[CONTRIBUTION FROM THE LABORATORY OF ORGANIC CHEMISTRY, UNIVERSITY OF ATHENS]

Dealkylation and Debenzylation of Triesters of Phosphoric Acid. Phosphorylation of Hydroxy and Amino Compounds

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Under mild conditions sodium or barium iodide brings about cleavage of a single primary alkyl–oxygen (or benzyl–oxygen) bond in a triester of phosphoric acid; the sodium or barium salt of the diester, and the alkyl or benzyl iodide are produced. The di-*p*-nitrobenzyl and di-*p*-iodobenzylphosphoryl chlorides which were prepared from the corresponding diester, can be used for phosphorylation, as they react readily with hydroxy and amino compounds; the *p*-substituted benzyl groups were removed by catalytic hydrogenation.

Introduction

Several years ago we introduced a new method of phosphorylation[1] based on the reaction of the silver salt of dibenzyl phosphate with suitable halides and subsequent displacement of the benzyl groups of the resulting triester by catalytic hydrogenation.[1-4] Later, Atherton, Openshaw and Todd[5] used the chloride of dibenzyl phosphate as a phosphorylating agent, thus giving the method a wider applicability.

$$(\mathrm{ArylCH_2O})_3\mathrm{PO} \xrightarrow[\boxed{\text{yield almost quantitative}}]{\text{NaI, 5–15 min., 55–80°}} (\mathrm{ArylCH_2O})_2\mathrm{P(O)ONa} + \mathrm{ArylCH_2I}$$

$$(\mathrm{ArylCH_2O})_3\mathrm{PO} \xrightarrow[\text{BaI}_2\text{, 2–5 min., 55°}]{\boxed{\text{yield almost quantitative}}} [(\mathrm{ArylCH_2O})_2\mathrm{P(O)O}]_2\mathrm{Ba} + \mathrm{ArylCH_2I}$$

$$(\mathrm{AlkylCH_2O})_3\mathrm{PO} \xrightarrow[\text{10 min., 100° (90\% yield)}]{\text{BaI}_2\text{(NaI), 60 min., 55° (10\% yield)}} [(\mathrm{AlkylCH_2O})_2\mathrm{P(O)O}]_2\mathrm{Ba} + \mathrm{AlkylCH_2I}$$

Dibenzyl phosphate can be prepared by the reaction of the triester with concentrated alkalies,[6] with hydrazine,[7] with tertiary bases and their halogenides,[8,9] with lithium chloride[10] or by the action of phosphorus pentoxide on benzyl alcohol.[3]

In our experience, the monodealkylation or monodebenzylation of triesters by alkali seldom proceeds satisfactorily. The tertiary bases produce only debenzylation,[9] whereas lithium chloride can also be used for the monodealkylation of trialkyl phosphates.[10] Although lithium chloride removes a benzyl group more readily than an alkyl group from a mixed triester, the rate of dealkylation is high under the conditions necessary for debenzylation.

A general and simpler method[7] for the preparation of the diesters of phosphoric acid consists in heating the triester in a suitable solvent with barium or sodium iodide for a short time.[11] The sodium or barium salt of the diester precipitates and even in the presence of excess iodide, the reaction does not proceed beyond the formation of the diester. By this method we have obtained the diethyl, dibenzyl, di-*p*-nitrobenzyl, di-*p*-iodobenzyl, di-*p*-bromobenzyl and di-*p*-phenylbenzyl phosphates from the corresponding triesters.[12]

Under the conditions employed (heating for a few minutes at 80–100° or at the most for one hour at 55°) the iodide salts remove only primary alkyl,[13] benzyl and substituted benzyl groups; esters of secondary alcohols, *e.g.*, triisopropyl phosphate or phenyl esters, *e.g.*, triphenyl phosphate, are practically unaffected. Presumably esters of tertiary alcohols will not be attacked either.

