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

RECEIVED JUNE 24, 1955

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 $\lambda_{min.}$ 280 mμ; $\lambda_{max.}$ 333 mμ; $\epsilon_{max.}$ 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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[1] The work described in this paper was sponsored by the U. S. Atomic Energy Commission.

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

give a yellow oily product just as in preceding preparations. However, repeated attempts to obtain crystalline thioctic acid from this oil by hexane extraction and crystallization failed. A perfectly clear hot hexane extract of the radioactive product was allowed to cool for several hours. A large percentage of the oily product which separated from the cool hexane would not redissolve in hot hexane. With each extraction and subsequent cooling, more of this insoluble oil was formed. After several crystallization attempts (identical to those successfully employed in isolating the less radioactive acid) the major product was a colorless, insoluble oil. The hexane soluble fraction, however, was still yellow.

It was found that a solution containing thioctic acid with no major radioactive impurities could be obtained in the following manner. The combined oily products from the crystallization attempts were extracted with 4 small portions of hot hexane, leaving behind a large insoluble residue (approximately 30 mc. S^{35}). This residue, examined spectrophotometrically, was found to contain almost no thioctic acid. The hexane extract, containing 3.6 mc. S^{35}, was immediately evaporated to dryness and extracted with several small portions of 0.01 *M* phosphate buffer (*p*H 6.75). Again a large residue remained. This residue contained 2.5 mc. S^{35} of which about 25% was thioctic acid, 25% was thioctic acid sulfoxide and 50% was composed of radioactive compounds, not yet identified.

Analysis of the buffer solution by one-dimensional paper chromatography (with butanol-*N*/2 ammonium hydroxide as solvent) showed 6% of the radioactivity on the origin, 7% as thioctic acid sulfoxide (R_f 0.25), and the remainder as thioctic acid (R_f 0.5), with no other radioactive impurity. This buffer solution (1.1 mc.) was used for the biological experiments described elsewhere.

A check on the chromatographic behavior of thioctic acid was made, using spectroscopically pure crystalline acid (1.1 μc./mg.). Three spots invariably appear, with the relative amounts varying with the solvent system used. In butanol-ammonia (*N*/2) solvent, the sulfoxide spot contains 4-8%, the origin contains 5-15% and the thioctic acid spot contains the remainder of the activity. Percentages of both of the derivative compounds increase when the thioctic acid applied to the origin is heated or allowed to stand in air before introduction of the solvent.

Radiation Decomposition.—Samples of thioctic acid have been subjected to γ-irradiation from a Co^{60} source, and to 4.5 Mev. electron-irradiation from a linear accelerator. The extent of decomposition under various conditions is listed in Table I. In order to estimate the rate of decomposition of thioctic-S^{35}_2 acid due to its own radiation, a sample of thioctic acid was irradiated with carbon-14 β-rays, whose average energy (0.05 Mev.) is approximately equal to that of sulfur-35.

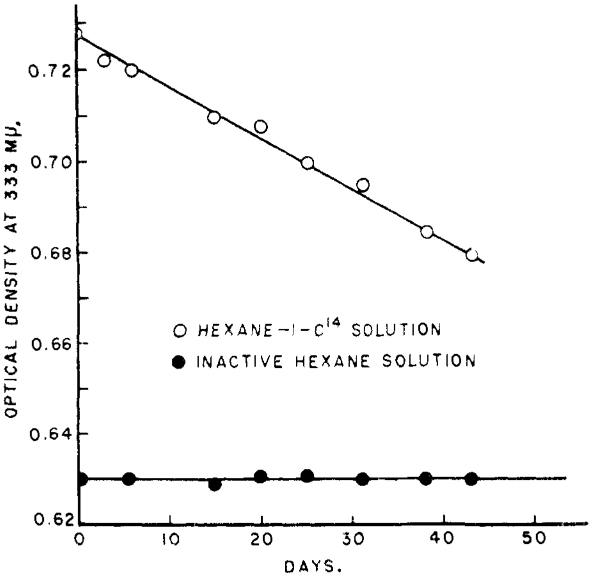


Fig. 1.—Decomposition of thioctic acid by carbon-14 β-rays.

