when the formation of water appeared to be at an end. Two distillations at 2 mm. gave a clean orange solid, which was dissolved with an equal weight of picric acid in benzene. After two crystallizations the picrate was obtained as purplish-black needles, m. p. 182.0–182.5°, corr. (5.9 g.). This was converted with ammonia into methylcholanthrene (a), which formed pale lemon yellow needles, m. p. 178.5–179.0°, corr.; yield, 3.2 g. (25%, based on the 4-bromo-7-methylhydrindene used). Samples of the hydrocarbon and its picrate prepared from desoxycholic acid by the known methods melted 0.5–1° lower than the synthetic samples and there was no melting point depression on admixture.

7-Methyl-8,9-dimethylene-1,2-benzanthracene (IV, b) was obtained by the similar pyrolysis of 4-(β-naphthoyl)-7-methylhydrindene (6.1 g.) at 400–405°, the hydrocarbon being crystallized directly from benzene–ether, as purification through the picrate was not found to be of advantage. Fine, very faintly yellow needles melting at 187.5°, corr. were obtained; yield, 2.9 g. (50%). The solution in concentrated sulfuric acid has an orange-green fluorescence. The picrate forms dark crimson needles from benzene, m. p. 164.5–165.5°, corr.

Anal. Calcd. for $C_{21}H_{16}$: C, 93.98; H, 6.02. Found: (a) C, 94.11; H, 6.26; (b) C, 94.23; H, 6.13. Calcd. for $C_{27}H_{19}O_7N_3$: N, 8.45. Found: (b) N, 8.75.

Summary

The cancer-producing hydrocarbon methylcholanthrene has been synthesized in 11% yield from *p*-bromotoluene in an eight-step process involving a modified Elbs condensation.

CONVERSE MEMORIAL LABORATORY
CAMBRIDGE, MASS. RECEIVED APRIL 3, 1935

[CONTRIBUTION FROM THE LABORATORY OF PHYSICAL CHEMISTRY OF THE UNIVERSITY OF UPSALA]

The Ultracentrifugal Study of Gliadin[1]

BY LAURA KREJCI AND THE SVEDBERG

Due chiefly to the work of Osborne,[2] gliadin, the alcohol-soluble constituent of wheat gluten, has generally been considered a simple homogeneous protein. His careful investigations threw doubt on the conclusions of Ritthausen[3] that it consisted of three proteins, gliadin, mucedin and glutenfibrin, which differed in their solubility in alcohol of varying concentration; and of Kutscher[4] that gliadin and mucedin were evidently the same protein, but that glutenfibrin contained a lower content of glutamic acid. Recently, however, evidence has been accumulated, chiefly by Haugaard and Johnson,[5] that gliadin does not behave as a simple homogeneous protein. Measurements of sedimentation in high centrifugal fields provide a sensitive test for homogeneity, and the present investigation is an attempt to give a conclusive answer to this question.

Experimental Procedure

In most cases alcohol of medium concentration was used as solvent because of the low solubility of gliadin in neutral aqueous solvents. This necessitated some slight modifications in the ultracentrifuge cells. The plate from which the sectorial aperture is cut was made of elastolite[6] rather than ebonite because of the greater impermeability to alcohol. Thin sheets of vulcanized rubber pressed tightly between this plate and the quartz windows prevented leakage of the solution. Ordinarily the whole was encased in a duralumin collar but for strongly alkaline solutions which attack duralumin a collar of the magnesium alloy electron was used instead.

In order to prevent convection currents, arising from diffusion into the alcohol of the paraffin oil used to cover the solution during centrifuging, each solution was previously agitated with a measured quantity of oil to ensure mutual saturation.

Specially milled Manitoba flour was used as source of the material. The extract resulting from treatment of the well-washed gluten with 64 volume per cent. alcohol was stored in a refrigerator for several days to precipitate the starch, glutenin, and gluten, and the solution decanted; this was used for the study of the stability range. For the remainder of the investigation a quantity of purified gliadin was prepared according to the method of Dill and Alsberg.[7]

Partial Specific Volume

The partial specific volume of gliadin was determined pycnometrically in aqueous solutions of low *p*H and low salt content. The results are collected in Table I. There is a definite downward drift with decreasing concentration; the value for the most concentrated solution is lower than that for practically any of the proteins so far studied. However, it is possible that at higher concentrations the partial specific volume approaches 0.745, the

(1) Original manuscript received February 13, 1934.

