properties were determined[7]: d° 0.7907, d^{20} 0.7764, $n^{20}D$ 1.4339, b.p. 89.8° (30 mm.), b.p. 109.6° (66 mm.), b.p. 124.4° (115 mm.), b.p. 148.0° (248 mm.), b.p. 163.0° (383 mm.), b.p. 187.4 (731 mm.), calculated b.p. 189.3° (760 mm.), infrared spectrum #2054.

Anal. Calcd. for $C_{10}H_{24}Si$: C, 69.66; H, 14.03; MR_D,[8] 58.06. Found: C, 71.0; H, 14.4; MR_D, 57.83.

Tri-*n*-propylethylsilane.—This compound was similarly prepared by reaction 166 g. (1.0 mole) of ethyltrichlorosilane with 3.8 moles of *n*-propylmagnesium bromide in one liter of ether. Fractional distillation of the dried crude product gave 94 g. (0.51 mole, 51% yield) of tri-*n*-propylethylsilane[9]; b.p. 202–202.5° at atmospheric pressure, $n^{20}D$ 1.4361–3. The product was freed of a pungent odor with activated alumina and decolorizing carbon and the following properties were determined[7]: d^{0} 0.7936, d^{20} 0.7795, $n^{20}D$ 1.4362, b.p. 104.8° (33 mm.), b.p. 129.0° (90 mm.), b.p. 151.4° (181 mm.), b.p. 164.4 (264 mm.), b.p. 179.4 (406 mm.), b.p. 202.2 (732 mm.), calculated b.p. 204.3° (760 mm.), infrared spectrum #2053.

Anal. Calcd. for $C_{11}H_{26}Si$: C, 70.87; H, 14.06; MR_D,[9] 62.69. Found: C, 71.2; H, 14.4; MR_D, 62.55.

Other Tetraalkylsilanes.—The other compounds needed in this investigation were well known[7] and were prepared by the Grignard reaction: tetraethylsilane, $n^{20}D$ 1.4269, infrared spectrum #2056; *n*-propyltriethylsilane, $n^{20}D$ 1.4313, infrared spectrum #2057; tetra-*n*-propylsilane, $n^{20}D$ 1.4384,[10] infrared spectrum #2055. Samples of triisopropylsilane and triisopropylphenylsilane were kindly loaned to us by Dr. Henry Gilman[11] for determination of their infrared spectra, #2589 and 2584, respectively.

Purification of Tetraalkylsilanes.—Many of the highly refined tetraalkylsilanes prepared during this and a previous investigation[7] possessed pungent, terpene-like odors. It was found possible to remove these odors by treatment with Norit A decolorizing carbon black; less effective were activated alumina and silica gel. $MeSiEt_3$ and Et_4Si retained a mild camphor odor through many treatments with absorbents. The pungent, terpene-like odors were apparently due to traces of decomposition products formed upon refluxing for several hours above about 150° in the presence of air; deodorized samples subjected to this treatment reacquired the pungent, terpene-like odor. This behavior was noted with $PrSiEt_3$, $BuSiEt_3$, $AmSiEt_3$, n-$C_6H_{13}SiEt_3$, n-$C_7H_{15}SiEt_3$, Pr_2SiEt_2, Pr_3SiEt and n-$C_7H_{15}SiMe_3$. Presence or absence of odors had no perceptible effects on physical properties.

Redistribution of Et_4Si and Pr_4Si.—In a 100-ml., round-bottom flask was placed 20.2 g. (0.14 mole) of tetraethylsilane and 30.1 g. (0.15 mole) of tetra-*n*-propylsilane together with 1.2 g. (0.009 mole) of C.P. anhydrous aluminum chloride. The reaction mixture turned yellow and then colorless during a five-hour reflux period at 175–180° after which it was found to have incurred no weight loss. The crude product was washed twice with water and dried over Drierite and anhydrous K_2CO_3. Fractional distillation in a Piros-Glover spinning band column gave the five products corresponding to Et_4Si, $PrSiEt_3$, Pr_2SiEt_2, Pr_3SiEt and Pr_4Si which by calculation from the distillation curve were obtained in 5, 24, 39, 26 and 7 mole % yields, respectively; these values agree well with those obtained previously both theoretically and experimentally.[4] The infrared spectra of many of the fractions, including some inter-fractions, were kindly determined by C. A. Hirt of the General Electric Research Laboratory with a Perkin–Elmer Recording Infrared Spectrophotometer. These were compared with the reference curves which were also determined by Mr. Hirt using 0.03-mm. cells, except for the isopropyl compounds which were done in 0.017–0.019 mm. cells. The infrared spectra of the redistribution products were the same as those of the reference compounds. None of the redistribution product fractions showed the absorption band at 11.37 μ characteristic of isopropylsilanes, even though it is a very strong band.

