substance to be identical with the product obtained through the acetylation of 1-*O*-mesitoyl-α-D-glucopyranose as described below.

Further elution of the alumina with acetone (500 ml.) gave 434 mg. of sirup. Neither this material nor any of the other fractions from the chromatography crystallized when seeded with 1,3,4,6-tetra-*O*-acetyl-2-*O*-mesitoyl-β-D-glucose.[4]

2,3,4,6-Tetra-*O*-acetyl-1-*O*-mesitoyl-α-D-glucose (VIII) from 1-*O*-Mesitoyl-α-D-glucose (VI).—1-*O*-Mesitoyl-α-D-glucopyranose (50 mg.) was added to a mixture of acetic anhydride (0.12 ml., 8.3 molar equivalents) and pyridine (1 ml.). The mixture was kept overnight at room temperature and then diluted with water and the product extracted with methylene chloride (2 × 10 ml.). The combined extracts were washed successively with 3 *N* sulfuric acid (2 × 10 ml.), saturated aqueous sodium bicarbonate (2 × 10 ml.) and water (2 × 10 ml.). Moisture was removed with sodium sulfate and the solution, after filtration through decolorizing carbon, concentrated *in vacuo* to dryness. From ethanol–pentane the residue yielded 42 mg. (55%) of 2,3,4,6-tetra-*O*-acetyl-1-*O*-mesitoyl-α-D-glucose melting at 119–120° either alone or in admixture with a sample made earlier[1] through the fusion of a mixture of α-D-glucopyranose pentaacetate, mesitoic acid and zinc chloride. The infrared spectra of the samples from the two sources were identical.

2-*O*-Mesitoyl-β-D-glucose (VII) from 1-*O*-Mesitoyl-α-D-glucopyranose (VI).—1-*O*-Mesitoyl-α-D-glucopyranose (102.7 mg.) was dissolved in 10 ml. of methanol and the solution treated with 1 ml. of 3.2 *N* methanolic ammonia. After 23 hr. at 20° the mutarotation had ceased. The solution was then evaporated to dryness and the residue crystallized from ethyl acetate–pentane to give 72 mg. (70%) of 2-*O*-mesitoyl-β-D-glucose melting at 188–190°. Two recrystallizations from ethyl acetate afforded pure material melting at 192–193°.

In earlier work[4] 2-*O*-mesitoyl-β-D-glucose was reported to melt at 180–187° and mutarotate in water (*c* 0.94) +24.0° (13.5 min.) → +45.5° (19 hr.). The sample prepared at that time was later found to melt at 188–190°. After two recrystallizations from ethyl acetate, it gave, with little loss, material melting at 192–193° and rotating $[\alpha]^{20}D$ +19.8° (16 min.) → +45.4° (28 hr., constant) (H_2O, *c* 0.66). This product did not depress the melting point of that obtained as described above through the rearrangement of 1-*O*-mesitoyl-α-D-glucopyranose. The infrared spectra of the two samples were identical.

Anal. Calcd. for $C_{16}H_{22}O_7$: C, 58.88; H, 6.80. Found: C, 58.91; H, 6.52.

Acknowledgment.—Analyses were carried out in the Institutes' Analytical Services Unit under the direction of Dr. W. C. Alford.

BETHESDA 14, MD.

COMMUNICATIONS TO THE EDITOR

CHEMICAL EVIDENCE FOR A TRIPLET GROUND STATE FOR METHYLENE

Sir:

We have found what we believe to be conclusive chemical evidence that the methylene molecule possesses a triplet electronic ground state. Herzberg and Shoosmith[1] already have obtained spectroscopic evidence for a triplet state of methylene. In more recent work,[2] results were obtained which suggest that the flash photolysis of diazomethane yields methylene molecules in highly excited singlet states which are subsequently energetically degraded to the triplet ground state through collisions with inert gas molecules. The most intense spectrum for the triplet state was obtained when a large excess of nitrogen was present. On the other hand, all of the previous evidence based on the observed chemistry of methylene was consistent only with a singlet (not necessarily ground) state for this molecule.[3]

Skell, *et al.*,[3] have found that the 1,2-dimethylcyclopropane formed from methylene and *cis*- or *trans*-2-butene in the gaseous or liquid phase is almost entirely the *cis* or *trans* isomer respectively. These workers felt that this stereospecificity implied a singlet state for methylene under these conditions.

