plexes have been subjected to complete structure determination, and hence we have carried out a study of Ag^+(Cyclooctatetraene)NO_3^- by the X-ray diffraction method. We anticipate a close relation between Ag^+(Olefin) complexes[1] and the ethylene-like Ag^+(COT) complex.[7]

The strongest bonding in the complex (Fig. 1) is between one Ag^+ and one COT; these Ag^+(COT) units are then joined more weakly into infinite

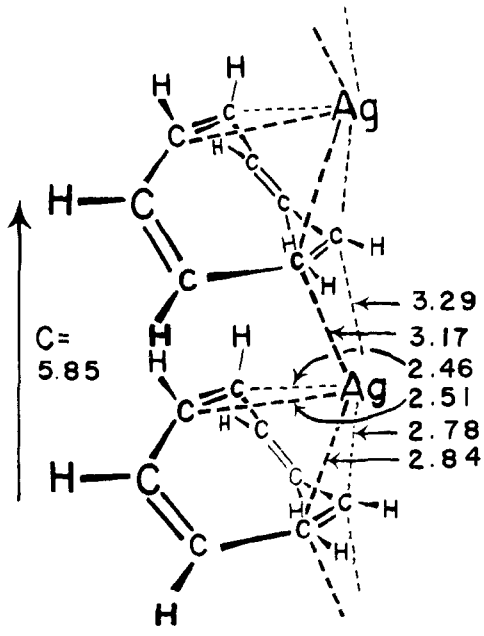

Fig. 1.—The Ag^+(COT) complex joined in infinite chains along the c axis; distances are in Å.

chains along the c axis of the crystal. The closest Ag. . .O distance to a NO_3^- is 2.43 Å., a value between the ionic and covalent sums of radii. The closest Ag. . .C distances of 2.5 Å., distinctly greater than the sum (2.3 Å.) of covalent radii, are sufficiently short that the bonding in COT may be perturbed. The COT molecule has the D_{2d} tub form, as expected from earlier studies,[8,9] and has average C=C = 1.37 and C—C = 1.46 distances, both ±0.04 Å. Corresponding distances are 1.31 and 1.46 (±0.01 Å.) in a refinement[10] of the COT crystal data, and 1.334 and 1.462 (optimistically ±0.001 Å.) in a recent electron diffraction study. Thus we claim plausibility, but not significance, for the increased C=C distance. In the infinite Ag^+(Benzene) complex[6] the average C—C distance is, however, 1.40 Å., essentially the same as in benzene itself. Ag. . .C distances[6] of 2.50 and 2.63 Å. in the Ag^+(Benzene) complex are comparable with those in Fig. 1 but indicate more distortion in the Ag^+(Benzene) complex.

The unit cell is monoclinic, the space group is $P2_1/a$ and there are four Ag^+(COT)NO_3^- in the cell. Parameters are a = 16.84, b = 8.94, c = 5.85 Å. and β = 91°7′. Values[11] of R = 0.113 and r = 0.056 for the 1136 observed reflections indicate that refinement is nearly complete.

We wish to thank Professor S. W. Fenton for suggesting this investigation, the Office of Naval Research for support, and the Minneapolis Honeywell Company for a fellowship to F.S.M.

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(11) R. E. Dickerson, P. J. Wheatley, P. A. Howell and W. N. Lipscomb, *J. Chem. Phys.*, **27**, 200 (1957).

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RECEIVED JULY 21, 1958

A NEW ADENOSINE DINUCLEOTIDE ISOLATED FROM MUSCLE EXTRACTS[1]

Sir:

A dinucleotide of adenosine (XAD) which has no color at *p*H 6 but which turns to green at *p*H 9 has been found in muscle. The adenine base was determined by its ultraviolet spectrum (values ϵ 280/ϵ260: *p*H 2, 0.368; *p*H 7, 0.122; reference values[2]: *p*H 2, 0.375; *p*H 7, 0.125) and by chromatography (Table I). The inference that the other base is a pteridine is supported by its blue fluorescence, ultraviolet spectrum and the indication that it contains 4 nitrogen per mole (calculated from the Am value worked out from the determination of phosphate and ribose). The spectral characteristics, composition and chromatographic behavior of XAD and its products of acid hydrolysis (mononucleotide (XRP), nucleoside (XR) and free pteridine (X)) are given in Fig. 1 and Table I.

