perchlorate. Davidson and French[3,4] have prepared diphenylboronium perchlorate $[(C_6H_5)_2BClO_4]$ in nitromethane solution by the reaction of diphenylboron chloride and silver perchlorate from which they were able to prepare and isolate an addition compound with 2,2′-dipyridyl. We have prepared a trimethylamine addition compound of boron dichloroperchlorate and have observed enhancement in stability. Direct reaction proceeds with explosive violence, but the reaction can be moderated with suitable solvents. The compound once formed is a white solid which does not melt below 330° and is less sensitive to moisture than the original perchlorate.

(3) J. M. Davidson and C. M. French, *J. Chem. Soc.*, 114 (1958).
(4) J. M. Davidson and C. M. French, *Chem. Ind.* (London), 750 (1959).

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Concerning the Steric Requirements for Allylic 1,3-Spin–Spin Fluorine–Proton Coupling[1]

Sir:

The steroid skeleton, by virtue of its rigidity, offers in many instances an ideal structure for determination of the steric requirements for proton–proton[2] or proton–fluorine[3] spin–spin coupling. Recently it has been demonstrated that in Δ^4-3-keto steroids the axial 6β-proton but not the equatorial 6α-proton is significantly 1,3-spin–spin coupled to the C-4 vinyl proton.[4]

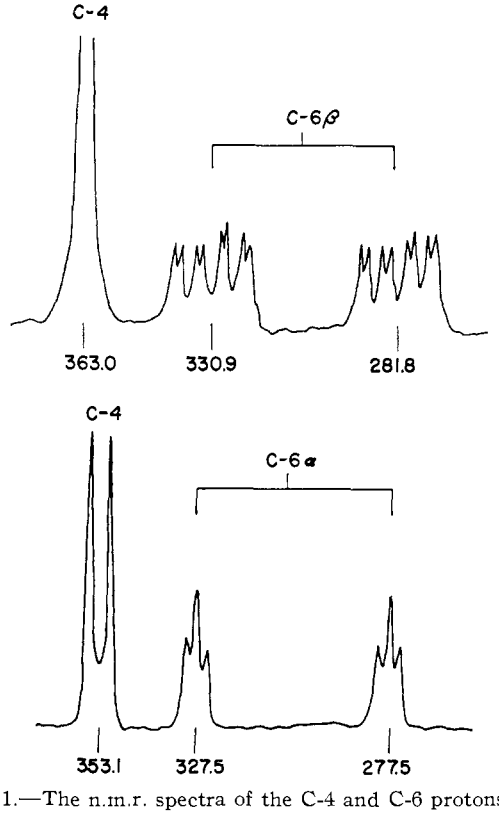
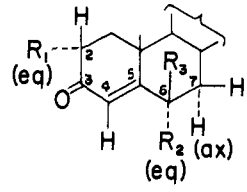


Fig. 1.—The n.m.r. spectra of the C-4 and C-6 protons of the isomeric 6-fluoroandrost-4-ene-3,17-diones determined in $CDCl_3$ with tetramethylsilane internal standard.[5] Upper curve, 6α-fluoro isomer; lower curve, 6β-fluoro compound.

This coupling is manifested in the nuclear magnetic resonance spectrum by the appearance of the vinyl proton as a doublet (J = 1.7–2.0 c.p.s.) when a bromo, chloro, or methyl substituent is present at C-6α and as merely a broad singlet (line width at half-height = 3.4 c.p.s.) when two protons are present at C-6.

We report herein that the spin–spin coupling of an allylic fluorine atom at C-6 with the C-4 vinyl proton appears to exhibit steric dependence identical with the proton–proton examples. A number of 6β-fluoro- (Ia) and 6α-fluoro- (Ib) -Δ^4-3-ketosteroids have been examined[5] and in each case the 6β-fluorosteroid exhibited for the C-4 proton a doublet centered at about 350 c.p.s.,[5] J = 5–5.5 c.p.s., attributable to fluorine–proton 1,3-coupling. In contrast the C-4 proton of the 6α-fluoro compounds appeared as a single peak at about 360 c.p.s. with an average line width at half-height of 4.5 c.p.s.,[6] the marked broadening being due primarily to unresolved coupling with the 6β-proton and probable coupling of low magnitude with the 6α-fluoro atom.