TABLE I

RADIATION DECOMPOSITION OF THIOCTIC ACID

Radiation	Reps. received	Solvent	Concn. of thioctic acid, *M*	% decomposed	G-$(-M)^a$
Co^{60} γ	1.5×10^7	None	....	2.6	9.7
Co^{60} γ	4.0×10^7	None	....	7	9.9
Co^{60} γ	3.0×10^5	Hexane	7.7×10^{-3}	20	9.2
Co^{60} γ	1.7×10^6	Hexane	4.1×10^{-3}	23	9.7
Co^{60} γ	1.7×10^5	Hexane	2.4×10^{-3}	43	10.3
Co^{60} γ	2.7×10^5	Ethanol	5.5×10^{-3}	18	5.4
Co^{60} γ	6.0×10^5	10% Benzene 90% Hexane	5.7×10^{-3}	12	1.8
Co^{60} γ	1.3×10^6	90% Hexane	5.7×10^{-3}	28	1.7
4.3 Mev. e^-	7.1×10^7	None	....	19	15.3
C^{14} β	1.8×10^4	Hexane	4.9×10^{-3}	6.6	31.0

[a] The rate of decomposition was calculated for crystalline thioctic acid as $G(-M) = \frac{\text{molecules decomposed}}{\text{100 e.v. absorbed}}$; for soln., as $G(-M) = \frac{\text{molecules solute decomposed.}}{\text{100 e.v. absorbed by total soln.}}$

The carbon-14 irradiation was accomplished by dissolving 2.4 mg. of thioctic acid in 2.4 ml. of *n*-hexane-1-C^{14} (90 μc./ml.) in a tightly stoppered Beckman spectrophotometer cell (the air volume above the solution was 0.6 ml.). The absorption at 333 mμ was determined every few days for 43 days (see Fig. 1). During the same period of time, a non-radioactive sample (stored and treated in a manner identical to that for the radioactive solution) showed no change in its ultraviolet spectrum. At the end of 43 days, an aliquot portion of the hexane solution was evaporated to dryness. Only traces of radioactivity remained with the non-volatile fraction. This non-volatile radioactivity corresponds to one molecule of hexane for eight molecules of thioctic acid decomposed. Since it represents less than one part in 10^6 of the original hexane activity, it may arise from adsorption of hexane on the thioctic acid residue.

Discussion

Since the *G* values for the decomposition of thioctic acid under γ-irradiation in hexane solutions do not change with varying concentration, we can conclude that almost all of the energy which causes the destruction of the thioctic acid has first been absorbed in the hexane. The $G(-M)$ value for crystalline thioctic acid is the same as for thioctic acid in hexane solution. If we assume that the energy required for rupture of the sulfur-sulfur bond is the same in the crystalline state as it is in hexane solution, we can infer that the energy absorbed by the hexane is quantitatively transferred to the thioctic acid for this rupture. Since no appreciable radioactivity from hexane-1-C^{14} was found associated with thioctic acid after irradiation, the process of decomposition of the solute must be one of energy transfer, rather than of reaction of an activated hexane with the thioctic acid molecule.

When 10% benzene is added to the solution, the *G* value is decreased from approximately 10 to 1.8. There is apparently a competition for radiation energy absorbed in the system between thioctic acid and benzene (present in approximately 200-fold mole excess over the thioctic acid). This decreased rate of decomposition shows that a very real advantage could be gained by storing radioactive thioctic acid in solutions containing benzene.

The self-decomposition rate of sulfur-35-labeled thioctic acid, either in the crystalline state or in solution in hexane, should be similar to that of the thioctic acid in carbon-14-labeled hexane. Therefore the $G(-M)$ for self-decomposition of the thioctic-S^{35}_2 acid should be approximately 30. A decomposition rate of 0.3% per day would then be expected for the high specific activity product, which produces 3×10^{16} e.v./day/mg. This rate is obviously too low to explain the difficulties encountered in crystallizing the product from the 100 mc. preparation, since visible amounts of hexane-insoluble oil were formed from a clear hexane solution during one day's standing. It is possible that some impurity, formed in the synthesis, and originally hexane soluble, is highly sensitive to radiation. Polymerization of this impurity during the periods of standing in hexane would inhibit crystallization of the thioctic acid and would explain the appearance of the insoluble oil.

BERKELEY, CALIFORNIA

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF NORTHWESTERN UNIVERSITY]

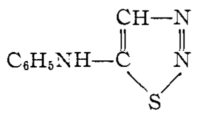
On Acylhydrazones and 1,2,3-Thiadiazoles

BY CHARLES D. HURD AND RAYMOND I. MORI

RECEIVED MAY 19, 1955

The preparation of carbethoxyhydrazones, acetylhydrazones and phenylsulfonylhydrazones of ketones and α-keto acids proceeds with good yields. The behavior of these hydrazones toward thionyl chloride was such that when there was a methylene group next to the hydrazono group then light-sensitive 1,2,3-thiadiazoles were formed in good yields. A mechanism is presented which explains their formation and also the formation of the intermediates which were isolated.

