II) as detected by DTNB[7] (5,5′-dithiobis-(2-nitrobenzoic acid)), or nitroprusside.[8] DPNH will not substitute for TPNH. Approximately two moles (1.7 to 2.2 corrected) of —SH appear per mole of TPNH oxidized by PrSS. Successive preparations of PrSS show 40 to 60 mμmoles of enzymatically reduced —SH per mg. of PrSS. We have not attained the expected value of 200 mμmoles sulfhydryl per mg. of PrSS (assumed mol. wt. 10,000) owing possibly to equilibrium considerations or its ready polymerization as evidenced by loss of activity in concentrated solutions. These results suggest a

TABLE II

STOICHIOMETRY OF PrSS REDUCTION

Treatment	TPNH oxidized,[a] (mμmoles)	–SH formed,[b] (mμmoles)
Complete[c]	25.1	37.5
Minus PrSS	3.2	0.7
Minus TPNH	0.0	1.0
Minus FAD	12.9	22.0
Boiled enzyme fraction A-1	0.0	−1.4

[a] TPNH was determined by its decrease in optical density at 340 mμ during 22 minutes of anaerobic incubation at 25°. [b] –SH was determined on separate aliquots of each reaction mixture after zero and 22 minutes of anaerobic incubation by the addition of 0.2 μmole of DTNB. [c] Complete reaction mixture per ml.: Tris, *p*H 8.0, 50 μmoles; $MgCl_2$, 5 μmoles; TPNH, 120 mμmoles; FAD, 6 mμmoles; PrSS, 0.9 mg.; Fraction A-1, 0.043 mg. protein. Fraction A-1 was obtained by chromatography of Fraction A on diethylaminoethylcellulose, treatment with Cγ gel, elution with phosphate buffer, and reprecipitation with $(NH_4)_2SO_4$.

$$\text{PrSS} + \text{TPNH} + \text{H}^+ \xrightleftharpoons{\text{Fraction A}} \text{Pr(SH)}_2 + \text{TPN}^+ \tag{1}$$

$$\text{PAPS} + \text{Pr(SH)}_2 \xrightleftharpoons{\text{Fraction B}} \text{SO}_3^{=} + \text{PAP} + \text{PrSS} \tag{2}$$

mechanism for PAPS reduction: Hilz[9] has postulated a similar sequence involving lipoic acid. However, analyses performed by Mr. J. Matthews and Dr. L. J. Reed show less than 0.001 μg. of lipoic acid per mg. of PrSS.

Fraction A also will act as a diaphorase, coupling to ferricyanide, and this reaction is accelerated by flavin adeninedinucleotide. Our reductase system resembles in this respect, as well as in the involvement of disulfide, the α-ketoglutarate, pyruvate and aldehyde dehydrogenases.[10,11,12,13]

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TETRAHYDRO-3,4-FURANDIONE. I. PREPARATION AND PROPERTIES

Sir:

During a study of the reactivity of α diones it became desirable to have available tetrahydro-3,4-furandione I (THFD). Since a method for preparation of I could not be found in chemical literature synthesis of the cyclic dione was attempted. Recently this has been achieved.

1,4-Dibromo-2,3-butandione[1] II with triethyl orthoformate and sulfuric acid affords the monoketal III, m.p. 40.5°, $C_8H_{14}O_3Br_2$. Found: C, 30.31; H, 4.51; λ_{max} 298 mμ ($\epsilon = 57$). In the infrared C═O band 1755 cm.$^{-1}$. Sodium methoxide in methanol converts III into 2-methoxy-3,3-diethoxy-4-bromobutene-1-oxide IV.[2] Acidification with CH_3OSO_3H yields 1-bromo-2,2-diethoxy-3,3-dimethoxy-4-butanol V.[2] Cyclization with KOH forms 3,3-dimethoxy-4,4-diethoxy THF VI, b.p. 53° (0.10 mm.), $n^{25}D$ 1.4382, found for $C_{10}H_{20}O_5$: C, 54.22; H, 9.35. Through II with trimethylorthoformate and III with sodium ethoxide in ethanol the 3,3-4,4-tetramethoxy and the tetraethoxy analogs of VI have been obtained. In water sulfur dioxide removes both ketal groups of VI and forms a monoadduct of THFD, VII. Ethanol with a few per cent. of water and lead carbonate replaces the SO_3H group with OC_2H_5 to give the monohemiketal of THFD, VIII. Distillation at 0.10 mm. dissociates VIII and affords I, yellow prisms or rhombohedra, m.p. 126°; found for $C_4H_4O_3$; C, 48.15; H, 4.02; ultraviolet and infrared C═O band 1780 cm.$^{-1}$, and n.m.r. spectra indicate the lack of enolization.

