trast, when dimethylamine-borine is heated, the principal product is dimethylaminoborine dimer.[2] However the preparation of trimeric dimethylaminoborine, $[(CH_3)_2NBH_2]_3$, has been accomplished by heating the dimer with higher boron hydrides. Both pentaborane(9) and the yellow boron hydride solids produced by the pyrolysis of diborane have been effective in this conversion.

A sample of $[(CH_3)_2NBH_2]_3$, prepared by heating the dimer at 100–110° with pentaborane(9), and purified on the vacuum line,[3] melted at 97.0–97.8°. The molecular weight (cryoscopic in benzene) was 165.3 (calcd. 170.7). In two experiments the acid methanolysis (16 hours at 85°) showed 3.48 and 3.50% active hydrogen, or an average of 5.91 moles of hydrogen produced per mole of $[(CH_3)_2NBH_2]_3$ (calcd. 6.00).

Anal. Calcd.: C, 42.25; H, 14.17; N, 24.62; B, 19.01. Found: C, 42.24, 42.14; H, 14.10, 14.21; N, 24.44, 24.35; B, 19.02 and 19.28.

The proton magnetic resonance spectrum of $[(CH_3)_2NBH_2]_3$ consisted of four signals of equal intensity with spacing of approximately 2 p.p.m., with a very strong superimposed single peak. An integration showed that the intensity of the large single peak was three times the sum of intensities of the four smaller peaks, corrected for 19% B^{10} concentration. The interpretation was that the large single peak was due to the C–H hydrogen, and that the $N(CH_3)_2$ protons are magnetically equivalent, whereas the smaller multiplet was due to the hydrogen atoms attached to B^{11}. The relative intensities, therefore, indicate that there are three hydrogen atoms bonded to carbon for each B–H hydrogen, which is in agreement with the formula $[(CH_3)_2NBH_2]_3$. This interpretation was substantiated by the B^{11} spectrum, run at 16.2 mc. in a field of 11,900 gauss. A simple triplet was observed, with a 1–2–1 intensity ratio, which strongly indicates that all boron atoms are magnetically equivalent and that each has two covalently bonded hydrogen atoms.

The dimethylaminoborine trimer has a camphor-like odor, and is quite unreactive in moist air and even when dissolved in wet acetone it does not hydrolyze measurably at room temperature. In this respect it is quite comparable to the methylaminoborine trimer.[1] In view of the nuclear magnetic resonance analysis, it appears that this compound has a cyclic structure, comparable to the phosphinoborine trimers.[4]

It was reported by Burg[5] that the reaction of pentaborane(9) with dimethylaminoborine (present in excess) produced the compound "$[(CH_3)_2N]_3B_3H_4$." No compound of this composition was obtained from this system. Since the physical properties of trimeric dimethylaminoborine were nearly identical to those of the reported "$[(CH_3)_2N]_3B_3H_4$," and the preparation process was identical to that described by Burg, it appears likely that trimeric dimethylaminoborine and "$[(CH_3)_2N]_3B_3H_4$" are the same compound.

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(2) A. B. Burg and C. L. Randolph, THIS JOURNAL, **73**, 953 (1951).

(3) The last traces of $[(CH_3)_2N]_2B_4H_8$ were removed from the trimeric dimethylaminoborine by slow distillation at 0 to 5°. The infrared spectrum of $[(CH_3)_2N]_2B_4H_8$ showed strong absorption at 4.08 μ, with a shoulder at 3.9 μ, whereas $[(CH_3)_2NBH_2)_3$ had low absorption at these wave lengths, but absorbed strongly at 4.14, 4.25 and 4.42 μ. Other absorption bands for $[(CH_3)_2N]_2B_4H_8$, which were useful in detecting its presence in trimeric dimethylaminoborine, were found at 8.68 μ and 10.35 μ. The complete absence of absorption at these wave lengths was prerequisite to further work with trimeric dimethylaminoborine.

(4) W. C. Hamilton, *Acta Cryst.*, **8**, 199 (1955).

(5) A. B. Burg, THIS JOURNAL, **79**, 2129 (1957).

ELECTRON TRANSFER FROM THE INDOLE NUCLEUS TO THE PYRIDINE COENZYMES

Sir:

We report the formation of charge transfer complexes among biologically active compounds and a concomitant strong support for the views of Mulliken[1] and Kosower[2] on the importance of charge transfer complexes in biochemical systems.

Addition of DPN[3] or TPN or of their model compound 1-benzyl-3-carboxamide pyridinium chloride to an aqueous solution of any of the indole derivatives available to us (these in Table I and yohimbine) developed *instantaneously* a faint yellow color. Spectroscopic examination indicates the appearance of a *new, quite diffuse* band as a long tail to the longer wave length side of the indole nucleus absorption.

