Study of the Dithiobiuret-Benzaldehyde Condensation Product

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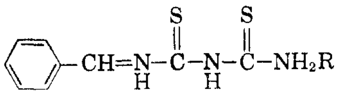
It was previously reported by Foye and Hefferen[2] that compounds of the Tibione type, *p*-AcNHC$_6$H$_4$-CH=NNHCSNH$_2$, could be prepared by the condensation of *para*-substituted benzaldehydes with dithiobiuret.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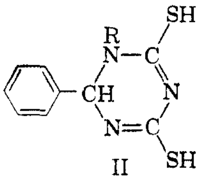
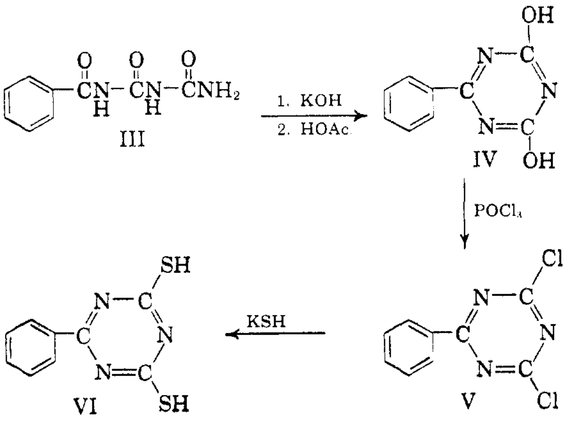

II

However, Fairfull and Peak[3] recently proved that the products of the reaction between aromatic aldehydes and substituted dithiobiurets were not benzaldithiobiurets, I, as indicated by Foye and Hefferen,[2] but instead the cyclized compound having an *s*-triazine structure, II. They further stated the unsubstituted biuret should behave in a similar manner but they made no attempt to prove this.

Because Fairfull and Peak[3] did not prove the structure of the condensation of benzaldehyde with unsubstituted dithiobiuret and because other groups had postulated the benzal dithiobiuret structure,[2,4] it was felt there was a reasonable doubt as to the structure of the product. The condensation product of benzaldehyde with dithiobiuret was studied and proved conclusively to be 2,4-dimercapto-6-phenyldihydro-*s*-triazine, II (R = H). The approach was to synthesize the known 2,4-dimercapto-6-phenyl-*s*-triazine, VI, by condensing benzoyl chloride with urea to give 1-benzoylbiuret, III.[5] This compound was then treated with potassium hydroxide to afford 2,4-dihydroxy-6-phenyl-*s*-triazine, IV,[5] which on treatment with phosphorous oxychloride gave 2,4-dichloro-6-phenyl-*s*-triazine, VI.[6] The desired product could then be obtained by treating the dichloro compound with potassium hydrosulfide. This yielded compound VI.



The product obtained from the reaction of benzaldehyde with dithiobiuret was then oxidized and proved to be identical with the known compound, VI, by mixed melting point.

The infrared spectra of 2,4-dimercapto-6-phenyl-*s*-triazine, VI, and the product obtained by the alkaline potassium ferricyanide oxidation of the condensation product were identical when run in KBr disks. These data indicate the dithiobiuret-benzaldehyde condensation product to be 2,4-dimercapto-6-phenyldihydro-*s*-triazine, II, (R = H).

EXPERIMENTAL

1-Benzoylbiuret. Essentially the method of Bloch and Sobotka was used involving the reaction of benzoyl chloride and urea. A white crystalline solid was obtained, m.p. 229–230° dec. (lit. 224–225°).[5]

2,4-Dihydroxy-6-phenyl-s-triazine. IV. This compound was prepared by Bloch and Sobotka by treating 1-benzoylbiuret with aqueous potassium hydroxide. The authors obtained a 74% yield, m.p. 299–300° dec. (lit. 297–300°).[5]

2,4-Dichloro-6-phenyl-s-triazine, V, was prepared by the method of Fairfull and Peak in 65% yield, m.p. 120–121° (lit. 119–120°).[3]

2,4-Dimercapto-6-phenyl-s-triazine, VI, was prepared by the method of Fairfull and Peak in 66% yield, m.p. 244–245° dec. (lit. 248–249°),[3] $\lambda_{\max}^{\mathrm{EtOH}}$ 235 (39,600), $\epsilon_{\max}$ 39,600.

