clude that reaction occurs between the C_9 position in the excited dye molecule and oxygen-containing species in the immediate solvation sphere, the chemical nature of the latter being strongly predetermined by pH. The minima for Rhodamine 6G then are construed to represent the transition between acidic and basic solvation spheres, either of which favors hydrol formation. That hydrol may be formed in either acidic or basic media has been confirmed previously.[11]

Registry No.—1, 2150-48-3; disodium Fluorescein, 518-47-8; Rhodamine 6G, 989-38-8.

(11) L. Lindqvist, *Ark. Kemi*, **16**, 79 (1960).

Preparation of Uniformly ^{14}C-Labeled *p*-Hydroxybenzoic Acid[1]

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It was found that *p*-hydroxybenzoic acid (HBA) is converted to the benzoquinone nucleus of coenzyme Q (CoQ) in *Rhodospirillum rubrum*.[2-4] The conversion of HBA to CoQ also occurs in the rat.[2b,5] After these initial studies in 1963–1964, the biosynthetic significance of HBA was studied in several laboratories, and the following additional citations are representative and pertinent to interests in the availability of uniformly labeled [^{14}C]-*p*-hydroxybenzoic acid.

The complete sequence of biosynthesis from HBA to CoQ was elucidated for *R. rubrum*, and it was projected that the same sequence or a very closely related sequence would exist in mammalian tissue according to Friis, *et al.*[6]

Rudney[7] described studies on the biosynthesis of CoQ and polyprenylphenols in cell-free preparations of *R. rubrum*, *E. coli*, and rat tissue, and reported that the first two enzymatic systems in the biosynthetic pathway from HBA to CoQ were characterized. Trumpower, *et al.*,[8] utilized labeled precursors of CoQ, including HBA, in studies on liver slices and identified 5-demethoxy coenzyme Q_9 as an intermediate in the biosynthesis of CoQ_9 in the rat. Momose and Rudney[9] reported on the biosynthesis of 3-polyprenyl-4-hydroxybenzoate in the inner membrane of mitochondria from HBA and isopentenyl pyrophosphate.

Whistance, *et al.*,[10-12] have reported on the biosynthesis of CoQ in yeasts, gram-negative bacteria, and animals, and utilized tracer techniques with labeled HBA.

Nilsson, *et al.*,[13-15] utilized labeled HBA in the determination of precursors, in the biosynthesis of CoQ in genetically dystrophic mice, and in the biosynthesis of CoQ_{10} in beating cell cultures from heart tissue.

The preparation of [^{14}C]-*p*-hydroxybenzoic acid by the alkaline fusion of [^{14}C]tyrosine has been described.[2,16] However, this preparation from relatively expensive [^{14}C]tyrosine has given erratic and disappointing yields in our experience. Consequently, the procedure which has evolved from our many preparations of uniform ^{14}C-labeled HBA is described. The ready availability of [^{14}C]-HBA is essential to continuing studies on the biosynthetic conversion of HBA to CoQ in various systems, including normal and diseased tissues from experimental animals and humans.

Experimental Section

The fusion was carried out in a nickel crucible with a handle which was wrapped with asbestos for easy handling. The crucible was 44 mm deep, 44 mm in top diameter, 25 mm in bottom diameter, and 25 ml in capacity.

Uniformly labeled [^{14}C]-L-tyrosine (100 μCi) with a specific activity of 507 mCi/mmol was purchased from the Amersham-Searle Corp., Chicago, Ill. This tyrosine was received in an aqueous solution containing 2% ethanol. The solution was pipetted from its container into the crucible. The container and cap were washed thoroughly with 0.01 *N* HCl, and the washings were added to the crucible. The solution was evaporated by a warm-water bath under a stream of nitrogen. The inside surface of the crucible was well washed with 0.01 *N* HCl and the solution was evaporated. This washing was repeated about three times, and each time with a diminished volume to assure that the tyrosine was concentrated in one area of the bottom of the crucible. Approximately 150 mg of NaOH and 150 mg of KOH were finely crushed together and immediately added to the crucible in the region of the tyrosine. The NaOH and KOH were melted by placing the crucible in a Wood's Metal bath at 270°. The melted alkali was swirled around the crucible to encompass all the tyrosine. The temperature of Wood's Metal bath dropped about 15° on initial contact with the crucible and slowly climbed to 270°. After 10 min, the crucible was removed from the bath and allowed to cool. The melted residue solidified. Slowly, 1 ml of 10 *N* H_2SO_4 was added to dissolve the residue, and the solution was transferred to a small separatory funnel. The crucible was washed twice with 1 ml of water and the washings were poured into the separatory funnel.

The reaction mixture was extracted with 10 ml of ether. The extract was transferred into another small separatory funnel and extracted with 1 ml of H_2O. The extraction of the reaction mixture with ether followed by a water extraction of the ether was repeated ten times. The combined ether extract, 100 ml, was evaporated under vacuum. The residue was purified by thin layer chromatography on 1 mm silica gel G plates. The mobile phase for development was absolute methanol. Pure HBA was used as reference material. The area corresponding to the R_f value of HBA was removed and eluted four times with 50 ml of absolute methanol each time; this extraction was necessary to

(1) Coenzyme Q. CLVII.

