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

third and fourth rows. The negative contribution to $\Delta\overline{\overline{H}}$ 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.

DEPARTMENT OF CHEMISTRY
CORNELL UNIVERSITY
ITHACA, NEW YORK

J. F. M. OTH
E. T. DUMITRU
O. K. SPURR, JR.
P. J. FLORY

RECEIVED MAY 7, 1957

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.

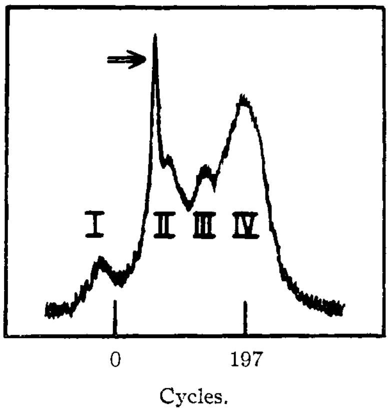


Fig. 1.—Nuclear magnetic resonance spectrum of ribonuclease.

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.

of ribonuclease, of the 664 non-exchangeable hydrogens 7.1% are aromatic and 45.0% are on aliphatic carbon atoms attached only to other aliphatic carbons. Of the remaining protons, 19.0% are on α carbons, 8.1% are on carbons attached to carboxyls and amides, 6.0% are on carbons with hydroxyl substituents, 5.4% are on carbons attached to sulfur, 4.2% are on carbons bonded to positively charged nitrogen, 3.9% are on carbons attached to aromatic nuclei, and 1.2% are on carbons bound to amide nitrogen. All of these categories could reasonably be expected to give rise to peaks between the extremes, but their positions in peaks II and III cannot be assigned with certainty at this time.

CONTRIBUTION NO. 1448 FROM THE
STERLING CHEMISTRY LABORATORY
YALE UNIVERSITY
NEW HAVEN, CONNECTICUT

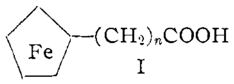
MARTIN SAUNDERS
ARNOLD WISHNIA
JOHN G. KIRKWOOD

RECEIVED MAY 20, 1957

FERROCENE BRIDGING AND HOMOANNULAR CYCLIZATIONS[1]

Sir:

The question of homoannular *vs.* heteroannular intramolecular acylation in substituted ferrocenes has been raised recently by Nesmeyanov.[2] We wish to report here examples of both of these types of cyclizations from suitable ω-ferrocenylaliphatic acids[3] (I), together with an instance of intermolecular acylation, and to present evidence that the product obtained from these ferrocenyl aliphatic acids is determined by the length of the polymethylene chain separating the ferrocene nucleus from the carboxyl group.



I

β-Ferrocenylpropionic acid[3] (Ia, n = 2), when treated with polyphosphoric acid, gave heteroannular cyclization to the bridged compound 1,1′-(α-ketotrimethylene)-ferrocene,[4] m.p. 144–144.5°.[5] *Anal.* Calcd. for $C_{13}H_{12}FeO$: C, 65.03; H, 5.04; Fe, 23.26; mol. wt., 240. Found: C, 64.50; H, 5.04; Fe, 23.98; mol. wt. 281 (ebull., benzene), 324 (ebull., butanone). The infrared spectrum contains ketone bands at 1682 and 1266 cm.$^{-1}$, but no bands near 1110 or 1004 cm.$^{-1}$. Presence of 1110 and 1004 cm.$^{-1}$ bands would indicate an unsubstituted cyclopentadienyl ring.[3,6] The ultraviolet spectrum of this ketone is highly characteristic in that it shows no maximum near 225 or 270 mμ, regions where maxima have been shown to occur for ketones conjugated with the ferrocene nucleus.[7] Such spectral behavior is reasonable for a compound of this structure since the stereochemistry of the 3-carbon bridge prevents coplanarity, and thus conjugation, of the carbonyl with the adjacent ring.

γ-Ferrocenylbutyric acid[3] (Ib, n = 3), when treated with either polyphosphoric acid or trifluoroacetic anhydride, gave as the major product the homoannular cyclized ketone, 1,2-(α-ketotetramethylene)-ferrocene,[8] m.p. 85.4–85.7°, λ_{max} 226, 270 mμ, ϵ_{226} 17,600, ϵ_{270} 7,500, infrared bands: 1679, 1281, 1109, 1003 cm.$^{-1}$. *Anal.* Calcd. for $C_{14}H_{14}FeO$: C, 66.19; H, 5.55; mol. wt., 254. Found: C, 66.59; H, 5.69; mol. wt., 286 (ebull., butanone).