From the above scheme, it is obvious that monodebenzylation occurs more readily than monodealkylation. Therefore, a benzyl group can be removed selectively and almost quantitatively from mixed triesters under suitable conditions; a *p*-iodobenzyl group was removed from di-*p*-iodobenzyl *n*-butyl phosphate by heating at 55° for about 20–30 minutes. Mixed esters containing benzyl and secondary (or tertiary) alkyl groups, *e.g.*, di-*p*-nitrobenzyl isopropyl phosphate and β-D-glucose tetraacetate dibenzyl 1-phosphate, can be selectively debenzylated even under conditions which brought about the monodealkylation of triethyl phosphate.

The diesters (di-*p*-nitrobenzyl and di-*p*-iodobenzyl phosphate) reacted with phosphorus pentachloride to give the corresponding phosphoryl chlorides (I and II). These are stable, crystalline compounds which may be used for the synthesis of monoesters, of mixed di- and triesters and of amino derivatives of phosphoric acid. I and II are more stable than dibenzylphosphoryl chloride and give derivatives of lower solubility and higher stability. The following reactions illustrate the use of the phosphoryl chlorides; similar reactions were carried out with II.

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[1] L. Zervas, *Naturwissenschaften*, **27**, 317 (1939); K. Panagopoulos, Thesis, University of Athens, 1940.

[2] M. L. Wolfrom, C. S. Smith, D. E. Pletcher and A. E. Brown, THIS JOURNAL, **64**, 23 (1942).

[3] F. Lynen, *Ber.*, **73**, 367 (1940).

[4] J. C. Sheehan and V. S. Frank, THIS JOURNAL, **72**, 1312 (1950).

[5] F. R. Atherton, H. T. Openshaw and A. R. Todd, *J. Chem. Soc.*, 382, 660 (1945).

[6] W. Lossen and A. Koehler, *Ann.*, **262**, 196 (1891).

[7] I. Dilaris-Papadimitriou, Thesis, University of Athens, 1948.

[8] J. Baddiley, V. M. Clark, J. J. Michalski and A. R. Todd, *J. Chem. Soc.*, 815 (1949).

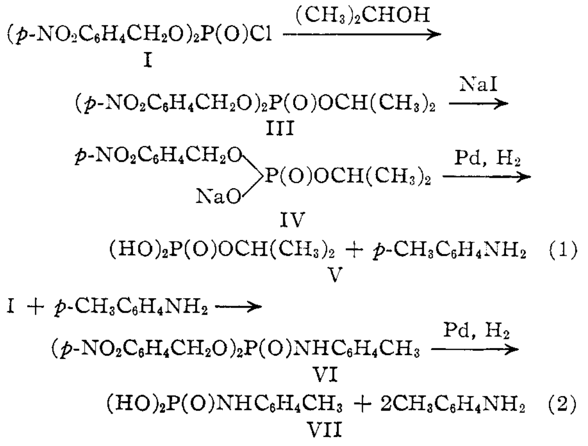
[9] V. M. Clark and A. R. Todd, *ibid.*, 2023 (1950).

[10] V. M. Clark and A. R. Todd, *ibid.*, 2030 (1950); J. Lecocq and A. R. Todd, *ibid.*, 2381 (1954).

[11] Debenzylation and dealkylation with lithium chloride require 2–3 hours heating at 100°.

[12] The triester need not be pure for this reaction; the iodide may be added to a solution of the crude triester, obtained by the reaction of an alkyl, benzyl or *p*-substituted benzyl halide with silver phosphate, or by the reaction of a dialkyl, dibenzyl or *p*-substituted dibenzyl phosphoryl chloride with hydroxy compounds.

