hexane) II as yellow-orange prisms, m.p. 98.5–100.5°. Found for $C_{14}H_{11}N$: C, 86.92; H, 5.82; N, 7.13. The ultraviolet spectrum of II in hexane showed λ_{max} in mμ (log ε) at 251 (4.34), 274 (4.38) and 286 (4.30). The visible spectrum had a single broad peak with λ_{max} at 432 mμ and log ε 3.30. On hydrogenation over a Rh–C catalyst II took up 4.0 moles of hydrogen readily to form VI (identified by ultraviolet and infrared spectra). A solution of II in concentrated sulfuric acid showed λ_{max} in mμ (log ε) at 220 (4.15), 254 (4.0) and 295 (4.08). The last two maxima also were obtained with a solution in glacial acetic acid. II was degraded slowly by acetic acid and was decomposed by alumina or silica gel. It was stable to alcoholic alkali.

The properties of II suggest that the orange impurity in the 1,5-pyrindine obtained by Robison[7] was 1-pyrindine. Further studies on I, II and related compounds are in progress.

(7) M. M. Robison, THIS JOURNAL, **80**, 6254 (1958).
(8) Standard Oil of California Fellow, summer, 1958.

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16-HYDROXYLATED STEROIDS. XI.[1] THE PREPARATION AND EPIMERIZATION OF 16β-ACETOXY-17α-HYDROXY-CORTICOIDS

Sir:

The important biological and therapeutic properties of triamcinolone (9α-fluoro-11β,16α,17α,21-tetrahydroxy-1,4-pregnadiene-3,20-dione, IX) and related 16α-hydroxy-compounds[2] have created interest in the preparation of the various 16β-hydroxy analogs. This report is concerned with the synthesis and properties of the 16β-acetoxy derivatives of 17α-hydroxy-corticoids.

Treatment of 21-acetoxy-16α,17α-epoxy-4,9(11)-pregnadiene-3,20-dione (I)[3] with sulfuric acid and acetic acid[4] yielded 16β,21-diacetoxy-17α-hydroxy-4,9(11)-pregnadiene-3,20-dione (II), m.p. 173–175°; λ_{max}^{EtOH} 239 mμ (ε 15,700), found C, 67.78; H, 7.56. Addition of the elements of hypobromous acid[5] afforded the amorphous bromohydrin III which was cyclized to 16β,21-diacetoxy-9β,11β-epoxy-17α-hydroxy-4-pregnene-3,20-dione (IV), m.p. 200–202°, λ_{max}^{EtOH} 243–244 mμ (ε 15,200), found C, 65.28; H, 7.28. The latter with hydrogen fluoride gave the fluorohydrin diacetate V, m.p. 239-241.5°, λ_{max}^{EtOH} 239 mμ (ε 16,500), ν_{max}^{KBr} 3540, 3420, 1755, 1738, 1718, 1669, 1627 cm.$^{-1}$, $[\alpha]^{24}D$ + 106° (acetone), found C, 62.66; H, 7.11; F, 4.05. Dehydrogenation of V with selenium dioxide in *t*-butyl alcohol produced 16β,21-diacetoxy-9α-fluoro-11β,17α-dihydroxy-1,4-pregnadiene-3,20-dione (VI), m.p. 233.5–236°, λ_{max}^{EtOH} 238 mμ (ε 13,000), ν_{max}^{KBr} 3490, 3320, 1755, 1733, 1713, 1660, 1620, 1608 cm.$^{-1}$, $[\alpha]^{25}D$ + 76.5° (acetone), found C, 63.13; H, 6.53; F, 3.62.

Saponification of the 9β,11β-oxide diacetate IV with potassium hydroxide in methanol in an inert atmosphere, yielded, most unexpectedly, 9β,11β-epoxy-16α,17α,21-trihydroxy-4-pregnene-3,20-dione (VII)[2a,c] identical in all respects with an authentic sample. Similarly 16β,21-diacetoxy-9α-fluoro-11β,17α-dihydroxy-4-pregnene-3,20-dione (V) was converted into 9α-fluoro-11β,16α,17α,21-tetrahydroxy-4-pregnene-3,20-dione (VIII),[2a,c] and 16β,21-diacetoxy-9α-fluoro-11β,17α-dihydroxy-1,4-pregnadiene-3,20-dione (VI) into triamcinolone (IX).[2a,c]

A further study of this epimerization revealed that treatment of 16β,21-diacetoxy-17α-hydroxy-4-pregnene-3,20-dione (X)[4] with potassium hydroxide, sodium methoxide, sodium carbonate or sodium bicarbonate gave in all cases 16α,17α,21-trihydroxy-4-pregnene-3,20-dione (XI).[6,7] Careful partition chromatography of the product in some of these experiments has revealed the presence of at least two additional products, designated as A and B, isomeric with XI.

Work is in progress to determine the structure of compounds A and B, and to establish a possible mechanism for the epimerization.