2,4-Dimercapto-6-phenyldihydro-s-triazine. To 135.0 g. (1.00 mole) of dithiobiuret, dissolved in 3 l. of hot glacial acetic acid, was added 170.0 g. (1.60 moles) of benzaldehyde. The mixture was refluxed for 12 hr. concentrated under reduced pressure to 1.5 l., and cooled to 0°. The yellow, crystalline solid was removed, dissolved in sodium hydroxide solution, filtered, reprecipitated with acetic acid, and recrystallized from absolute ethanol to give 84.0 g. (38%) of the condensation product, m.p. 235–236° dec. (lit. 236–238°)[3] $\lambda_{\max}^{\mathrm{EtOH}}$ 275, 298, $\epsilon_{\max}$ 20,200; 21,500.

Anal. Calcd. for C$_9$H$_9$N$_3$S$_2$: C, 48.40%; H, 4.06; N, 18.82; S, 28.72. Found: C, 48.85; H, 3.56; N, 18.60; S, 28.31.

Oxidation of 2,4-dimercapto-6-phenyldihydro-s-triazine to 2,4-dimercapto-6-phenyl-s-triazine. One gram (0.0045 mole) of 2,4-dimercapto-6-phenyldihydro-*s*-triazine was dissolved in a minimum amount of 5% sodium hydroxide. A solution of potassium ferricyanide (2.96 g. of potassium ferricyanide in 10.0 ml. of water) was added dropwise to the above solution at room temperature with constant agitation. After addition was complete the mixture was filtered and acidified with acetic acid to afford a yellow material. This material was washed with water and mixed with 15.0 ml. of dimethyl formamide. The resulting slurry was filtered and the yellow solution was chromatographed on a chloroform-silicic acid column. It was eluted with pure chloroform to give a pure product which on crystallization from water-ethanol (9:1) gave long thin needles, 0.12 g. (12%) m.p. 244–245° dec. (lit. 248–249° dec.).[3]

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(1) In partial fulfillment for the Master of Science Degree, University of Wisconsin.

(2) W. O. Foye and J. J. Hefferen, *J. Am. Pharm. Assoc.*, **42**, 31 (1953).

(3) A. E. S. Fairfull and D. A. Peak, *J. Chem. Soc.*, 803 (1955).

(4) A. Claus, *J. prakt. Chem.*, **47**, 135 (1893).

(5) E. Bloch and H. Sobotka, *J. Am. Chem. Soc.*, **60**, 1656 (1938).

(6) A. Ostrogovich, *Chem. Ztg.*, **36**, 739 (1912).

Anal. Calcd. for $C_9H_7N_3S_2$: C, 48.84; H, 3.19; N, 18.99. Found: C, 48.51; H, 2.92; N, 18.53.

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Nitrous Acid Oxidation of Triacyl Pyridoxamine[1]

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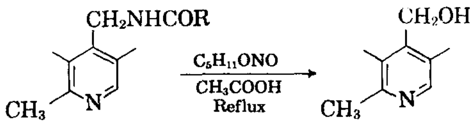
Based on elemental analysis, it appeared probable that the nitrous acid oxidation product (MDP) of *N,O,O*-tripalmitoylpyridoxamine was a mixture of mono- and di-palmitoylpyridoxine.[2] Since this reaction represented direct conversion of the acylated amino to a hydroxyl group, further studies were carried out with various acyl derivatives of pyridoxamine.

Several workers have synthesized *N*-nitroso derivatives of acylated primary amines and their reactions have been investigated.[3] Heyns *et al.* observed that the nitroso derivatives of *N*-*n*-propylacetamide, *N*-*n*-butylacetamide, and *N*-benzylacetamide could be thermally decomposed to form acetates of the corresponding alcohols.[4]

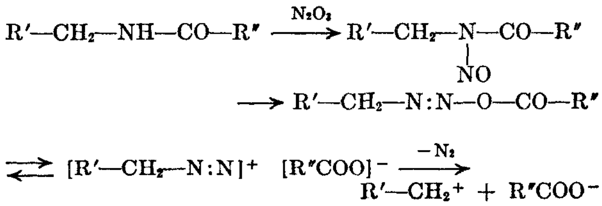
The transformation of free pyridoxamine to pyridoxine under mild treatment with nitrous acid has been reported.[5,6] The test compounds used were triacetyl-, tridecanoyl, tripalmitoyl-, tribenzoyl- and tri-*p*-nitrobenzoyl-pyridoxamine. When the acyl pyridoxamine was refluxed in a mixture of isoamyl nitrite and glacial acetic acid (1:2 v/v) for 30–60 *min.*, complete replacement of the acylated amino group with a hydroxyl group took place. Evidence for this conversion has been obtained by paper chromatography and microbiological assay using a minute quantity of the test compound.