[13] Lithium chloride removes also secondary alkyl groups, ref. 10.



$$\underset{\text{I}}{(p\text{-}NO_2C_6H_4CH_2O)_2P(O)Cl} \xrightarrow{(CH_3)_2CHOH}$$

$$\underset{\text{III}}{(p\text{-}NO_2C_6H_4CH_2O)_2P(O)OCH(CH_3)_2} \xrightarrow{NaI}$$

$$\underset{\text{IV}}{\begin{matrix} p\text{-}NO_2C_6H_4CH_2O \\ NaO \end{matrix}\!\!>\!P(O)OCH(CH_3)_2} \xrightarrow{Pd,\ H_2}$$

$$\underset{\text{V}}{(HO)_2P(O)OCH(CH_3)_2} + p\text{-}CH_3C_6H_4NH_2 \quad (1)$$

$$\text{I} + p\text{-}CH_3C_6H_4NH_2 \longrightarrow$$

$$\underset{\text{VI}}{(p\text{-}NO_2C_6H_4CH_2O)_2P(O)NHC_6H_4CH_3} \xrightarrow{Pd,\ H_2}$$

$$\underset{\text{VII}}{(HO)_2P(O)NHC_6H_4CH_3} + 2CH_3C_6H_4NH_2 \quad (2)$$

The mixed triesters prepared by the phosphorylation of hydroxy compounds with dibenzyl phosphoryl chloride (I or II) are rarely obtained in a pure crystalline state. Therefore, to avoid impurities in the hydrogenated product we have first debenzylated the triester as shown in (1), and then hydrogenated the diester; the latter were always obtained as pure crystalline compounds.

Acknowledgments.—This Laboratory, which lost practically its entire equipment during the war and the post-war troubled period, has been rehabilitated largely through the "American Aid to Greece" following suggestions of the Greek Ministry of Coördination. We take this opportunity to express our gratitude.

The authors thank Dr. P. Katsoyannis and Mr. A. Stavropoulos for their assistance.

Experimental

The solvents and sodium and barium iodide were anhydrous. Silver phosphate was prepared by the procedure of Lipmann and Tuttle.[14] Prior to analysis, the sodium or barium salts were dried at 78°; other compounds were dried at 56°. Phosphorus was determined colorimetrically on the alkyl phosphates after hydrolysis with concentrated hydrochloric acid and on the benzyl phosphates after catalytic hydrogenation; the mixed phosphates were hydrogenated and the product hydrolyzed prior to the phosphorus determination. Hydrogenations were carried out in methanolic sodium hydroxide with palladium black as the catalyst. Hydrogenolysis was usually complete within 10–15 minutes and the yield of phosphoric acid was quantitative.

Dialkyl, Dibenzyl and *p*-Substituted Dibenzyl Phosphates.—The diesters of phosphoric acid were prepared by heating the corresponding triester with sodium or barium iodide[15] in a suitable solvent, followed by acidification of the salt of the diester. The following procedures (A and B) are typical. Some of the diesters prepared are listed in Table I; those triesters which were prepared by procedure B and were isolated are included. The iodides which were isolated from the corresponding reaction mixtures are given in Table II.

(A).—A solution of 3.7 g. (0.01 mole) of tribenzyl phosphate and 1.65 g. of sodium iodide (0.011 mole) in 20 ml. of acetone[16] was refluxed over a steam-bath for about 20 minutes; with methyl ethyl ketone as the solvent, the boiling time can be reduced to 5–6 minutes. Precipitation of the anhydrous sodium salt of dibenzyl phosphate began within the first minutes. After the mixture, which had been concentrated to half of its original volume, had stood for several hours in the ice-box, the salt was collected and washed with acetone. The filtrate was distilled *in vacuo*, water was added and the mixture extracted with ether. After removal of the ether and distillation, 1.7 g. of benzyl iodide was obtained.

Upon acidification of the aqueous solution of the sodium salt with hydrochloric acid, dibenzyl phosphate precipitated almost quantitatively (m.p. 79°); an additional 0.3–0.4 g. was obtained by acidification of the aqueous layer after extraction with ether. The silver salt (m.p. 212–216° dec.)[3,4] was prepared from an aqueous solution of the sodium salt.

(B).—For the preparation of the diester, especially in large quantities, the crude triester can be used. Thus, 38 g. (0.3 mole) of benzyl chloride, 46 g. of silver phosphate (10% excess) and 150 ml. of chloroform were heated on the steam-bath for 7–8 hours. The precipitated silver salts were repeatedly washed with chloroform and the combined filtrate evaporated to dryness *in vacuo*.[17] To the residue dissolved in 120 ml. of acetone was added 16.5 g. of sodium iodide and the solution heated on the steam-bath for 20–30 minutes. Upon cooling 19 g. (65%) of the sodium salt of dibenzyl phosphate was obtained.

