the reaction of tertiary phosphines with polyhalomethanes.[4]

(4) Acknowledgment is made to the Donors of the Petroleum Research Fund administered by the American Chemical Society (Grant 286-A), and to the National Cancer Institute of the National Institutes of Health (Grant CY-4769) for support of this research.

(5) Alfred P. Sloan Fellow, 1961–1963.

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RECEIVED FEBRUARY 22, 1962

EFFECT OF SOLVENT ON THE OPTICAL ROTATORY DISPERSION OF UNCHARGED MOLECULES CONTAINING THE PEPTIDE GROUP[1]

Sir:

It has been reported[2,3] that the optical rotatory dispersion of many native globular proteins does not resemble the optical rotatory dispersion of polypeptides with an α-helical structure, and that the change in going from a native to a denatured state is not the change expected for a helix-coil transition. In terms of the Moffitt–Yang equation[4,5]

$$\frac{3}{n^2+2}\cdot\frac{M_0}{100}[\alpha] = \frac{a_0\lambda_0^2}{\lambda^2-\lambda_0^2} + \frac{b_0\lambda_0^4}{(\lambda^2-\lambda_0^2)^2} \quad (1)$$

it is found that typical right-handed α-helical structures have $b_0 \simeq -650°$ (with λ_0 assigned a value of 212 mμ), whereas unfolded structures have $b_0 \simeq 0$. In the many "non-helical" globular proteins, by contrast, b_0 is close to zero in both native and unfolded states. Only a_0 changes when the protein becomes denatured.

A possible explanation of these results is the existence of a specific structure, as yet unidentified, which all "non-helical" proteins have in common. An alternative suggestion[2] is that no particular structure is needed to account for the observed results: that the difference between the optical rotation of native and denatured proteins may be partly (sometimes entirely) a quasi-solvent effect, reflecting the fact that the peptide groups of the native protein are largely in the interior of the globular structure, whereas in an unfolded conformation they are in a medium consisting largely of water.

A necessary part of any proof for such a hypothesis is a demonstration that the optical rotatory properties of *independent* peptide groups depend strongly on the solvent. We have accordingly made measurements, in a variety of solvents, on several simple molecules which contain peptide groups. Only uncharged molecules were used, so that changes in state of ionization (which are known to have important effects on the optical rotation of amino acids and small peptides) cannot occur.

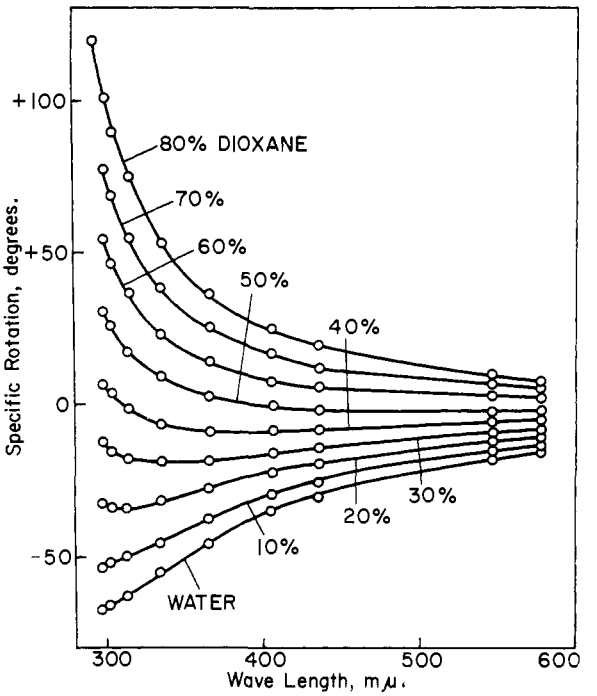

Fig. 1.—Optical rotatory dispersion of N-acetyl-L-glutamic acid, at 25°, in dioxane–water mixtures. Concentration of dioxane is per cent by volume. A Rudolph spectropolarimeter, with a mercury lamp as light source, was used.