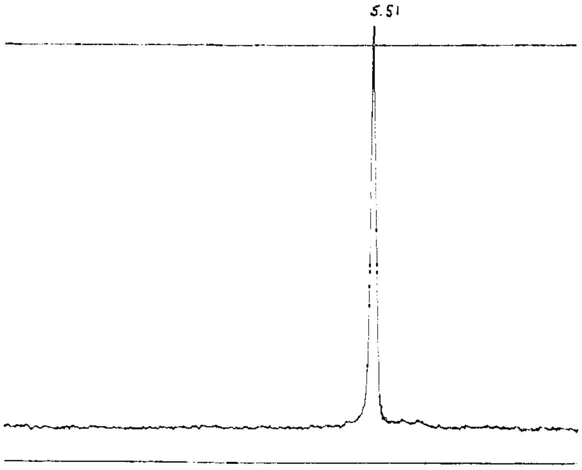

Fig. 1.

The over-all yield from II through VI is about 90%, from VI to I about 70%. THFD forms monohemiketals with primary and secondary alcohols, and with two molecules of water, 3,3-4,4-THF-tetrol IX, m.p. 85°, found for $C_4H_8O_5$; C, 35.33; H, 5.83; sp. gr. 1.578, no absorption in C═O region of ultraviolet or infrared. X-Ray dif-

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fraction indicates a unit cell of 8 molecules, mol. wt. 136. When heated or held under a reduced pressure of 0.05 mm. IX does not lose water and revert to I. Di- and polymeric derivatives are formed. In alcohols one hydroxyl group of IX is replaced with OR. The adjacent ketone no longer can remain hydrated, probably for steric reasons, and VIII is formed. IX and *o*-phenylenediamine give 3,3 4,4-THFD quinoxaline, m.p. 155°, found for $C_{10}H_8N_2O$: C, 69.88; H, 4.61; N, 16.07. With two equivalents of HIO_4 or H_2O_2 and Na_2CO_3 IX yields diglycolic acid.

3,4-Dione analogs of I with carbon or sulfur in place of oxygen in the ring exist largely in enolized form.[3] The mechanism by which I is stabilized in the dione structure is not evident; its disclosure will require further searching investigation. In ultraviolet the λ_{max} of the single C=O band of THFD at 520 mμ closely coincides with the 518 mμ band of its 2,2-5,5-tetramethyl analog.[4]

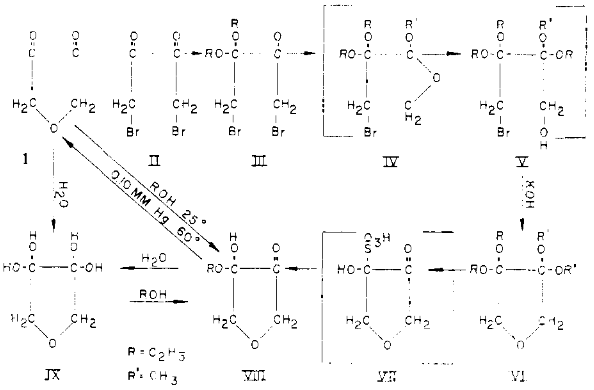

Fig. 2.