(2) T. B. Osborne, "Proteins of the Wheat Kernel," Carnegie Institute of Washington, Publication No. 84, 1907.

(3) H. Ritthausen, "Die Eiweisskörper der Getreidearten, Hülsenfrüchte und Ölsamen," Max Cohen & Son, Bonn, 1872.

(4) F. Kutscher, *Z. physiol. Chem.*, **38**, 111 (1903).

(5) Haugaard and Johnson, *Compt. rend. trav. lab. Carlsberg*, **18**, 2 (1931).

(6) Dr. Rudolf Signer had previously found elastolite cells satisfactory for use with other organic solvents, Signer and Gross, *Helv. Chim. Acta*, **17**, 59 (1934).

(7) Dill and Alsberg, *J. Biol. Chem.*, **65**, 279 (1925).

mean value for most proteins. Indeed, inspection of the data of Haugaard and Johnson[5] (p. 112) on the density of gliadin solutions in alcohol of varying concentration indicates that this is so at least in alcohol solution; the specific volume is normal when the gliadin concentration is 7% or higher, and drops to about 0.710 in the neighborhood of 1% concentration.

TABLE I

PARTIAL SPECIFIC VOLUME OF GLIADIN

Concentration of gliadin, %	Solvent KCl, *M*	Solvent HCl, *M*	Partial specific volume
1.97	0.020	0.010	0.738[a]
0.985	.020	.010	.730[a]
.493	.020	.010	.720[a]
1.96	.008	.008	.736
1.55	.008	.008	.727
0.98	.008	.008	.730
.25	.008	.008	.712

[a] Determinations by Inga-Britta Eriksson-Quensel, to whom grateful acknowledgment is made.

Sedimentation Velocity Measurements.—Determinations of the sedimentation velocity were made throughout the *p*H scale. The results are summarized in Table II and represented graphically in Fig. 1. The solvent was in every case 64 volume per cent. alcohol. The total molarity of electrolytes present varies from about 0.01 to 0.20 *M;* these concentrations are sufficient to depress the Donnan effect.[8] The sedimentation constants have been corrected to the basis of sedimentation in pure water at 20° by means of the relation

$$s_{20} = s \cdot \eta/\eta_0 \cdot (1 - v\rho_0)/(1 - v\rho)$$

where η is viscosity of the solvent,[9] η_0 is viscosity of water at 20°; V is partial specific volume of the solute (= 0.715); ρ is density of the solvent; and ρ_0 is density of water at 20°. For 64% alcohol the viscosity correction is greater in magnitude than the density correction, and opposite in sign. The actual sedimentation is therefore less rapid than in water. The diffusion is also depressed; there is little blurring of the boundary after three hours of centrifuging.[10]

The sedimentation constant of gliadin is unusually low, 2.10×10^{-13}. In addition to these lighter molecules, which predominate, the gliadin also contains some heavier molecules which were not resolved in the centrifugal fields available. The *p*H stability range is unusually wide. The protein is stable far into the acid region; the sedimentation constant is unchanged even at *p*H 1.46, the lowest *p*H studied. In the far alkaline region, beyond *p*H 12, there is a drop in the sedimentation constant accompanied by an increase in light absorption and the disappearance of the heavier constituents.

TABLE II

SEDIMENTATION VELOCITY MEASUREMENTS IN 64% ALCOHOL

Centrifugal force 200,000–250,000 times gravity; thickness of column of solution 1.0 cm.; source of light, mercury lamp; light filter, chlorine and bromine; plates, Imperial Process; time of exposure, 45 sec. to 4 min.; metol hydroquinone developer, 1 min.