The results of one such study are shown in Fig. 1, which gives the measured rotation for N-acetyl-L-glutamic acid in mixtures of dioxane and water. It is seen that a striking solvent effect is indeed observed. When the data are plotted according to equation 1, with $\lambda_0 = 212$ mμ, the values of a_0 and b_0 given in Table I are obtained. A change in solvent is seen to influence a_0 but not b_0. The total

TABLE I

PARAMETERS OF THE MOFFITT-YANG EQUATION[a] (Degrees of Rotation)

		a_0	b_0
Ac-L-Glu[b]	In water	−165	+67
	In 10% dioxane	−140	+62
	In 20% dioxane	−113	+65
	In 30% dioxane	− 89	+68
	In 40% dioxane	− 60	+67
	In 50% dioxane	− 28	+67
	In 60% dioxane	+ 6	+64
	In 70% dioxane	+ 35	+66
	In 80% dioxane	+ 64	+67
β-Lactoglobulin[c]	Native	−169	−66
	Denatured	−623	−77
γ-Globulin[d]	Native	−280	0
	Denatured	−600	−20

[a] Using $\lambda_0 = 212$ mμ. The dispersion of refractive index was taken into account in the calculations of this paper. [b] Ac-L-Glu = N-acetyl-L-glutamic acid. [c] Ref. 2. [d] C. Tanford, C. E. Buckley III, P. K. De and E. P. Lively, *J. Biol. Chem.*, **237**, 1168 (1962).

(1) This work was supported by research grant G 17477 from the National Science Foundation, and by research grant A-4576 from the National Institute of Arthritis and Metabolic Diseases, U. S. Public Health Service.

(2) C. Tanford, P. K. De and V. G. Taggart, *J. Am. Chem. Soc.*, **82**, 6028 (1960).

(3) B. Jirgensons, *Tetrahedron*, **13**, 166 (1961).

(4) W. Moffitt and J. T. Yang, *Proc. Nat. Acad. Sci., U. S.*, **42**, 596 (1956). In this paper this equation is to be regarded as purely empirical.

(5) In equation 1, $[\alpha]$ is the rotation at any wave length λ, M_0 the molecular weight (in proteins it is the molecular weight per residue), n the refractive index of the solvent, and λ_0 an absorption wave length, which has been assigned a value of 212 mμ in this paper. The parameters a_0 and b_0 are derived from experiment.

change in a_0 in going from water to 80% dioxane is approximately half as large as the difference in a_0 between denatured and native "non-helical" proteins, data for two of which are shown in the table for comparison.

That these results are not due to the presence of a small number of ionized carboxyl groups in the purely aqueous solution, with suppression of the ionization as dioxane is added, was demonstrated by repeating some of the experiments in the presence of 0.1 *M* HCl. No significant change was observed. The general trend of the data is also independent of the choice of λ_0, within narrow limits. With λ_0 = 234 mμ, for example, b_0 = 45° and a_0 goes from −138 to +53°.

Data similar to those shown here have been obtained with N-acetyl-L-leucine, N-benzoyl-L-glutamic acid, and N-benzoyl-L-leucine amide, and with a wide variety of solvents. For each substance, the effect of the solvent is to change a_0 and not b_0. In each case the non-polar solvents lie at one extreme and the more polar ones at the other. The results will be reported in full at a later date. The conclusion to be drawn from all the data is that a change in environment of peptide groups of a protein, such as accompanies denaturation, is likely to make a substantial contribution to the over-all change in optical rotation, and that this contribution is likely to appear as a change in a_0 when the data are analyzed by use of equation 1.

(6) The able technical assistance of Mrs. P. M. Hudson is acknowledged.

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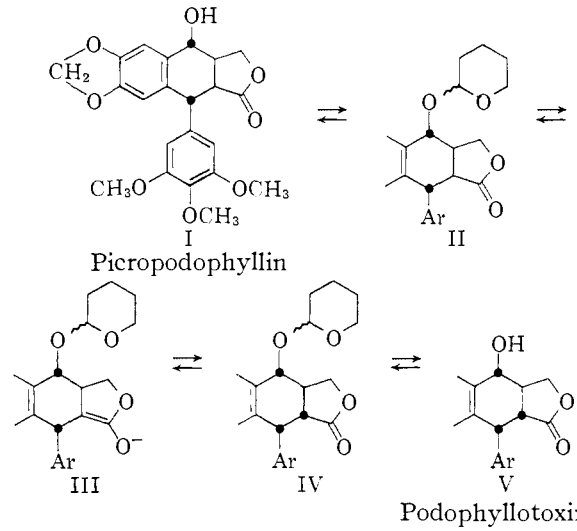
RECEIVED FEBRUARY 15, 1962

SYNTHESIS OF PODOPHYLLOTOXIN[1]

Sir:

Podophyllotoxin (V), containing a strained lactone system, is easily and essentially completely isomerized with alkaline catalysts to the thermodynamically more stable picropodophyllin (I).[2] We now find that protonation of the enolate[3] of a picropodophyllin derivative makes possible the regeneration of podophyllotoxin (V) from picropodophyllin (I).

