TABLE I

Reactant concns. in mm.				Product ratios[a]	
cis-2-Butene	*trans*-2-Butene	Diazomethane	Nitrogen	*cis*-1,2-Dimethylcyclopropane	*trans*-1,2-Dimethylcyclopropane
370	0	400	0	100	12
1.1	0	1.6	101	100	75
0.6	0	5.9	560	15	100
0	357	293	0	10[b]	100
0	2.1	5.1	563	18[b]	100

[a] As determined from the relative band areas in the chromatography experiments. These are typical runs; all have been done at least in duplicate. Under all conditions the dimethylcyclopropanes make up approximately 50% of the C_5 fraction, and this fraction is the major one apart from unreacted, *unisomerized* butene. [b] These represent upper limits for *cis*-1,2-dimethylcyclopropane. A correction for the small amount of 2-methyl-2-butene present was not made in these two cases.

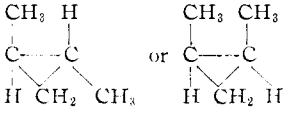
itself in a triplet state. In this case, as pointed out by Skell[3] the final bond formation step to form the cyclopropane ring may proceed only after the intermediate has suffered sufficient collisions to undergo a triplet-to-singlet transition. The life-time of the intermediate in the triplet state is of sufficient duration to allow for its isomerization.[4] Therefore, the reaction may be envisaged as

(1) $CH_2N_2 \xrightarrow{h\nu} N_2 + CH_2^*[\uparrow\downarrow]$ (electronically and vibrationally excited)

(2) $CH_2^*[\uparrow\downarrow] \xrightarrow[\text{numerous collisions}]{N_2} CH_2[\uparrow\uparrow]$

(3) $CH_2[\uparrow\uparrow]$ + *cis*- or *trans*-2-butene $\longrightarrow$ $\dot{C}H_2$—$CH(CH_3)$—$\dot{C}H(CH_3)[\uparrow\uparrow]$

(4) $\dot{C}H_2$—$CH(CH_3)$—$\dot{C}H(CH_3)[\uparrow\uparrow] \xrightarrow{N_2}$



Molecular orbital theory predicts that the methylene molecule if bent should possess a singlet ground state ($^1A_1$) while if it is linear (or nearly so) it should possess a triplet ground state ($^3\Sigma_g^-$).[5]

(4) R. J. Cvetanović [*Can. J. Chem.*, **36**, 623 (1958)] has already observed that the reaction of oxygen atoms in the triplet state with either *cis*- or *trans*-2-butene produces a mixture of the *cis* and *trans* epoxides.

(5) A. D. Walsh, *J. Chem. Soc.*, 2260 (1953).

The present evidence then favors the linear structure for the ground state of methylene.

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YEAST SULFATE REDUCTASE[1]

Sir:

Previous studies demonstrated[2,3] the presence in yeast extracts of enzymes catalyzing the reduction of 3′-phosphoadenosine-5′-phosphosulfate (PAPS) to sulfite. Analogous systems have now been reported for *D. desulfuricans*.[4,5] We have subsequently reported[6] the participation of a heat-stable, non-dialyzable factor.

As shown in Table I, reduction of PAPS to sulfite involves at least two heat-labile fractions and the heat-stable factor (PrSS). PrSS is purified from yeast acetone powder extracts by heat and acid treatments, 96 hour dialysis, Dowex-50 chromatography and paper ionophoresis. It migrates as a pure protein (12% N) on ionophoretograms as detected by brom phenol blue or ninhydrin and is homogeneous in the ultracentrifuge, $S_{20} = 1.2$ for a 1% solution in water. Absorption maxima are at 276 and 325 mμ; the 280/260 ratio is 1.2.

