

bonds is indicated by the fact that treatment of chromatophore preparations with reagents known to cleave such bonds converts the preparation quantitatively into serologically univalent fragments. The chromatophore cleavage reaction can be effected either by treating with sulfite and ammoniacal copper in 8*M* urea by the method of Swan³ or by reducing with thioethanol according to White's⁴ procedure used on ribonuclease. If the latter method is used, dialysis of the reaction product against 0.02*M* potassium iodoacetate is required to prevent reformation of the disulfide bonds and consequent regeneration of the precipitable chromatophore. If sulfite or thioethanol is omitted from the reaction mixture, no univalent fragments are obtained. Data from a typical experiment in which the chromatophore preparation was converted to univalent antigen by sulfite cleavage are given in Table I.

TABLE I
INHIBITION OF CHROMATOPHORE PRECIPITIN REACTION BY
SULFITE-TREATED CHROMATOPHORES^a

A chromatophore preparation from *R. rubrum* in 0.01 *M* phosphate pH 7.2 having an O.D. at 880 m μ of 12.5 was treated as described by Pechere, *et al.*,⁵ for 1 hour at 25°. The preparation was dialyzed extensively at 2° against Versene and water to remove the reagents. Precipitin titrations were made using 1-ml. aliquots of a 1:5 dilution of an antiserum to *R. rubrum* chromatophores which had been absorbed previously at the equivalence point with an extract of dark-grown *R. rubrum*.⁶ Aliquots of the antigen preparations, standardized on their bacteriochlorophyll contents, were added to a series of tubes of serum as indicated. The tubes were incubated at 2° for 48 hours, the precipitates were collected by centrifugation, washed, and dissolved in dilute alkali; the bacteriochlorophyll contents were determined spectrophotometrically at 880 m μ . Blank tubes of antigens in saline contained negligible precipitates.

Chromatophores	Antigen preparation added—		Precipitate formed
	Sulfite-treated	Sulfite-omitted	
13	0	0	12
26	0	0	23
52	0	0	27
78	0	0	22
26	30	0	9
26	15	0	13
26	7.5	0	17
26	3.7	0	21
0	7.5	0	0
0	15	0	1
0	30	0	1
26	0	26	19
26	0	13	21
26	0	7	19
26	0	3	22
0	0	26	12
0	0	13	6

^a All data are millimicromoles of bacteriochlorophyll.

The sulfite-treated chromatophore preparation becomes a competitive inhibitor of chromatophore precipitation, although it can no longer form a precipitate with the serum. That the inhibition is specific for chromatophore antibodies is indi-

cated by the absence of any effect on unrelated immune systems. Furthermore, the relative inhibition is less in the region of antibody excess, as is characteristic of hapten inhibition.

These and other⁶ data show that *R. rubrum* elaborates unique antigenic components on its chromatophores during photosynthetic growth and that the antigenic groups formed are placed between disulfide bridges in the chromatophore. Rupture of the bridges, which are presumably of the "interchain" type such as that present in insulin, separates the antigenic sites so that lattice formation with bivalent antibodies can no longer occur.

A metabolic generation of antigen fragments from *R. rubrum* chromatophores was previously described.⁶ The present results provide a chemical basis for such antigen fragmentation. Evidence for separation of univalent fragments of rabbit antibody by disulfide bond cleavage has been reported recently,⁷ and these two observations taken together may have some bearing on the chemical mechanism of antibody synthesis.

Bergeron⁸ proposed a model of the chromatophore in which the subunits were held together by disulfide bonding. The present results provide direct experimental support for this concept, as well as for the existence of specific antigen complexes covalently bonded together as a repeating substructure in a photochemical unit. Characterization of these subunits is in progress.

(7) A. Nisonoff, *et al.*, *Biochem. Biophys. Research Commun.*, **1**, 318 (1959).

(8) J. Bergeron, in "The Photochemical Apparatus," Brookhaven Symposium in Biology No. 11, Office of Technical Services, Department of Commerce, Washington, D. C., 1959.

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1,5-HYDROGEN SHIFT IN A DECAHYDRODIMETHANONAPHTHALENE SYSTEM¹ Sir:

Since the unsaturated alcohol I was available from the study of the octahydrodimethanonaphthyl non-classical homocyclopropenyl cation² II also, this made available the saturated alcohol III-OH, m.p. 124–126°, by hydrogenation over palladium on charcoal. Since the simple 7-norbornyl system³ is exceedingly slow to ionize and structures such as III possess unique hydrogen congestion,⁴ system III is an instructive one for the study of anchimeric effects of 5-hydrogen in carbonium ion reactions.

