, **73**, 1636 (1957).

(5) E. Schnabel and C. H. Li, *ibid.*, **82**, 4576 (1960).

ester method[6] with Z-His(Bz)-Phe-OH[7] (Z-His(Bz)-Phe-$OC_6H_4NO_2$: m.p. 152–155°, $[\alpha]^{25}D$ −19.0° (*c* 1, dimethylformamide)). The resulting protected pentapeptide was saponified, hydrogenated, and then condensed with Z-Glu(OBz)-$OC_6H_4NO_2$ (m.p. 114–115°; $[\alpha]^{25}D$ −32.5° (*c* 1, methanol)). The product was purified by countercurrent distribution in the system $CHCl_3$-CCl_4-CH_3OH-H_2O (3:1:3:1, by volume). The main fraction, K = 0.16, was crystallized from dimethylformamide and recrystallized from 90% aqueous dioxane, m.p. 193–195°; $[\alpha]^{26}D$ −19° (*c* 1, dimethylformamide).

Anal. Calcd.: C, 62.85; H, 5.74; N, 12.93; Found: C, 62.79; H, 5.87; N, 12.86.

The protected tetrapeptide, Z-Lys(Tos)-Pro-Val-Gly-OH, (III), was made as described: Z-Pro-Val-OH[8] was coupled with H-Gly-OCH_3 by the dicyclohexylcarbodiimide method.[9] This tripeptide ester (m.p. 111–112°; $[\alpha]^{25}D$ −90.4° (c 1.6, methanol))[9a] was then hydrogenated and the product allowed to react with Z-Lys(Tos)-$OC_6H_4NO_2$.[10] After saponification, the crystalline protected peptide III had m.p. 109–110°; $[\alpha]^{25}D$ −73° (*c* 1, methanol).

Anal. Calcd.: C, 57.6; H, 6.60; N, 10.2; S, 4.66; Found: C, 57.5; H, 6.59; N, 10.4; S, 4.60.

The protected pentapeptide, Z-Lys(Tos)-Lys(Tos)-Arg(Tos)-Arg-Pro-OCH_3 (IV) was synthesized in a stepwise manner starting with the COOH-terminal amino acid ester, H-Pro-OCH_3. This ester was coupled with Z-Arg(Tos)-OH[5] with the use of dicyclohexylcarbodiimide.[9] The crystalline protected dipeptide (m.p. 152–153°, $[\alpha]^{25}D$ −39.0 (*c* 1, methanol)) was then hydrogenated and the product was again coupled with Z-Arg(Tos)-OH by the same method.[9] The resulting protected tripeptide (m.p. 112–120°, $[\alpha]^{25}D$ −31.0° (*c* 1, methanol)) was then hydrogenated and the product coupled with Z-Lys(Tos)-$OC_6H_4NO_2$ for the next two steps (Z-Lys(Tos)-Arg(Tos)-Arg-Pro-OCH_3: m.p. 110–115°, $[\alpha]^{25}D$ −30.5 (*c* 1, methanol)) by means of the *p*-nitrophenyl ester method.[6] The amorphous peptide (IV) had m.p. 109–112°; $[\alpha]^{25}D$ −29° (*c* 1, methanol).

Anal. Calcd.: C, 54.7; H, 6.19; N, 12.6; S, 8.85. Found: C, 54.5; H, 6.19; N, 12.7; S, 8.84. Countercurrent distribution in the solvent system $CHCl_3$–$C_6H_5CH_3$–CH_3OH–H_2O (5:5:8:2, by volume) showed IV to travel as one single peak with K = 0.45.

The carbobenzoxy group of IV was removed by hydrogenation and the base then was allowed to react with the crystalline *p*-nitrophenyl ester of III which was obtained by the dicyclohexylcarbodiimide method[9,10a] and had m.p. 152–153°, $[\alpha]^{25}D$ −40.5 (*c* 2, dimethylformamide). Countercurrent distribution in the toluene system described above indicated the resulting nonapeptide ester (Va) to be homogenous with K = 0.26; m.p. 119–121°; $[\alpha]^{25}D$ −43.2° (*c* 1, methanol); yield, 96%.

Anal. Calcd.: C, 55.1; H, 6.40; N, 12.7; S, 8.08. Found: C, 54.9; H, 6.24; N, 12.7; S, 8.08.

Saponification of Va yielded the protected nonapeptide acid (Vb) as an amorphous product with m.p. 135–137°; $[\alpha]^{25}D$ −37.4° (*c* 1, methanol).

Anal. Calcd.: C, 54.9; H, 6.34; N, 12.8. Found: C, 55.1; H, 6.41; N, 12.8. This material was homogenous according to the results of countercurrent distribution with K = 0.75 in the toluene system.

Peptide Vb was next submitted to hydrogenation and the resulting base was coupled with II by the mixed anhydride procedure with isobutyl chlorocarbonate,[11] to give the protected pentadecapeptide (Vc). Peptide Vc was purified by countercurrent distribution in the toluene system and distributed with K = 0.34; m.p. 135–140°; $[\alpha]^{25}D$ −25.3° (*c* 0.5, dimethylformamide); yield, 35%.

Anal. Calcd.: C, 57.58; H, 6.14; N, 13.48. Found: C, 57.39; H, 6.13; N, 13.49.

Peptide I was converted to the azide and then condensed with the product obtained by the hydrogenation of Vc. The resulting protected nonadecapeptide, Vd, was purified by repeated precipitation from dimethylformamide–ether and methanol–ethyl acetate; m.p. 165–170°; $[\alpha]^{26}D$ −25° (*c* 0.5, dimethylformamide); yield, 56%.

Anal. Calcd.: C, 54.91; H, 6.26; N, 13.96. Found: C, 54.50; H, 6.36; N, 13.71.