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(7) The methods have been described: F. C. Whitmore, L. H. Sommer, P. D. George, W. A. Strong, R. E. Van Strien, D. L. Bailey, H. K. Hall, E. W. Pietrusza and G. T. Kerr, THIS JOURNAL, **68**, 475 (1946).

(8) Calculated with bond refractions given by K. G. Denbigh, *Trans. Faraday Soc.*, **36**, 936 (1940), and by E. L. Warrick, THIS JOURNAL, **68**, 2455 (1946). Similar results may be obtained by the method of R. O. Sauer, *ibid.*, **68**, 954 (1946).

(9) A. D. Petrov and V. F. Mironov, *Izvest. Akad. Nauk SSSR, Otdel. Khim. Nauk*, **4**, 635 (1952); *C. A.*, **47**, 10471f (1953). and **48**, 4462h (1954). These workers obtained the compound from ethylmagnesium bromide and 2-bromopropyltri-*n*-propylsilane through a beta-elimination reaction; their product had b.p. 207–210 (748 mm.), $n^{20}D$ 1.4375, d^{20} 0.7824.

(10) This refractive index has not previously been reported. Used with d^{20} 0.7845 (S. Sugden and H. Wilkins, *J. Chem. Soc.*, 126 (1931)) it gives MR_D 67.07 compared with calcd.[9] MR_D 67.32.

(11) H. Gilman and R. N. Clark, THIS JOURNAL, **69**, 1499 (1947).

On the Sublimation of Amino Acids and Peptides

BY D. GROSS AND G. GRODSKY

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Kempf,[1] Werner,[2] and Brown[3] have studied the sublimation of some amino acids. These reports, along with others,[4–6] however, have included little information on the recovery, extent of decomposition, diketopiperazine formation and change in optical rotation. In view of the possible ease of purification of some radioactive amino acids by sublimation, a large variety of amino acids was investigated for the mentioned properties. In the course of these studies it also was discovered that some peptides were capable of subliming with little decomposition.

Experimental

Chemicals.—The free amino acids and related compounds were commercial preparations which were chromatographically pure, and had optical rotations close to values[7–9] given in the literature. The peptides were obtained from the following sources: Mann Research Labs., N. Y., glycyl-L-leucine, DL-alanyl-DL-norvaline; Fischer Collection, University of California, L-alanyl-L-leucine, leucyl-alanine (configuration unknown); H. M. Chemical Co., California, glycyl-glycine; synthetic preparation, L-leucyl-glycine. All chromatographed as single spots, except leucyl-glycine. This was chromatographically identical to a known sample of leucyl-glycine (Mann Research Labs.), but had a trace of leucine which did not interfere with the determinations.

Apparatus and Methods.—The glass sublimation apparatus used had a 7 mm. gap distance between the flat bottom pot (4 cm. dia.) and the glass sleeve (2.5 cm. dia.) which fitted snugly on the water-cooled condenser. The sleeve made the apparatus convenient for manipulation and determination of yield. This size apparatus was convenient for 200 to 500 mg. of amino acid or peptide. The apparatus was heated in a clear oil-bath. A vacuum of 0.3 mm. was used. Sublimation was conducted usually 30–60° above the temperature at which a sublimate first was noted. Sublimation to completion usually took two to three hours, though the majority of the material sublimed in a shorter period. The sublimation temperature was determined in this apparatus whose temperature was raised approximately one degree per minute. The table records the initial sublimation temperature and the temperature at which the majority of the sublimate passed over quickly without decomposition.