Two methods are known at present for the synthesis of monocyclic 1,2,3-thiadiazoles. One involves reaction of diazomethane and an isothiocyanate such as phenyl isothiocyanate, which yields 5-anilino-1,2,3-thiadiazole[1] (I). The other involves reaction of 1,2,3-oxadiazoles with ammonium hydrosulfide. The oxadiazoles are known also as diazoanhydrides[2] or α-keto diazo compounds[3] and are prepared by diazotization of α-amino ketones. Using this method, Wolff was able to synthesize 1,2,3-thiadiazole-4-carboxylic acid[4] (II). It is obvious that neither of these methods is well suited for synthetical purposes.



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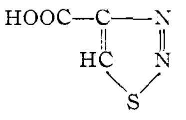
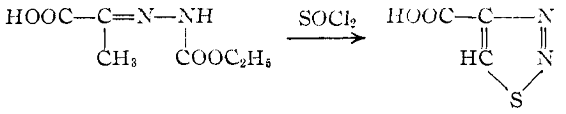


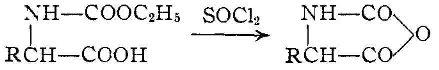
II

In the work to be described it will be shown that good yields of 1,2,3-thiadiazoles are obtainable in an unexpected way by interaction of thionyl chloride and acylhydrazones of the general structure $RCH_2CR'{=}N{-}NX$, wherein R′ = COOH, C_6H_5, etc., and X = $COOC_2H_5$, $COCH_3$, $SO_2C_6H_5$, etc. The following synthesis of II is illustrative, the yield being 53%.



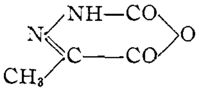
III II

The half ester III may be compared with the half ester of an azasuccinic acid (IV) which is known[5] to yield the acid anhydride on reaction with thionyl chloride

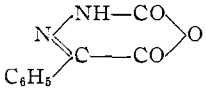


IV

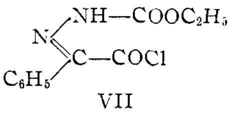
Anhydride V would be the expected product if III had behaved analogously. Some of it was indeed formed but its quantity was small. The comparable phenyl compound VI was produced in about 90% yield, however, and the acyl chloride VII was shown to be isolable as an intermediate.



V



VI



VII

No thiadiazole could result in the phenyl series because no methylene group was present in the original half ester. Evidently the presence of a methylene group adjacent to a hydrazono group favors thiadiazole formation in the reaction with thionyl chloride, rather than production of the cyclic acid anhydride.

III was prepared from pyruvic acid in quantitative yield by reaction with carbethoxyhydrazine: $CH_3COCOOH + NH_2NHCOOC_2H_5 \rightarrow III + H_2O$. The phenyl analog was prepared similarly from phenylglyoxylic acid.

A sample of 1,2,3-thiadiazole-4-carboxylic acid (II), prepared in powdered potassium bromide, was placed in a Baird double beam spectrophotometer, and these major bands were found in the infrared spectrum (medium intensity unless noted as weak (w) or strong (s)): 2.95 w, 3.25–4.00, 5.21 w, 5.98 s, 6.78 s, 7.14 s, 7.56, 8.20 s, 9.15, 10.25 s, 10.75, 11.20, 11.45, 12.55, 12.84, 13.80 μ. The assignments for carboxylic acid are 3.25–4.00, 5.98, 7.14, 8.20. Hence, the carboxylic acid structure for II is supported rather than a dipolar structure with a carboxylate ion.

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(1) H. Pechmann and A. Nold, *Ber.*, **29**, 2588 (1896).

(2) L. Wolff, H. Kopitzsch and A. Hall, *Ann.*, **333**, 1 (1904).

(3) H. Staudinger and J. Seigwart, *Ber.*, **49**, 1918 (1916).

(4) L. Wolff and co-workers, *Ann.*, **325**, 129 (1902).

(5) H. Leuchs, *Ber.*, **39**, 857 (1906); C. Hurd and C. Buess, THIS JOURNAL, **73**, 2409 (1951).