While bromobenzenesulfonate⁵ III-OBs, m.p.

(1) Research sponsored by the Office of Ordnance Research, U. S. Army.

(2) S. Winstein and R. L. Hansen, *Tetrahedron Letters*, in press.

(3) (a) S. Winstein, M. Shatavsky, C. Norton and R. B. Woodward, *THIS JOURNAL*, **77**, 4183 (1955); (b) C. J. Norton, Thesis, Harvard University, 1955.

(4) L. de Vries and S. Winstein, *THIS JOURNAL*, **82**, 5363 (1960).

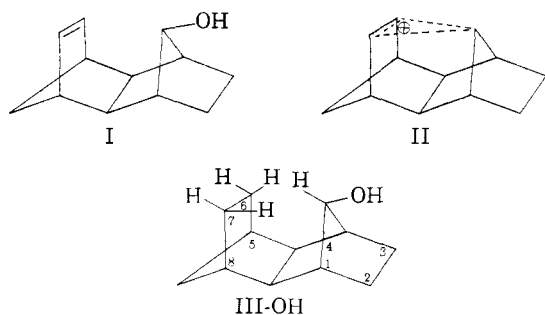
(5) This compound, as well as the other indicated materials, had a satisfactory C,H analysis.

(3) J. M. Swan, *Nature*, **180**, 643 (1957).

(4) F. H. White, *J. Biol. Chem.*, **235**, 383 (1960).

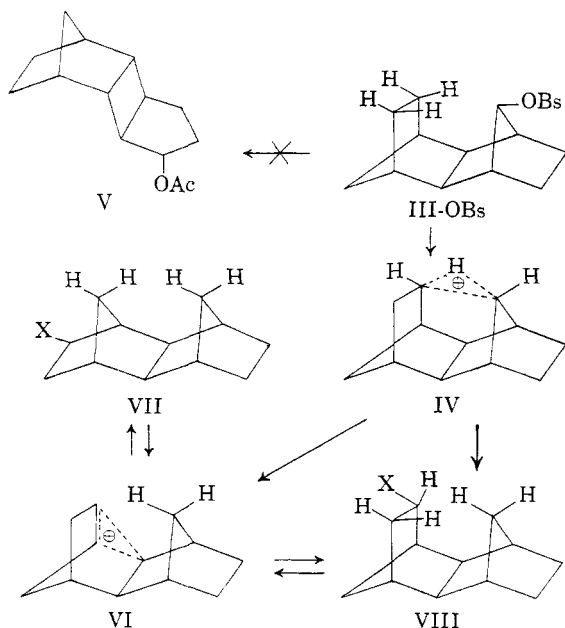
(5) J. F. Pechere, *et al.*, *ibid.*, **233**, 1364 (1958).

(6) J. W. Newton, *Biochim. et Biophys. Acta*, **42**, 34 (1960).



109–110°, acetolyzes quite slowly by absolute standards, it is nevertheless very much more reactive than is the 7-norbornyl analog. The observed first order rate constant for acetolysis of III-OBs in 0.01 *M* sodium acetate in acetic acid solvent is $(4.27 \pm 0.12) \times 10^{-5} \text{ sec.}^{-1}$ at 127.6° and $(1.94 \pm 0.05) \times 10^{-6} \text{ sec.}^{-1}$ at 100.0°, leading to an extrapolated value of $2.42 \times 10^{-11} \text{ sec.}^{-1}$ at 25.0°. Thus, rate of ionization of III-OBs exceeds that of the 7-norbornyl system³ by a factor of slightly more than 10^3 .

The product of acetolysis of III-OBs, analyzed by vapor phase chromatography and confirmed by infrared spectrum, is an 83:17 mixture of the *exo-exo*-fused and *endo-exo*-fused acetates VII-OAc and VIII-OAc, respectively (Table I). The *exo-exo*-fused alcohol VII-OH, m.p. 74–76°, has been described previously by Soloway,⁶ while the *endo-exo*-fused isomer⁵ VIII-OH, m.p. 95.5–97.5°, was available⁷ from hydroboration-oxidation of the corresponding *endo-exo*-fused monoene described earlier.⁸ Analogously, the product of hydrolysis



of III-OBs is an 80:20 mixture of VII-OH and VIII-OH, respectively (Table I). Only the hydrogen-shifted products were observed, there being no evidence of the ring contraction which occurs

(6) S. B. Soloway, *THIS JOURNAL*, **74**, 1027 (1952).

(7) D. Thompson and R. L. Hansen, unpublished work.

