TABLE II
IODIDE-131 EXCHANGE REACTIONS

Reaction	1	2
4′,5′-Diiodo-4-amino-fluorescein	60 mg.	61 mg.
Acetic acid solvent	2.0 ml.	2.0 ml.
Sodium iodide-131 aqueous[a]	0.40 ml.	0.40 ml.
Initial activity[b]	4.36 mc.	6.02 mc.
Reaction conditions	8.5 hr. at 95–102°	19 hr. at 78–94°
Decay time[c]	46 hr.	29 hr.
Activity of product[d]	37 μc./mg.	88 μc./mg.
Per cent exchange	60%	99%

[a] Carrier free. [b] Activity at start of exchange reaction. [c] Time elapsed from start of exchange to time of counting. [d] Measured activity at time of counting.

phenolic function. However, it also is known[20–22] that halogenated quinones readily undergo nucleophilic displacement of their halogens. The organic iodide in our fluorescein has the character of an atom *ortho* to a quinoid as well as phenolic function, and the possibility of nucleophilic displacement by iodide-131 ion should not be dismissed.

Experimental[23]

4′,5′-Diiodo-4-nitrofluorescein.—A mixture of 1.00 g. of 4-nitrofluorescein, 894 mg. of iodine monochloride, and 25 ml. of glacial acetic acid was stirred for 5 hr. The original suspension of 4-nitrofluorescein gradually dissolved and a new orange solid precipitated. After standing overnight, 200 ml. of water was added and the precipitate became red. It was filtered, redissolved in cold 1 *N* sodium hydroxide, reprecipitated with acetic acid, collected, washed with water, and dried. The crude red 4′,5′-diiodo,4-nitrofluorescein, 1.56 g., had m.p. 228–240° dec. This crude product was purified somewhat through its diacetate.

Acetylation.—The crude 4′,5′-diiodo-4-nitrofluorescein was refluxed with sodium acetate in acetic anhydride for an hour. Working up the product with recrystallization from acetic anhydride gave 4′,5′-diiodo-4-nitrofluorescein diacetate as fine crystals with a slight yellow tinge, m.p. 296–298°.

Anal. Calcd. for $C_{24}H_{13}NO_9I_2$: C, 40.41; H, 1.84; I, 35.59. Found: C, 40.43; H, 1.96; I, 35.55.

Deacetylation.—A suspension of the above diacetate in a saturated solution of sodium hydroxide in 90% ethanol was stirred and warmed gently until all dissolved. Dilution with water and acidification with glacial acetic acid gave a purified 4′,5′-diiodo-4-nitrofluorescein monohydrate, m.p. 262–264° dec. On drying under vacuum at 110° this solid lost weight equivalent to an equimolar portion of water. On exposure to air the weight again gradually increased.

Anal. Calcd. for $C_{20}H_9NO_7I_2 \cdot H_2O$: C, 37.11; H, 1.71; I, 39.24. Found: C, 37.0; H, 1.38; I, 39.55.

4′,5′-Diiodo-4-aminofluorescein. A. Iodination of 4-Aminofluorescein in Hydrochloric Acid.—A warm solution of 470 mg. of iodine monochloride in 10 ml. of 1 *N* hydrochloric acid was added to a warm stirred solution of 501 mg. of 4-aminofluorescein in 60 ml. of 0.7 *N* hydrochloric acid. A solid precipitated immediately. The mixture was heated nearly to boiling and cooled, and the solid was collected, washed well with water, and dried. The bright orange 4′,5′-diiodo-4-aminofluorescein, 860 mg. (99%), m.p. 213–218° dec., gave a reasonable elemental analysis and was used in the exchange reactions.

Anal. Calcd. for $C_{20}H_{11}NO_5I_2$: C, 40.09; H, 1.85; I, 42.36. Found: C, 40.78; H, 2.03; I, 42.60.

