Anal. Calcd. for $C_{21}H_{32}O_2F_2$: C, 71.07; H, 9.03; F, 10.13. Found: C, 70.89; H, 9.12; F, 10.21.

Saponification of the isomeric difluoro acetate obtained from pregnenolone acetate (Ia) under similar conditions gave the same substance as above. This compound does not appear to be a 5,6-difluoro compound, since the corresponding diketone [m.p. 174–176° (from methylene dichloride–ether), $[\alpha]_D$ +73°, ν_{max} 1710 and 1715 cm.$^{-1}$ (C=O). *Anal.* Calcd. for $C_{21}H_{30}O_2F_2$: C, 71.55; H, 8.52; F, 10.70. Found: C, 71.19; H, 8.52; F, 10.70] obtained by oxidation with 8 *N* chromic acid gave neither 6α- nor 6β-fluoroprogesterone on attempted elimination with sodium acetate in methanol or hydrogen chloride in ethyl acetate.

5α,6α-Difluoropregnane-3,20-dione (III).—A solution of 5α,6α-difluoropregnane-3β-ol-20-one (IIb, 150 mg.) in acetone (40 ml.) was treated with a slight excess of 8 *N* chromium trioxide at 0°. After 2 minutes, the mixture was poured into water and the crude diketone III (120 mg.) was collected. After two recrystallizations from acetone–hexane it had m.p. 224–227°, $[\alpha]_D$ +80°.

Anal. Calcd. for $C_{21}H_{30}O_2F_2$: C, 71.55; H, 8.52; F, 10.70. Found: C, 71.29; H, 8.57; F, 9.42.

6α-Fluoroprogesterone (IV).—A solution of 5α,6α-difluoropregnane-3,20-dione (30 mg.) in methanol (3 ml.) containing sodium acetate (90 mg.) was heated under reflux for 3 hours. The cooled solution was poured into water (20 ml.) and the product, m.p. 134–139°, λ_{max} 236 mμ, log ε 4.14, was collected. Recrystallization from acetone–hexane gave 6α-fluoroprogesterone (IV), m.p. 145–147°, λ_{max} 236 mμ, log ε 4.16. A mixed melting point with an authentic sample[4] was undepressed and the infrared spectra were identical.

5α-Fluoropregnane-3β-ol-20-one Acetate (Va).—A cooled solution of pregnenolone acetate (Ia, 25.0 g.) in methylene dichloride (300 ml.) was added to a mixture of hydrogen fluoride (176.5 g.) and tetrahydrofuran (296 g.) at −75° and the solution was left at 0° for 20 hr. The mixture was poured into iced water (3.0 l.) and neutralized with sodium carbonate. The organic layer was separated and the aqueous phase was extracted with methylene dichloride (2 × 250 ml.). Evaporation of the dried organic extracts gave a semi-crystalline residue (24.8 g.) which was chromatographed in hexane–benzene (70:30) on alumina (750 g.). Elution with the same solvent mixture first gave recovered pregnenolone acetate (Ia) (18.2 g.), followed by mixed fractions (2.5 g.) and finally 5α-fluoropregnane-3β-ol-20-one acetate (Va), 2.2 g., m.p. 172–177°. Rechromatography of the mixed fractions gave a further 1.0 g. of Va. Recrystallization from acetone–methanol gave a total of 2.36 g., m.p. 194–196°, $[\alpha]_D$ +86°; ν_{max} 1733 and 1248 (acetate) and 1704 cm.$^{-1}$ (C=O).

Anal. Calcd. for $C_{23}H_{35}O_3F$: C, 72.97; H, 9.39; F, 5.09. Found: C, 73.06; H, 9.36; F, 4.04.

5α-Fluoropregnane-3β-ol-20-one (Vb).—A solution of the above acetate Va (420 mg.) in methanol (20 ml.) containing potassium hydroxide (200 mg.) was heated on the steam-bath for 2 hr. The cooled solution was neutralized with acetic acid, concentrated and poured into water. The precipitate of the 3β-alcohol Vb was collected, washed well with water and dried (360 mg., m.p. 178–180°). A recrystallized sample had m.p. 188–189°, $[\alpha]_D$ +105°, ν_{max} 3530 (OH) and 1704 cm.$^{-1}$ (C=O).

Anal. Calcd. for $C_{21}H_{33}O_2F$: C, 74.96; H, 9.88; F, 5.63. Found: C, 75.12; H, 9.80; F, 4.76.

5α-Fluoropregnane-3,20-dione (VI).—To a solution of 5α-fluoropregnane-3β-ol-20-one (290 mg.) in acetone (20 ml.) at 0° was added a slight excess of 8 *N* chromium trioxide in acetone. After 1 minute, the solution was poured into water and the dione VI was collected (245 mg., m.p. 200–203°). After two recrystallizations from acetone–hexane, the product had m.p. 204–205°, $[\alpha]_D$ +100°.

