TABLE I
CHROMATOGRAPHIC DATA[a]

Compd	Paper chromatography[b] R_p[A d]	R_p[B]	R_f[C]	R_p[D]	R_f[E]	Paper electrophoresis,[c] borate, $R_{R\text{-}5\text{-}P}$
D-Ribose		1.65	0.70		0.36	
D-Arabinose					0.30	
Ribose 5-phosphate	0.43	1.02	0.43	4.0		1.00
α-Ribofuranose 1-phosphate	0.8	1.21	0.47	2.3		0.92
β-Ribofuranose 1-phosphate	0.6	1.23	0.48	2.5		0.98
Ribopyranose 1-phosphate	0.8	1.07	0.47			0.93
$H_2P_2O_7^{2-}$	0.4	0.79		0.2		
HPO_4^{2-}	1.0	1.0		1.0		

[a] Whatman 3MM paper was used. Sugars and sugar phosphates were detected with an aniline–phthalic acid spray (I. M. Hais and K. Macek, "Paper Chromatography," House of the Czechoslovak Academy of Science, Prague, and Academic Press, New York, N. Y., 1963, p 793). Sugar phosphates and inorganic phosphates were detected by spraying with ammonium molybdate–perchloric acid, followed by uv irradiation to develop the blue color of the phosphomolybdate complex (same reference, p 819). [b] Chromatographic solvents: A, isopropyl alcohol, concentrated ammonia, 0.1 *M* sodium borate (7:1:2); B, *n*-propyl alcohol, concentrated ammonia, water (11:2:7); C, *n*-propyl alcohol, concentrated ammonia, 0.1 *M* sodium borate (11:2:7); D, Methyl Cellosolve, methyl ethyl ketone, 3 *N* ammonia (7:2:3); E, *n*-butyl alcohol, glacial acetic acid, water (4:1:1). [c] Sodium borate buffer (0.05 *M*, pH 9.0); 1000 V during 2 hr. [d] Movement of spots: R_p, relative to orthophosphate; R_f, relative to the solvent front; $R_{R\text{-}5\text{-}P}$, relative to ribose 5-phosphate.

C. Enzymatic Dephosphorylation.—The amount of orthophosphate released from the purified product (see section E) by alkaline phosphatase was within experimental error (about 2%), equal to that released during hydrolysis in hydrochloric acid (0.1 *N*, 10 min, 100°). This confirms that no substantial part of the product is an acid-stable phosphate ester.

D. Chromatographic Analyses.—Samples from reaction mixtures containing D-ribose-1-^{14}C were analyzed by paper chromatography in solvent B. The resulting paper strips were analyzed on a radiochromatogram scanner and the ratio of ^{14}C activity in the ribose to that in the ribose phosphate regions was determined. In a run initially 0.1 *M* in ribose and phosphate and 0.05 *M* in cyanogen (pH 7.0 or 8.0; 3 hr at 24°) the yield of ribose phosphate was 9%. With 0.2 *M* cyanogen under the same conditions the yield was 20%.

Cochromatography of the reaction mixtures with the different ribose phosphates in system A for 6 days gave coincidence of the ^{14}C activity only with a spot corresponding to β-ribofuranose 1-phosphate.

E. Isolation of the Ribose Phosphate.—In one run, the reaction mixture (containing initially 0.2 *M* each of sodium phosphate, D-ribose, and cyanogen at pH 8.0), after standing overnight at 25°, was treated with barium acetate to remove the unreacted orthophosphate. Crude barium ribose phosphate was then precipitated by addition of ethanol as a brownish sticky powder (yield 27%, after overnight vacuum drying). In a similar experiment, the crude reaction mixture was passed through a column of Dowex 2-X8 anion-exchange resin in the formate form and was eluted with a 0.1 *M* formate buffer (pH 5.0).[10] The effluent was treated with barium acetate and then ethanol, as above, yielding a small amount of purified product. After twice dissolving the product in water, precipitating with ethanol, and drying overnight under vacuum, shiny white crystals were obtained. *Anal.* Calcd for $C_5H_9O_8PBa \cdot 1H_2O$:[7] P, 8.1. Found: P, 8.0. The product gave only one spot, identical with that given by β-ribofuranose 1-phosphate, on descending paper chromatography in solvent A.