***p*-Phenylbenzyl Bromide.**—A solution of 33.6 g. (0.2 mole) of *p*-phenyltoluene[18] and 35.6 g. (0.2 mole) of bromosuccinimide in 150 ml. of carbon tetrachloride was boiled gently for 18 hours. The precipitated succinimide was filtered. The filtrate was washed first with dilute alkali and then with water, dried with sodium sulfate and distilled to dryness *in vacuo* at 40–45°. Cold petroleum ether (30–60°) was added to the crystalline residue and the mixture filtered; yield 22 g. (50%). After two recrystallizations from alcohol or petroleum ether, the material melted at 85° (reported[19] m.p. 87°).

Anal. Calcd. for $C_{13}H_{11}Br$: Br, 32.34. Found: Br, 32.52.

A bromide melting at 80° was used for the preparation of the iodide and tri-*p*-phenylbenzyl phosphate.

Di-*p*-nitrobenzyl- (I) and Di-*p*-iodobenzylphosphoryl Chloride (II).—To a suspension of the diester (0.04 mole) in 50 ml. of chloroform was added a 10% excess of phosphorus pentachloride in 2 or 3 portions with stirring and moderate cooling. After 15 minutes stirring the reagents went into solution. In the case of I, petroleum ether (b.p. 30–60°) was added directly to the chloroform solution; in the case of II the chloroform solution was distilled *in vacuo* at 20–25° almost to dryness and then petroleum ether added to the residue. The chlorides were collected and washed with petroleum ether. They were dried and stored in a vacuum desiccator over phosphorus pentoxide and sodium hydroxide; under these conditions they are stable for a long time.

I (96% yield) was recrystallized from chloroform–petroleum ether, m.p. 107°.

Anal. Calcd. for $C_{14}H_{12}O_7N_2PCl$: N, 7.26; Cl, 9.16. Found: N, 7.4; Cl, 9.3.

II was obtained in 75% yield, m.p. 89°.

Anal. Calcd. for $C_{14}H_{12}O_3ClI_2P$: Cl, 6.46; Cl + I, 52.76. Found: Cl, 6.57; Cl + I, 52.98.

As expected, when the phosphoryl chlorides were stirred with dilute alkali at room temperature (prior to the determination of chlorine), only the chlorine was split off.

***p*-Nitrobenzyl Isopropyl Hydrogen Phosphate.**—A solution of I (9.7 g., 0.025 mole) in 30 ml. of chloroform was added to a mixture of 5.4 ml. of isopropyl alcohol and 6 ml. of pyridine in 10 ml. of chloroform. The solution was allowed to stand for 4 hours at room temperature. It was then washed successively with potassium hydrogen carbonate, water, dilute hydrochloric acid and again with water. It was dried over sodium sulfate and distilled to dryness *in vacuo;* a few ml. of alcohol was added. After several hours in the cooler, a small amount of precipitate was filtered and the filtrate distilled to dryness. The resulting sirupy tri-

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[14] F. Lippmann and L. C. Tuttle, *J. Biol. Chem.*, **153**, 571 (1944).

[15] Calcium and potassium iodide were also used for the preparation of dibenzyl phosphate.

[16] Dibenzyl phosphate was obtained in 95% yield with pyridine as the solvent by heating for 1–2 minutes at 100°; it must be assumed that the benzyl iodide formed reacts with the pyridine.

[17] For the isolation of pure crystalline tribenzyl and tri-*p*-phenylbenzyl phosphates ether–petroleum ether was added; the other *p*-substituted benzyl phosphates were obtained by adding alcohol and recrystallizing from the same solvent.

[18] W. E. Bachmann and R. A. Hoffmann in "Organic Reactions," Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1944, p. 247. M. Gomberg and J. C. Pernert, THIS JOURNAL, **48**, 1372 (1926).

[19] H. Dahn and P. Zoller, *Helv. Chim. Acta*, **35**, 1348 (1952).