TABLE I

YEAST FRACTIONS REQUIRED FOR SULFITE FORMATION FROM PAPS

Fraction added[a]	Sulfite formed,[b] (mμmoles)
Fraction A + boiled B + PrSS	0.3
Fraction B + boiled A + PrSS	0.2
Fraction A + fraction B + PrSS	7.6
Fraction A + fraction B − PrSS	0.9

[a] Reaction mixture of 0.75 ml. contained in μmoles: Tris, *p*H 7.5, 25; ethylenediaminetetraacetate, 0.65; $MgCl_2$, 2.5; glucose-6-phosphate, 2.5; TPN^+ (oxidized triphosphopyridine nucleotide), 0.31; Na_2SO_3, 2.5; PAPS[35] (1015 cpm/mμmole), 0.50; glucose-6-phosphate dehydrogenase (9.4 units/mg. protein), 0.8 unit; Fraction A, 0.234 mg. protein; Fraction B, 0.070 mg. protein; PrSS, 1.67 mg. Fractions A and B were prepared from extracts of yeast acetone powder treated with 4 volumes of 0.1 *N* H_2SO_4 saturated with $(NH_4)_2SO_4$. After removal of denatured protein from the resuspended precipitate, Fraction A was obtained by precipitation from 0.57 to 0.80 saturated $(NH_4)_2SO_4$. Fraction B was obtained by precipitation from 0.35 to 0.55 saturated $(NH_4)_2SO_4$ and reprecipitated from 15 to 22% ethanol. [b] Sulfite formed in 2 hr. at 37° was determined as previously described.[2]

Incubation of PrSS with Fraction A or a further purified Fraction A-1 and TPNH leads to the oxidation of TPNH and the appearance of —SH (Table

(1) Report of work supported in part by the National Science Foundation. We are indebted to Dr. J. R. Brunner and Mr. M. P. Thompson for ultracentrifuge studies and to Dr. S. Shiftin for detecting the 325 mμ absorbency of PrSS.

(2) L. G. Wilson and R. S. Bandurski, THIS JOURNAL, **80**, 5576 (1958).

(3) H. Hilz and M. Kittler, *Biochem. biophys. acta*, **30**, 650 (1958).

(4) M. Ishimoto, *J. Biochem. (Tokyo)*, **46**, 105 (1959).

(5) H. D. Peck, Jr., *Proc. Nat. Acad. Sci. (U. S.)*, **45**, 701 (1959).

(6) L. G. Wilson, T. Asahi and R. S. Bandurski, *Fed. Proc.*, **19**, 2 (1960).

II) as detected by DTNB[7] (5,5′-dithiobis-(2-nitrobenzoic acid)), or nitroprusside.[8] DPNH will not

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 $(NH_4)_2SO_4$.

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

$$\text{PrSS} + \text{TPNH} + \text{H}^+ \underset{\longleftarrow}{\overset{\text{Fraction A}}{\longrightarrow}} \text{Pr(SH)}_2 + \text{TPN}^+ \quad (1)$$

$$\text{PAPS} + \text{Pr(SH)}_2 \underset{\longleftarrow}{\overset{\text{Fraction B}}{\longrightarrow}} \text{SO}_3^= + \text{PAP} + \text{PrSS} \quad (2)$$

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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(8) R. R. Grunert and P. H. Phillips, *ibid.*, **30**, 217 (1951).
(9) H. Hilz, M. Kittler and G. Knape, *Biochem. Z.*, **332**, 151 (1959).
(10) M. Koike and L. J. Reed, THIS JOURNAL, **81**, 505 (1959).
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(13) V. Massey, *Biochem. et biophys. acta*, **30**, 205 (1958).

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 (ϵ = 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

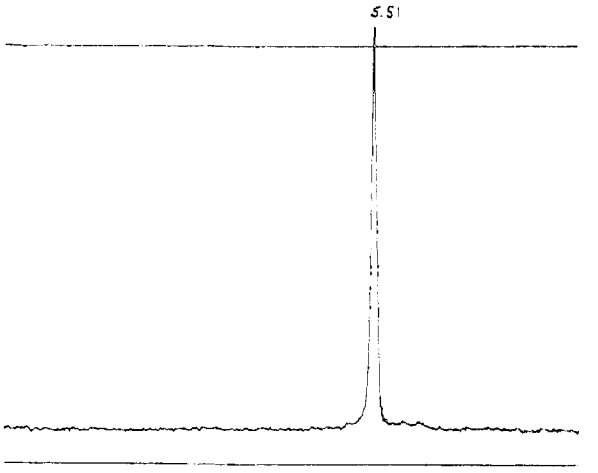

Fig. 1.

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.

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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(2) C. L. Stevens, W. Malik and R. Pratt, THIS JOURNAL, **72**, 4758 (1950); C. L. Stevens, R. L. McLean and A. J. Weinheimer, *ibid.*, **80**, 2276 (1958).