After hydrolysis overnight in 0.01 *N* hydrochloric acid at room temperature, chromatography of the hydrolysate (in solvent E) gave only one spot, that of D-ribose. No D-arabinose could be detected.

F. Rate of Acid Hydrolysis.—Rates of hydrolysis were measured by dissolving weighed portions of each sugar phosphate in 0.010 *N* hydrochloric acid and maintaining the solutions at 25.0°. Aliquots were analyzed for orthophosphate by measuring the absorption at 700 mμ of the phospho–molybdate complex.[8]

The half-life of our purified product at 25° in 0.010 *N* hydrochloric acid was 3 ± 1 hr compared with 2.0 ± 0.4 hr for α-ribofuranose 1-phosphate, 2.4 ± 0.4 hr for β-ribofuranose 1-phosphate and 70 ± 20 hr for ribopyranose 1-phosphate. The rates for the α- and β-ribofuranose 1-phosphates are similar, and within experimental error equal to that of our product. They are much larger than the rate for acid hydrolysis of ribopyranose 1-phosphate, in agreement with a previous report.[7] Consequently ribopyranose 1-phosphate is not a substantial component of our product.

G. Attempt to Phosphorylate 2-Deoxy-D-ribose.—2-Deoxy-D-ribose and orthophosphate (both 0.1 *M*, at pH 7.0 or 8.8 and at 25 or 65°) did not undergo phosphorylation in the presence of cyanogen (0.22 *M*).

Condensation with Cyanamide.—Dilute aqueous solutions of D-ribose, orthophosphate, and cyanamide at pH 7.0 were placed in a bath at 65°. When the three components were each initially 0.02 *M* in concentration, formation of a ribose phosphate could be detected by paper chromatography (solvent B) after 7 days of reaction. The product was identified as an aldose 1-phosphate by its acid lability and alkali stability. With 0.25 *M* cyanamide, 0.1 *M* D-ribose (with added D-ribose-1-^{14}C), and 0.1 *M* orthophosphate, the yields of ribose 1-phosphate after 21 and 72 hr were 6 and 8%, respectively. They did not increase after that. The product was identified as β-ribofuranose 1-phosphate by descending development in solvent A for 6 days.

Registry No.—D-Ribose, 58-91-3; β-ribofuranose 1-phosphate, 21317-51-1.

Acknowledgment.—We are grateful to Professor H. G. Khorana for a sample of ribopyranose 1-phosphate and to Dr. Ch. Degani for carrying out some of the chromatographic separations. This work was supported in part by Grant No. GB 5303 from the National Science Foundation.

(10) "Methods in Enzymology," Vol. III, S. P. Colowick and N. O. Kaplan, Ed., Academic Press Inc., New York, N. Y., 1957: (a) P. E. Plesner and H. Klenow, p 181; (b) A. A. Benson, p 123.

Chlorinolysis of Cysteine Ethyl Ester Hydrochloride. An Efficient Route to Certain Chloramino Acid Derivatives[1]

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Chlorinolysis of crystine diester derivatives with molecular chlorine to give the corresponding 2-amino-3-chloropropionates (**4**) in high yield was first reported

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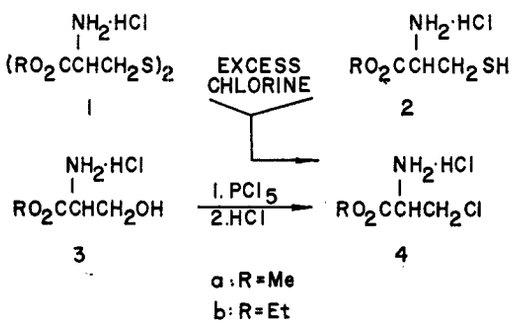
in 1960.[2] Fischer and Raske[3] earlier had prepared small quantities of methyl 2-amino-3-chloropropionate (**4a**) in 80–85% yield by treating serine methyl ester hydrochloride **3a** conventionally with phosphorous pentachloride and hydrogen chloride. These chloramino acids provide convenient entry into sulfur-35-crysteines[4] and seleniferous amino acids.[5] Since this chloro derivative was required in quantity in this laboratory, an improved preparation of ethyl 2-amino-3-chloropropionate hydrochloride (**4b**) was developed, and is reported here.

