indicates that the complexes are formed in a 1:1 ratio. Values of the association constant, K, and of the molar extinction coefficient ϵ are reported in Table I.

The spectra of these change transfer complexes are similar in both contour and intensity to the spectrum of GPD in presence of DPN.[6-9] This suggests that in the GPD–DPN complex the pyridinium moiety of the coenzyme molecule interacts with indole side chains of the enzyme[10]; this interaction should almost certainly occur in the case of GPD–APDPN complex, since iodoacetate only slightly reduces the absorption in the 360 mμ region.[9]

Complexing said to be associated with electron transfer from the indole nucleus to flavines and pteridines has been reported recently.[11]

Acknowledgment.—We are indebted to the Rockefeller Foundation and to the Brazilian "Conselho Nacional de Pesquisas" for grants, and to the latter also for a fellowship granted to P. G.

(6) E. Racker and I. K. Krimsky, *J. Biol. Chem.*, **198**, 731 (1952).
(7) S. F. Velick, *ibid.*, **203**, 563 (1953); in "Mechanism of Enzyme Action," W. D. McElroy and B. Glass, eds., The John Hopkins Press, Baltimore, Md., 1954, p. 500–505.
(8) J. B. Fox, Jr., and W. B. Dandliker, *J. Biol. Chem.*, **221**, 1005 (1956).
(9) N. O. Kaplan, M. M. Ciotti and F. E. Stolzenbach, *Archiv. Biochem. Biophys.*, **69**, 441 (1957).
(10) It is true that –SH reagents abolish the 365 mμ interaction in the DPD–DPN complex (ref. 6–8 and also B. Chance in "Mechanism of Enzyme Action," W. D. McElroy and B. Glass, eds., The Johns Hopkins Press, Baltimore, Md., 1954, p. 434–438), but as pointed out by Chance and by Velick (ref. 7) this does not necessarily indicate a direct binding of coenzyme to sulfur.
(11) H. A. Harbury and K. A. Foley, *Proc. Natl. Acad. Sci.*, **44**, 662 (1958); I. Isenberg and A. Szent-Györgyi, *ibid.*, **44**, 857 (1958); E. Fujimori, *ibid.*, **45**, 133 (1959); B. Pullman and A. Pullman, *ibid.*, **44**, 1197 (1958); **45**, 136 (1959).

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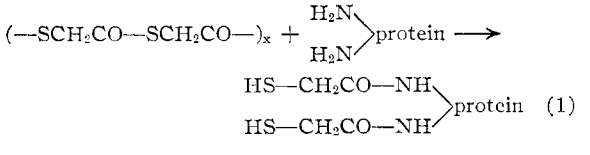
RECEIVED MAY 5, 1959

A NEW METHOD FOR THE INTRODUCTION OF THIOL GROUPS INTO PROTEINS

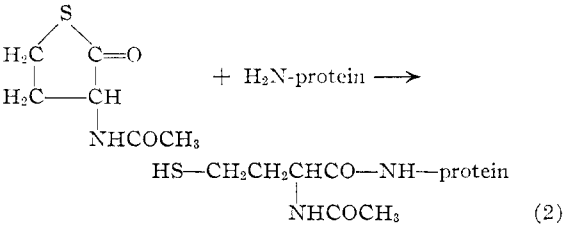
Sir:

For fundamental and technical reasons many attempts have been made to introduce sulfur *de novo* into proteins. Using thioglycolides, Schöberl[1] prepared highly thiolated casein and ovalbumin



(1)

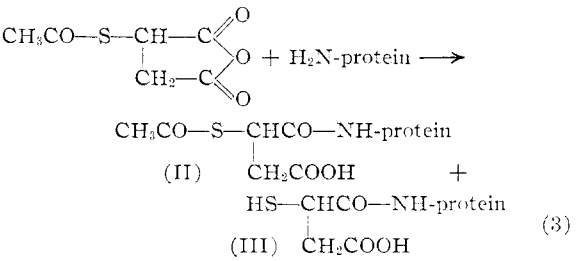
Since Schöberl's reagent is difficult to characterize, the recent method of Benesch and Benesch[2,3] using N-acetylhomocysteine thiolactone is more attractive



(2)

Although direct reaction is not especially useful,[4] with Ag^+ as adjuvant[3] thiolation of gelatins has been effected rapidly at *p*H 7.5. However, the presence of silver, and subsequently 1 *M* thiourea for its removal, presents problems particularly with disulfide-containing proteins.

