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).

ratios identical with those of guanylic acid under similar conditions. Further analyses are listed in the table.

COMPOSITION OF FRACTION III

Analyses are on a total eluate concentrated to 50 ml.

Component	mmol./liter
Guanine	0.41
Total phosphate	0.82
Labile phosphate	0.39
Pentose	0.39
Total amino acids	0.35
Acid labile amino acids	0.38

It is apparent that guanine, pentose and amino acids (ninhydrin on a hydrolysate with 8 *N* HCl for 24 hr. at 105°) were present in a 1:1:1 ratio. Total phosphate was in a ratio of 2:1 with the other components but half was released by hydrolysis with 1 *N* HCl for 30 min. Guanosine diphosphate was the major nucleotide found in an alkaline hydrolysate with a formate column.[5] The arrangement of amino acids cannot be as a protein or as a single peptide; hydrolysis with 1 *N* HCl for 60 min. at 100° led to the release of the amino acids (acid labile amino acids). Thus the amino acids must be combined in some type of labile linkage with guanine nucleotides.

These results support the concept that polynucleotide, non-protein materials have catalytic activity in the hydrolysis of peptides. Uridine nucleotides and hexosamine, previously reported as minor components of an active fraction from Dowex columns, have not been found in this material; otherwise, the composition is much as was reported.[6]

(5) R. B. Hurlbert, H. Schmitz, A. F. Brumm and V. R. Potter, *J. Biol. Chem.*, **209**, 23 (1954).

(6) F. Binkley, C. K. Olson and C. Torres, Abstracts, 128th Meeting, American Chemical Society, Minneapolis, 1955.

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SYNTHESIS OF A BIOLOGICALLY ACTIVE ANALOG OF OXYTOCIN, WITH PHENYLALANINE REPLACING TYROSINE[1]

Sir:

We have studied the significance of one of the few free functional groups of oxytocin, the phenolic hydroxyl group of the tyrosyl residue, by synthesis of an analog of oxytocin with tyrosine replaced by phenylalanine. This analog, 2-phenylalanine oxytocin,[2] was prepared by a method recently used for the synthesis of oxytocin.[3]

Methyl S-benzyl-N-carbobenzoxy-L-cyteinyl-L-phenylalaninate (I), m.p. 106–107°, $[\alpha]^{20}D$ −37° (*c* 2, dimethylformamide), (*Anal.* Calcd for $C_{28}H_{30}O_5N_2S$: C, 66.4; H, 5.97; N, 5.53. Found: C, 66.2; H, 6.08; N, 5.63), was prepared by coupling S-benzyl-N-carbobenzoxy-L-cysteine with methyl L-phenylalaninate by the dicyclohexylcarbodiimide procedure[4] and also by the reaction of *p*-nitrophenyl S-benzyl-N-carbobenzoxy-L-cysteinate[3,5,6] with methyl L-phenylalaninate. I was hydrolyzed to give S-benzyl-N-carbobenzoxy-L-cysteinyl-L-phenylalanine (II), m.p. 157–158°, $[\alpha]^{20}D$ −22° (*c* 2, pyridine), (*Anal.* Calcd. for $C_{27}H_{28}O_5N_2S$: C, 65.8; H, 5.73; N, 5.69; neut. equiv., 492.6. Found: C, 65.9; H, 5.92; N, 5.76; neut. equiv., 491). II was converted to a mixed anhydride[7] by the action of isobutyl chloroformate and brought into reaction with L-isoleucyl-L-glutaminyl-L-asparagine (III).[3] The protected pentapeptide S-benzyl-N-carbobenzoxy-L-cysteinyl-L-phenylalanyl-L-isoleucyl-L-glutaminyl-L-asparagine (IV), m.p. 235–238°, $[\alpha]^{20}D$ −27° (*c* 0.5, dimethylformamide), (*Anal.* Calcd. for $C_{42}H_{53}O_{10}N_7S$: C, 59.5; H, 6.30; N, 11.6; neut. equiv., 848. Found: C, 59.7; H, 63.0; N, 11.6; neut. equiv., 848), thus obtained was linked[4] to S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide[3,8,9] (V) to give the protected nonapeptide S-benzyl-N-carbobenzoxy-L-cysteinyl-L-phenylalanyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide (VI), m.p. 247–248°, $[\alpha]^{22}D$ −52° (*c* 1, dimethylformamide), (*Anal.* Calcd. for $C_{65}N_{86}N_{12}O_{13}S_2$: C, 59.7; H, 6.63; N, 12.9. Found: C, 59.6; H, 6.77; N, 12.7). The protecting groups were removed from VI with sodium in liquid ammonia and the resulting nonapeptide oxidized by aeration to form the cyclic disulfide (VII), an octapeptide, the 2-phenylalanine analog of oxytocin. One milligram of VII gave in this procedure 18 units of avian depressor activity[10] and 0.08 unit of pressor activity in the rat.[11] VII was purified by extraction with alcohol and precipitation by ethyl acetate followed by extraction with pyridine and reprecipitation by ethyl acetate. The solid thus obtained assayed about 25 units/mg. of avian depressor activity and was further purified by countercurrent distribution in a solvent system of butanol–ethanol–0.05% acetic acid (4:1:5). The distribution curve obtained by Folin color assay[12] was in excellent agreement with the curve showing biological activity and also with a curve calculated for the *K* value 0.68. This agreement indicates the presence of a single compound. After this purification VII showed an avian depressor activity[10] of about 60–70 units per mg. and about 30 units per mg. of oxytocic activity when assayed on the isolated rat uterus.[13] No pressor activity[11] was found in the rat at a total

(1) This work was supported in part by a grant from the National Heart Institute, U. S. Public Health Service, Grant No. H-1675.

(2) In order to designate various analogs of oxytocin, this numbering is proposed (using the reduced form):

CySH-Tyr-Ileu-Glu(NH_2)-Asp(NH_2)-CySH-Pro-Leu-Gly(NH_2).
1 2 3 4 5 6 7 8 9

(3) M. Bodanszky and V. du Vigneaud: in press.

(4) J. C. Sheehan and G. P. Hess, THIS JOURNAL, **77**, 1067 (1955).

(5) M. Bodanszky, *Nature*, **175**, 685 (1955); *Acta Chim. Hung.*, **10**, 335 (1957); M. Bodanszky, M. Szelke, E. Tomorkeny and E. Weisz, *Chem. and Ind.*, 1517 (1955); *Acta Chim. Hung.*, **11**, 179 (1957).

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(7) J. P. Vaughan, Jr., THIS JOURNAL, **74**, 6137 (1952).

(8) C. Ressler and V. du Vigneaud, *ibid.*, **76**, 3107 (1954).

(9) M. Zaoral and J. Rudinger, *Chem. Listy*, **49**, 745 (1955).

(10) J. M. Coon, *Arch. Intern. Pharmacol.*, **62**, 79 (1939).

(11) J. Dekanski, *Brit. J. Pharmacol.*, **7**, 567 (1952).

(12) O. H. Lowry, N. V. Rosebrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.*, **183**, 265 (1951).

(13) J. H. Burn, D. J. Finney and L. G. Goodwin, "Biological Standardization," Oxford University Press, New York, N. Y., 1950.