CHART I



The chlorinolysis of cystine diethyl ester hydrochloride (**1b**) is the more practical synthesis of the two recorded in the literature. However, the ready availability of cysteine ethyl ester hydrochloride (**2b**) suggested that it might provide a more convenient route to the desired product **4b**. A thorough literature survey failed to reveal reports of such a chlorinolytic reaction, although the initial step in the chlorinolysis of **1** has been shown to be the formation of **2** equiv of a sulfenyl halide, **5**.[2]

Other chlorinolysis reactions of sulfides have been shown to lead primarily to single or multiple substitution of chlorine for the hydrogens attached to the carbon atom or atoms bearing the sulfur.[6] For instance, the chlorinolysis of dimethyl sulfide leads smoothly to a monoalkyl derivative of sulfur tetrachloride, methyl sulfur trichloride.[7] This compound spontaneously forms chloromethyl sulfenyl chloride at room temperature. Similar tetrasubstituted sulfur derivatives have been proposed as occurring in the course of the chlorinolysis of **1**.[2] Their insolubility in paraffin and halogenated paraffin solvents suggest an alkyldichlorosulonium chloride structure, such as **6**.[8]

This report describes the chlorinolytic cleavage of the HS–C bond in mercaptans possessing a quaternized amino function vicinal to the sulfur bond, and the accompanying extrusion of sulfur dichloride; a reaction closely related to those of other sulfides and disulfides with molecular chlorine, but the course (and products) of which is greatly changed because of the adjacent $-NH_3^+$ function.

(2) H. Baganz and G. Dransch, *Ber.*, **93**, 782 (1960).
(3) E. Fischer and K. Raske, *ibid.*, **40**, 3717 (1907).
(4) J. B. Melchior and H. Tarver, *Arch. Biochem.*, **12**, 301 (1947).
(5) E. P. Painter, *J. Amer. Chem. Soc.*, **69**, 229 (1947).
(6) D. L. Tuleen and T. B. Stephens, *J. Org. Chem.*, **34**, 31 (1969), and references quoted therein, particularly ref 4.
(7) K. R. Bower and I. B. Douglass, *J. Amer. Chem. Soc.*, **73**, 5787 (1951).
(8) H. Kwart and P. S. Strilko, *Chem. Commun.*, 767 (1967); H. Kwart, R. W. Body, and D. M. Hoffman, *ibid.*, 765 (1967).

Results and Discussion

When **2** was subjected to a vigorous stream of chlorine in a methylene chloride slurry, the slightly soluble organic salt formed a dense yellow-white precipitate characteristic of the sulfenyl chloride–chlorine adduct **6**. After 2 days of refrigeration at −20°, work-up of the red-orange solution gave **4b** in 70% yield, and sulfur dichloride was qualitatively identified in the vacuum distillate of the solvent-free filtrate.

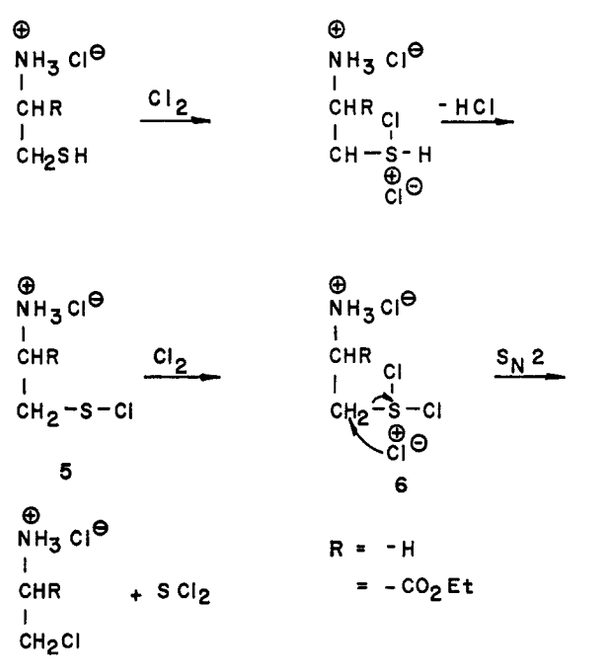
Under our conditions, 3-mercaptopropionic acid and its ethyl ester gave the corresponding disulfides, and *n*-dodecyl mercaptan, benzyl mercaptan, an equimolar mixture of dodecyl mercaptan and *n*-butylamine hydrochloride, and thiophenol gave mixtures from which no desired product could be detected by comparative vapor phase chromatography. Cysteamine hydrochloride was the only other compound amenable to the desired chlorinolytic cleavage under our conditions. The greater yields obtained with a solvent of higher dielectric constant support the suggestion of an ionic intermediate for the reaction (Table I), and the ease of reaction at lower temperatures suggests the formation of a sulfonium chloride as a key intermediate in the reaction.

