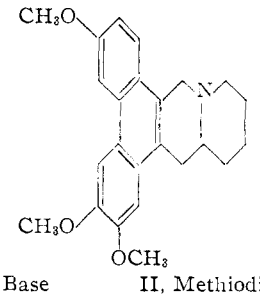
57.29; H, 5.08; N, 3.71. Found: C, 57.11; H, 4.77; N, 3.90). Reduction yielded the corresponding amino derivative (m.p. 206–207°) which when diazotized underwent the Pschorr cyclization to yield 2,3,6-trimethoxyphenanthrene-9-carboxylic acid (m.p. 222°; Calcd. for $C_{18}H_{16}O_5, H_2O$: C, 65.44; H, 5.49. Found: C, 65.54; 5.36). The phenanthroic acid was converted to the ethyl ester (m.p. 136.5–137°), which was reduced to the corresponding carbinol (m.p. 184–187°; *Anal.* Calcd. for $C_{18}H_{18}O_4$: C, 72.56; H, 6.17. Found: C, 72.46; H, 6.08) by the action of lithium aluminum hydride.



I, Base II, Methiodide

The bromide obtained from 2,3,6-trimethoxyphenanthryl-9-carbinol was not purified, but allowed to react immediately with pyridine-2-aldehyde. The salt thus obtained was cyclized by heating it in polyphosphoric acid at 80° for five hours. The cyclization product, presumably a 2,3,6-trimethoxydibenzo[h,j]acridizinium salt, could not be recrystallized satisfactorily and therefore was converted to the chloride by means of ion exchange and hydrogenated directly using platinum oxide catalyst. The product, purified by chromatography, melted at 199–200° (lit.[4] m.p. 197–198°; *Anal.* Calcd. for $C_{24}H_{27}NO_3$: C, 76.36; H, 7.21; N, 3.71. Found: C, 75.85; H, 7.17; N, 4.00) did not depress the melting point of an authentic sample,[7] and had ultraviolet and infrared spectra like those reported for cryptopleurine. The methiodide (II, m.p. 272–274°, lit.[4,8] 270–272° did not depress the melting point of an authentic sample of ±-cryptopleurine methiodide, and the infrared spectra (potassium bromide plate method) of the two substances were identical.

(7) We are indebted to Dr. E. Gellert for making this comparison for us and for furnishing us with a sample of ±-cryptopleurine methiodide.

(8) E. Gellert, *Australian J. Chem.*, **9**, 489 (1956). Since Dr. Gellert's method for obtaining the ± base from natural (optically active) cryptopleurine is somewhat involved, he has taken pains to demonstrate rigorously that the optically inactive methiodide is actually ±-cryptopleurine methiodide.

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RECEIVED MAY 11, 1957

PHASE EQUILIBRIUM IN THE HYDROTHERMAL SHRINKAGE OF COLLAGEN

Sir:

The shrinkage of collagen has been shown to denote a first order transition between crystalline and amorphous phases.[1,2] This being the case, the stress τ_{eq} required to maintain a state of equilibrium between the phases should be related to the temperature T according to an analog of the Clausius–Clapeyron equation[3,4]

$$d(\tau_{eq}/T)/d(1/T) = (\Delta\overline{\overline{H}}/V_c)/(\Delta\overline{\overline{L}}/L_c) \qquad (1)$$

where $\Delta\overline{\overline{H}}$ and $\Delta\overline{\overline{L}}$ are the latent enthalpy and length changes accompanying melting and subsequent swelling in the excess of surrounding solvent, V_c and L_c are the volume and length of the (dry) crystalline fiber, and τ_{eq} is the stress referred to the cross section of the fiber in the same state. Tobolsky, Haselkorn and Catsiff[5] have attempted recently to apply this relationship to the spontaneous shrinkage temperature T_s for bovine tendon at various stresses τ_s, the sample being immersed in water. Their results, like those similarly obtained a number of years ago by Wöhlisch,[6] yield a value of about 1.7 cal. $g.^{-1}$ for the latent enthalpy.[7]

We wish to point out that the temperature for the onset of shrinkage is substantially higher than that required for equilibrium between crystalline and amorphous phases. The values of τ_s and T_s which pertain to the shrinkage phenomenon as ordinarily determined should not therefore be employed in the foregoing equation, which is applicable only at thermodynamic equilibrium. We have found, however, that the equilibrium stress τ_{eq} at a given temperature T_m may be determined readily by the procedure outlined below.