Ia, R_1=H; R_2=H; R_3=F
b, R_1=H; R_2=F; R_3=H
c, R_1=F; R_2=H; R_3=H

The five 6β-fluoro and eleven 6α-fluoro steroids examined gave essentially similar patterns for the spectral peaks of the C-4 and C-6 protons, but only in the case of the isomeric 6-fluoroandrost-4-ene-3,17-diones was solubility sufficient to allow complete elucidation of the C-6 proton pattern for both isomers. These spectra are therefore reported in detail (Fig. 1). In the 6β-fluoro substance the C-4 proton exhibited a doublet at 350.5 and 355.8 c.p.s. while the C-6α proton appeared as two triplets centered at 277.5 and 327.5 c.p.s. A first-order approximation allows the assignment of $J_{6\beta F,4H}$ = 5.3 c.p.s., $J_{6\alpha H,F}$ = 50 c.p.s., $J_{6\alpha H,7\alpha H}$ = 2.9 c.p.s., and $J_{6\alpha H,7\beta H}$ = 2.9 c.p.s. The 6β-proton of the 6α-fluoro compound appeared as a pair of quartets centered at 281.8 and 330.9 c.p.s.: $J_{6\beta H,6\alpha F}$ = 49.1 c.p.s., $J_{6\beta H,7\alpha H}$ = 11.5 c.p.s., $J_{6\beta H,7\beta H}$ = 5.7 c.p.s. Each of the eight peaks for the 6β proton was further split into a doublet (J = 1.7 c.p.s.) by 1,3-coupling[4] with the C-4 proton. The C-4 proton itself appeared as a single peak at 363 c.p.s.

The 6βF–4H coupling of 5–5.5 c.p.s. is clear-cut and requires no further interpretation. The appearance of the C-4 proton as a broad singlet in the 6α-fluoro case, however, leaves unresolved the degree, if any, of masked 6αF–4H coupling, since it is apparent that even the 6βH–4H coupling of 1.7 c.p.s. has been masked. The possible degree of 6αF–4H interaction has been derived in the following manner. Treating the 6βH, 4H, 6αF atoms as an ABX system with fluorine the X portion, J_{AB} = 1.7 c.p.s. and J_{AX} = 49 c.p.s., the line pattern for the C-4 proton has been calculated[7] assuming various values for J_{BX} (*i.e.*,

(1) Supported in part by grants A-4044 and CY-4550, U. S. Public Health Service, and T-185, American Cancer Society.

(2) *Cf.* K. L. Williamson and W. S. Johnson, *J. Am. Chem. Soc.*, **83**, 4623 (1961); F. J. Schmitz and W. S. Johnson, *Tetrahedron Letters*, 647 (1962); H. J. Ringold, M. Gut, M. Hayano, and A. Turner, *ibid.*, 835 (1962).

(3) A. D. Cross and P. W. Landis, *J. Am. Chem. Soc.*, **84**, 1736 (1962); **84**, 3784 (1962).

(4) D. J. Collins, J. J. Hobbs, and S. Sternhell, *Tetrahedron Letters*, 197 (1963); T. A. Wittstruck, S. K. Malhotra, and H. J. Ringold, *J. Am. Chem. Soc.*, **85**, 1699 (1963).

(5) Spectra were obtained using a Varian 4300 n.m.r. spectrometer, with field homogeneity control unit, at a frequency of 60 Mc.p.s. The samples were dissolved in deuteriochloroform and the spectra were calibrated using the side-band technique. Peak positions are reported in c.p.s. downfield from tetramethylsilane (internal reference).