These problems are avoided by a suitable extension of the reaction of acid anhydrides with proteins.[5] For example with S-acetylmercaptosuccinic anhydride



(3)

Solid anhydride[6] is added to protein solution at, for example, *p*H 7, over from 0.25–1 hour, depending on the amount of reagent. The *p*H is maintained by adding sodium hydroxide. Air is excluded throughout with nitrogen. (Coupling can be performed over a range of *p*H and temperature.) Hydrolyzed anhydride (I) is removed with an anion exchanger, salts with a mixed-bed exchanger or by dialysis. The mercaptosuccinylated protein is isolated by lyophilization.

Typical results at room temperature are summarized in Table I.

TABLE I

MERCAPTOSUCCINYL PROTEINS

Protein	Moles (I) added per 10^5 grams of protein	*p*H of reaction	Moles introduced per 10^5 grams of protein: Mercaptosuccinyl (III)	Moles introduced per 10^5 grams of protein: Acetylmercaptosuccinyl (II)
Gelatin	30	7	6	12
Gelatin	120	8	12	17
Gelatin	360	8	14	23
Bovine serum albumin	45	8	5	21
Bovine serum albumin	360	8	8	54
Ribonuclease	140	7	2	6

A reaction analogous to (3) giving mercaptosuccinylated esters has been achieved with polyhydroxylic molecules (*e.g.*, dextran, polyvinyl alcohol).

(1) A. Schöberl, *Angew. Chem.*, **60**, 7 (1948).
(2) R. Benesch and R. E. Benesch, THIS JOURNAL, **78**, 1597 (1956).
(3) R. Benesch and R. E. Benesch, *Proc. Natl. Acad. Sci.*, **44**, 848 (1958).
(4) It has been successfully applied at high *p*H (10.7) by S. J. Singer, J. E. Fothergill and J. R. Shainoff, THIS JOURNAL, **81**, 2277 (1959).
(5) H. Frankel-Conrat, R. S. Bean and H. Lineweaver, *J. Biol. Chem.*, **177**, 385 (1949); P. H. Maurer and H. Lebovitz, *J. Immunol.*, **76**, 335 (1956); A. F. S. A. Habeeb, H. G. Cassidy and S. J. Singer, *Biochim. Biophys. Acta*, **29**, 587 (1958).
(6) B. Holmberg and E. Schjånberg, *Arkiv Kemi Mineral. Geol.*, **14A**, No. 7 (1940).

That the mercapto groups are really bound to protein, and not merely the result of inefficient separation, was shown by ultracentrifugation with a colored azomercurial. At 60,000 r.p.m., the schlieren and color boundaries moved together.

The acetyl-S linkage of (II) is stable for days in aqueous solution at *p*H's as high as 9.5. Conversion of (II) into (III), if desired, can be accomplished in a few minutes in dilute sodium hydroxide, *p*H 11.5. Preliminary experiments indicate that some nitrogenous bases also work at *p*H's much nearer 7 (*e.g.*, imidazole).

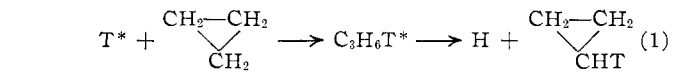
DEPARTMENT OF CHEMISTRY
NORTHWESTERN UNIVERSITY — IRVING M. KLOTZ
EVANSTON, ILLINOIS — RICHARD E. HEINEY

RECEIVED MAY 25, 1959

HIGH ENERGY EXCHANGE REACTION OF TRITIUM ATOMS WITH CYCLOPROPANE

Sir:

Our recent experiments with tritium atoms slowing down from very high energies in the presence of cyclopropane show a substantial incorporation into the organic molecule by reaction (1), in which the asterisk designates an energetic species



(1)

Previous experiments with thermal deuterium atoms have failed to show any exchange of D for H in the cyclopropane molecule.[1]

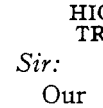
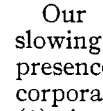
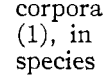
Gaseous mixtures of He^3 and cyclopropane, with oxygen or He^4 sometimes added, have been irradiated with thermal neutrons to produce tritium by the reaction $He^3(n,p)H^3$. The resulting radioactive products have been separated and measured with a proportional counter on the outlet end of a gas chromatographic column.[2] The percentage of radioactivity incorporated in each radioactive product is shown in Table I for several runs, both with and without added gases.

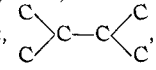
In these systems, O_2 serves as a very effective radical scavenger.[3] The essentially unchanged yield of cyclopropane in its presence indicates that free radicals are not involved, and that the reaction goes through an intermediate as indicated in (1). Presumably the absence of observable exchange with thermal deuterium atoms is the result of a high activation energy for this reaction; the recoil tritium atoms react as "hot" atoms before reaching thermal energies. Moderating collisions with He^3 or He^4 serve to reduce the average energy of the tritium atom at the time of reaction,[4] and hence reduce the possibility of exchange during collision. This is reflected in the lower yield of cyclopropane in the He^4 experiments.