TABLE I
SOLVENT AND TEMPERATURE EFFECTS[a] ON YIELD OF 4

Chlorine addition temp, °C	Induction period temp, °C	Yield,[a] %	Solvent
R.T.[b]	R.T.	23	$HCCl_3$
0	R.T.	30	$HCCl_3$
−12	0–2	30–34	$HCCl_3$
−78	−10	42	$HCCl_3$
−78	−10	68	H_2CCl_2
−78	−20	71	H_2CCl_2
−78	−20	0	CCl_4

[a] All reactions were run for the same time. [b] Room temperature.

CHART II



Unfortunately, available model examples of the reaction system are few. Several attempts to extend the

generality of the reaction failed; a number of other mercaptans were tried without success. The structural requirements for the chlorinolytic cleavage without accompanying substitution or oxidation are readily perceived, but the role of the quaternized amino group as a necessary component of the reaction mechanism is not immediately obvious.

The reported reaction provides a straightforward and high-yield synthesis of certain chlorinated amino acid derivatives commonly needed as starting materials for various biologically related compounds containing sulfur or selenium.

Experimental Section[9]

Ethyl 2-Amino-3-Chloropropionate Hydrochloride (4b).—A slurry of cysteine ethyl ester hydrochloride (2)[10] (50.0 g, 0.27 mol) in 300 ml of dry methylene chloride was stirred at −78° for 1 hr while a vigorous stream of chlorine was bubbled through the reaction mixture. The slurry became homogeneous and yellow within 10 min. The solution was held at −20° for 2 days, and then warmed to room temperature. Excess chlorine and approximately one-half of the solvent were removed at room temperature by water aspirator vacuum, and an equal volume of diethyl ether was added. Fine crystals of **4b** formed immediately After being chilled to 0°, the mixture was filtered. The white crystals were washed with ether and air dried: yield 28.9 g (71%); mp 143° dec (sealed evacuated capillary) (lit.[2] mp 141°).

2-Chloroethylamine Hydrochloride.—Cysteamine[10] (2-aminoethanethiol) hydrochloride (10.0 g, 0.088 mol) was stirred in 100 ml of dry methylene chloride for 2 hr at −78° while subjected to a vigorous stream of chlorine gas. After standing for 5 days at −10°, the red-orange heterogeneous reaction mixture was filtered to remove 2.6 g of unreacted cysteamine hydrochloride.

The filtrate was warmed to reflux for 15 min. A white precipitate immediately appeared. An equal volume of diethyl ether was added, and the mixture was cooled to 0°. Filtration of the white crystalline solid followed by ether washing and air drying produced 6.2 g (61% yield, 81% conversion) of 2-chloroethylamine hydrochloride, mp 117–121° (lit.[11] mp 119–123°), confirmed by comparison of the infrared spectrum of this compound with that of an authentic sample.[12]

Registry No.—2b, 868-59-7; 4b, 21615-66-7.

Acknowledgment.—We are grateful to the Joint Awards Council of the Research Foundation of the State University of New York for generous support of this work by Research Grant JA-67-40-006.

(9) All temperature readings were uncorrected. Ir spectra were determined on a Perkin-Elmer Infracord spectrophotometer. Vpc analyses were performed on a Hewlett-Packard chromatograph, Model 5750. Methylene chloride was dried over sodium sulfate.

