do not form rapidly on the outside of the diver the simplified method described can be applied to weighing tissues much smaller than embryonic hearts. The improvement in the equation given by Zeuthen makes accurate weighings possible under conditions where the diver is or is not suspended at atmospheric pressure.

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On the Structure of Morphine and its Derivative Metopon

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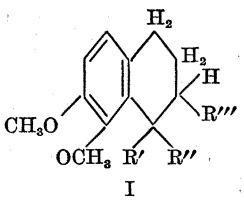
Because the unique and valuable analgesic properties of the morphine derivative Metopon (methyldihydromorphinone) (*4*) may be due, in large measure, to the influence of the new methyl group, information regarding the latter's position in the molecule is of some moment. With a view toward elucidating this point, a method has been developed for degrading the more accessible dihydrothebaine (Metopon's precursor) to an optically active dimethoxytrialkyltetrahydronaphthalene Ia or Ib.



I

(*a*) $R' = C_2H_5$; $R'' = C_2H_5$; $R''' = C_2H_5$

(*b*) $R' = CH_3$; $R'' = C_2H_5$; $R''' = C_3H_7$

Analysis. Calculated for $C_{18}H_{28}O_2$; C, 78.21; H, 10.21; OCH_3, 22.46. Found: C, 78.22; H, 10.20; OCH_3, 22.22. $[\alpha]_D^{20} - 52.7°$ (c, 0.927, ethanol); $n_D^{26} = 1.5295$; $d_{20}^{20} = 1.027$; bp (evaporative distillation) 97–104° C/0.4 mm.

It is hoped to distinguish between the two possible isomers on the basis of results of synthetic experiments which are now nearly complete.

Degradation of Metopon according to this new scheme should lead to a methyl homologue of either Ia or Ib, distinguishable by synthetic processes.

Of equal and perhaps greater importance is the fact that this method of degradation now affords a complementary means of rigorously proving whether C^{13} is one of the points of closure of the heterocyclic nitrogen ring in morphine. The important synthetic approaches of Grewe (*2*, *3*) and of Gates (*1*) both favor C^{13} as one point of ring closure; the other point, C^9, appears well established.

Intimately linked with the above are the stereochemical implications associated with carbon atoms 9, 13, and 14 in the morphine molecule. All the foregoing will be fully reported in papers to be published elsewhere.

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Streaming Birefringence of Denatured Ovalbumin[1]

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That the denaturation of globular proteins consists essentially of an unfolding of the polypeptide chain or chains is generally conceded, but evidence for the nature, or even existence, of the unfolded structure in solution is limited and indirect. Intrinsic viscosity data, which have been most used, are difficult to interpret since they yield only a measure of the effective hydrodynamic volume of the solute. Streaming birefringence, which yields essentially a measure of the particle length and is dependent only to a minor extent on molecular asymmetry and hydration, would appear to be a method of great potential value in the study of the denaturation process. Furthermore, results obtained over a wide range of velocity gradients provide an insight into the homogeneity (with regard to length) of the solute (*1*, *2*).

The authors have completed more than 100 flow birefringence runs, in the concentric cylinder apparatus used by one of us previously (*3*), on ovalbumin denatured in various ways. The orientation angle χ (the angle between the optic axis and the direction of streaming) was measured, as well as the birefringence. The recently computed numerical solutions of Edsall and co-workers (*5*) for the flow orientation equations developed by Peterlin and Stuart (*6*, *7*) were used for determining α (the ratio of the velocity gradient G to the rotary diffusion constant β) from the χ values obtained. The apparent length of the denatured ovalbumin molecules was calculated by applying the Perrin (*5*) theory for the case of an elongated ellipsoidal structure.