Solvent	Total molar	*p*H of soln.	$s_{20} \times 10^{13}$[a]
HCl, KCl	0.23	1.46	2.07
HCl	.08	1.56	2.17
HCl	.08	1.56	2.16
HCl, KCl	.21	1.58	2.17
HAc, KCl	.10	4.59	2.06
KCl	.17	5.89	2.09
KCl	.18	5.90	2.16
KCl	.14	5.90	2.16
KCl	.14	5.90	2.32
KCl	.07	6.20	2.01
Na_2HPO_4, KH_2PO_4	.01	6.63	2.11
Na_2HPO_4, KH_2PO_4	.01	7.14	2.27
Na_2HPO_4, KH_2PO_4	.01	7.20	2.13
Na_2HPO_4, KH_2PO_4	.01	7.35	2.12
Na_2HPO_4, KCl	.13	7.90	2.09
Na_2HPO_4, NaOH	.04	8.20	2.06
Na_2HPO_4, NaOH	.01	8.55	2.19
Na_2HPO_4, NaOH	.01	8.93	2.24
Na_2HPO_4, NaOH	.01	9.44	2.17
Na_2HPO_4, NaOH	.02	12.02	1.96
Na_2HPO_4, NaOH	.02	13.20	1.70

[a] Corrected to water basis.

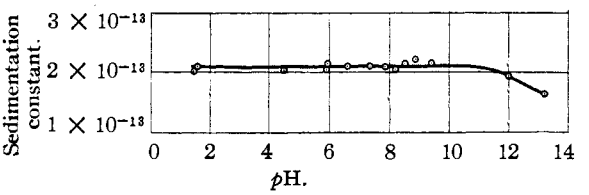


Fig. 1.—The *p*H stability range of gliadin.

Sedimentation in Aqueous Solution.—The stability of gliadin in the presence of acid was taken advantage of to determine the sedimentation constant directly in water. The results are shown in Table III. Agreement with the constant determined in alcohol and corrected to a water basis is good; the differences are perhaps less than the experimental error of the method.

TABLE III

SEDIMENTATION VELOCITY MEASUREMENTS IN AQUEOUS SOLUTION

Experimental conditions as in Table II. (Exposure item 15–45 sec.)

Solution	Solvent HCl, *M*	Solvent KCl, *M*	*p*H of soln.	$s_{20} \times 10^{13}$
Crude gliadin	0.008	0.008	2.40	2.01
	.010	.021	2.23	2.20
	.015	.004	2.05	2.22
Purified gliadin	.008	.008	2.46	2.25
	.047	.008	1.48	2.19

The two runs with purified gliadin are normal and indicate that the ether used in the purification had caused no harmful effect. The purified product appeared to differ from the crude extract only in a slightly lower content of heavy molecules. Determinations were made at *p*H 2.40 and *p*H 1.48; agreement between the two is good and

(8) *Cf.* Tiselius, *Kolloid-Z.*, **19**, 308 (1932).

(9) The data of Traube [*Ber.*, **19**, 880 (1886)] were used for the viscosity correction.

(10) The magnitude of the effect of viscosity over and above that of density can be seen by comparison of A and B, Fig. 2.

there is no evidence of either dissociation or aggregation at the lower *p*H.[11]