The triacylpyridoxamine was hydrolyzed with 2*N* ethanolic potassium hydroxide by refluxing for 30 min. The resulting hydrolysate contained two fractions detectable on the papergram with *N*, 2,6-trichloro-*p*-quinoneimine. One was free pyridoxamine and the other was a high R_f fraction, which was probably the corresponding *N*-monoacylpyridoxamine (Table 1). The ester linkages at the 3- and 5-positions must have been cleaved during the alkali treatment, since complete hydrolysis of various *O,O,O*-triacylpyridoxines resulted under identical conditions. If the triacylpyridoxamine was treated with nitrous acid prior to alkali hydrolysis, the only vitamin B_6 component present in the final hydrolysate was found to be pyridoxine (Table 1). Oxidation with nitrous acid under milder conditions gave a mixture of pyridoxamine, pyridoxine, and a high R_f fraction (Table 1). In all cases, pyridoxal was found to be absent.



The theoretical amount of pyridoxine moiety in MDP was calculated from the nitrogen analysis. A portion of MDP was also subjected to microbiological assay. The two values thus obtained were in complete agreement indicating that the nitrogen present in MDP accounted for all of the nitrogen in the pyridoxine molecule. By paper chromatography, it also became clear that MDP contained pyridoxine free from pyridoxamine. However, free pyridoxine, pyridoxal, pyridoxamine as well as the pyridoxamine moiety present in the alkali hydrolysate of tridecanoylpyridoxamine[7] was totally destroyed by refluxing in a mixture of isoamyl nitrite and acetic acid for one hour. This was shown by color test with *N*, 2,6-trichloro-*p*-quinoneimine and by microbiological assay. Protection of one or both of the 3-hydroxyl and 5-hydroxymethyl groups in vitamin B_6, therefore, appeared to be essential in order to prevent destruction of the vitamin fragment. When 3-monopalmitoylpyridoxal[2] or 5-monopalmitoylpyridoxine[2] was similarly treated, the vitamin B_6 moiety withstood the nitrous acid treatment.

The following equations may be of value to explain the conversion of the acylated amino group to



$$R'{-}CH_2{-}NH{-}CO{-}R'' \xrightarrow{N_2O_3} R'{-}CH_2{-}\underset{\displaystyle NO}{N}{-}CO{-}R''$$

$$\longrightarrow R'{-}CH_2{-}N{:}N{-}O{-}CO{-}R''$$

$$\rightleftarrows [R'{-}CH_2{-}N{:}N]^+ \ [R''COO]^- \xrightarrow{-N_2} R'{-}CH_2^+ + R''COO^-$$

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(3) (a) H. v. Pechmann and L. Frobenius, *Ber.*, **27**, 651 (1894); (b) E. Bamberger, *Ber.*, **27**, 914 (1894); (c) G. F. D'Alelio and E. E. Reid, *J. Am. Chem. Soc.*, **59**, 109 (1937); (d) Von R. Husigen and J. Reinertshofer, *Z. Naturforsch.*, **6b**, 395 (1951); (e) K. Heyns and O. Woyrsch, *Ber.*, **86**, 76 (1953); (f) Von R. Husigen and H. Nakaten, *Ann.*, **586**, 84 (1954).

(4) K. Heyns and W. V. Bebenburg, *Ber.*, **86**, 278 (1953).

(5) S. A. Harris, D. Heyl, and K. Folkers, *J. Am. Chem. Soc.*, **66**, 2088 (1944).

(6) E. E. Snell, *J. Biol. Chem.*, **157**, 491 (1945).

(7) Tridecanoyl pyridoxamine was refluxed in 2*N* ethanolic potassium hydroxide for 30 min. After neutralization with ethanolic hydrogen chloride, the precipitate of potassium chloride was removed by filtration and the solvent was evaporated until dryness *in vacuo*. The residue was then used for the nitrous acid oxidation.