B. Hydrogenation of 4′,5′-Diiodo-4-nitrofluorescein.—The hydrogenation of 286 mg. of purified 4′,5′-diiodo-4-nitrofluorescein was carried out in 10 ml. of ethanol at room temperature for 1 hr. using hydrogen at 53 p.s.i. and about 2 g. of Raney nickel catalyst. The catalyst was filtered, and the ethanol solution was reduced in volume by gentle heating in a stream of nitrogen. The addition of 100 ml. of water produced an unfilterable suspension which was extracted into ether. Evaporation gave 156 mg. of a crude bright orange 4′,5′-diiodo-4-aminofluorescein, m.p. 205–210° dec., which was low in iodine. Under identical conditions the iodines of 4′,5′-diiodofluorescein were not affected.

4′,5′-Diiodofluorescein.—A mixture of 3.32 g. of fluorescein and 3.24 g. of iodine monochloride in 25 ml. of glacial acetic acid was warmed to 115° for 1 min., cooled, and stirred for 8 hr. at room temperature. The solid was collected and washed successively with 10 ml. of acetic acid and 100 ml. of water. It was then suspended in acetone, collected, and dried giving 3.54 g. of crude orange 4′,5′-diiodofluorescein as a powder, m.p. 220–235°. An Eastman Kodak grade material had m.p. 247–250° dec. Thin layer chromatography on Merck silica gel G using methanol showed these compounds to be identical except for several minor spots in our product, which may be the chloride or other polyhalogenated material.[13] No purification of this product was attempted.

Exchange Reactions with Iodide-131.—The 4′,5′-diiodo-4-aminofluorescein was suspended in acetic acid in a centrifuge tube. Aqueous sodium iodide-131 was added and the mixture was stirred and heated to about 100° using a silicone bath. At this temperature nearly all of the solid dissolved. After the reaction time indicated in Table II, the mixture was cooled, diluted with water, and centrifuged. The solid was washed three times with water, then dried, and stored in a vacuum desiccator. There was no change in the melting point.

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(20) Ng. Ph. Buu-Hoi, R. Royer, and M. Hubert-Habart, *Rec. trav. chim.*, **73**, 188 (1954).

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(22) J. W. Hancock, C. F. Morrell, and D. Rhum, *Tetrahedron Letters*, 987 (1962).

(23) Melting points were taken on a calibrated Fischer-Johns hot-stage block.

The Reaction of Alkyl Borinates with α-Amino Acids

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The stabilization of boron compounds through complex formation with amines is well-established. By this method the air-sensitive alkyl- or aryl-substituted boron derivatives can be transformed into stable solids. For instance, a diarylborinic acid readily condenses with ethanolamine or 8-hydroxyquinoline to form an internally stabilized compound.[1]

Formation of mixed anhydrides of borinic and amino acids also should result in stabilization. A recent British patent[2] disclosed that heating trialkylborines with amino acids produces this type of compound, but no examples were given for the triarylborines.

(1) (a) J. Douglass, *J. Org. Chem.*, **26**, 1312 (1961); (b) R. Letsinger and I. Skoog, *J. Am. Chem. Soc.*, **77**, 2491 (1955).

(2) K. Lang, F. Schubert, and K. Nutzel, British Patent 905,093 (September 5, 1962).

During the investigation of biologically active boron compounds in this laboratory, compounds such as 2-aminoacetic diphenylborinic anhydride were formed by heating butyl diphenylborinate with glycine.

$$(C_6H_5)_2BOC_4H_9 + H_2NCH_2COOH \longrightarrow (C_6H_5)_2BOCCH_2NH_2 + C_4H_9OH$$

The low-molecular weight amino acids, such as glycine, react well without a solvent. A solvent, such as toluene, is more convenient with the higher molecular weight, more soluble amino acids. The white solid products appear to be stable and have not undergone change on storage in air for 4 years. These anhydrides may be recrystallized from alcohol–water without change, but they react readily with ethanolamine to form 2-aminoethyl diphenylborinate.

Experimental

All melting points were obtained on a Fischer-Johns melting point apparatus and were corrected by comparison with standard compounds.

Reaction with Glycine.—A mixture of 0.50 g. (0.0067 mole) of glycine and 1.59 g. (0.0067 mole) of butyl diphenylborinate[3] was boiled and stirred for 5 min. The mixture became solid. Toluene (5 ml.) was added, and the mixture was boiled for a few minutes. After cooling, the solid was collected on a filter and was washed with ether. The anhydride was boiled with 25 ml. of distilled water and was collected on a filter after cooling, 0.82 g., m.p. 244–245°, 51% (based on butyl diphenylborinate). A 0.33-g. portion was dissolved in 5 ml. of 95% ethanol, filtered while hot, cooled, and recrystallized once more from ethanol to yield 0.13 g., m.p. 244–245°.