(6) The full line widths were measured at half-height and the average line width of the tetramethylsilane reference peak was 1.2 c.p.s. The uncertainty in measurement is estimated to be ±0.2 c.p.s.

(7) K. B. Wiberg and B. J. Nist, "The Interpretation of NMR Spectra," W. A. Benjamin, Inc., New York, N. Y., 1955, p. 25.

$J_{4H,6\alpha F}$). 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 6αF–4H coupling is between 0.5 and 1.2 c.p.s.

The axial 6β-fluoro C–F bond is approximately perpendicular to the plane passing through the C-4, 5, and 6 carbon atoms and parallels the π-orbitals of the double bond. Expressed in other terms, the dihedral angle of the projection of the C 4–4H bond and the C-6–6βF bond approximates 90° while the corresponding angle with the C-6–6αF bond is about 15°. Although the magnitude of 6αF–4H coupling cannot be determined with exactitude it is clearly minimal compared to the 5–5.5 c.p.s. of the axial 6β-fluoro atom. Thus, just as in the examples[4] 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 σ-bond with the π-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 π-electron system. For example, the C-4 proton of 2α-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 2α-fluorine, possibly *via* the π-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° than to 90°.

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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,[1] although the *p*-nitrophenyl ester method[2,3] was a key to the synthesis. The recently reported esters of N-hydroxyphthalimide[4,5] appear to be more reactive than *p*-nitrophenyl esters, but have some disadvantages.[5] 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,[6] 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 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[7,8] and N-hydroxyphthalimide[4,5] 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[9] 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°, $[\alpha]^{25}D$ −9.1° (*c* 10, EtOH); lit.[10] 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.[11] yield 68%, m.p. 155°, *via* a thiophenyl ester. The N-hydroxyphthalimide ester of carbobenzoxyglycine[5] 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-phenylalanylglycine[12] 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-phenylalanylglycine; recrystallization from water–ethanol yielded 4.12 g. (80%), m.p. 154–155°, $[\alpha]^{25}D$ −27.6° (*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.

(1) R. Schwyzer and P. Sieber, *Nature*, **199**, 172 (1963).
(2) M. Bodanszky, *ibid.*, **175**, 685 (1955).
(3) M. Bodanszky, *Ann. N.Y. Acad. Sci.*, **88**, 655 (1960).
(4) G. H. L. Nefkens and G. I. Tesser, *J. Am. Chem. Soc.*, **83**, 1263 (1961).
(5) G. H. L. Nefkens, G. I. Tesser, and R. J. F. Nivard, *Rec. trav. chim.*, **81**, 683 (1962).
(6) Beilstein, Vol. 21, p. 380; R. Wegler, F. Grewe, and K. Mehlhose, U. S. Patent, 2,816,111 (1957).
(7) D. F. Elliot and D. W. Russell, *Biochem. J.*, **66**, 49P (1957).
(8) M. Rothe and F. W. Kunitz, *Ann.*, **609**, 88 (1957).
(9) E. Fischer, *Ber.*, **34**, 433 (1901).
(10) J. R. Vaughan, Jr., and R. Osato, *J. Am. Chem. Soc.*, **74**, 676 (1956).
(11) H. N. Rydon and P. W. G. Smith, *J. Chem. Soc.*, 1956, 3643.
(12) L. Zervas and D. M. Theodoropoulos, *J. Am. Chem. Soc.*, **78**, 1359 (1956).

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

Sir:

Metal bearing enzymes such as leucine amino peptidase[1] 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.[2] Hydroxide gels of highly charged ions such as Ce(IV) and La(III) are

(1) E. L. Smith and R. L. Hill in "The Enzymes," Vol. 4, edited by P. D. Boyer, H. Lardy, and K. Myrback, Academic Press, New York, N. Y., 1960, p. 37.
(2) L. Meriwether and F. H. Westheimer, *J. Am. Chem. Soc.*, **78**, 5119 (1956).