Anal. Calcd. for $C_{21}H_{31}O_2F$: C, 75.41; H, 9.34; F, 5.68. Found: C, 75.08; H, 9.04; F, 5.76.

Treatment of 5α-Fluoropregnane-3,20-dione (VI) with Sodium Acetate.—A solution of the 5α-fluoro-3,20-dione VI (50 mg.) in methanol (5.0 ml.) containing sodium acetate (150 mg.) was heated under reflux for 2 hr. It was then concentrated to small volume and diluted with water (50 ml.). Extraction with methylene dichloride (2 × 20 ml.) afforded a product which was dissolved in hexane–benzene (20:30) and adsorbed onto alumina (1.0 g.). Elution with hexane–benzene (50:50) (60 ml.) gave progesterone (27 mg., m.p. 112–119°) which after crystallization from methylene dichloride–hexane had m.p. 126–128°, λ_{max} 240–242 mμ, log ε 4.22, and was identical in all respects with an authentic specimen.

3β-Fluoro-Δ^5-pregnene-20-one (Ic).—A solution of pregnenolone (Ib) (20.0 g.) in methylene dichloride (260 ml.) was cooled to −40° and added to a mixture of anhydrous hydrogen fluoride (167 g.) and tetrahydrofuran (281 g.) at −75°. The mixture was stirred at 6° for 18 hr., poured into iced water (3 l.) and neutralized with sodium carbonate. The aqueous layer was separated and extracted with methylene dichloride (2 × 250 ml.). The combined organic extracts were dried and evaporated. The residue was chromatographed in hexane–benzene (50:50) on alumina (500 g.). Elution with the same solvent pair gave first fractions of m.p. 145–155° (8.5 g.), then low melting material (m.p. 110–140°) and finally recovered pregnenolone (Ib, m.p. 185–189°, 9.2 g.). The first eluted fractions were rechromatographed on alumina (300 g.) in hexane–benzene (80:20) to give 6.1 g. of 3β-fluoro-Δ^5-pregnene-20-one (Ic), m.p. 161–162°, and a further 1.1 g. of recovered pregnenolone (m.p. 186–189°). Recrystallization of the 3β-fluoro-Δ^5-pregnene-20-one raised the m.p. to 167–168°, $[\alpha]_D$ +14°, ν_{max} 1705 cm.$^{-1}$ (C=O). Jacobson and Jensen[35] report m.p. 164–165°, $[\alpha]_D$ +14° and Shoppee and Summers[36] erroneously report m.p. 170–172°, $[\alpha]_D$ +114°.

COMMUNICATIONS TO THE EDITOR

THE USE OF REMOTE DEUTERATION FOR THE DETERMINATION OF COUPLING CONSTANTS AND CONFORMATIONAL EQUILIBRIA IN CYCLOHEXANE DERIVATIVES

Sir:

The application of high-resolution proton nuclear magnetic resonance (n.m.r.) to the study of conformational equilibria is often made difficult or impossible by the complexity of the spectra. For example, cyclohexanol[1] (see also Fig. 1) and its alkyl derivatives[2,3] and cyclohexyl halides[4] give a broad unresolved band for the tertiary ring proton (H1) even though H1 is well chemically shifted from all the other protons in the molecule. It is therefore not possible to make full use of the difference in the magnitude of coupling constants which should exist[2,5] between *gauche* (a,e or e,e) and conformationally *trans* (a,a) vicinal protons.

(1) A. C. Huitric and J. B. Carr, *J. Org. Chem.*, **26**, 3648 (1961).

(2) R. U. Lemieux, R. K. Kullnig, H. J. Bernstein and W. G. Schneider, *J. Am. Chem. Soc.*, **80**, 6098 (1958).

(3) S. Brownstein and R. Miller, *J. Org. Chem.*, **24**, 188 (1959); J. I. Musher, *J. Am. Chem. Soc.*, **83**, 1146 (1961).

(4) L. W. Reeves and K. O. Stromme, *Can. J. Chem.*, **38**, 1241 (1960).

(5) M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959); H. Conroy, *Advances in Organic Chemistry*, **2**, 311 (1960).

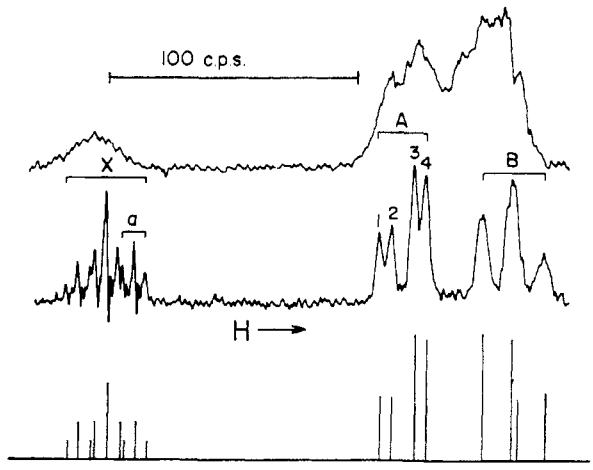

Fig. 1.—N.m.r. spectra (60 Mc./sec.) of cyclohexanol (top) and 3,3,4,4,5,5-hexadeuteriocyclohexanol (I) (middle) in D_2O. The OH band (actually of HDO) occurs at lower field and is not shown. The calculated spectrum (bottom) is that for an $(AB)_2X$ system with ν_{AB} = 38.0, J_{AB} = 12.2, J_{AX} = 4.09 and J_{BX} = 10.17 cps. The calculated intensities of the X lines are not on the same scale as the AB lines.