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TABLE I

SUBLIMATION DATA OF AMINO ACIDS AND PEPTIDES

	Subl. temp., °C.	Temp. of subl., °C.	Recov. %	Time per 100 mg., hr.	$[\alpha]^{25}D$, deg. Before	After	Nitrogen, % Calcd.	Found	Diketopiperazine, %
L-Alanine	138	193–200	98.5	0.25	+12.8	+12.8[a]			
L-Ethionine	133	196–216	99.1	.25	+23.6	+23.3[b]			
L-Hydroxyproline	150	190–223	99.1	.9	−75.0	−75.4[c]			
L-Isoleucine	105	170–181	99.7	.5	+38.2	+38.9[b]			
L-Leucine	109	180–188	99.1	.3	+15.2	+15.1[b]			
L-Methionine	130	197–208	99.8	.2	+22.9	+22.9[b]			
L-Phenylalanine	130	176–184	98.2	.2	−33.7	−33.7[b]			
L-Proline	119	182–187	99.4	.25	−84.1	−84.4[b]			
L-Threonine	170	200–226	99.6	.4	−27.8	−27.8[b]			
L-Tryptophan	180	220–230	99.0	1.6	−29.4	−29.4[d]			
L-Tyrosine	186	235–240	99.2	0.9	− 7.58	− 7.63[d]			
L-Valine	102	178–188	99.5	.1	+26.7	+26.6[b]			
β-Alanine	111	170–180	98.2	.3			15.7	15.9	
DL-Allylglycine	110	172–178	99.0	.2			12.1	12.1	
L-Aspartic acid		230–237							
L-Cysteine	124	170–180	97.5	.3			11.5	11.5	
Glycine	118	145–150	99.0	4.0			18.7	18.8	
L-Histidine		200							
L-Lysine[e]		160							
L-Serine		160–170							
Sarcosine	120	180–185	99.1	0.5			15.7	15.8	
Glycyl-glycine		190–200	30.0	0.7					Present
Glycyl-L-leucine		180–215[f]	98	1.0			14.9	15.0	7
DL-Leucyl-glycine		165–210[f]	99	1.3			14.9	14.8	4
L-Alanyl-L-leucine		150–170	99	1.6			13.8	13.3	6
Leucyl-alanine		150–180	90	4.0			13.8	13.9	Less than 1
DL-Alanyl-DL-norvaline		180–215[f]	98	1.0			14.9	14.3	2

[a] 2% in 5 *N* HCl. [b] Concentration and solvent as cited in literature.[7-9] [c] 1% in water. [d] Rotation at 23°. [e] A small quantity of lysine monohydrochloride was dissolved in water, neutralized to *p*H 9.8 with dilute carbonate free NaOH, and lyophilized. The material sublimed at 160° (0.15 mm.) to yield a greasy white solid in low yield. [f] Predominantly over 200°.

Analysis was conducted on twelve of the amino acids by their optical rotations before and after sublimation. Measurements were made in thermostated tubes on a polarimeter accurate to 0.01° using a sodium lamp. Others were analyzed by the Kjeldahl method. Chromatograms were made from all specimens before and after sublimation, using a solvent system consisting of two parts *n*-butanol, one part water and 0.4 part glacial acetic acid. Whatman paper #1 or #4 was used. Spots were developed by 0.2% ninhydrin in alcohol and by the chlorination method[10] on parallel strips taken from the same chromatogram.

Quantitative determination of diketopiperazine was conducted as follows: 10 to 50-mg. quantities of sublimate were dissolved in 20 ml. of hot water and passed through 5 g. of Dowex-50 in the acid form washed previously with water. An additional 10 ml. of water was used to wash the resin. The combined filtrate was evaporated to dryness on a steam-bath, taken up in 6 ml. of 3 *N* HCl, and hydrolyzed in sealed test-tubes in the autoclave at 120° for three hours. The solutions were taken to dryness, 4 ml. of dilute NaOH added, boiled to drive off ammonia, and adjusted to *p*H 5. The solutions were made up to 10 ml., and aliquots were analyzed by the quantitative ninhydrin method,[11] using glycine as the standard. Separation was complete by the first wash.