The protecting groups of Vd were removed by treatment with sodium in liquid ammonia[12] and the crude product was submitted to countercurrent distribution in the system 0.1% HOAc–1-butanol–pyridine (11:5:3) for 1188 transfers. When the

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material with $K = 0.082$ was isolated, it was found to be the desired nonadecapeptide V. Redistribution of V in 2-butanol/0.5% trichloroacetic acid for 214 transfers gave a band with $K = 0.58$.

Quantitative amino acid analysis of the 24-hour hydrolysate of V by both the chromatographic procedure[13] and the paper-fluorodinitrobenzene method[14] gave this composition in molar ratios: $Ser_{1.8}Tyr_{1.0}Met_{1.1}Glu_{1.0}His_{1.1}Phe_{1.1}Arg_{3.1}Try_{1.0}Gly_{2.0}Lys_{2.9}Pro_{2.2}Val_{1.0}$. Tyrosine and tryptophan were determined by a spectrophotometric method.[15] Digestion of V successively with trypsin, chymotrypsin and leucine aminopeptidase produced the expected constituent amino acids by quantitative analysis.[14] NH_2-terminal amino acid analysis by the fluorodinitrobenzene procedure[14,16] disclosed serine as the NH_2-terminal residue, with traces of glutamic acid and lysine.

The synthetic nonadecapeptide,[17] according to the results of bioassay by the *in vitro* adrenal method,[18] had an ACTH activity of 31 U.S.P. units per mg. Estimation of ACTH activity by the usual adrenal ascorbic acid depletion procedure[19] gave a potency[20] of 29 U.S.P. units per mg. A single dose of 0.1 microgram of the peptide caused a change in melanophore index in hypophysectomized *Rana pipiens*[21] from 1+ to 3+ within one hour, an MSH potency comparable to that of the native adrenocorticotropins.[22]

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CONDUCTANCES OF SOME LANTHANIDE COBALTICYANIDES IN DIOXANE–WATER: A RE-ASSESSMENT

Sir:

The conductances of four lanthanide cobalticyanides in water and in 10% and 20% dioxane–water at 25° have been measured by Atkinson[1] and the corresponding Λ_0 and K values evaluated by the method of Shedlovsky.[2] For $LaCo(CN)_6$ in water, the given answers are $\Lambda_0 = 168.36$ and $K = 3.835 \times 10^{-4}$ but while the former is in excellent agreement with a previous estimate,[3] K is much higher than that which James and Monk[3] obtained by the method of Davies,[4] which makes use of the limiting forms of the equations of Onsager and Debye and Hückel

$$\Lambda_i = \Lambda_0 - S(c_i\Lambda/\Lambda_i)^{1/2} \quad (1)$$

$$-\log f_i = Az_i^2I^{1/2} \quad (2)$$

S and A are numerical constants[5] under given physical conditions, Λ_i is the equivalent conductance for an equivalent ionic concentration c_i and I is the ionic strength ($= 3c_i$ here).[5] These equations are solved by applying successive approximations to (1) till Λ_i is constant, taking $\Lambda_i = \Lambda_0$ on the right-hand side for a start. Some of the data of Atkinson have been recalculated along these lines and are summarized by the table where c is in equivs./l.; the original Λ_0 values were used.

DISSOCIATION CONSTANTS DERIVED BY THE METHOD OF DAVIES ($K \times 10^4$)

$10^2c^{1/2}$	0.50	1.0	1.5	2.0	2.5	3.0
$LaCo(CN)_6$ in water	1.25	1.64	1.73	1.75	1.67	1.72
$LaCo(CN)_6$ in 10% dioxane	0.43	0.55	0.59	0.59	0.59	0.55
$LaCo(CN)_6$ in 20% dioxane	0.25	0.28	0.27	0.22	0.19	0.16
$NdCo(CN)_6$ in water	0.36	0.53	0.61	0.64	0.71	0.70

Considering firstly the figures for $LaCo(CN)_6$ in water (with omission of that at $c^{1/2} = 0.005$—it is a common feature of conductance that measurements below $c^{1/2} = 0.01$ are often too low, probably because of adsorption effects), the average of $K = 1.70 \times 10^{-4}$ is in good accord with the result of James and Monk,[3] namely, 1.73×10^{-4}. The most likely explanation why Atkinson's answer is so much higher is that Shedlovsky's method,[2] which was devised for 1:1 electrolytes, needs a slight modification when applied to higher valent symmetrical electrolytes since for 3:3 types

$$K = c_i^2f_i^2/3(c - c_i) \quad (3)$$

and the appropriate plot is $1/\Lambda S'(z)$ against $c\Lambda f_i^2S'(z)/3\Lambda_0^2$, where $S'(z)$ is a special function.[2] By dividing the original answer by the extra factor of 3, one does in fact find an answer reasonably close to the average of the Table.

The value for 10% dioxane is also now of the same order as James[6] obtained for the very similar system of $LaFe(CN)_6$ in 9.67% dioxane, namely, $K = 0.76 \times 10^{-4}$ ($\Lambda_0 = 138.0$). On the other hand it is to be seen that consistent results cannot be obtained with $\Lambda_0 = 116.6$ for $LaCo(CN)_6$ in 20% dioxane although the average for $c^{1/2} = 0.01$ and 0.015 of 0.27×10^{-4} is in general agreement with $K = 0.26 \times 10^{-4}$ obtained[6] for $LaFe(CN)_6$ in 18.1% dioxane. It would be possible to remove the drift by increasing Λ_0 but K would then be $<0.15 \times 10^{-4}$.

The position is much less satisfactory when the other results of Atkinson are analyzed by the present method. This is illustrated by the results for

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