Anal. Calcd. for $C_{14}H_{14}O_2NB$: C, 70.4; H, 5.91; N, 5.86; mol. wt., 239. Found: C, 70.2; H, 6.0; N, 5.76; mol. wt., 241.

If the anhydride is heated with ethanolamine in alcohol–water solution, 2-aminoethyl diphenylborinate crystallizes upon cooling. The melting point and mixture melting point with 2-aminoethyl diphenylborinate were 187–189°.

If toluene is used as a solvent for this reaction, the insolubility of the glycine is a problem, and the yield is lowered.

Similar compounds were prepared from DL-alanine and L-leucine. These are summarized in Table I.

TABLE I

PREPARATION AND PROPERTIES OF $(C_6H_5)_2BOC(O)CH(NH_2)R$

R	Solvent	Yield, %	M.p., °C.	Analysis, % N— Calcd.	Found
—H	None	51	244–245	5.86	5.76
	Toluene	39			
—CH_3	None	57	231–232	5.54	5.52
—$CH_2CH(CH_3)_2$	Toluene	88	180–181	4.75	4.62

(3) Distilled from the ammonia complex, see ref. 1b.

Hydrolysis of Cysteamine S-Phosphate

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In a recent paper in this journal, Dittmer, Ramsay, and Spalding[1] reported on the hydrolysis of cysteamine S-phosphate [S-(2-aminoethyl)phosphorothioate] and they found two rate maxima of hydrolysis, at pH 2–3 and at pH 8–9. The rate of hydrolysis at pH 2–3 was found to be approximately four times as large as that at pH 8–9.

In an earlier paper by Åkerfeldt,[2] cysteamine S-phosphate was reported to have only one rate maximum, at pH 3. The rate profiles for fifteen other compounds containing the $-SPO_3^{-2}$ group also were investigated.[3,4] In all instances a single rate maximum was found and its position was in the pH range 2–4. It could be shown conclusively that the most easily hydrolyzable ionic species of all compounds studied was the monoanion.[3,5]

In view of the finding by Dittmer, *et al.*,[1] of a second rate maximum at pH 8–9 in the case of cysteamine S-phosphate, a re-examination of the hydrolysis of this compound has been carried out. The investigation was performed (a) under experimental conditions practically identical with those used by Dittmer, *et al.*, and using the same analytical procedure as these authors, (b) using the same technique of hydrolysis as in a but using the analytical procedure of Gomori,[6] and (c) using a low ionic strength incubation medium combined with the analytical procedure of Gomori.[6]

The results obtained with a 99% pure preparation of cysteamine S-phosphate (4.0 mmolar) showed practically the same low rate of hydrolysis at pH 7.0, 8.0, and 9.0. The first-order rate of hydrolysis constants at 35.0° were $k_{obsb} = (2.0 \pm 0.2) \times 10^{-5}$ sec.$^{-1}$ at ionic strength of 0.1 M, and $k_{obsb} = (1.0 \pm 0.2) \times 10^{-5}$ sec.$^{-1}$ at ionic strength of 1 M. The existence of a rate maximum from pH 8–9 has thus not been confirmed.

Dittmer, *et al.*, found at 37° and 1 M ionic strength the following rate constants (sec.$^{-1}$): 3.8×10^{-5} (pH 7.02), 13.1×10^{-5} (pH 8.06), and 9.9×10^{-5} (pH 9.08).

The rate constants reported in the present communication are thus lower than those found by Dittmer, *et al.* This implies the presence of an impurity in their preparation of cysteamine S-phosphate, which is the likely explanation for the observed rate increase at pH 8–9.

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The Influence of Dicyclohexylcarbodiimide Concentration on the Rate of Phospho Diester Bond Formation

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Dicyclohexylcarbodiimide (DCC) has proven to be an efficient condensing agent for the synthesis of phos-

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