Protonation of the enolate of picropodophyllin (as in III) could lead either to podophyllotoxin or to picropodophyllin. Examination of models suggested that the front of the enolate is somewhat more accessible to an approaching proton donor than the back. Conceivably, therefore, frontside protonation, which would give podophyllotoxin, could compete effectively with backside protonation, which would give picropodophyllin.



Test of this possibility required formation of the enolate of picropodophyllin. To avoid complications arising from the presence of hydroxyl hydrogen, picropodophyllin (I) was converted by combination with dihydropyran to O-tetrahydropyranylpicropodophyllin (II). One of the two crystalline diastereoisomers of II[4] was treated at room temperature with just over one molar equivalent of triphenylmethylsodium in ether.[5] Then to neutralize enolate III in the resulting tan mixture, cold acetic acid containing a trace of sulfuric acid was added in one portion. After preliminary fractionation of the protonated material, the tetrahydropyranyl derivative (IV) of podophyllotoxin was treated with hot hydrochloric acid in alcohol to remove the protecting group. Crystallization and chromatography of the product furnished pure podophyllotoxin (V) in 23% yield.[6] Approximately 40% of the protonated product consisted of the tetrahydropyranyl derivative of picropodophyllin (II) mixed with some picropodophyllin. It is expected that proton sources bulkier than acetic acid will increase the ratio of podophyllotoxin to picropodophyllin.

Since picropodophyllin has been synthesized,[7] the work described here completes a total synthesis

(1) This report, describing a portion of the Doctoral research of C. D. Gatsonis, represents paper No. XIII in the series on "Compounds Related to Podophyllotoxin"; the preceding paper is by W. J. Gensler, F. Johnson, and A. D. B. Sloan, *J. Am. Chem. Soc.*, **82**, 6074 (1960).

(2) A comprehensive review is given by J. L. Hartwell and A. W. Schrecker, *Fortschr. Chem. Org. Naturstoffe*, **17**, 83 (1958).

(3) The rate controlled protonation of enols has been studied carefully by Zimmerman and his associates. Pertinent references may be found in a paper by H. E. Zimmerman and T. W. Cutshall, *J. Am. Chem. Soc.*, **81**, 4305 (1959). In the present note, we have made no distinction between enol and enolate; the same stereochemical arguments apply to both.

(4) Some of the properties of this form are: m.p. 203–204°; $[\alpha]^{25}D$ +103 (c = 1 in chloroform); ultraviolet absorption maximum as a 10^{-4} *M* solution in alcohol, 291 mμ (log ε 3.51); no infrared absorption for hydroxyl at 3700–3125 cm.$^{-1}$; *Anal.* Calcd. for $C_{27}H_{30}O_9$: C, 65.05; H, 6.07. Found: C, 65.31; H, 6.10. Exploratory work by Dr. S. C. Chakravarti showed that the reaction of podophyllotoxin with dihydropyran and hydrochloric acid catalyst gave the crude tetrahydropyranylpodophyllotoxin in low yield. Later, R. G. McInnes obtained the tetrahydropyranyl derivatives of podophyllotoxin and of picropodophyllin, as mixtures, by using dihydropyran as solvent and a trace of phosphorus oxychloride as catalyst. In the present work, the tetrahydropyranyl derivatives of picropodophyllin were prepared with dihydropyran in chloroform solvent with *p*-toluenesulfonic acid as catalyst.

(5) W. B. Renfrew, Jr., and C. R. Hauser, "*Org. Syntheses*," Collective Volume 2, 607 (1943); C. R. Hauser and B. E. Judson, Jr., *Org. Reactions*, **1**, 286 (1942).

(6) Some of the data are: m.p. 160–161°; the synthetic podophyllotoxin showed m.p. 156–157° when mixed with authentic material (m.p. 156–157°); $[\alpha]^{25}D$ −132° (c = 1 in chloroform); the infrared absorption curves of synthetic and authentic podophyllotoxin are the same; treatment of synthetic podophyllotoxin with piperidine causes isomerization to picropodophyllin.

(7) W. J. Gensler, C. M. Samour, Shih Yi Wang and F. Johnson, *J. Am. Chem. Soc.*, **82**, 1714 (1960).