In order to obtain spectra in which the bands of Hl consist of sharp lines, we have prepared[6] 3,3,4,4,5,5-hexadeuteriocyclohexanol (I), its acetate (II), and *cis*- and *trans*-3,3,4,5,5-pentadeuterio-4-*t*-butylcyclohexanol (III and IV, respectively).

In each of the above compounds (I-IV) the band of Hl in the n.m.r. spectrum consisted of a number of sharp well-resolved lines (*e.g.*, Fig. 1). The methylene protons, although somewhat broadened by coupling with deuterium atoms, were readily recognizable as the AB part of an ABX system. The band of Hl is the X part of two ABX systems. Analysis of the spectra gave the data in Table I.

TABLE I

VICINAL COUPLING CONSTANTS[a] OF CYCLOHEXANE DERIVATIVES

Compound[b]	State	$J_{a,a}$	$J_{a,e}$	$J_{e,e}$
IIA	25% in CS_2	11.43	4.24	
IIB	at −110°		(2.71)[c]	(2.71)[c]
III	8% in CCl_4		3.00	2.72
IV	10% in CCl4	11.07	4.31	
		J_{large}	J_{small}	K[d]
I	5% in D_2O	10.17	4.09	8.09[e]
I	25% in CCl_4	9.77	3.93	5.25[e]
II	25% in CS_2	9.26	3.89	3.00[f]

[a] The coupling constants are considered to be accurate to better than 0.1 cps. [b] IIA and IIB are the equatorial-acetoxy and axial-acetoxy conformations of II; the ratio of IIA to IIB is about 10 to 1 at −110°. [c] Average of $J_{a,e}$ and $J_{e,e}$. [d] K = [equatorial OH conformer]/[axial OH conformer] and is calculated on the basis of K_{large}. [e] Calculated using the coupling constants found for III and IV above. [f] Calculated using the coupling constants found for IIA and IIB above.

It is surprising that the *gauche* coupling constants in IIA and IV are much larger than in IIB and III. It does not seem likely that changes in dihedral angles could be entirely responsible for this. On the whole, however, the values of the coupling constants are very much as expected.[1,2,5,7]

The relative signs of the coupling constants were determined for I in D_2O. From the relative intensities (2 > 1, 3 > 4) of the lines of the A spectrum (Fig. 1), it follows that J_{AX} and J_{BX} have the same sign. As anticipated[8,9] J_{AB} is of opposite sign to J_{BX} because the *high-field* triplet (a) of X collapsed to a single line when A was simultaneously irradiated[10] with a magnetic field of 1.5 milligauss at a frequency corresponding to the center of the *low-field* doublet of A.

It is possible, once accurate coupling constants are available for III and IV, to deduce conformational equilibria for I under various conditions. From the data in Table I the A value[11] of I is calculated to be 1.25 kcal./mole in D_2O and 1.0 kcal./mole in CCl_4 at 28°. By using the coupling constants found at low temperatures the A value of II in CS_2 at 28° is found to be 0.66 kcal./mole. The present A values are thought to be reliable, although they are somewhat higher than some of those previously reported.[11,12]

Measurements are being undertaken of the coupling constants of I, III, IV and their derivatives and of related compounds at different temperatures and in various solvents.

(6) The reasons for using the particular deuterated derivatives and the method used for their syntheses will be discussed in a full paper. See also F. A. L. Anet, *Can. J. Chem.*, **39**, 2262 (1961).

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STUDIES ON POLYPEPTIDES. XXII. HIGH ADRENOCORTICOTROPIC ACTIVITY IN THE RAT AND IN MAN OF A SYNTHETIC EICOSAPEPTIDE AMIDE[1-3]

Sir:

In 1956 Boissonnas, *et al.*,[4] announced a synthesis of the eicosapeptide (I) and reported that their preparation possessed low *in vitro* adrenocorticotropic activity (2 to 3 U./mg.). Recently, Li, *et al.*,[5] described a preparation of the nonadecapeptide (II) which exhibited *in vivo* adrenocorticotropic

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

(2) Peptides and peptide derivatives mentioned in this communication are of the L-variety.

(3) See *J. Am. Chem. Soc.*, **83**, 2294 (1961), for paper XXI in this series.

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