

$J_{4H,6\alpha F}$ ). This pattern should consist of four lines of relative intensity 0.95, 0.98, 1.05, and 1.02 with a line separation varying from 0.01, 1.7, and 0.01 c.p.s. when  $J_{BX} = 0$  c.p.s. to 1.7, 0.3, and 1.7 c.p.s. when  $J_{BX} = 2$  c.p.s. At maximum resolution with our instrument, tetramethylsilane exhibits a width at half-height of 1.2 c.p.s., which will lead to the appearance of the four lines as a doublet when  $J = 0-0.5$  c.p.s., as a triplet when  $J = 1.2-2$  c.p.s. and as merged peaks (at half-height) at intermediate  $J$  values. Since the C-4 proton actually appeared as an unsplit peak, the most probable value for the  $6\alpha F-4H$  coupling is between 0.5 and 1.2 c.p.s.

The axial  $6\beta$ -fluoro C-F bond is approximately perpendicular to the plane passing through the C-4, 5, and 6 carbon atoms and parallels the  $\pi$ -orbitals of the double bond. Expressed in other terms, the dihedral angle of the projection of the C-4-4H bond and the C-6- $6\beta F$  bond approximates  $90^\circ$  while the corresponding angle with the C-6- $6\alpha F$  bond is about  $15^\circ$ . Although the magnitude of  $6\alpha F-4H$  coupling cannot be determined with exactitude it is clearly minimal compared to the 5-5.5 c.p.s. of the axial  $6\beta$ -fluoro atom. Thus, just as in the examples<sup>4</sup> of allylic 1,3-proton-proton coupling, this type of fluorine-hydrogen long-range coupling and transmission of spin information probably depends upon maximal overlap of the C-F  $\sigma$ -bond with the  $\pi$ -orbitals of the double bond. Caution should be exercised, however, in extrapolating these results to other long-range fluorine-proton coupling situations when the fluoro atom is adjacent to a  $\pi$ -electron system. For example, the C-4 proton of  $2\alpha$ -fluorotestosterone (Ic) is strongly 1,3-coupled (doublet at 342.5 and 347.5 c.p.s.,  $J_{2\alpha F,4H} = 5$  c.p.s.) to the equatorial  $2\alpha$ -fluorine, possibly *via* the  $\pi$ -electrons of the adjacent ketone function. In this case the dihedral angle of the projection of the pertinent C-F and C-H bonds is much closer to  $0^\circ$  than to  $90^\circ$ .

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### N-Hydroxysuccinimide Esters in Peptide Synthesis

Sir:

The need for better methods of forming the peptide bond has been emphasized in a recent report of the synthesis of a 39-amino acid ACTH peptide,<sup>1</sup> although the *p*-nitrophenyl ester method<sup>2,3</sup> was a key to the synthesis. The recently reported esters of N-hydroxyphthalimide<sup>4,5</sup> appear to be more reactive than *p*-nitrophenyl esters, but have some disadvantages.<sup>5</sup> One disadvantage of both is the insolubility of by-products (*p*-nitrophenol and N-hydroxyphthalimide) in water except under alkaline conditions. In consideration of the ready water solubility of N-hydroxysuccinimide,<sup>6</sup> we have prepared esters of this compound with a number of N-acylamino acids; these are crystalline, highly reactive compounds which have given promising results in peptide synthesis. In several examples, yields in

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peptide synthesis averaged better than those with comparable literature methods, and purification was simplified. These new esters are particularly promising for the addition of an acylamino acid to the salt of a peptide in aqueous solution. Illustrative examples are given subsequently, and a more detailed paper is in process.

Analogous to the synthesis of *p*-nitrophenyl<sup>7,8</sup> and N-hydroxyphthalimide<sup>4,5</sup> esters, dicyclohexylcarbodiimide in dioxane or dimethoxyethane was used to make the N-hydroxysuccinimide esters; the products were obtained in yields of 50-90% after recrystallization from 2-propanol. Typical N-hydroxysuccinimide esters are those of carbobenzoxy-L-phenylalanine (I), m.p. 140-140.5°, carbobenzoxyglycine (II), m.p. 113-114°, and carbobenzoxy-L-proline (III), m.p. 90°. Reaction of I with an equivalent of ethyl L-tyrosinate<sup>9</sup> in dimethoxyethane for 40 min. at 25° followed by the addition of water yielded ethyl carbobenzoxy-L-phenylalanyl-L-tyrosinate, yield 85% after recrystallization from ethanol, m.p. 156-158°,  $[\alpha]^{25D} -9.1^\circ$  (*c* 10, EtOH); lit.<sup>10</sup> yield 46% by a mixed anhydride procedure. A solution of 1.53 g. of II in 10 ml. of dimethoxyethane was added to a solution of 0.87 g. of proline and 0.63 g. of sodium bicarbonate in 8 ml. of water; after an hour, acidification gave carbobenzoxyglycyl-L-proline (IV), yield 75% after recrystallization from ethyl acetate, m.p. 157-158°; lit.<sup>11</sup> yield 68%, m.p. 155°, *via* a thiophenyl ester. The N-hydroxyphthalimide ester of carbobenzoxyglycine<sup>5</sup> was similarly treated with proline; slow acidification gave a poor fractional separation of N-hydroxyphthalimide, and IV was obtained in 45% yield, m.p. 145-150°. A solution of 3.46 g. of III in 30 ml. of ethanol was added to a solution of 2.79 g. of glycyl-L-phenylalanyl-glycine<sup>12</sup> and 1.68 g. of sodium bicarbonate in 50 ml. of water plus 25 ml. of ethanol; after standing 18 hr., acidification and removal of ethanol by vacuum distillation gave 4.53 g. (89%) of crystalline carbobenzoxy-L-prolylglycyl-L-phenylalanyl-glycine; recrystallization from water-ethanol yielded 4.12 g. (80%), m.p. 154-155°,  $[\alpha]^{25D} -27.6^\circ$  (*c* 2, dioxane). *Anal.* Calcd. for  $C_{26}H_{30}N_4O_7$ : C, 61.16; H, 5.92; N, 10.98. Found: C, 61.45; H, 6.11; N, 11.01.

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### Hydrolytic Cleavage of N-Terminal Peptide Bonds by a Cobalt Chelate

Sir:

Metal bearing enzymes such as leucine amino peptidase<sup>1</sup> catalyze the hydrolysis of N-terminal peptide bonds through a process involving chelation between the enzyme, the substrate, and the metal ion. Divalent transition metal cations have also been shown to accelerate the hydrolysis of peptides.<sup>2</sup> Hydroxide gels of highly charged ions such as Ce(IV) and La(III) are

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