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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(2) R. N. Lewis and A. E. Newkirk, THIS JOURNAL, **69**, 701 (1947).
(3) V. Ipatieff and B. N. Dolgov, *Ber.*, **62**, 1220 (1929).
(4) All melting points and boiling points are uncorrected.
(5) R. N. Meals, *J. Org. Chem.*, **9**, 211 (1944).
(6) H. Gilman and F. Schulze, THIS JOURNAL, **47**, 2002 (1925).
(7) The silicon analyses were carried out by the procedure of H. Gilman, B. Hofferth, H. W. Melvin and G. E. Dunn, *ibid.*, **72**, 5767 (1950). A qualitative test for silicon was positive; see H. Gilman, R. K. Ingham and R. D. Gorsich, *ibid.*, **76**, 918 (1954).

(67%, based on the silicon tetrachloride) of tetra-*n*-hendecylsilane distilling at 248–251° (0.15 mm.), $n^{25}D$ 1.4608.

Anal. Calcd. for $C_{44}H_{92}Si$: C, 81.39; H, 14.28; Si, 4.32. Found: C, 81.60, 81.69; H, 14.30, 14.40; Si, 4.14, 4.20.

The *n*-docosane was recrystallized from absolute ethanol to give 1.3 g. (37%) of platelets melting at 43–44°.[8]

Di-*n*-octadecyldiphenylsilane, di-*n*-hendecyldiphenylsilane and di-10-hendecenyldiphenylsilane (see Table I) were prepared by a similar procedure.

Tetra-(*m*-fluorobenzyl)-silane.—To 0.076 mole of *m*-fluorobenzylmagnesium chloride (prepared in 76% yield from the corresponding chloride and magnesium) in 195 ml. of ether was added 2.4 g. (0.014 mole) of silicon tetrachloride in 50 ml. of ether. After refluxing, hydrolysis and separation as before, vacuum distillation of the residue gave 1.1 g. (50%) of 3,3′-difluorobibenzyl distilling at 60–63.5° (0.2 mm.) and 4.6 g. (71%) of tetra-(*m*-fluorobenzyl)-silane distilling at 238–240° (0.2 mm.). The 3,3′-difluorobibenzyl solidified on standing and melted at 33–34°.[9]

The tetra-(*m*-fluorobenzyl)-silane solidified on standing; recrystallization from petroleum ether (b.p. 60–70°) gave 4.0 g. (62%) of colorless rhombic crystals melting at 62–63°.

Anal.[10] Calcd. for $C_{28}H_{24}F_4Si$: C, 72.39; H, 5.21; F, 16.36. Found: C, 72.38, 72.34; H, 5.25, 5.22; F, 16.21, 16.44.

(8) The reported m.p. for *n*-docosane is 43.5–44.5°; see G. Egloff, "Physical Constants of Hydrocarbons," Vol. V, Reinhold Publ. Corp., New York, N. Y., 1953, p. 242.

(9) The reported m.p. for 3,3′-difluorobibenzyl is 34–35°; see M. Szwarc and J. S. Roberts, THIS JOURNAL, **70**, 2831 (1948).

(10) The silicon analyses of this compound were erratically low possibly due to loss of silicon as SiF_4. A similar difficulty in the analysis of fluorine-containing organosilicon compounds has been reported; see H. Gilman, A. G. Brook and L. S. Miller, *ibid.*, **75**, 3757 (1953).

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The Synthesis of Propanediol Phosphate-2-C^{14} from Pyruvamide-2-C^{14} and the Purification of Labeled Pyruvic Acid[1]

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Recently evidence[4,5] has been obtained in this Laboratory that 1,2-propanediol-1-phosphate (PDP) is formed from pyruvate in appreciable amounts by glycolyzing rat tissue homogenates under anaerobic conditions. The synthesis and purification of PDP-2-C^{14} was undertaken in order to test the significance of PDP in the metabolism of normal and neoplastic tissues. A procedure for preparing PDP-2-C^{14} for use as a biological tracer is presented, and a chromatographic procedure for the purification of C^{14}-labeled pyruvic acid is included.