δ-Ferrocenylvaleric acid[3] (Ic, n = 4), with either polyphosphoric acid or trifluoroacetic anhydride, gave the homoannular product, 1,2-(α-ketopentamethylene)-ferrocene, m.p. 65.5–66.5°, λ_{max} 227, 269 mμ, ϵ_{227} 15,500, ϵ_{269} 7,400, infrared bands: 1655, 1290, 1110, 1004 cm.$^{-1}$. *Anal.* Calcd. for $C_{15}H_{16}FeO$: C, 67.19; H, 6.02; mol. wt., 268. Found: C, 66.99; H, 6.18; mol. wt., 314 (ebull., butanone). The shift in the infrared carbonyl band to lower frequency[9] and the reduction in the extinction coefficient in the ultraviolet spectrum,[10] when compared to similar spectral features of the lower, homologous, ketone from IIb, containing a 6-membered ring, are in agreement with like shifts found between the corresponding 7- and 6-membered benzocyclanones.[9,10]

The principal product from the treatment of ϵ-ferrocenylcaproic acid[3] (Id, n = 5) with polyphosphoric acid was a red-brown solid, apparently polymeric,[11] insoluble in usual organic solvents, decomposing slowly above 200°. The material exhibits strong infrared ketonic absorption (1660 cm.$^{-1}$), weaker carboxyl absorption (1725, 2700, 2620 cm.$^{-1}$) and gives analytical data consistent with those for a condensation trimer, as $H[C_{10}H_8\text{-}Fe(CH_2)_5CO]_3OH$, though it is more likely a mixture of compounds of varying molecular weight. *Anal.* Calcd. for $C_{48}H_{56}Fe_3O_4$: C, 66.68; H, 6.53; Fe, 19.38. Found: C, 66.76; H, 6.58; Fe, 18.95.

---

(1) Presented in part at the 131st Meeting of the American Chemical Society, Miami, Florida, April 7–12, 1957; *cf.* Abstracts, p. 46-O.

(2) A. N. Nesmeyanov, N. A. Volkenau and V. D. Vilchevskaya, *Doklady Akad. Nauk S.S.S.R.*, **111**, 362 (1956).

(3) K. L. Rinehart, Jr., R. J. Curby, Jr., and P. E. Sokol, THIS JOURNAL, **79**, in press (1957).

(4) Ferrocene substituents on the same cyclopentadienyl ring are numbered "1, 2, 3," etc., as in the benzenoid compounds, while substituents on the second cyclopentadienyl ring are indicated by an accent, as "1′, 2′, 3′." etc. [*cf.*, *e.g.*, P. L. Pauson, THIS JOURNAL, **76**, 2187 (1954)].

(5) Woodward and Csendes obtained what is apparently the same compound (m.p. 141–142°) from treatment of β-ferrocenylpropionic acid with trifluoroacetic anhydride, though the ketone was incompletely characterized (R. B. Woodward and E. Csendes, personal communication; *cf.* ref. 6, Appendix).

(6) M. Rosenblum, Ph.D. Thesis, Harvard University, August, 1953.

(7) K. L. Rinehart, Jr., K. L. Motz and S. Moon, THIS JOURNAL, **79**, 2749 (1957).

(8) Nesmeyanov[2] has recently assigned a di-homoannular structure similar to this to the polyphosphoric acid cyclization product from 1,1′-ferrocenedibutyric acid.

(9) W. M. Schubert and W. A. Sweeney, THIS JOURNAL, **77**, 4172 (1955).

(10) G. D. Hedden and W. G. Brown, *ibid.*, **75**, 3744 (1953).

(11) Homoannular cyclization of Id would require the formation of an 8-membered ring expected to form with difficulty. A similarly difficult cyclization would be anticipated from ferrocenylacetic acid[3] (I, n = 1), which must give either a highly strained 4-membered benzocyclobutanone ring or a 2-carbon bridge, for which the interannular distance probably is too great.[12] In preliminary experiments in polyphosphoric acid, no ketonic product has been obtained.

(12) P. F. Eiland and R. Pepinsky, THIS JOURNAL, **74**, 4971 (1952); J. D. Dunitz and L. E. Orgel, *Nature*, **171**, 121 (1953).