Fresh rat-tail tendons were cross-linked (*i.e.*, tanned) with formaldehyde in order to suppress flow in the amorphous phase. A single tanned fiber about 10 cm. in length was suspended vertically between a fixed lower clamp and an upper clamp attached to a strain gage. The fiber and clamps were immersed in thermostated 3 *M* KCNS solution and the fiber was allowed to undergo partial contraction, such that one or more totally amorphous (shrunken) regions formed adjacent to (native) crystalline regions. The length was then adjusted to yield a force which remained constant for at least 30 minutes. Close approach to equilibrium is indicated by the fact that a small change in force, induced by slight alteration of the length, was succeeded by a change in the force toward the equilibrium value. The observed force is independent of the degree of shrinkage of the fiber, provided that a crystalline section remains. By measuring the thus established τ_{eq} as a function of temperature, we have obtained the results summarized below for two samples differing in degree of cross-linking.

The first two rows of the table emphasize the disparity between T_s and T_m^i, the equilibrium melting temperature established by extrapolation to zero force. The latent enthalpies $\Delta\overline{\overline{H}}/V_c$ per unit volume of collagen given in the fifth row have been calculated from eq. (1) using the results in the

(1) R. R. Garrett and P. J. Flory, *Nature*, **177**, 176 (1956).

(2) P. J. Flory, *Science*, **124**, 53 (1957).

(3) G. Gee, *Quart. Rev.*, **1**, 265 (1947).

(4) P. J. Flory, THIS JOURNAL, **78**, 5222 (1956).

(5) A. Tobolsky, R. Haselkorn and E. Catsiff, *ibid.*, **78**, 5957 (1956).

(6) E. Wöhlisch, *Naturw.*, **28**, 305, 326 (1940).

(7) Professor Tobolsky has informed us that the results in their paper (ref. 5) are referred to 1 g. of *swollen* collagen, and hence that a factor of approximately 4 must be applied to convert their results to a "per unit of dry collagen" basis. Such correction has been introduced in the value 7 cal. $g.^{-1}$ quoted above.

T_s (f_s = 0), °C.	28	35
T_m^1 (f_{eq} = 0), °C.	20	28
$-d(\tau_{eq}/T)/d(1/T)$ dynes cm.$^{-2}$	9.1 × 10^8 (40°)	11 × 10^8 (50°)
$-\Delta\bar{L}/L_0$	0.48 (40°)	0.57 (50°)
$(\Delta\bar{H}/V_0)$ cal. cc.$^{-1}$	10.5	15.0
$(\Delta H/V_0)$ cal. cc.$^{-1}$	17.5	22
ΔH_u, cal./peptide unit	1200	1500

third and fourth rows. The negative contribution to $\Delta\bar{H}$ from the heat of dilution of collagen, estimated from the temperature coefficient of the degree of swelling in the amorphous state, has been deducted to obtain the latent heats of fusion expressed in cal. cc.$^{-1}$ and in cal. per mole of peptide units, respectively, in the last two rows of the table.

The values of ΔH_u are in satisfactory agreement with previous results derived from calorimetric measurements (1180 cal.[8]) and from depression of the melting point of collagen by glycol (2200 cal.[1]). The heat and entropy of fusion compare favorably with those for other crystalline polymers, contrary to the conclusions reached by Tobolsky and co-workers.[5]

Tanning with quinone yields more stable cross linkages, thus permitting investigation of the phase equilibrium in pure water at temperatures up to 70° under stress. Experiments on this system are in progress.