Results

The twelve amino acids analyzed by optical rotation sublimed rapidly (except tyrosine) and quantitatively, yielding white crystalline products. Threonine occasionally gave a clear glass-like product. Histidine, serine and aspartic acid were characterized by an extremely slow sublimation and low recovery, extensive darkening of the material in the pot after a few hours, and a glass-like sublimate. All the amino acids (except lysine) gave single spots when developed by ninhydrin and the chlorination method, the latter being very sensitive to diketopiperazines.[10] Tolbert[5] reports that diketopiperazine formation occurred when glycine, but not when alanine was sublimed. In this work, a pure crystalline glycine with no detectable diketopiperazine was obtained. On the other hand chromatograms of the lysine sublimate gave three unidentified spots (ninhydrin and chlorination) in addition to the predominant lysine spot.

The nitrogen analysis, optical rotation and chromatograms indicated a lack of diketopiperazine formation for the amino acids investigated.

L-Arginine, L-asparagine, L-citrulline, L-cystine, L-diiodotyrosine, L-glutamic acid, L-glutamine, glutathione and taurine decomposed when subjected to the sublimation procedure. Preparations of radioactive methionine, alanine, ethionine and leucine have been purified by sublimation in this Laboratory from dried salt solutions which have previously been adjusted to *p*H 6–7.[5] These preparations sublimed readily in excellent yield to give pure compounds.

Peptides.—Quantities of 100–200 mg. of peptides were sublimed. The sublimate initially formed a clear glass on which in some cases white crystals developed as sublimation continued. As the sublimates formed glasses, no attempt to obtain a sublimation temperature was made. Chromatograms of the sublimed material gave single spots of the

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(11) S. Moore and W. H. Stein, *J. Biol. Chem.*, **176**, 367 (1948).

peptide with ninhydrin identical with the controls. Parallel chromatograms developed by the chlorination method gave (in addition to the peptide spot) a second spot with a higher R_f in all cases except leucyl-alanine, which gave no spot. In the case of glycyl-glycine this corresponded to a known sample of the diketopiperazine of glycine. The extra spots on the leucyl-glycine and glycyl-leucine chromatograms were identical. When quantitative determinations for diketopiperazine were made, the assumed diketopiperazine (in the water wash) gave the same spot on chromatograms as the extra spot of the sublimed peptides. The material was ninhydrin negative which is expected for diketopiperazines.[10] Chromatograms of the hydrolyzed materials had spots corresponding to the amino acids of the original peptide. The quantitative data explained the lack of a diketopiperazine spot for leucyl-alanine.

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Some Tetraorganosilanes

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Tetraphenylsilane has been reported[1] to distil undecomposed above 530°; more recently, investigators have indicated[2] the boiling point to be 428°. It also has been reported[3] that this compound is not acted upon when heated at 450° for 200 hours with hydrogen at a pressure of 75 atmospheres. Tetrabenzylsilane similarly has been reported[1] to distil undecomposed above 550°; a rough determination has indicated this boiling point to be approximately 100° too high, but nevertheless indicates the compound to possess remarkable thermal stability.

A number of long-chained alkylsilanes have been prepared in connection with a study of their thermal stabilities and other properties. The methods of preparation may be illustrated by the equations

$$4RMgX(excess) + SiCl_4 \xrightarrow{150\text{–}160^\circ} R_4Si + 4XMgCl$$

$$2RMgX(excess) + (C_6H_5)_2SiCl_2 \longrightarrow R_2Si(C_6H_5)_2 + 2XMgCl$$

Since an excess of the Grignard reagent was employed, some coupling and hydrolysis products were also obtained.