TABLE I

Phosphate esters	Yield, %	M.p., °C.	Formula	Analyses, %	
				Calcd.	Found
Dibenzyl-Na	75[a]		$C_{14}H_{14}O_4PNa$	P, 10.32	10.25
Dibenzyl	96[b]	79[c]			
Dibenzyl-Ba	97[a]		$C_{28}H_{28}O_8P_2Ba$	Ba, 19.85	19.65
Diethyl-Ba[d]	90[a]		$C_8H_{20}O_8P_2Ba$	P, 13.98; Ba, 30.96	13.89;30.75
Diethyl-Pb[d,e]	90[a]	182–183[f]	$C_8H_{20}O_8P_2Pb$	P, 12.08	12.17
Tri-*p*-nitrobenzyl	84	130[g]	$C_{21}H_{18}O_{10}N_3P$	N, 8.35	8.45
Di-*p*-nitrobenzyl-Na	96[a]		$C_{14}H_{12}O_8N_2PNa$	P, 7.94; N, 7.17	7.82;7.05
Di-*p*-nitrobenzyl	75[b]	175[h]	$C_{14}H_{13}O_8N_2P$	N, 7.6	7.45
Di-*p*-nitrobenzyl-Ba	98[a]		$C_{28}H_{24}O_{16}N_4P_2Ba$	Ba, 15.75	15.86
Tri-*p*-bromobenzyl	83	133[i]			
Di-*p*-bromobenzyl-Na	96[a]		$C_{14}H_{12}O_4Br_2PNa$	P, 6.76	6.65
Di-*p*-bromobenzyl	96[b]	161–162[j]	$C_{14}H_{13}O_4Br_2P$	Br, 36.65; P, 7.11	36.82;7.2
Di-*p*-bromobenzyl-Ba	98[a]		$C_{28}H_{24}O_8Br_4P_2Ba$	Ba, 13.63	13.85
Tri-*p*-iodobenzyl	85	164	$C_{21}H_{18}O_4I_3P$	I, 51.03; P, 4.15	51.25;4.2
Di-*p*-iodobenzyl-Na[k]	91[a]		$C_{14}H_{12}O_4I_2PNa$	I, 45.98; P, 5.61	45.72;5.7
Di-*p*-iodobenzyl	93[b]	178	$C_{14}H_{13}O_4I_2P$	I, 47.88; P, 5.84	47.53;5.89
Tri-*p*-phenylbenzyl[l]	85	173	$C_{39}H_{33}O_4P$	P, 5.19	5.1
Di-*p*-phenylbenzyl-Na[k]	97[a]		$C_{26}H_{22}O_4PNa$	P, 6.85	6.8
Di-*p*-phenylbenzyl	92[b]	148–150	$C_{26}H_{23}O_4P$	P, 7.2	7.1

[a] By procedure A. [b] By acidifying the sodium salt. [c] M.p. 78–79°, ref. 6. [d] Acetoacetic ester used as the solvent. [e] Since the sodium salt of diethyl phosphate is soluble in acetoacetic ester or acetone, the phosphate ester was isolated as the lead salt. [f] M.p. 180°; "Beilstein," 4th edition, Vol. I, J. Springer, Berlin, 1918, p. 322. [g] M.p. 127–128°, ref. 8. [h] M.p. 173–174°, ref. 8. [i] M.p. 132–133°, ref. 8. [j] M.p. 155–156°, ref. 8. [k] Methyl ethyl ketone was used as the solvent; the salt is soluble only in hot water (1:25). [l] Upon catalytic hydrogenation, the tri-*p*-phenylbenzyl phosphate yielded *p*-phenyltoluene (m.p. 47–48°) and phosphoric acid quantitatively.