The n.m.r., expressed in terms of shielding numbers,[5] was determined in deuteriochloroform on a 60 megacycle Varian Associates instrument with benzene as an external reference. Only a single sharp resonance was observed at 5.51 τ. This indicates that the four protons are equivalent. The position of the resonance is in accord with expectation and the absence of a peak in the olefinic region is strong evidence against any appreciable enolization.

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TETRAHYDRO-3,4-FURANDIONE. II. DIOXOLANE AND DIOXANE DERIVATIVES

Sir:

Synthesis of tetrahydro-3,4-furandione[1] (THFD) has revealed a high degree of stabilization of the heterocycle in the dione form with little or no enolization. The molecule rapidly adds water or alcohol and tends to exist with one or both ketone groups doubly substituted as a tetrol IVb, hydroxydioxane V, hydroxydioxolane VI and VII, or a hemiketal IX.

A small per cent. of water with 0.10 *N* sulfuric acid in acetic acid hydrolyzes one ketal group of THF-3,3-4,4-tetramethoxydiketal[1] I to yield the monoketone-monoketal of THF II, λ_{max} 325 mμ $\epsilon = 40$, C=O band in infrared 1780 cm.$^{-1}$. Water and methanol add to the ketone 78 and 8%, respectively, as indicated by the value of ε. The hydrate or hemiketal III cannot be isolated.

Hydrolysis of the ketal groups of THF-3,3-4,4-tetraethoxydiketal[1] IVa with sulfur dioxide in water and removal of the SO_3H group with lead carbonate affords the dioxane derivative V, slightly soluble in acetone, and the bisdioxolane VI, crystallized from ethyl acetate. Dehydration of V to give VII and hydration of VII to give V are essentially quantitative reactions. Dioxane and dioxolane rings of V, VI, and VII are broken with primary alcohols and water. For VII ε in ethanol is almost twice the value of ε in ethyl acetate.

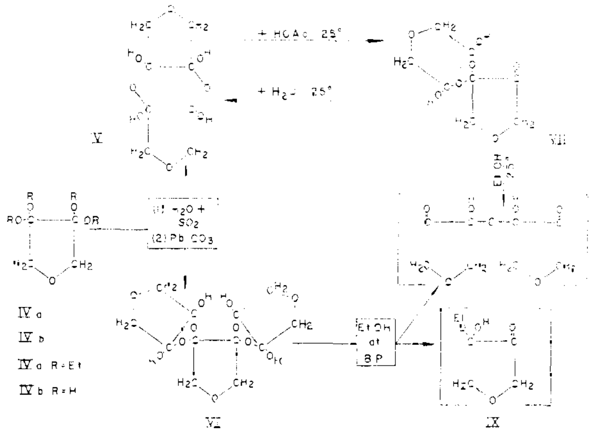

Fig. 1.

V, VI, and VII decompose when heated. The melting points 125°, 205°, and 160° respectively are not sharp. V, found for $C_8H_{12}O_8$: C, 40.60; H, 5.15; strong OH band 3250 cm.$^{-1}$ in infrared; VI, found for $C_{12}H_{16}O_{11}$: C, 42.84; H, 5.15; strong OH band 3333 cm.$^{-1}$ in infrared. VII, found for $C_8H_{10}O_7$: C, 44.04; H, 4.73; C=O band 1777 cm.$^{-1}$ in infrared; ultraviolet λ_{max} 332 mμ, $\epsilon = 31$. VII in acetone with acetic anhydride and sulfuric acid forms a diacetate, m.p. 156–158°, found for $C_{12}H_{14}O_9$: C, 47.61, H, 4.64; infrared C=O band 1785 cm.$^{-1}$. The diacetate of VII does not make a quinoxaline with *o*-phenylenediamine. IVb, V, VI and VII form the same quinoxaline derivative in theoretical yield.[1]

The dioxolane structure of VII, its λ_{max} in ultraviolet, and the value of ε agree with the corre-

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