TABLE I

DATA FOR CHARGE TRANSFER COMPLEXES OF 1-BENZYL-3-CARBOXAMIDE PYRIDINIUM CHLORIDE WITH INDOLE AND DERIVATIVES[a]

		Association constant l. mol.$^{-1}$	Molar extinction coefficient ϵ	mμ
Indole	Water	2.5	540	370
L-Tryptophan	Water	2.2	860	370
Glycyl-L-tryptophan	Water	2.9	500	400
Indole-3-acetic acid	1.7×10^{-2} *M* phosphate *p*H 6.7	4.1	1220	370
Serotonin[b]	*p*H 6.5	1.8	1410	380
Acetyltryptophan	*p*H 6.5	4.0	510	400

[a] Room temperature (25 ± 2°). [b] As creatinine sulfate.

Under comparable conditions, no other amino acid is able to replace tryptophan in this kind of interaction.

Chymotrypsinogen after preincubation with urea for a few hours also develops the charge transfer band by addition of DPN or its model, even at low *p*H's where reactivity of other amino acids is out of question.[4]

Application of the equation of Foster,[5] *et al.*,

(1) R. S. Mulliken, THIS JOURNAL, **74**, 811 (1952).

(2) E. M. Kosower, *ibid.*, **78**, 3497 (1956).

(3) These abbreviations are used: DPN, diphosphopyridine nucleotide; TPN, triphosphopyridine nucleotide; GPD, glyceraldehyde phosphate dehydrogenase; APDPN, the acetyl analogue of DPN.

(4) See J. van Eys, *J. Biol. Chem.*, **233**, 1203 (1958)

(5) R. Foster, D. Ll. Hammick and A. A. Wardley *J. Chem. Soc.*, 3817 (1953).

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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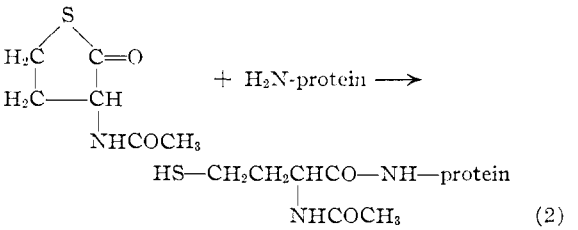
A NEW METHOD FOR THE INTRODUCTION OF THIOL GROUPS INTO PROTEINS

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

$$(\text{—SCH}_2\text{CO—SCH}_2\text{CO—})_x + \begin{matrix}\text{H}_2\text{N}\\ \text{H}_2\text{N}\end{matrix}\!\!>\text{protein} \longrightarrow \begin{matrix}\text{HS—CH}_2\text{CO—NH}\\ \text{HS—CH}_2\text{CO—NH}\end{matrix}\!\!>\text{protein} \quad (1)$$

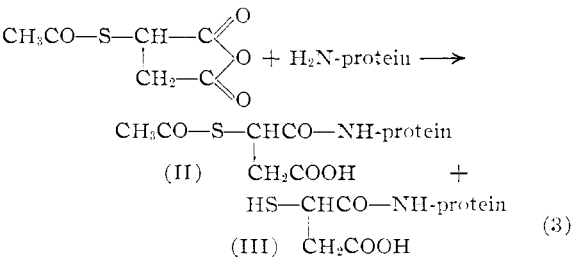
Since Schöberl's reagent is difficult to characterize, the recent method of Benesch and Benesch[2,3] using N-acetylhomocysteine thiolactone is more attractive



$$+ \text{H}_2\text{N-protein} \longrightarrow \text{HS—CH}_2\text{CH}_2\text{CHCO—NH—protein}$$
$$\text{NHCOCH}_3 \qquad (2)$$

Although direct reaction is not especially useful,[4] with Ag^+ as adjuvant[3] thiolation of gelatins has been effected rapidly at pH 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



$$\text{CH}_3\text{CO—S—CHCO—NH-protein} \quad (3)$$
$$\text{(II)}\quad \text{CH}_2\text{COOH} \quad + \quad \text{HS—CHCO—NH-protein}$$
$$\text{(III)}\quad \text{CH}_2\text{COOH}$$

Solid anhydride[6] is added to protein solution at, for example, pH 7, over from 0.25–1 hour, depending on the amount of reagent. The pH is maintained by adding sodium hydroxide. Air is excluded throughout with nitrogen. (Coupling can be performed over a range of pH 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	pH 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 pH (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).