TABLE II

Iodide	Yield, %	M.p., °C.	Formula	Analyses for iodine, %	
				Calcd.	Found
Benzyl		24			
p-Nitrobenzyl	91	124[a]	$C_7H_6O_2NI$	48.26	48.42
p-Bromobenzyl	80	74–75[b]	C_7H_6BrI	42.73	42.56
p-Iodobenzyl	90	88–89	$C_7H_6I_2$	73.81	73.54
p-Phenylbenzyl	85	102[c]			

[a] M.p. 124°; H. Finkelstein, *Ber.*, **43**, 1531 (1910). [b] M.p. 80–81°; A. Hantzsch and O. W. Schultze, *ibid.*, **29**, 2253 (1896). We also prepared *p*-bromobenzyl iodide by the treatment of *p*-bromobenzyl bromide with sodium iodide; this sample melted at 74–75° after 2 recrystallizations from alcohol. The melting point of a mixture of this iodide and the sample, obtained from the debenzylation of tri-*p*-bromobenzyl phosphate, was not depressed. [c] *p*-Phenylbenzyl iodide, prepared by the treatment of *p*-phenylbenzyl bromide with sodium iodide, also melted at 102°.

ester III (6.1 g., 60%) was dissolved with 2.5 g. of sodium iodide in 30 ml. of acetone and the solution heated on a steam-bath for about one hour; with acetoacetic ester as solvent the heating time can be reduced to 15 minutes. After cooling, the crystals of the sodium salt (IV) were collected and washed with acetone; yield 4 g. (90%, if it is assumed that the sirupy residue is exclusively the triester III).

Anal. Calcd. for $C_{10}H_{13}O_6NPNa$: N, 4.7; P, 10.43. Found: N, 4.5; P, 10.5.

The acetone solution yielded *p*-nitrobenzyl iodide, m.p. 124°.

When the aqueous solution of the sodium salt was acidified with hydrochloric acid, *p*-nitrobenzyl isopropyl hydrogen phosphate was precipitated almost quantitatively; it was recrystallized from alcohol; m.p. 69–70°.

Anal. Calcd. for $C_{10}H_{14}O_6NP$: N, 5.08. Found: N, 5.3.

IV can also be prepared by debenzylation of the triester III obtained by the reaction of the silver salt of di-*p*-nitrobenzyl phosphate and isopropyl bromide. The yield (50%) calculated on the basis of the isopropyl bromide, is low due to the formation of di-*p*-nitrobenzyl phosphate (m.p. 175°) and propylene by a side reaction.

Isopropyl Phosphate (V).—Methanol was added to 3 g. (0.01 mole) of IV dissolved in a small amount of water and the solution catalytically hydrogenated. The hydrogenation was completed after 20 minutes, when 1000 ml. of hydrogen had been absorbed (27°, 753 mm.). To the filtrate were added 20 ml. of *N* sodium hydroxide and then 4 g. of barium iodide dissolved in methanol. The barium salt of V which separated was filtered and washed with methanol; yield 2.6 g.

Anal. Calcd. for $C_3H_7O_4PBa$: P, 11.25; Ba, 49.87. Found: P, 11.3; Ba, 50.02.

The mother liquor was evaporated to dryness. Upon the addition of dilute alkali *p*-toluidine precipitated (m.p. 44–45°).

n-**Butyl Phosphate**.—Di-*p*-iodobenzyl *n*-butyl phosphate, which was prepared as described above from II and *n*-butyl alcohol, was heated at 55° with sodium iodide for 20–30 minutes; the sodium salt of *p*-iodobenzyl *n*-butyl phosphate which precipitated was used for the preparation of the barium salt of *n*-butyl phosphate as described for V. Recrystallization from water gave the crystalline dihydrate.[20]

Anal. Calcd. for $C_{11}H_{15}O_4IPNa$: I, 32.35; P, 7.9. Found: I, 32.5; P, 7.85. *Anal.* Calcd. for $C_4H_9O_4PBa$: P, 10.7; Ba, 47.46. Found: P, 10.65; Ba, 47.65.