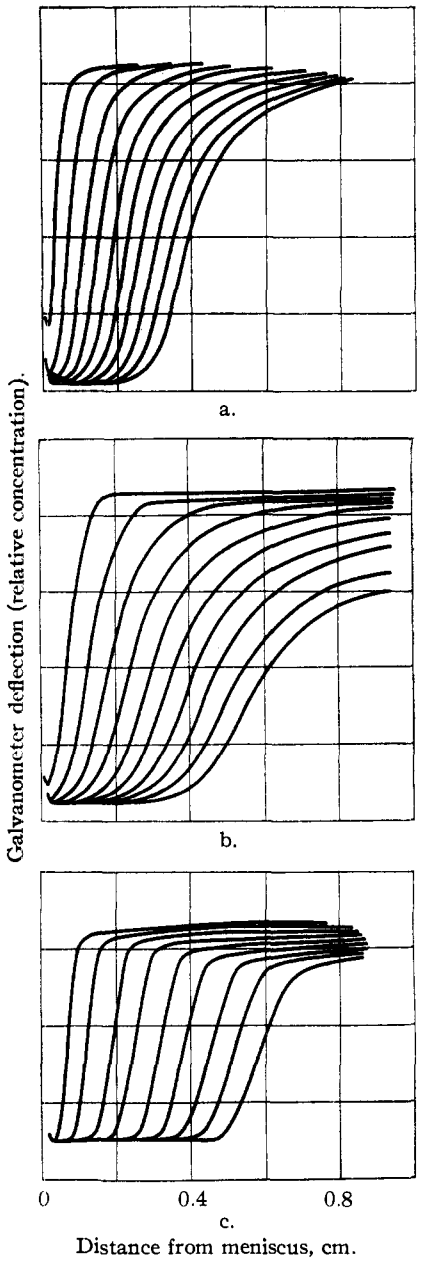


Fig. 2.—(a) Fraction IV in alcohol solution; 56,000 r. p. m.; 20 min. between exposures. (b) Fraction IV in aqueous solution; 56,000 r. p. m.; 15 minutes between exposures. (c) Fraction I in aqueous solution; 74,000 r. p. m.; 10 minutes between exposures.

(11) *p*H is not strictly comparable in aqueous and alcoholic solution. The isoelectric point of gliadin is 5.76 in aqueous solution and 7.11 in 52.2% alcohol [Gortner, "Colloid Symposium Monograph," 1924, Vol. II, p. 209]. However, solutions which give the same potential difference above the hydrogen electrode are assumed to have the same H-ion concentration. See Haugaard and Johnson, Reference 5, p. 8.

Sedimentation Equilibrium.—In order to determine the weight of the predominant gliadin molecule, five determinations of the sedimentation equilibrium were made in aqueous solution at different conditions of *p*H and temperature. The results are shown in Table IV. In each case there is a drift in molecular weight with distance from the center of rotation, an indication of the heterogeneity of the system. At *p*H 2.23 and above, when the temperature is 20° or lower, the molecular weight of the predominating species is about 26,000; this might represent a mixture of whole and half molecules of weight 34,500 and 17,250, respectively. At higher temperatures and higher acidities dissociation appears to be complete; the molecular weight is about 17,500.

TABLE IV

SEDIMENTATION EQUILIBRIUM MEASUREMENTS (AQUEOUS SOLUTION)

Approximate speed of rotation in all runs: 12,000 r. p. m.

Material	*p*H	Temp., °C.	Molecular weight
Unfractionated gliadin	1.48	20	18,100
	2.23	20	27,500
	2.46	20	25,300
	2.46	30	17,150
	2.46	9	25,800
Fraction I (see fractionation of purified gliadin)	2.46	20	26,750

Fractionation of Purified Gliadin.—Haugaard and Johnson[5] (p. 108) found definite physical and chemical differences in the fractions obtained by cooling a concentrated solution of gliadin in 60 volume per cent. alcohol first to 0°, then to −11°. This type of fractionation was repeated in the present investigation, and the fractions were studied in the high-speed ultracentrifuge.

One-third of a gram of gliadin was dissolved in 5 cc. of 64% alcohol. The solution was kept at 0° for several days; the protein thus precipitated corresponds to fraction IV of Haugaard and Johnson. The solution remaining above it was decanted and cooled in turn to −10.2° for several days; the protein thus precipitated corresponds to fraction II of Haugaard and Johnson. The clear solution above it is fraction I, and the unfractionated gliadin fraction III. From a comparison of the light absorption, based on the assumption that the different molecular species present possess the same extinction coefficient, I appeared to contain about 8%, II 34%, and III 58% of the original gliadin.

The results of the sedimentation velocity measurements are given in Table V, and microphotometric records in Fig. 2. Even in a centrifugal field 400,000 times gravity fraction I appears to be homogeneous, with no trace of heavier molecules. Fractions II, III, and IV, on the other hand, contain increasing quantities of heavy molecules.

Because fraction I gave such strong evidence of homogeneity, a determination of the sedimentation equilibrium was made at *p*H 2.40 and 20°. A drift in the molecular weight with distance from the center of rotation indicated heterogeneity. However, since the larger molecules had not been detected in the velocity determinations, the concentration in the solution must have been less than 5–8% of that of the total protein.