Experimental and Results

Synthesis of PDP-2-C^{14}.—Pyruvamide-2-C^{14} [6] was used as the starting material because of its commercial availability. The purity of the pyruvamide-2-C^{14} was determined by carrier crystallization with unlabeled pyruvamide since contamination with non-pyruvamide-C^{14} is usually present. This permitted a calculation of the true specific activity of the pyruvamide-2-C^{14}, a figure used to determine purity of the PDP-2-C^{14}. The procedure routinely used was as follows: 22 mg. of pyruvamide-2-C^{14} was stirred with 10 ml. of anhydrous diethyl ether and added to 3 ml. of an approximately 0.5 *M* solution of $LiAlH_4$ (Metal Hydrides Co., Beverly, Mass.) at a rate just necessary to produce gentle reflux.[7] The mixture of pyruvamide-2-C^{14} and $LiAlH_4$ was refluxed for four hours, and allowed to stand overnight at room temperature. The ether was removed by evaporation of the mixture to 3–5 ml.; 10 ml. of anhydrous $CHCl_3$ was added and the evaporation repeated. Then 0.6–0.7 ml. of $POCl_3$ (Eastman Technical grade) in 10 ml. of anhydrous $CHCl_3$ was introduced. The mixture was refluxed for 4–5 hours, and allowed to stand overnight at room temperature. Fifteen ml. of water was added, and the $CHCl_3$ removed by evaporation on a steam-bath. The water solution was approximately 1.3–1.5 *M* with respect to HCl, which was liberated from the $POCl_3$ used. In order to hydrolyze possible contaminating phosphate esters in the PDP-2-C^{14} preparation, the water solution was heated for two hours at 100°. PDP is insensitive to hydrolysis under these conditions.[8] The solution was cooled, and neutralized to *p*H 7 with small additions of 10 *M* KOH. Then 12–14 ml. of 1 *M* barium acetate was added to precipitate inorganic phosphate as the barium salt. PDP is not precipitated.[8] The milky suspension of barium phosphate was heated for 5 minutes at 100° with constant stirring. The barium phosphate was removed by centrifuging, and washed five times with 25-ml. portions of water, resuspending the barium phosphate and heating for five minutes at 100° with each wash. The combined supernatant solutions were allowed to percolate through a 2 × 20 cm. column of Dowex-50 (H form). The column was washed with five successive 10-ml. portions of water. The combined eluates were evaporated *in vacuo* at room temperature to approximately 5 ml. Organic phosphorus was measured as an estimate of PDP-2-C^{14} present.[9] The yields ranged from 65–70% in several test runs.

Purification of PDP-2-C^{14}.—The PDP-2-C^{14} was subjected to two-dimensional paper chromatography for purification. The eluate from the Dowex-50 column (above) was streaked along the top edge of a $22\frac{1}{2}$ × 18 inch sheet of Schleicher and Schüll #589 filter paper and allowed to dry at room temperature. Approximately 30 micromoles of organic phosphorus was used on each sheet. The paper was developed in a tank designed for descending paper chromatography. The following solvent system was employed: 6 parts isopropyl alcohol, 2 parts concentrated ammonia, 2 parts water saturated Versene solution.[5] The PDP-2-C^{14} was located by placing the dried paper in contact with sensitive X-ray film. The labeled material corresponded in migration rate to samples of known unlabelled PDP used as standard. The region of the paper containing the PDP-2-C^{14} was cut out and eluted by percolating water down the strip of filter paper. The solution was then streaked upon a second sheet of S. and S. #589 paper and developed using the following solvent system: 7 parts isopropyl alcohol–2 parts H_2O saturated with Versene–1 part 2 *M* monochloroacetic acid.[5] Upon isolation as above, the PDP-2-C^{14} had a specific activity (counts per minute per micromole phosphorus) which was equal to the specific activity of the original pyruvamide-2-C^{14} used in the synthesis. The yields from the purification of the PDP-2-C^{14} by the filter paper chromatographic procedure were as follows: isopropyl alcohol–ammonia–Versene system 77–92%; isopropyl alcohol–monochloroacetic acid–Versene system 50–61%. The total over-all yields of highly purified PDP-2-C^{14} (based upon the pyruvamide-2-C^{14}) were 25–39%.

The Purification of C^{14}-Labeled Pyruvic Acid.—In the course of investigations upon the anaerobic metabolism of pyruvate,[8] it was observed that pyruvamide-2-C^{14} and py-

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(3) The studies reported here were used as partial fulfillment of the requirements for the degree of Doctor of Philosophy, University of Wisconsin, June, 1954.

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