Experimental[4]

Tetra-*n*-octadecylsilane.—*n*-Octadecylmagnesium bromide[5] was prepared, under a nitrogen atmosphere, from *n*-octadecyl bromide (15.3 g., 0.05 mole) and 1.21 g. (0.05 g. atom) of magnesium turnings, with the addition of a crystal of iodine to initiate the reaction. The yield, by titration, was 86% (0.043 mole). To the Grignard solution was added 1.2 g. (0.007 mole) of silicon tetrachloride in 25 ml. of ether and the resulting mixture was refluxed overnight. The ether was then distilled off and the residue was heated at 150–160° for 4 hours. After cooling, 100 ml. of ether was added and the mixture was refluxed for an additional 4-hour period. At this time Color Test I[6] was still positive. The mixture was hydrolyzed with 5% hydrochloric acid and subsequent filtration gave 2.1 g. of a white solid which melted from 65–72°. The two layers of the filtrate were separated and the ethereal layer was dried over anhydrous sodium sulfate. Evaporation of the ether left a semi-solid residue which when recrystallized from petroleum ether (b.p. 60–70°) gave 1.5 g. of a white solid melting at 70–73°. This and the solid obtained from the initial filtration of the reaction mixture were combined and shaken with 75 ml. of cold ether; filtration gave 2.9 g. of solid melting at 75–76°. Recrystallization from petroleum ether (b.p. 60–70°) gave 2.2 g. (58%, based on 0.015 mole excess of the Grignard reagent) of hexatriacontane, m.p. 76–77°; a mixed m.p. with an authentic sample[5] was not depressed.

The petroleum ether filtrate from the first recrystallization was evaporated leaving a white residue which melted from 40–48°. Three recrystallizations from ethyl acetate gave 3.6 g. (49%, based on the silicon tetrachloride) of tetra-*n*-octadecylsilane melting at 50–50.5°.

Anal.[7] Calcd. for $C_{72}H_{148}Si$: C, 82.99; H, 14.32; Si, 2.69. Found: C, 83.11, 83.00; H, 14.46, 14.43; Si, 2.74, 2.77.

Tetra-*n*-hexadecylsilane (see Table I) was prepared by a similar procedure.

TABLE I

Products, silanes	Yield,[a] %	B.p. °C.	B.p. Mm.	M.p.	Analyses, % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Silicon Calcd.	Silicon Found
Tetra-*n*-hexadecyl[b]	57	*c*		38.5–40	82.67	82.60	14.31	14.22	3.02	3.12
Di-*n*-octadecyldiphenyl[d]	67	303–306	0.15	*e*	83.64	83.49	12.30	12.48	4.07	4.02
Di-*n*-hendecyldiphenyl[f]	78	262–264	0.1	*g*	82.85	82.78	11.45	11.63	5.69	5.72
Di-10-hendecenyldiphenyl[h]	49	258–260	0.15	*i*	83.54	83.37	10.72	10.78	5.74	5.87

[a] The yields of alkylsilanes are based on the chlorosilanes; the yields of hydrocarbons are based on the excess of Grignard reagents. [b] A 72% yield of dotriacontane was also obtained; a mixed m.p. determination with an authentic sample (see ref. 5) was not depressed. [c] Recrystallized from ethanol–ethyl acetate solution. [d] A 24% yield of *n*-octadecane, b.p. 99–104° (0.15 mm.), m.p. 25–27°, was obtained. The reported m.p. for *n*-octadecane is 27–28°; see ref. 8, p. 227. [e] $n^{25}D$ 1.4945. [f] A 40% yield of *n*-docosane, b.p. 101–107° (0.1 mm.), m.p. 40–42°, was obtained; see ref. 8 for reported m.p. of *n*-docosane. [g] $n^{25}D$ 1.4960. [h] No other pure products were isolated. [i] $n^{25}D$ 1.5057.

Tetra-*n*-hendecylsilane.—To 125 ml. (0.061 mole) of an ethereal solution of *n*-hendecylmagnesium bromide was added 1.6 g. (0.009 mole) of silicon tetrachloride in 50 ml. of ether. The mixture was then heated as in the preceding experiment; after hydrolysis no insoluble material remained. Following separation of the layers and removal of the solvent from the ethereal layer, the residue was distilled to give 1.6 g. (44%, based on the excess Grignard reagent) of *n*-docosane distilling at 110–115° (0.15 mm.) and 4.1 g.

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(3) V. Ipatieff and B. N. Dolgov, *Ber.*, **62**, 1220 (1929).
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(5) R. N. Meals, *J. Org. Chem.*, **9**, 211 (1944).
(6) H. Gilman and F. Schulze, THIS JOURNAL, **47**, 2002 (1925).
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