TABLE V

FRACTIONATION OF PURIFIED GLIADIN. SEDIMENTATION VELOCITY MEASUREMENTS

Centrifugal force 200,000–250,000 times gravity (400,000 times gravity second, third, tenth and eleventh runs); thickness of column of solution 1.0–1.2 cm.; exposure time 15 sec.–2 min.; other experimental conditions as in Table I.

Fraction	Solvent		M		M	pH of soln.	% heavy molecules	$s_{20} \times 10^{13}$
I	Water	HCl	0.008	KCl	0.008	2.46	0	2.03
I	Water	HCl	.008	KCl	.008	2.46	0	1.99
I	Water	HCl	.008	KCl	.008	2.46	0	2.09
II	Alcohol	KCl	.160	Na_2HPO_4	.010	7.90	25	1.92
II	Water	HCl	.008	KCl	.008	2.46	20	2.04
III	Alcohol	KCl	.050	Na_2HPO_4	.010	8.00	37	2.27
III	Water	HCl	.008	KCl	.008	2.46	37	2.25
IV	Alcohol	KCl	.160	Na_2HPO_4	.010	7.90	49	2.04
IV	Water	HCl	.008	KCl	.008	2.46	46	1.99
IV	Water	HCl	.008	KCl	.008	2.46	40	2.30
IV	Water	HCl	.008	KCl	.008	2.46	44	2.36

Discussion

The results of this investigation, especially of the fractionation just described, show that gliadin is not a simple homogeneous protein. The constituent which is present in greatest concentration, and which forms the bulk of normal gliadin, has a sedimentation constant of 2.10×10^{-13}, and might consist of a mixture of whole and half molecules weighing 34,500 and 17,250.

The sedimentation constant for a protein of molecular weight 34,500 possessing spherical molecules is 3.54×10^{-13}. The theoretical sedimentation constant for a protein of molecular weight 17,250 can be calculated from the relation

$$s = MD(1 - V\rho)/RT$$

where M is the molecular weight, V the partial specific volume of the solute, ρ the density of the solvent, R the gas constant, T the absolute temperature, and D the diffusion constant, which can be evaluated from the equation

$$D = RT/N \times 1/6\pi\eta \times (4\pi N/3MV)^{1/3}$$

in which the symbols have their usual significance. The result is 2.54×10^{-13}. The unusually slow sedimentation of gliadin must be a result of the dissymmetry of both the whole and the half molecules.

The molar frictional constant, $f = M(1 - V\rho)/s$ is 4.71×10^{16} for the whole molecule of gliadin, and 2.35×10^{16} for the half molecule. The molar frictional constant, f_0, for spherical molecules of the same weight and specific volume as gliadin, can be calculated from the relation

$$f_0 = 6\pi\eta N\ (3MV/4\pi N)^{1/3}$$

The result is 2.45×10^{16} for 34,500, and 1.95×10^{16} for 17,250. The dissymmetry factor, f/f_0, which for a spherical molecule would be unity, is 1.92 for the whole gliadin molecule, and 1.21 for the half molecule. The latter value is high, the former unusually high.

Similar behavior has been met with in the investigations on some of the low molecular respiratory blood proteins. The erythrocruorins from *Thyone*, *Myxine* and *Petromyzon*[12] have molecular weights ranging from 23,600 to 19,100 and sedimentation constants from 2.6 to 1.9; they consist of mixtures of molecules of weight 34,500 and 17,250, containing two and one hemin groups per molecule, respectively. Myoglobin,[13] the respiratory protein of muscle fiber, behaves similarly. The sedimentation constant is 2.5×10^{-13}. At 20° it consists of dissymmetrical molecules of weight 34,000; at 30° these are dissociated into submolecules of weight 17,000. Myoglobin has been found to pass through collodion membranes. It is interesting to notice that Haugaard and Johnson[5] (p. 95) found the same to be true of gliadin in their measurements of osmotic pressure. A similar observation was made by Dill and Alsberg[7] (p. 293) for collodion membranes, though not for parchment membranes.