(8) P. Bruck, D. Thompson and S. Winstein, *Chemistry and Industry* 405, (1960).

TABLE I
PRODUCTS OF SOLVOLYSIS

ROBs	Temp., °C.	Product, % ±0.3 VII	Product, % ±0.3 VIII
AcOH; 0.01 <i>M</i> NaOAc			
VII	50	96.1	3.9
VIII	50	96.3	3.7
VII	100	92.7	7.3
III	100	82.5	17.5
III-D ₈	100	82.5	17.5
90% Me ₂ CO			
VII	50	94.1	5.9
VIII	50	94.1	5.9
70% Me ₂ CO; 0.02 <i>M</i> NaOAc			
III	100	80.5	19.5

with 7-norbornyl⁹ and which would give rise to V in the present instance.

When the shifting hydrogen atom in III-OBs is replaced by deuterium, an appreciable kinetic isotope factor is observed. Deuterated III-OH containing 86% of the theoretical 8 deuterium atoms per molecule was prepared through the Diels-Alder reaction between hexachlorocyclopentadiene and *anti*-7-norbornenol, dechlorination with lithium and *t*-BuOD in tetrahydrofuran,⁸ and hydrogenation with deuterium over palladium on charcoal. The (k_H/k_D) isotope factor in acetolysis at 100°, obtained with the deuterated III-OBs, was 1.24. The composition of the acetolysis product from the deuterated material was the same as that from ordinary III-OBs (Table I).

The two solvolysis products, VII-OAc and VIII-OAc, or VII-OH and VIII-OH, are also obtained from the related bromobenzenesulfonates,⁵ VII-OBs and VIII-OBs. However, these derivatives give rise to a much smaller proportion of the *endo-exo*-fused¹⁰ VIII-product, only several per cent. of this being formed from the norbornyl-type¹¹ non-classical carbonium ion VI (Table I).

Inspection of models, as well as the occurrence of an anomalously high C-H stretching frequency⁴ in the infrared spectrum of III-OH, makes it evident that non-bonded H-H distances between the methano-carbon atom and C-6 or C-7 of the decahydrodimethanonaphthalene system III-OH are much below ordinary van der Waals distances. Thus, great steric acceleration of ionization of III-OBs could be expected. The observed rate factor between III-OBs and 7-norbornyl is larger than that observed in the accelerated solvolysis of cyclodecyl toluenesulfonate¹² and smaller than the one observed for the tri-*t*-butylcarbonyl system.¹³ For completely rearranging cases, it is not easy to distinguish^{13,14} between steric acceleration without neighboring group participation in the rate-determining step, and that which involves neighboring

(9) S. Winstein, F. Gadiant, E. T. Stafford and P. E. Klinedinst, Jr., *THIS JOURNAL*, **80**, 5805 (1958).

(10) See also S. Cristol, W. Seifert and S. B. Soloway, *ibid.*, **82**, 235 (1960), and P. Schleyer and M. M. Donaldson, *ibid.*, **82**, 4645 (1960).

(11) S. Winstein and D. S. Trifan, *ibid.*, **71**, 2953 (1949); **74**, 1154 (1952).

(12) R. Heck and V. Prelog, *Helv. Chim. Acta*, **38**, 1541 (1955).

(13) (a) P. D. Bartlett and E. B. Lefferts, *THIS JOURNAL*, **77**, 2804 (1955); (b) P. D. Bartlett and M. Stiles, *ibid.*, **77**, 2806 (1955).

(14) S. Winstein, *et al.*, *ibid.*, **74**, 1113 (1952).

group participation and which we include, therefore, under anchimeric acceleration. In the present case, neither the large rate factor, nor even the isotope factor,¹⁵ are as convincing as the absence of products like V that hydrogen participation is occurring in the ionization step for III-OBs. Also, the geometry of III-OBs is such that it seems difficult for ionization to occur at C α without some C-H electron delocalization in the transition state.

The solvolysis products from III-OBs apparently arise mostly from the rearranged norbornyl-type ion VI. However, the distinctly higher proportion of the VIII-structure in the products from III-OBs than from VII-OBs and VIII-OBs, suggests that some of the product arises at a stage earlier than ion VI. One of the simplest possibilities is that a hydrogen-bridged species such as IV intervenes between III-OBs and VI, and this intermediate can either lead to VIII-product or rearrange to VI, this in turn leading to its characteristic mixture of VII plus a little VIII.

(15) (a) V. Prelog, *Chimia*, **11**, 257 (1957); (b) L. S. Bartell, *Tetrahedron Letters*, **6**, 13 (1960); (c) K. T. Leffek, J. A. Llewellyn and R. E. Robertson, *Chemistry and Industry*, 588 (1960).