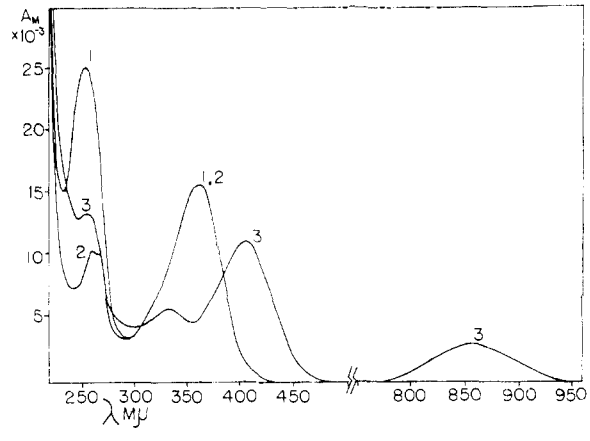

Fig. 1.—Absorption spectra of XAD and XRP: 1, spectrum of XAD at *p*H 2; 2, spectrum of XRP at *p*H 2; 3, spectrum of XRP at *p*H 12.

Adenine and the pteridine derivatives were isolated from the products of hydrolysis of XAD by electrophoresis (by this method adenine remains in the origin) and purified by chromatography with the solvents Iso and Eth. The spectrum of XRP at *p*H 2 shows a peak at 256 mμ (Am = 10.0 × 10^3) and another at 366 mμ (Am = 15.5 × 10^3). When

(1) Supported by research grants from the National Institute of Health, U. S. Public Health Service (Nos. A-1174 and H-1889).

(2) W. E. Cohn in "Methods in Enzymology," S. P. Colowick and N. O. Kaplan, Editors, Academic Press, Inc., New York, N. Y., 1957, Vol. III, p. 740.

TABLE I

COMPOSITION AND CHROMATOGRAPHIC CHARACTERISTICS OF XAD AND ITS DERIVATIVES

Compound	Ribose mole/mole base 15 min.	Ribose mole/mole base 75 min.	Phosphate mole/mole base 10 min.	Phosphate mole/mole base Total	R_f in solvents[a] Iso[b]	Eth[c]	Ph[d]	But[e]
XAD	1.50	2.05		2.04[i]	0.21	0.10	0.55–.65	
XRP[f]	0.56	0.95	0.10	1.02	.21	.18	.42–.49	
XR[f]		1.16	.00	0.00	0.40–.45	.62		
X[g]		0.00	.00	.00	.62			
Adenine[f,h]	.00	.00	.00	.00	.90(.90)			0.41(.41)
5′-AMP	1.00	1.00						

[a] X derivatives were located with a Mineralight lamp (Max. emission 253 mμ). [b] Isobutyric acid, NH_3, H_2O (66:1:33). [c] Ethanol, 1 *M* ammonium acetate *p*H 7.5 (7:3). [d] Phenol, H_2O (8:2 v:v). [e] Butanol, acetic acid, H_2O (4:1:5). [f] Obtained from XAD after 10 min. hydrolysis in 1 *N* HCl at 100°. [g] Obtained from XAD after 1 hour hydrolysis with 1 *N* HCl at 100°. [h] Values in parentheses correspond to adenine isolated from 5′ AMP. [i] Initial inorganic phosphate was zero.

X passes from *p*H 6 to 12 the 366 mμ band shifts to 405 mμ (Am at *p*H 12 = 11.2 × 10^3). At *p*H above 9, XRP has another band with maximum at 854 mμ (Am = 2.87 × 10^3).

The Am values for XRP were calculated assuming the ratio X:ribose (or X:phosphate) = 1:1. When these values were subtracted from the spectrum of XAD, the differential spectrum corresponded to 1 mole of adenosine monophosphate per mole of XRP (Am at 257 mμ for AMP in XAD, calcd. = 14.9 × 10^3; reference value[3] for 5′ AMP Am = 15 × 10^3).

XAD was obtained from the Ba soluble fraction of rabbit muscle extract, which was absorbed on Dowex 1 (formate form), eluted with 4 *M* formic acid and purified by paper chromatography and electrophoresis. The average yield was 1 μmole per kg. of muscle.

(3) R. M. Bock, *et al.*, *Arch. Biochem. and Biophys.*, **62**, 253 (1956).