Similar experiments have been carried out on the shrinkage of racked rubber cross-linked by gamma radiation.[9] The heat of fusion obtained through the use of eq. (1) stands in good agreement with independent values.

(8) A. Künzel and K. Doehner, *Angew. Chem.*, **52**, 175 (1939).
(9) J. F. M. Oth and P. J. Flory, to be published.

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THE NUCLEAR MAGNETIC RESONANCE SPECTRUM OF RIBONUCLEASE[1]

Sir:

We wish to report the observation of a nuclear magnetic resonance spectrum of the protein ribonuclease. In previous investigations[2] of macromolecules in solution, it had been reported that deoxyribonucleic acid markedly altered the proton magnetic resonance spectrum of water. The two proteins studied concurrently, hemocyanin and ovalbumin, neither modified the water spectrum nor exhibited independent peaks attributable to protein protons, at the concentrations used. We have found that concentrated aqueous solutions of bovine serum albumin (20%) gave no protein peaks, when the solutions were treated as below. An observable spectrum for ribonuclease might be attributed to internal flexibility of the molecule, absent in the other proteins, although there are other possible explanations.

Bovine pancreatic ribonuclease (Armour Lot No. 381-059) was dialyzed for 12 hours against several changes of glass-redistilled water and then equilibrated for 20 hours with a similar dialysis bag containing only water. Both solutions were deionized by passage through a column of ion-exchange resins.[3] The final ribonuclease concentration was about 15%, as determined spectrophotometrically. Investigation of the ribonuclease solution in a Varian High Resolution NMR Spectrometer operating at 40 Mc. showed a small protein peak in addition to the large water peak. Exchange of the protein solution with deuterium oxide removed the interfering protons of water, as well as the exchangeable protons of ribonuclease. Using a sample tube 4.9 mm. in inner diameter, we obtained the detailed spectrum shown in Fig. 1. The sharp peak indicated by the arrow decreased in relative intensity with successive exchanges and hence is due to residual water protons. The control solution described above showed only an unmodified water peak before exchange with D_2O and no spectrum at all afterward, making it unlikely that the peaks are due to low molecular weight contaminants in the ribonuclease preparation, the dialysis casing, or the resin column.

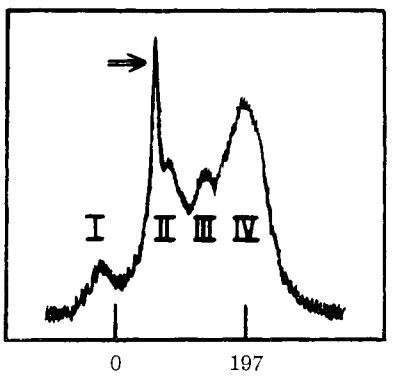

Fig. 1.—Nuclear magnetic resonance spectrum of ribonuclease.

The scale shown was established using the aromatic peak of toluene in an introduced capillary as zero. Since the spectrum range is the range of usual proton chemical shifts and since it does not change noticeably with concentration, it seems that we have an essentially unbroadened spectrum of the non-exchangeable protons of ribonuclease.

Because of their location, peaks I and IV can be assigned tentatively to aromatic hydrogens and to hydrogens on aliphatic carbon atoms attached only to other aliphatic carbons. The relative areas of each peak, after subtracting the estimated contribution of water protons, obtained from several spectra of two independently prepared samples were: I, 9 ± 1%, II, 26 ± 2%, III, 18 ± 3%, IV, 47 ± 3%. From the known composition

(1) This work was carried out partly with the help of funds provided by the Office of Naval Research, Contract No. Nonr-609(24).
(2) B. Jacobson, W. A. Anderson and J. T. Arnold, *Nature*, **173**, 772 (1954).
(3) H. M. Dintzis, Ph.D. Thesis, Harvard University, 1952.