(10) Aldrich Chemical Co., Inc.

(11) S. Gabriel, *Ber.*, **21**, 573 (1888).

(12) Sadtler Standard Spectrum No. 24369, Sadtler Research Laboratories, Inc., Philadelphia, Pa., 1964.

Synthetic Intermediates Potentially Useful for the Synthesis of Tetrodotoxin and Derivatives

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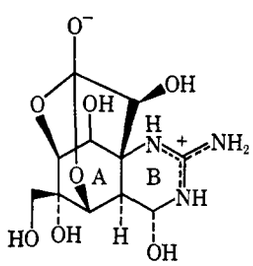
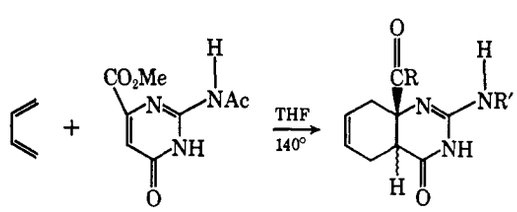
For some time we have been engaged in studies directed toward the total synthesis of the California newt (Taricha Torosa)[1] and Japanese Fugu (puffer fish)[2] poison tetrodotoxin **1** and closely related structural modifications. In the course of this work, we have developed a simple three-step synthetic sequence which permits construction of an intermediate possessing many features of the tetrodotoxin skeleton together with functional groups which ought to be readily alterable to produce ultimately a tetrodotoxin derivative. This paper describes the synthesis of the key intermediate **3**, thereby establishing a new synthetic route to 9-substituted hydroquinazolines.

We envisaged construction of the tetrodotoxin skeleton by means of a Diels–Alder reaction between a diene component which would become the carboxylic ring A and a heterocyclic dienophile containing a preformed guanidine ring system which would become ring B of tetrodotoxin (**1**). Heterocycle **2**[3] was readily prepared



1

by condensation of guanidine with dimethyl acetylenedicarboxylate followed by acetylation[4] or, better, by condensation of acetylguanidine with dimethyl acetylenedicarboxylate. When allowed to react with butadiene in tetrahydrofuran solvent at 140° for 2 days, dienophile **2** afforded crystalline adduct **3** in 72% yield. Nmr, ir, mass spectral, and elemental analysis data are all in accord with expression **3** (see Experimental Section for spectral data on all pertinent compounds). Hydrolysis of adduct **3** with aqueous potassium hydroxide in methanol afforded acid amine **4** in high yield, which could be reconverted into starting adduct **3** by esterification with methanolic hydrogen chloride to produce amino ester **5** followed by acetylation with acetic anhydride. Amino ester **5** could also be pre-



2

3, R = OMe, R′ = Ac
4, R = OH, R′ = H
5, R = OMe, R′ = H
6, R = OH, R′ = Ac

(1) H. S. Mosher, F. A. Fuhrman, H. D. Buchwald, and H. G. Fischer, *Science*, **144**, 1100 (1964).

(2) K. Tsuda, *Naturwissenschaften*, **53**, 171 (1966); C. Y. Kao, *Pharmacol. Rev.*, **18**, 997 (1966); R. B. Woodward and J. Z. Gougoutas, *J. Amer. Chem. Soc.*, **86**, 5030 (1964); K. Tsuda, S. Ikuma, M. Kawamura, R. Tachikawa, K. Sakai, C. Tamaura, and O. Amakasu, *Chem. Pharm. Bull* (Tokyo), **12**, 1357 (1964); T. Goto, Y. Kishi, S. Takahashi, and Y. Hirata, *Tetrahedron*, **21**, 2059 (1965); C. Tamura, O. Amakasu, Y. Sasada, and K. Tsuda, *Acta Crystallogr.*, **21**, 219, 226 (1966).

(3) It has not been established which double bond tautomer involving the nitrogen atoms in the heterocyclic rings of **2**, **3**, **4**, **5**, and **6** is the preferred one.

(4) S. Ruhemann and H. Stapleton, *J. Chem. Soc.*, **77**, 804 (1900).