(2) (a) H. Rudney and W. W. Parson, *J. Biol. Chem.*, **238**, PC 3137 (1963). (b) W. W. Parson and H. Rudney, *Proc. Nat. Acad. Sci. U. S.*, **51**, 444 (1964).

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remove all the [^{14}C]-HBA. An aliquot was counted by liquid scintillation and showed that a yield of 55% of [^{14}C]-HBA was obtained. The purity of the HBA from fusion was tested by tlc in three additional systems: 1-propanol:H_2O:NH_4OH (8:1:1), benzene:methanol (1:4), and benzene:methanol:acetic acid (90:16:8). In all three systems, only one radioactive peak was detected by a Dünnschicht-Scanner, LB2721 (Berthold), and each had the same R_f value as reference HBA. Pauly's reagent was used to detect the areas of reference HBA.

Registry No.—[^{14}C]-*p*-Hydroxybenzoic acid, 33875-99-9; [^{14}C]tyrosine, 18875-48-4.

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Nitriles from Aldoximes. A New Reaction of Phosphonitrilic Chloride[1]

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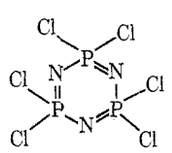
In previous work[2] we have reported that hexachlorocyclotriphosphazatriene (phosphonitrilic chloride) (**1**) can be used as an activator of the type RCOOX=Y[3] in the conversion of the carboxylic functions into amides and hydrazides in high yields and under very mild conditions.

Continuing the study of the chemical behavior of phosphonitrilic halides, we have examined the response of aliphatic, aromatic, and olefinic aldoximes toward phosphonitrilic chloride.[4]



1

We found that nitriles are produced at room temperature in a process that is exceptionally mild, comparable to procedures recently reported, which involve dehydration of aldoximes.[5]

The method involves addition of a solution of triethylamine (3 mol) to a solution of phosphonitrilic chloride (1 mol) and oxime (1 mol) followed by isolation of the nitrile after 2–24 hr, usually by chromatography. Some results given by our process are shown in Table I,

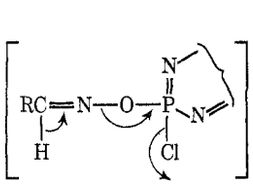
TABLE I

Oxime[a]	Yield of nitrile, %	Time of reaction, hr
(*E*)-Benzaldoxime	72 (88)[d]	24
(*Z*)-Benzaldoxime	74	24
(*E*)-*p*-Chlorobenzaldoxime	76 (60.8)[e]	18
(*Z*)-*p*-Chlorobenzaldoxime	78 (42.4)[e]	20
(*E*)-Cinnamaldoxime	98 (95)[d]	12
(*Z*)-Cinnamaldoxime	97	12
Undecaldoxime[b]	95	8
Heptaldoxime[b]	93 (42.7)[e]	8
(*Z*)-Furaldoxime	89 (60)[d]	18
p-Phenylbenzaldoxime	69	24
Pyridine-2-aldoxime	95	12
3,7-Dimethyl-2,6-octadienaldoxime	98	12
3-Indolecarboxaldehyde[c]	98	2

[a] Nomenclature of J. E. Blackwood, C. L. Gladys, K. L. Leoning, A. E. Petrarca, and J. E. Rush, *J. Amer. Chem. Soc.*, **90**, 509 (1968). [b] Stereochemistry unknown. [c] Reaction performed in THF. [d] Reference 5b. [e] Reference 5a.

in which the yields refer to analytically pure products obtained from reactions performed in diethyl ether. The yields given using other mild procedures are also reported.

Aldoximes react with phosphonitrilic chloride at room temperature to give nitriles, and no *O*-phosphonitrilic chloride derivative of type **2** has been observed during the reaction, whereas the cyclohexanone oxime reacts with compound **1** under the same conditions to give the compound **3** in 63% yield.



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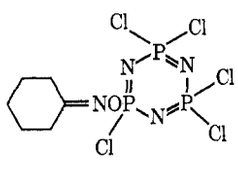

3

This fact induced us to hypothesize that aldoximes also react with phosphonitrilic chloride in the presence of triethylamine to give intermediates of type **2** that successively undergo 1,4 fast elimination of hydrogen chloride to form the corresponding nitriles.

Tlc analysis (silica gel, benzene as eluent) revealed the formation of nitrile and the disappearence of oxime during the time of the reaction, which was longer for the conversion of aromatic aldoximes (10–24 hr) than for the aliphatic and olefinic ones (2–8 hr). The stereochemistry of aldoximes (*E*, *Z*) has little effect on the reaction. The reaction can be performed in a variety of solvents (benzene, ethyl acetate, chloroform, THF) in very good yields.

The possibility of using a variety of solvents for the reaction, the simplicity of the operations involved, the high yields together with the mild conditions, and the ready availability of the reagent **1** recommend this new route to nitriles.

(1) This work was done with financial support from the Italian National Research Council (C. N. R.).

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