Both 9α-fluoro-16β,21-diacetates, V and VI, were inactive in the rat liver glycogen assay at a 500 μg. dose level.[8]

(1) Paper X, S. Bernstein, J. J. Brown, L. I. Feldman and N. E. Rigler, THIS JOURNAL, in process of publication.
(2) (a) S. Bernstein, R. H. Lenhard, W. S. Allen, M. Heller, R. Littell, S. M. Stolar, L. I. Feldman and R. H. Blank, *ibid.*, **78**, 5693 (1956); (b) S. Bernstein, M. Heller, R. Littell, S. M. Stolar, R. H. Lenhard and W. S. Allen, *ibid.*, **79**, 4555 (1957); (c) S. Bernstein, *Recent Progress in Hormone Research*, **14**, 1 (1958); (d) R. H. Freyberg, C. A. Berntsen, Jr., and L. Hellman, *Arthritis and Rheumatism*, **1**, 215 (1958).
(3) L. B. Barkley, M. W. Farrar, W. S. Knowles and H. Raffelson, THIS JOURNAL, **76**, 5017 (1954); W. S. Allen, S. Bernstein, L. I. Feldman and M. J. Weiss, in preparation for publication.
(4) K. Heusler and A. Wettstein, *Chem. Ber.*, **87**, 1301 (1954).
(5) J. Fried and E. F. Sabo, THIS JOURNAL, **75**, 2273 (1953); **76**, 1455 (1954); **79**, 1130 (1957).
(6) W. S. Allen and S. Bernstein, *ibid.*, **78**, 1909 (1956).
(7) Heusler and Wettstein[4] first reported this reaction and assumed the product to be a D-homo rearrangement compound. We wish to thank Dr. Wettstein for sending us a sample of his compound, the infrared spectrum of which revealed it to be identical to authentic XI. J. Romo and A. R. De Vivar, *J. Org. Chem.*, **21**, 902 (1956), also have assumed the product obtained by treatment of 21-acetoxy-4,16-pregnadiene-3,20-dione with osmium tetroxide and then decomposition of the osmate complex with sodium sulfite in an alcohol medium to be a D-homo product since it was identical to the Heusler–Wettstein compound. Dr. Romo kindly sent us a sample of his product, which proved to be identical to authentic XI.
(8) We are indebted to L. Bortle, E. Heyder, J. Perrine, E. Ross and I. Ringler of the Experimental Therapeutics Research Section for these results.

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ALKALINE REARRANGEMENT OF PHENYL GROUPS LINKED TO SILICON

Sir:

It long has been recognized that strong bases may cause cleavage of phenyl groups attached to silicon,[1] as well as rearrangement of siloxane bonds.[2] Bailey and Pines[3] have reported that sodium ethoxide brings about disproportionation of crotyl-

(1) F. S. Kipping and A. G. Murray, *J. Chem. Soc.*, 1427 (1928).
(2) M. J. Hunter, J. F. Hyde, E. L. Warrick and H. J. Fletcher, THIS JOURNAL, **68**, 667 (1946).
(3) D. L. Bailey and A. N. Pines, *Ind. Eng. Chem.*, **46**, 2363 (1954).

and allyltriethoxysilanes. Base-induced rearrangements in which phenyl groups shift from one silicon atom to another do not appear in the literature, although such rearrangement was reported as a side reaction in a recent manuscript,[4] from which its discussion was stricken in deference to the opinion of one referee.

The present communication reports evidence which has accumulated in a variety of experiments that bases which frequently cause siloxane rearrangement of phenyl group cleavage may also produce rearrangement of phenyl groups involving their interchange with oxygen on silicon.

When in the manner of the general preparation of low-polymer silsesquioxanes[4] the hydrolyzate of $PhSiCl_3$ was heated with sodium hydroxide up to 500° (2 mm.), phenyl rearrangement occurred as indicated by the presence in the distillate of compounds in which three or four phenyl groups were bonded to a single silicon atom. One fraction comprised about 5 g. of a mixture of oil and crystals. Double recrystallization from toluene yielded 0.7 g. of crystals which were shown by infrared spectrum to consist of 75–80% Ph_4Si, identified by characteristic absorption at 13.50, 14.15 and 14.23 μ; and 20–25% of $(Ph_3Si)_2O$, identified by absorption at 9.27 and 14.00 μ, and evaluated from the former band. The crystal mixture melted at about 210°.[5]

Another example of this type of rearrangement was observed when methylphenylpolysiloxane, heated in the presence of bases, gave copious amounts of $(Ph_2MeSi)_2O$ and Ph_3SiMe, among other products insufficient for isolation. Fractional distillation of a run wherein 35 kg. $(PhMeSiO)_n$ was heated with 0.1% LiOH to 300° pot temperature at 1 mm. afforded 3.15 kg. $(Ph_2MeSi)_2O$, identified via infrared absorptions at wave lengths (microns): 3.28 (w), 7.00 (m), 7.98 (w), 8.95 (s), 9.40 (s), 12.61 (s), 12.85 (w), 13–55–13.82 (s), 14.38 (s).

