unusually great resonance stabilization. Refinements of these calculations are under way, as are studies of the reactions of these anions and attempts to prepare additional members of the series.

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RECEIVED OCTOBER 26, 1960

A NEW SYNTHESIS OF PHOSPHORODITHIOATE ESTERS

Sir:

Esters of O,O-dialkyl phosphorodithioic acids are of marked interest because of their exceptional insecticidal properties and low mammalian toxicity.[1] S-Alkyl esters have been obtained by alkylation of salts of the acids[2] or addition of the acids to double bonds,[1a] while S-aryl phosphorodithioates have been prepared with greater difficulty by reaction of thiol salts with O,O-dialkyl phosphorochloridothioates[3] or decomposition of aryl diazonium salts in the presence of the acids.[4] No simple S-alkenyl or S-alkynyl phosphorodithioates have been prepared by these methods.[5]

I now wish to report a new procedure which allows the preparation of S-alkyl, aryl, alkenyl and alkynyl phosphorothioates in high yields, with complete absence of side reactions.

Disulfides of O,O-dialkyl phosphorodithioic acids[6] react very rapidly and exothermally with Grignard reagents or alkyl or aryl lithium reagents to give excellent yields of the S-substitution products (equation 1). The reactions are run conveniently in ether or hydrocarbon solvents at room temperature[7] and usually are complete within 2–3 minutes, but may require a somewhat longer reaction time if the metallo-organic reagent is insoluble in the solvent employed. The order of addition of the reagents is unimportant. S-Alkenyl and S-alkynyl phosphorodithioates are prepared similarly in somewhat lower yields. No attempt was made to obtain maximum yields for any reaction.

All of the products were identified by elementary analysis or by comparison with compounds synthesized by independent routes. The infrared spectra of alkenyl and alkynyl phosphorodithioates exhibit the expected peaks at 6.3 and 4.6μ, respectively, and undergo typical addition reactions of alkenes and alkynes.

Careful examination of the crude and distilled products revealed no evidence of attack by the organo-metallic reagent at a phosphorus atom rather than at sulfur, or of any rearrangements occurring during the reaction.

TABLE I

PREPARATION OF O,O-DIALKYL PHOSPHORODITHIOIC ESTERS

$$(RO)_2\overset{S}{\overset{\|}{P}}-S-S-\overset{S}{\overset{\|}{P}}(OR)_2 + R'\ominus M\oplus \longrightarrow (RO)_2\overset{S}{\overset{\|}{P}}-S-R' + (RO)_2\overset{S}{\overset{\|}{P}}-S\ominus M\oplus \quad (1)$$

R	R'	M	Yield (%) $(RO)_2\overset{S}{\overset{\|}{P}}-SR'$	B.p. °C.	B.p. Mm.	n_D^{25}
Et	Ph	MgBr	78	88–95[a]	0.01	1.558
Et	*sec*-Bu	MgBr	88	65–75[a]	0.01	1.499
Et	*i*-Bu	MgBr	100	65–75[a]	0.01	1.497
Et	*n*-Bu	Li	86	60–70[a]	0.01	1.497
Et	CH_2Ph	MgCl	93	87–94[a]	0.01	1.555
Et	$CH{=}CH_2$	MgBr	62	108–112	0.7	1.512
Me	$CH{=}CH_2$	MgBr	58	74–77	2.0	1.533
Et	CH=CHPh	MgBr	59	110–122[a]	0.01	1.582
Et	$C{\equiv}CCH_3$	Li	79	95–99	0.8	1.524
Me	$C{\equiv}CCH_3$	Li	57	115–117	2.3	1.539
Et	C≡CPh	Li	72	85–89[a]	0.01	1.592

[a] Evaporation temp. during molecular distillation.

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BERNARD MILLER

RECEIVED OCTOBER 10, 1960

DISULFIDE BONDING OF ANTIGEN SUBUNITS IN THE PHOTOCHEMICAL APPARATUS OF BACTERIA

Sir:

Disulfide bonds are known to be important cross-linking groups for maintenance of protein structure.[1] Their role in preserving the structural integrity of certain cellular components, such as the mitotic apparatus, is well documented.[2] In this communication I wish to report chemical and immunochemical evidence for disulfide bonding of repeating subunits in another cellular structure, the chromatophores of photosynthetic bacteria, which contain the photochemical apparatus of the cell.

An antiserum was prepared to chromatophores which were first purified by differential centrifugation from extracts of light-grown *Rhodospirillum rubrum*. An extract of *R. rubrum* grown aerobically in the dark was added to the serum at the equivalence point so that antibodies reactive to the extract would be absorbed quantitatively. The supernatant serum obtained reacted only with components formed by the cells during photosynthetic growth, *i.e.*, the antigenic components of the chromatophores elaborated specifically as a consequence of photosynthesis. Although the chemical nature of these unique chromatophore antigens is unknown, that they are held together by disulfide

(1) (a) G. A. Johnson, J. H. Fletcher, K. G. Nolan and J. T. Cassaday, *J. Econ. Entomol.*, **45**, 279 (1952); (b) D. E. H. Frear, "Chemistry of the Pesticides," Third Ed., D. Van Nostrand Co., New York, N. Y., 1955, pp. 86–90.

(2) E. I. Hoegberg and J. T. Cassaday, THIS JOURNAL, **73**, 557 (1951).

(3) G. Schrader, German Patent 855,176 (1953).

(4) (a) N. N. Mel'nikov, A. F. Grapov and K. D. Shvestsova-Shilovskaya, *Zhur. Obshchei Khim.*, **27**, 1905 (1957); (b) G. Bianchetti, *Rend. ist. lombardo sci.*, Pt. I, **91**, 68 (1957) [*Chem. Abs.*, **52**, 11769b (1958)].

(5) A phosphorodithioate of *p*-dioxene, which is formally an S-alkenyl phosphorodithioate, has been prepared: W. R. Diveley, *et al.*, THIS JOURNAL, **81**, 139 (1959).

(6) The disulfides were prepared by oxidation of phosphorodithioic acids with bromine or nitrous acid. *Chemical Abstracts* names these disulfides thioperoxydiphosphates, which is vague, confusing and incorrect. A referee has suggested the name O,O-dialkyl phosphorothionyl disulfides, which is closely related to accepted organic chemical practice (*e.g.*, acetyl disulfide), but unfortunately does not seem generally applicable to more complex phosphorothioates. The name used above, while somewhat inelegant, seems clear and of general utility.

(7) External cooling is recommended for large scale reactions.

(1) R. Benesch, *et al.*, editors, "Symposium on Sulfur in Proteins," Academic Press, Inc., New York, N. Y., 1959.

(2) D. Mazia, *ibid.*, p. 367.

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 Swan[3] or by reducing with thioethanol according to White's[4] 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 *p*H 7.2 having an O.D. at 880 mμ 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 mμ. Blank tubes of antigens in saline contained negligible precipitates.

	Antigen preparation added		
	Chromatophores		
Chromatophores	Sulfite-treated	Sulfite-omitted	Precipitate formed
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 indicated 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 other[6] 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.

Bergeron[8] 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.

(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).
(7) A. Nisonoff, *et al.*, *Biochem. Biophys. Research Communs.*, **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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J. W. NEWTON

RECEIVED AUGUST 29, 1960

1,5-HYDROGEN SHIFT IN A DECAHYDRODIMETHANONAPHTHALENE SYSTEM[1]

Sir:

Since the unsaturated alcohol I was available from the study of the octahydrodimethanonaphthyl non-classical homocyclopropenyl cation[2] 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 system[3] 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 bromobenzenesulfonate[5] 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.