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SELECTIVE CLEAVAGE OF PEPTIDE BONDS. II. THE TRYPTOPHYL PEPTIDE BOND AND THE CLEAVAGE OF GLUCAGON[1]

Sir:

When the action of N-bromosuccinimide on indole derivatives such as carbobenzyloxy(Cbz)-tryptophan, acetyltryptophan, Cbz-tryptophylglycine and tryptophan-containing peptides and proteins, such as gramicidin D, chymotrypsin(ogen) and lysozyme, in dilute aqueous solutions (2 × 10^{-4} *M*) is followed *in situ* with a self-recording ultraviolet spectrophotometer, one notices the disappearance of the indole absorption at 280 mμ and the concomitant appearance of a new band at 240-250 mμ and a low-intensity band at 307 mμ. The effect of added N-bromosuccinimide on the indole spectrum is instantaneous and linear up to the consumption of *ca.* 1.5 moles/mole of tryptophan with optimal conditions at *p*H 4 in aqueous acetate buffer. Multiplication of the decrease in optical density at 280 mμ by an empirical factor (1.31) gives the extinction due to tryptophan in the peptide or protein. The "titration" of tryptophan in representative proteins yielded 5.7% for chymotrypsin,[2] 5.7% for chymotrypsinogen[3] and 7.8% for lysozyme.[4] The use of differential ultraviolet spectrophotometric recording allows the detection

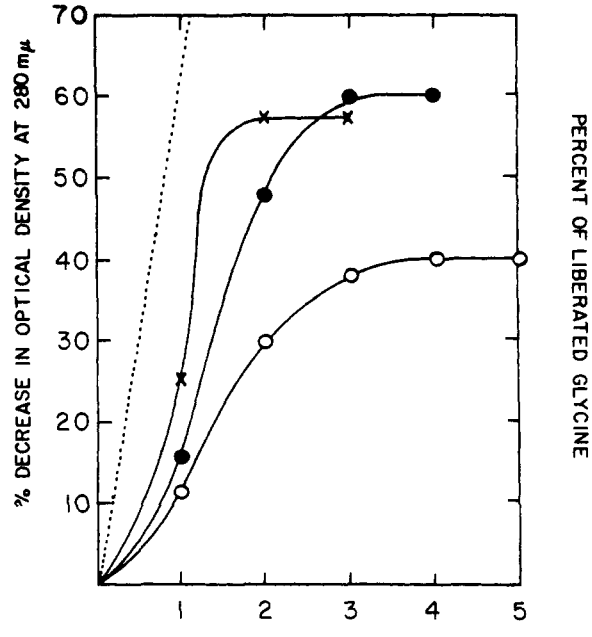

Fig 1. The liberation of glycine from N-benzoyltryptophylglycine (●), indole-3-propionylglycine (×) and carbobenzyloxytryptophylglycine (○) as a function of the addition of N-bromosuccinimide to the solution of the peptides in acetate formate buffer at *p*H 4. The decrease in optical density at 280 mμ (.....) reaches 100% after the addition of 1.53 moles of NBS.

and determination of tryptophan bound in protein on a micro scale and offers advantages over known spectral methods.[5]

After determination of the tryptophan content

(1) Presented in part before the Division of Biological Chemistry at the 134th ACS Meeting in Chicago, Sept. 7-12, 1958.

(2) Reported 5.7%: J. L. Weil and A. R. Buchert, *Arch. Biochem. Biophys.*, **46**, 266 (1953).

(3) Reported 5.6%: cited in J. H. Northrop, M. Kunitz and R. M. Herriott, "Crystalline Enzymes," 2nd Ed., Columbia University Press, New York, N. Y., 1948, p. 26.

(4) Reported 7.1 and 9.1%: C. Fromageot and M. Privat de Garilhe, *Biochim. et Biophys. Acta*, **4**, 509 (1950), and J. C. Lewis, N. S. Snell, D. J. Hirschmann and H. Fraenkel-Conrat, *J. Biol. Chem.*, **186**, 23 (1950). *Cf.* the decrease of ε at 280 mμ in the oxidation of lysozyme with periodate: K. Maekawa and M. Kushibe, *Bull. Agricultural Chemical Society of Japan*, **19**, 28 (1955).

(5) *Cf.* W. L. Bencze and K. Schmid, *Anal. Chem.*, **29**, 1193 (1957).