Three similar runs with $NaO(Me_2SiO)_2Na$, $KO(Me_2SiO)_2K$ and $CsO(Me_2SiO)_xCs$ as catalysts gave $(Ph_2MeSi)_2O$ as above but also produced Ph_3SiMe, in 4, 9 and 13 weight percentages, respectively. Interestingly, the relative activities of the catalysts in promoting these phenyl rearrangements were in the same order as observed for siloxane rearrangement: NaOH < KOH < CsOH.[6] Some phenyl cleavage was indicated by generation of benzene during the rearrangement. Triphenylmethylsilane was also recovered in 4.6% yield from the volatile by-products of the KOH-catalyzed copolymerization of $(PhMeSiO)_n$ with methyl-containing siloxanes.[7] In these experiments, Ph_3SiMe was identified by infrared spectra showing absorptions at wave lengths (microns): 3.28 (w), 7.02 (m), 8.02 (w), 9.00 (s), 12.70 (s), 13.70 (m, shoulder), 13.80 (s), 14.40 (s). Its spectrum is distinguished from $(PhMeSiO)_3$ and $(Ph_2MeSI)_2O$ by the peak at 13.80 μ. Its identity was further established by melting point, 66–67°,[8] and by analysis: Si, 10.2, 9.6; C, 83.6, 83.1; H, 7.3, 7.0 (calcd. for Ph_3SiMe: Si, 10.2; C, 83.2; H, 6.6).

The mechanism of the observed phenyl-oxygen interchange in siloxanes probably involves a nucleophilic attack by base at the positive silicon atom quite like that proposed for siloxane rearrangement.[6]

(4) A. J. Barry, W. H. Daudt, J. J. Domicone and J. W. Gilkey, THIS JOURNAL, **77**, 4252 (1955).

(5) Literature values: Ph_4Si, 230–232° [H. Gilman and H. W. Melvin, Jr., *ibid.*, **71**, 4050 (1949)]; 234–235° [J. S. Peake, W. H. Nebergall and Chem Yun Ti, *ibid.*, **74**, 1526 (1952)]; $(Ph_3Si)_2O$, 222–224° [H. Gilman, B. Hofferth, H. W. Melvin and G. E. Dunn, *ibid.*, **72**, 5767 (1950)]; 219° [H. H. Szmant and G. A. Brost, *ibid.*, **72**, 5763 (1950)].

(6) D. T. Hurd, R. C. Osthoff and M. L. Corrin, *ibid.*, **76**, 249 (1954).

(7) R. W. Soderberg, unpublished work.

(8) Literature values: 66–67° [H. Marsden and F. S. Kipping, *J. Chem. Soc.*, **93**, 198 (1908)]; 67–68° [R. A. Benkeser and D. J. Foster, THIS JOURNAL, **74**, 5314 (1952)].

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ISOLATION AND CHARACTERIZATION OF A RESISTANT PEPTIDASE[1]

Sir:

From a concentrate of peptidases prepared from a digest of kidney tissue,[2] an apparently homogeneous cysteinylglycinase has been isolated. A concentrate containing 300,000 units[3] of cysteinylglycinase was eluted from a column of Ecteola-cellulose,[4] 6.5 by 60 cm., by gradient elution from *p*H 8 to 7 and to 0.5 *M* NaCl with a total of 14 l. of eluate. Four active fractions were obtained: the first with gradient near 0.2 *M*,, the second near 0.3 *M*, the third and fourth near 0.5 *M*. The first fraction was poorly resolved from a fraction of relatively high peptide content and the second active fraction was poorly resolved from an inactive fraction that contained 99% of the absorption at 260 mμ. Fractions III and IV eluted several liters past the bulk of the material absorbing in the ultraviolet, emerged in a symmetrical manner with a constant ratio of absorption with activity. Fraction III contained 60,000 units of activity; on the basis of total nitrogen, the specific activity was 30,000 units representing a purification of 10,000-fold. In paper electrophoresis the material migrated as a single component in the range of *p*H 7 to 9 with no dissociation of the absorption in the ultraviolet from the activity. Rechromatography on small Ecteola columns with greatly varied gradients indicated homogeneity and no dissociation of the ultraviolet absorption from the activity.

The ratios of absorption in the ultraviolet, 250/260 and 280/260, were 0.90 and 0.61, respectively,

(1) These studies were supported by grants from the U. S. Public Health Service and the Rockefeller Foundation. Kidney tissue contains two types of peptidases; one type, easily soluble in water and destroyed by proteolysis, has been called labile. Another type, found in insoluble particles and released into solution by, but fully resistant to, proteolysis, has been designated as resistant.

(2) F. Binkley, V. Alexander, F. E. Bell and C. Lea, *J. Biol. Chem.*, **228**, 559 (1957).

(3) This corresponds to about 30 lb. of kidney tissue and to 10,000,000 units in the assay of Semenza (G. Semenza, *Biochim. et Biophys. Acta*, **24**, 401 (1957)). The purified material would have a specific unitage of 1,000,000 in his assay.

(4) E. A. Peterson and H. A. Sober, THIS JOURNAL, **78**, 751 (1956).