The effect of the dissociation of the gliadin molecules on the sedimentation constant is a question which naturally arises. In the determinations of the sedimentation velocity the temperature varied between 23 and 37°. At the lower temperature it is perhaps safe to assume a fair proportion of undissociated molecules; at the higher temperature dissociation must be nearly complete. Yet there is no systematic variation of the sedimentation constant with temperature,

(12) Svedberg and Eriksson-Quensel, THIS JOURNAL, **56**, 1700 (1934).

(13) Theorell, *Biochem. Z.*, **252**, 1 (1932).

either from run to run or from exposure to exposure during the course of a single run. Because of the high dissymmetry of both the whole and half molecules, it is possible that the sedimentation of the submolecule is so nearly that of the undissociated molecule that the difference is less than the experimental error of the method. This presumably have an absolute significance. Carpenter[15] has reported the recovery of only 81.3% of tryptophan purposely added to casein before digestion. Since it seems reasonable that only 81.3% would be recovered in any careful analysis, all the tryptophan figures have been corrected for this loss. The results are shown in Table VI.

TABLE VI

CALCULATION OF MOLECULAR WEIGHT

Method	Reference	Amt. of constituent, present, %	Corr. for loss, %	Min. mol. wt.	No. mols. assumed	Calcd. mol. wt.
Tryptophan content	Fraction I, Haugaard and Johnson	3.62	4.45	4590	4	18,760
	Fraction III, Haugaard and Johnson	2.59	3.19	6400	1	6,400
	Folin and Looney, quoted by Cohn, Hendry and Prentiss	1.14	1.40	14,560	1	14,560
	Abderhalden and Samuely, quoted by Cohn, Hendry and Prentiss	1.0	1.23	16,600	1	16,600
Tyrosine content	Fraction I, Haugaard and Johnson	1.96	..	9240	2	18,480
	Fraction III, Haugaard and Johnson	2.8	..	6470	4	25,870
	Cohn, Hendry and Prentiss	3.5	..	5170	4	20,700

would account for the constancy of the value of the sedimentation constant through *p*H 1.46 even though it has been shown by equilibrium runs that at some point between *p*H 2.23 and 1.48 the protein is completely dissociated into half molecules even at 20°. Since, however, this dissociation occurs at higher temperatures under conditions of *p*H at which the molecule is otherwise stable, it is permissible to define the limit of the stability range as the *p*H at which even the half molecules are attacked.

Cohn, Hendry and Prentiss[14] have estimated the minimal molecular weight of gliadin from the amino acid content, the maximal base-combining capacity, and the sulfur content. The mean of these estimates is about 20,000; but in view of the heterogeneity of gliadin the figure has little significance. Haugaard and Johnson[5] (p. 101) determined the distribution of total nitrogen in fractions I, II, III and IV. Their values for the tyrosine and tryptophan content of fraction I, which in the ultracentrifuge was found to be very nearly homogeneous, have been utilized to calculate the molecular weight by Cohn's method. The authors state that the values are accurate only for a comparison of the fractions and not for the absolute content of tyrosine and tryptophan in each; a similar calculation, therefore, has been made for fraction III, the unfractionated gliadin, to provide a basis for comparison with the estimates of Cohn, Hendry and Prentiss, which

The tryptophan content of fraction I, assuming four molecules per molecule of gliadin, gives a molecular weight of 18,760. The tyrosine content, assuming two molecules per molecule of gliadin, gives 18,480. Both figures are higher than the weight found by centrifuging, but they fall less wide of the mark than the estimate based on unfractionated gliadin. However, neither can be given much confidence because of the lack of agreement between fraction III and Cohn's estimates.

The present investigation has fully substantiated the conclusion of Haugaard and Johnson[5] (p. 96) based on osmotic pressure measurements, that "it actually appears as if the large gliadin molecules have a lower solubility than the small ones." It has further demonstrated that the method of fractionation employed by these authors is a surprisingly efficient means of separating the small molecules from the larger ones.

The expenses connected with the study have been defrayed by grants from the Rockefeller Foundation, the Nobel Foundation, and the foundation "Therese och Johann Anderssons Minne."

Summary

1. An ultracentrifugal study has been made of wheat gliadin.

2. The stability range has been determined in alcohol solution by means of sedimentation veloc-

(14) Cohn, Hendry and Prentiss, *J. Biol. Chem.*, **68**, 744 (1925).

