UCRL 2949

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THIOCTIC – S_2^{32} ACID : SYNTHESIS AND RADIATION DECOMPOSITION

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UCRL-2949
Unclassified Chemistry

UNIVERSITY OF CALIFORNIA
Radiation Laboratory
Berkeley, California

Contract No. W-7405-eng-48

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April 8, 1955

Printed for the U.S. Atomic Energy Commission

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ABSTRACT

Thioctic-S^{35}_2 acid has been prepared with a specific activity of 1 μc/mg in 10% to 15% yield from amorphous sulfur-35. In a preparation using 200 mc of S^{35} (specific activity of product, 200 μc/mg) no crystalline product could be isolated. Preliminary investigation into the extent of the radiation decomposition of thioctic acid has been made.

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The cyclic disulfide of 6,8-dithiooctanoic acid (α-lipoic acid, 6-thioctic acid) is a compound of great biological interest. Since it is present in animal and plant tissues in minute concentration, classical analytical methods are not sensitive enough to follow its behavior in the biological system. Analysis for biological activity with a S. faecalis growth assay[1] is sensitive to very small quantities of the acid, but is time-consuming and relatively inaccurate.

The preparation of labeled thioctic acid provides a sensitive analytical tool which enables one to follow, with great accuracy, not only thioctic acid but also any of its conjugates in the biological system. Attempts to label the molecule with carbon-14 have so far proven unsatisfactory. In any scheme of synthesis now known, the isotopic carbon must be introduced early in the sequence of reactions. The many subsequent steps result in very poor yields with regard to the label. In addition, these subsequent steps require purification of high-boiling, rather unstable intermediates, difficult to handle on the small scale necessary for isotopic synthesis.

The synthesis of thioctic acid reported by Reed and Niu[2] presents a method of introducing radioactivity into the thioctic acid molecule through the use of S^{35}. This method is well suited to the use of isotopic sulfur because the required intermediate, α-toluenethiol, may be prepared in good yield from the commercially available amorphous S^{35}, the sulfur is introduced into the molecule quite late in the synthetic scheme, and the subsequent steps proceed in good yield even on a small scale.

By the following series of reactions, thioctic-$S^{35}{}_2$ acid has been prepared on a 2.5-millimole scale from amorphous S^{35}:

$$S^* + C_6H_5CH_2MgCl \longrightarrow C_6H_5CH_2S^*MgCl \xrightarrow{H_2O} C_6H_5CH_2S^*H$$

$$\xrightarrow{NaOC_2H_5} C_6H_5CH_2S^*Na;$$

$$CH_2Br\,CH_2CHBr(CH_2)_4COOC_2H_5 \xrightarrow[2)\ KOH]{1)\ 2\,C_6H_5CH_2S^*Na}$$

$$C_6H_5CH_2S^*CH_2CH_2CH_2\underset{\underset{S^*CH_2C_6H_5}{|}}{CH}(CH_2)_4COOH;$$

$$\xrightarrow[liq.\ NH_3]{Na} HS^*CH_2CH_2\underset{\underset{S^*H}{|}}{CH}(CH_2)_4COOH \xrightarrow[FeCl_3]{O_2} \underset{\underset{S^*}{|}}{CH_2}CH_2\underset{\underset{S^*}{|}}{CH}(CH_2)_4COOH.$$

$$S^* - S^*$$

Three preparations of thioctic-S^{35}_2 acid (specific activity of 1 μc/mg) were completed according to the experimental procedure detailed below, with over-all yields of 8%, 10%, and 16%. In a very high-specific-activity preparation, no crystalline product could be isolated. It is believed that radiation damage to the product during its preparation was responsible for this anomalous behavior.

EXPERIMENTAL

α-Toluenethiol-S^{35}

An ethereal solution of benzylmagnesium chloride (10 millimoles in 25 ml of ether) was added with stirring to a slurry of 160 mg (5 millimoles) 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 millimoles (85%). Attempts to isolate this thiol through distillation resulted in loss of product through oxidation to benzyldisulfide.

6, 8-Dibenzylmercapto-S^{35}_2-octanoic Acid

The ethereal thiol solution was heated at 80° under a nitrogen atmosphere until the volume was reduced 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-dibromooctanoate (738 mg, 2.2 millimoles) in 10 ml of ethanol, followed by 102 mg (4.4 millimoles) 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}. Crystallization of the acid at this point resulted in loss of product and did not improve the yield on subsequent steps. Consequently 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 pH 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 its specific activity was

1.12 μc/mg (theory, 1.0 μc/mg). The compound showed the characteristic ultraviolet absorption spectrum for the 5-membered disulfide ring with λ_{min}: 280 mμ; λ_{max}: 333 mμ; ϵ_{max}: 149.

Thioctic-$S^{35}{}_2$ Acid: High Specific Activity

In order to obtain material more useful for biological experiments, a synthesis utilizing 200 mc S^{35} (specific activity of product = 200 μc/mg) was undertaken. The yield of α-toluenethiol was 88%. The crude dibenzylmercaptooctanoic acid contained 150 mc (75%). Sodium ammonia reduction, followed by oxygen oxidation to the disulfide, proceeded smoothly to 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 solution of the radioactive thioctic acid was allowed to cool for several hours. A large percentage of the oily product that separated from the cool hexane would not redissolve in hot hexane. With each attempt at crystallization, more of this insoluble oil was formed. Since these crystallization attempts were identical to those successfully employed in isolating the less radioactive acid, it was concluded that radiation from the S^{35} was destroying the product in solution so rapidly that crystallization was impossible.

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 several crystallization attempts were extracted with 4 small portions of hot hexane, leaving behind a large insoluble residue. 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 (pH 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 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 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% to 8%, the origin contains 5% to 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 of Thioctic Acid

Samples of thioctic acid have been subjected to γ-irradiation from a Co^{60} source. The rate of decomposition for crystalline thioctic acid is quite normal for organic compounds: 2.6% decomposition for 1.5×10^7 rep absorbed, 7% decomposition for 4×10^7 rep;

$G = \frac{\text{molecules decomposed}}{\text{100 ev absorbed}} = 10$. For a 7.7×10^{-3} M solution of thioctic acid in hexane, absorption of 3×10^5 rep resulted in 20% decomposition of the acid; $G = \frac{\text{molecules solute decomposed}}{\text{100 ev absorbed by total solution}} = 9$. The near identity of these two G values indicates that almost all of the energy absorbed in the hexane solution is used for the decomposition of the thioctic acid.

With the S^{35} β-radiation producing 3×10^{16} ev/day/mg of the high-specific-activity thioctic acid, and using the value of G = 10 from the γ-irradiation experiment, we calculate a thioctic acid decomposition rate of 0.1%/day. The self-decomposition rate appears to be greater than this since visible amounts of hexane-insoluble oil were formed during one day's standing, and since 0.1%/day impurity formation would not inhibit crystallization of the thioctic acid.

This work was done under the auspices of the U.S. Atomic Energy Commission.

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2. L. J. Reed and C. Niu, J. Am. Chem. Soc. 77, 416 (1955). See also Patricia T. Adams, Chemistry Division Quarterly Report, Sept.-Oct. 1954, UCRL-2841, January 1955, p. 25.