We have repeated the work of Skell and are in full agreement with his conclusions. However, we have extended the study of the photolysis of diazomethane in the gas phase to systems containing only small partial pressures of diazomethane and an alkene in the presence of a large excess of nitrogen. These are the conditions found by Herzberg to be most favorable to the formation of methylene molecules in the triplet ground state. Under these circumstances the stereospecificity of the reaction is entirely lost. In fact, in the reaction with *cis*-2-butene there is a complete reversal of the proportion in which the *cis*- and *trans*-1,2-dimethylcyclopropanes are formed.

It is evident from the data in Table I that the reaction becomes less stereospecific as the amount of nitrogen present during the decomposition is increased. This is most noticeable in the reactions with *cis*-2-butene. Our interpretation of the observations is presented. The methylene molecule when first formed is in a singlet state and thus gives rise to reactions which are almost entirely stereospecific as the two new bonds may be formed simultaneously.[3] When a large excess of nitrogen is present the methylene molecules undergo numerous collisions with the nitrogen molecules before striking an alkene. The collisions with the inert gas degrade the methylene molecules through their excited states to the ground state which in view of the loss of stereospecificity is a triplet. The combination of a methylene molecule in a triplet state ($CH_2[\uparrow\uparrow]$) and a butene molecule must give rise to an intermediate which is

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[1] G. Herzberg and J. Shoosmith, *Nature*, **183**, 1801 (1959).

[2] G. Herzberg, paper presented at the R. A. Welch Foundation, Conference on Chemical Research, Nov., 1959. We are grateful to Dr. Herzberg for a copy of this manuscript prior to its publication and for further unpublished information concerning the conditions which are necessary for the observation of methylene in the triplet state.

[3] R. C. Woodworth and P. S. Skell, THIS JOURNAL, **81**, 3383 (1959): and references therein. In some substituted methylenes there is evidence of triplet states [R. M. Etter, H. S. Skovronek and P. S. Skell, *ibid.*, **81**, 1008 (1959); P. S. Skell and J. Klebe, *ibid.*, **82**, 248 (1960)]. In none of these cases has the ground state been characterized.

TABLE I

Conventional high vacuum line techniques were used to purify and transfer the gases. All manometric measurements were performed with a Bourdon spoon gauge to avoid any contamination with mercury vapor. All of the reactions were carried only a few per cent. to completion; most of the diazomethane decomposed on the walls of the flask. The products of the reaction were separated by vapor-liquid partition chromatography using both silicone oil and silver nitrate in propylene glycol columns. The products were identified (a) by preparing authentic samples and comparing their elution times on the two columns and (b) by infrared analysis of the individual bands obtained by the chromatographic separation. The nature and proportion of the other products formed will be discussed in a future publication.

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.

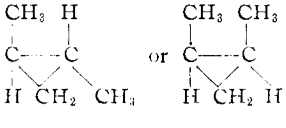
itself in a triplet state. In this case, as pointed out by Skell[3] the final bond formation step to form the cyclopropane ring may proceed only after the intermediate has suffered sufficient collisions to undergo a triplet-to-singlet transition. The life-time of the intermediate in the triplet state 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] The present evidence then favors the linear structure for the ground state of methylene.

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[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).

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 0.57 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

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[1] Report of work supported in part by the National Science Foundation. We are indebted to Dr. J. R. Bronner and Mr. M. P. Thompson for ultracentrifuge studies and to Dr. S. Shifrin 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).