(15) Carpenter, THIS JOURNAL, **53**, 1812 (1931).

ity measurements. Gliadin is stable even at *p*H 1.46, the lowest *p*H studied. Beyond *p*H 12 there is a drop in the sedimentation constant.

3. A number of measurements have been made in aqueous solution at low *p*H within the stability range. The results are in good agreement with those obtained in alcohol solution and corrected to a water basis.

4. The protein is inhomogeneous with respect to molecular weight. The sedimentation constant of the predominant constituent is 2.10×10^{-13}.

5. The molecular weight of the principal constituent was determined by measurement of the sedimentation equilibrium in aqueous solution. At *p*H 2.23 and above, when the temperature is 20° or lower, there is probably a mixture of whole and half molecules of weight 34,500 and 17,250. At higher temperatures and higher acidities dissociation into half molecules is complete.

6. The molecules are not spherical. The dissymmetry number is 1.21 for the half molecule, 1.92 for the whole molecule.

7. Purified gliadin was fractionated by the method used by Haugaard and Johnson. The least soluble fraction was found to contain a high concentration of heavy molecules, while the most soluble fraction consisted almost entirely of the constituent of lowest molecular weight.

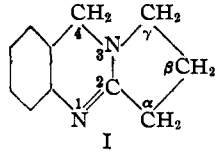
UPSALA, SWEDEN RECEIVED APRIL 5, 1935

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF ILLINOIS]

Structure of Vasicine. III. Position of the Hydroxyl Group[1]

R. C. MORRIS,[2] W. E. HANFORD AND ROGER ADAMS

With the structure of desoxyvasicine (I) definitely established through synthesis,[1,3] the position of the hydroxyl group in vasicine remains to be determined.



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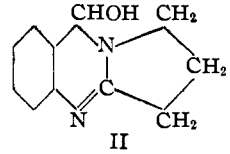
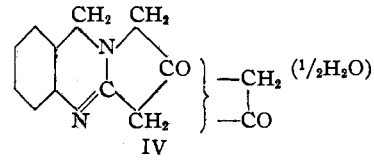


II

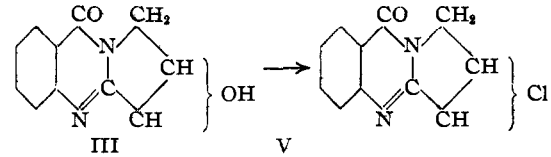
The reactions of vasicine previously described exclude the possibility of the hydroxyl group being in the γ position. It might be present, however, in the α-, β- or 4-position. The 4-position (II) would appear to be very unlikely since a molecule of this structure is a carbinol base and acid would very probably convert it into a quaternary ammonium salt with the elimination of water. The salts produced experimentally indicate no loss of water during formation.

Experimental evidence is presented in this communication in regard to the location of the hydroxyl group. Ghose[4] described the oxidation of vasicine by means of hydrogen peroxide. He reported two products (1) m. p. 213–214°, which involved the loss of two hydrogens and addition of one oxygen to vasicine, and (2) m. p. 168°, which involved the loss of two hydrogens with the retention of one-half molecule of water of crystallization. On the basis of the present established basic structure for vasicine, assuming the hydroxyl to be in the α- or β-position, these two oxidation products might possibly be represented by III and IV. Formula IV, for the compound m. p. 168°, however, would not coincide with Ghose's observation that it could be oxidized to III.

A restudy of the oxidation of vasicine by means of hydrogen peroxide has given results not identical with those of Ghose. Three per cent. hydrogen peroxide even on long heating left vasicine unchanged but with 30%, oxidation occurred. There was obtained by this procedure a mixture of unchanged vasicine and the product m. p. 213° reported by Ghose, which could be separated by fractional crystallization. The presence of the



IV



III V

(1) For the previous paper in this field see THIS JOURNAL, **57**, 921 (1935).

(2) Submitted as part of a thesis for the Degree of Doctor of Philosophy in Chemistry.

(3) Späth, Kuffner and Platzer, *Ber.*, **68**, 497 (1935).

(4) Ghose, Krishna, Narang and Rây, *J. Chem. Soc.*, 2740 (1932).