Such irradiations cause degradation of the parent molecules by ordinary radiation effects. In the 70.4 cm. Hg Δ run of Table I. the final gaseous mixture contained about 1% other hydrocarbons than the parent, principally ethane and propane. Runs for higher *nvt* irradiations showed a higher percentage of radiation damage. Quantitative explanations of the distribution of radioactivity will require separation of the energetic tritium atom reactions from the accompanying macroscopic radiation damage.

TABLE I

RADIOACTIVE PRODUCTS OF THE GASEOUS REACTION OF ENERGETIC TRITIUM ATOMS WITH CYCLOPROPANE

Gas Pressure, cm.	70.4Δ 2.0 He^3	31.4Δ 1.9 He^3	21.5Δ 1.9 He^3 8.6 O_2	10.1Δ 1.5 He^3 66.7 He^4	8.4Δ 1.9 He^3 24.3 He^4
Irradiation conditions n./cm.²/sec.	6 days at 3×10^9	12 hr. at 2×10^{12}	12 hr. at 2×10^{12}	6 days at 3×10^9	12 hr. at 2×10^{12}
Product	Per cent. total observed tritium[a]				
Δ	22.1	16.4	15.3	10.9	7.4
HT	31.2	47.4	58.3	30.1	54.9
CH_3T	2.5	6.8	6.0	1.8	6.8
C-C	6.2	4.7	4.0	7.8	6.4
C=C	1.5	1.8	1.8	2.0	2.0
C-C-C	12.0	6.5	4.1	13.7	6.8
C-C=C	2.1	2.5	2.2	1.8	1.7
	3.0	1.5	1.0	3.1	1.8
C-C-C-C	8.6	4.9	1.8	13.8	5.0
	Low			Low	
	5.4	4.0	1.5	7.8	3.7
C-C-C-C-C	1.4	1.7	0.7	1.8	0.9
	0.7	0.5	0.1	0.5	<0.1

[a] Smaller amounts (<1% each) have been observed for C≡C, C=C=C, C—C≡C,  , *i*-C_6, *n*-C_6, and others.

In these antibacterial substances.

The major compound isolated from oxamycin-treated cells had a slower mobility in several solvents than UDP-GNAc-lactyl-(L)ala-(D)glu-(L)-lys-(D)ala-(D)ala,[4,5] the principal compound which

DEPARTMENT OF CHEMISTRY
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RECEIVED MAY 15, 1959

OXAMYCIN, A COMPETITIVE ANTAGONIST OF THE INCORPORATION OF D-ALANINE INTO A URIDINE NUCLEOTIDE IN *STAPHYLOCOCCUS AUREUS*

Sir:

Oxamycin (D-4-amino-3-isoxazolidone, D-cycloserine), like penicillin, bacitracin, novobiocin and gentian violet, induces uridine nucleotide accumulation in *S. aureus*.[1] The nucleotides which accumulate are bacterial cell wall precursors.[2] Their accumulation, as well as protoplast formation,[3] is the consequence of inhibition of cell wall synthesis by these antibacterial substances.

(1) H. I. Schiff and E. W. R. Steacie, *Canad. J. Chem.*, **29**, 1 (1951).
(2) R. Wolfgang and F. S. Rowland, *Anal. Chem.*, **30**, 903 (1958).
(3) J. K. Lee, B. Musgrave and F. S. Rowland, 134th A. C. S. Meeting, Chicago, Sept., 1958.
(4) See, for example, M. El-Sayed, P. Estrup and R. Wolfgang, *J. Phys. Chem.*, **62**, 1356 (1958).

(1) J. Ciak and F. E. Hahn, *Antibiotics and Chemo.*, **9**, 47 (1959).
(2) J. T. Park and J. L. Strominger, *Science*, **125**, 99 (1957).
(3) J. Lederberg, *J. Bacteriol.*, **73**, 144 (1957).
(4) In this abbreviation UDP refers to uridine diphosphate and GNAc-lactyl to an ether of acetylglucosamine and lactic acid (acetylmuramic acid). The peptide, for which the usual abbreviations are employed, is linked to the carboxyl group of the lactic acid. Its sequence recently has been determined.[5]
(5) (a) J. L. Strominger, *Compt. Rend. Trav. Lab. Carlsberg*, **31**, 181