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SYNTHESIS OF A STABLE BIRADICAL¹

Sir:

We wish to report the analysis of a stable biradical (I) which differs from common organic biradicals.² Common organic biradicals, such as Chichibabin hydrocarbon, contain two uncoupled electrons in separate π -electron systems and the electrons are essentially independent of each other.³ Compound I contains two uncoupled electrons in the same π -electron system. Therefore, it may exist in a triplet ground state, a postulate which is in agreement with preliminary MO calculation.^{4,5}

Condensation of 2,6-di-*t*-butylphenol (II) with Compound III⁶ in H₂SO₄-HOAc gave a mixture of tris-(3,5-di-*t*-butyl-4-hydroxyphenyl)-methane (IV) (Found: C, 82.33; H, 10.34; m.p. 240–241°; γ_{\max} 3580 cm.⁻¹; λ_{\max} 277 m μ , log ϵ 3.79) and its corresponding quinone methide (V) (Found: C, 82.13; H, 10.03; m.p. 278–279°, γ_{\max} 3580 cm.⁻¹ and 1583 cm.⁻¹; λ_{\max} 449 m μ , log ϵ 4.41). V is the oxidation product of IV by III during the condensa-

(1) Supported by a PHS grant No. A-3444(A) from the National Institute of Arthritis and Metabolic Diseases, Public Health Service.

(2) For a review on common organic biradicals, see G. W. Wheland "Advanced Organic Chemistry," 3rd Edition, John Wiley and Sons, New York, N. Y., 1960, p. 813.

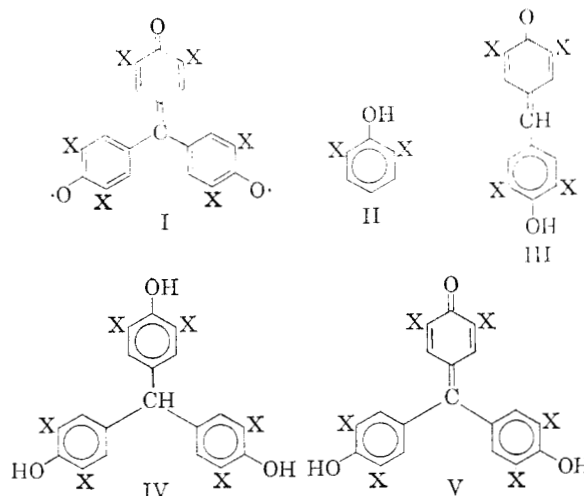
(3) D. C. Reitz and S. I. Weissman, *J. Chem. Phys.*, **33**, 700 (1960).

(4) H. C. Longuet-Higgins, *ibid.*, **18**, 265 (1950).

(5) D. Kearns, unpublished result.

(6) M. S. Kharasch and B. Joshi, *J. Org. Chem.*, **22**, 1435 (1957).

X Is *t*-BUTYL GROUP



tion and it may be reduced quantitatively to IV by zinc and acetic acid. The oxidation of V with two equivalents of potassium ferricyanide gave I (Found: C, 83.01; H, 9.69). I crystallized from benzene in deep purple prisms with metallic luster, undergoes a series of color changes when heated and liquefies at 280°. Compound I exhibits an absorption maximum at 442 m μ in benzene (log ϵ 5.15), a strong infrared band at 1562 cm.⁻¹ which has been attributed to phenoxyl radical,⁷ no absorption in the OH region and 1580–2800 cm.⁻¹ in the infrared, and no detectable proton magnetic resonance within $\pm 2,500 \tau$,⁸ indicating an abnormal shielding of the proton resonance by the uncoupled electrons. Hydrogenation of I in the presence of Adams catalyst consumed the calculated amount of hydrogen to give a mixture of IV and V in 96% yield. The reduction indicated no fragmentation during the ferricyanide oxidation, thus, the structure of I is confirmed.

The paramagnetic properties and a detailed quantum mechanical calculation of I are being investigated, respectively, by Professor C. A. Hutchison, Jr., and Dr. D. Kearns at the University of Chicago.

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(7) (a) C. D. Cook and B. E. Norcross, *THIS JOURNAL*, **78**, 3797 (1956); (b) G. M. Coppinger, *ibid.*, **79**, 501 (1957); and (c) I. E. Mueller, K. Ley and W. Kiedaisch, *Ber.*, **88**, 1819 (1955).

(8) G. V. D. Tiers, *J. Chem. Phys.*, **62**, 1151 (1958).

(9) Alfred P. Sloan Foundation Fellow.