N-Di-*p*-nitrobenzylphosphoryl-*p*-toluidine (VI).—A solution of 3.8 g. of I (0.01 mole) in 10 ml. of chloroform was added to a cold solution of 2.4 g. of *p*-toluidine (0.022 mole) in 10 ml. of chloroform. In a short time, toluidine hydrochloride separated. After standing for 6 hours at room temperature, the mixture was washed with dilute hydrochloric acid, water and potassium hydrogen carbonate. It was then dried over sodium sulfate and distilled *in vacuo*. The crystalline residue was recrystallized from alcohol; yield 3.3 g. (65%), m.p. 155°.

Anal. Calcd. for $C_{21}H_{20}O_3NI_2P$: N, 2.26. Found: N, 2.1.

The catalytic hydrogenation of either VI or VIII resulted in the formation of N-phosphoryl-*p*-toluidine (VII) which was precipitated as the barium salt; yield from VIII, 81%.

Anal. Calcd. for $C_7H_8O_3NPBa$: N, 4.34; Ba, 42.6. Found: N, 4.1; Ba, 42.8.

β-D-Glucose Tetraacetate Benzyl Hydrogen 1-Phosphate.—The sodium (or barium) salt of this acid was obtained almost quantitatively by the monodebenzylation of β-D-glucose tetraacetate dibenzyl 1-phosphate[1,2] with sodium (or barium) iodide in acetone or acetoacetic ester solution.

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(20) F. R. Atherton, H. T. Howard and A. R. Todd, *J. Chem. Soc.* 1106 (1948).

Anal. Calcd. for $C_{21}H_{26}O_{13}PNa$: P, 5.73. Found: P, 5.6.

The free acid (yield 90%, m.p. 110–111°) and the silver salt were prepared.

Anal. Calcd. for $C_{21}H_{27}O_{13}P$: P, 5.98. Found: P, 5.85. *Anal.* Calcd. for $C_{21}H_{26}O_{13}PAg$: Ag, 17.28. Found: Ag, 17.1.

ATHENS, GREECE

[CONTRIBUTION FROM THE RADIATION LABORATORY, UNIVERSITY OF CALIFORNIA, BERKELEY]

Thioctic-S^{35}_2 Acid: Synthesis and Radiation Decomposition[1]

BY PATRICIA T. ADAMS

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Thioctic-S^{35}_2 acid has been prepared with a specific activity of 1 μc./mg. in 10–16% yield. When material of 200 μc./mg. was prepared by the same method, no crystalline product could be isolated. The radiation sensitivity of thioctic acid has been investigated under a variety of circumstances. In Co^{60} γ-irradiation, crystalline thioctic acid is decomposed at the same rate as thioctic acid in hexane solution: $G(-M) = 10$. This value is decreased when benzene is added to the solution. When thioctic acid is irradiated by 4.3 Mev. electrons, $G(-M) = 15$. When it is irradiated by carbon-14 β-rays in hexane solution, $G(-M) = 31$.

The synthesis of thioctic acid labeled with sulfur-35 of high specific activity was undertaken in order to provide material for tracer studies in biological systems where the natural concentration of thioctic acid is very low. Because results of the synthesis using high specific activity sulfur-35 differed markedly from those where little or no radioactivity was used, an investigation into the stability of thioctic acid toward radiation decomposition was undertaken.

The method of Reed and Niu[2] for preparing thioctic acid through the intermediate dibenzylmercaptoöctanoic acid presented a convenient method for introducing sulfur-35 into the molecule. α-Toluenethiol-S^{35} was prepared by the reaction of benzylmagnesium chloride with amorphous sulfur-35. Thioctic acid was isolated from the subsequent reaction mixture as a yellow oil which was then recrystallized from *n*-hexane.

Three preparations of thioctic-S^{35}_2 acid (specific activity 1 μc./mg.) were completed according to the experimental procedure detailed below, with over-all yields of 10–16%. In order to obtain material useful for biological experiments, a synthesis utilizing 100 mc. sulfur-35 (calculated specific activity of product = 200 μc./mg.) was undertaken. In this preparation, no crystalline material could be isolated, although chromatographic analysis showed thioctic acid to be present in the reaction product. Pure high specific activity thioctic-S^{35}_2 acid was obtained by a series of solvent extractions.