Denaturation of ovalbumin was carried out under such conditions that no precipitation, gelation, or appreciable turbidity occurred. Heat denaturation was studied in the pH range 1–4 using glycine-buffered and unbuffered solutions, and in the pH range 6–9 in the presence of veronal and phosphate buffers. Urea denaturation was followed in the pH range 6–9. The effect of cationic detergents on the acid side of the isoelectric point and of anionic detergents on the alkaline side was also examined. In a typical heat denaturation experiment 0.100 g of ovalbumin in 15.0 ml of buffer was heated at 100° C for a given period of time; the solution was cooled rapidly and diluted with 42.0 g of 95% glycerol. (The gly-

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TABLE 1

Concentration of ovalbumin during denaturation, %	Denaturing medium	pH after heating	Temperature, C	Heating time	Length, A	
					$G\eta/T = 0.823$*	$G\eta/T = 2.82$*
0.60	0.100 *N* HCl	1.0	100	5 min	1,330	1,110
0.60	Glycine + HCl buffer	2.3	100	5 "	640	630
0.60	" " " "	2.3	100	60 "	940	850
0.60	" " " "	4.0	100	5 "	2,000	1,600
2.40†	" " " "	2.3	100	5 "	830	720
2.40†	" " " "	2.3	100	60 "	1,130	1,010
2.40†	" " " "	2.3	100	240 "	1,560	1,300
1.20	Phosphate buffer containing 40% urea	7.5	37	24 hr	1,230	1,070
1.20	Veronal buffer containing 40% urea	9.0	37	24 hr	740	680
0.60	Veronal buffer	8.0	100	5 min	1,710	1,420
0.60	Veronal buffer containing 18% urea	8.0	100	10 "	650	700
1.20	Glycine + HCl buffer containing 0.53% alkyl dimethyl benzyl ammonium chloride	2.3	100	5 "	600	580
0.60	Glycine + HCl buffer containing 0.17% alkyl dimethyl benzyl ammonium chloride	2.0	100	15 "	620	620
0.60	Veronal buffer + 0.30% dodecyl benzene sodium sulfonate	8.0	100	5 "	Too low to measure	

* Velocity gradient (sec^{-1}) times viscosity (poise) divided by absolute temperature.
† Diluted with buffer to 0.60% before diluting 15-ml aliquot with glycerol.

cerol was added to increase the viscosity of the solvent and hence promote orientation in the streaming gradient.) The solutions were filtered through sintered glass, deaerated by evacuation, and immediately run at 25° C. Some typical results obtained under a variety of conditions are presented in Table 1 in the form of apparent lengths calculated for a high and a low velocity gradient.

It will be noted that the apparent lengths obtained are highly variable, depending on conditions of denaturation. In particular, it is seen that protein concentration is important, a reaction of higher than first order being indicated. (The unaltered molecule is so short as not to influence the results appreciably.) Also, in general, the systems are heterogeneous, the apparent length decreasing markedly with increasing gradient.

There are a few runs, however, in which good agreement with the flow theory for homogeneous, rigid molecules is obtained. In all cases these correspond to lengths of about 600 A. Such results are obtained only at low protein concentration and, in the case of heat denaturation, only after brief heating. Samples denatured at pH 2.0–3.0 are more homogeneous and shorter than those at lower or higher pH in the acid range. Urea-denatured samples appear homogeneous only above pH 8.0 and in the more dilute protein systems.

It is concluded that aggregation is playing an important part in the over-all denaturation process for the following reasons: (1) The increase in apparent length and birefringence is concentration-dependent. (2) Lengths can be obtained which are much longer than could be accounted for on the basis of an unaggregated molecule of the molecular weight of ovalbumin. (3) The samples of greater apparent length are decidedly inhomogeneous. (4) Cationic detergents in the acid range and anionic detergents in the alkaline range slow the development of high lengths, a result that would be expected on the assumption that the cations (or anions) would be strongly bound and enhance intermolecular repulsive forces.

Frederieq (*4*) has reported lengths of 900–2,400 A for denatured ovalbumin using flow birefringence. Our results would indicate that aggregation was severe under the conditions he used.

It thus appears that the denaturation of ovalbumin under a wide range of conditions is complicated by aggregation, and interpretation of the results obtained by flow birefringence or any other technique must be made only with care. The fact that the only apparently homogeneous systems obtained under very different conditions yield lengths of about 600 A seems significant. This length corresponds to 1.5 A per residue, assuming no change in molecular weight, and could correspond to the unfolded unaggregated unit.

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