Samples of thioctic acid were subjected to external radiation under a variety of circumstances. Results of these irradiations indicate that the labeled thioctic acid of 200 μc./mg. would be decomposed at a rate of about 0.3% per day from its own radiation. This value indicates that radiation damage to the thioctic acid itself is not extensive enough to explain the difficulties encountered in crystallization of the high specific activity product.

Experimental

α-Toluenethiol-S^{35}.—An ethereal solution of benzylmagnesium chloride (10 mmoles in 25 ml. of ether) was added with stirring to a slurry of 160 mg. (5 mmoles) of amorphous sulfur containing 0.5 mc. of S^{35} in 15 ml. of dry benzene. The mixture was heated under reflux for 4 hours, cooled and treated with water and acid to decompose the Grignard complex. The toluenethiol was extracted into ether solution and this solution was carefully dried over sodium sulfate. An aliquot portion of the ethereal solution was titrated against standardized iodine solution. The yield of thiol was 4.4 mmole (85%). Attempts to isolate this thiol through distillation resulted in loss of product through oxidation. A nitrogen atmosphere was maintained during all subsequent reactions.

6,8-Dibenzylmercapto-S^{35}_2-octanoic Acid.—The ethereal thiol solution was concentrated to approximately 10 ml. Absolute ethanol (20 ml.) was added and the solution was again concentrated to 10 ml. A solution of ethyl 6,8-dibromoöctanoate (738 mg., 2.2 mmoles) in 10 ml. of ethanol, followed by 102 mg. (4.4 mmoles) freshly cut sodium, was added to the thiol solution. After 4 hours heating at reflux, a precipitate of sodium bromide had appeared. Potassium hydroxide (340 mg.) was added, and the mixture was allowed to stand overnight. The reaction mixture was diluted with water, acidified and extracted with ether. The ethereal solution was washed with water, dried and distilled to dryness; the oily residue contained 0.4 mc. S^{35}. In previous experiments the dibenzylmercaptoöctanoic acid was isolated by crystallization from benzene–hexane in approximately 50% yield. However, this crystallization of the acid results in loss of product and does not improve the yield on subsequent steps. Consequently, for this synthesis the crude acid was used for the following reduction.

Thioctic-S^{35}_2 Acid: Low Specific Activity.—The crude dibenzylmercapto acid was extracted into 5 ml. of dry toluene. A toluene-insoluble polymeric material contained 0.08 mc., 16% of the starting activity. The clear toluene solution was added very slowly with stirring to a solution of sodium in 50 ml. of liquid ammonia. Additional pieces of sodium were added to the reaction mixture until a dark blue color was maintained for 30 minutes. At this time the blue color was discharged with ammonium chloride and the ammonia was allowed to evaporate. The residue was dissolved in water and extracted with ether. The clear aqueous solution was diluted to 80 ml. and the *p*H was adjusted to 7.0. One ml. of 1% ferric chloride solution was added and oxygen was bubbled through the solution for 20 minutes. The solution was acidified and extracted with chloroform. Evaporation of the chloroform left a viscous yellow oil. The product was extracted into hot hexane, allowed to crystallize, isolated and recrystallized from hexane. The thioctic acid was isolated as light yellow crystals. The pure product weighed 54 mg. (10% yield from S^{35}) and had a specific activity of 1.12 μc./mg. (theory 1.0 μc./mg.). The compound showed the characteristic ultraviolet absorption spectrum for the 5-membered disulfide ring with $\lambda_{\text{min.}}$ 280 mμ; $\lambda_{\text{max.}}$ 333 mμ; $\epsilon_{\text{max.}}$ 149.

Thioctic-S^{35}_2 Acid: High Specific Activity.—In order to obtain material more useful for biological experiments, a synthesis utilizing 100 mc. of sulfur-35 (specific activity of product = 200 μc./mg.) was undertaken. The yield of toluenethiol was 88%. The crude dibenzylmercaptoöctanoic acid contained 75 mc. Sodium–ammonia reduction, followed by oxidation with oxygen proceeded smoothly to

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[2] L. J. Reed and C. Niu, THIS JOURNAL, **77**, 416 (1955).