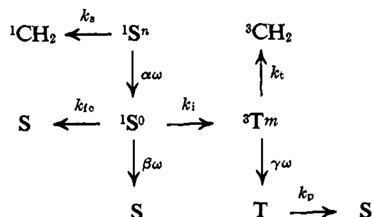
Scheme I



we will accept it as a useful basis for discussion of diazomethane also. Here $^1S^n$ is the lowest excited singlet at the level n of vibrational excitation; intersystem crossing occurs from lower vibrational levels; S and T are unreactive states; α, β, and γ are efficiency factors which could be unity. The original mechanism[8] has been modified by addition of the collisional stabilization rate,[9] $\beta\omega$. A maximum ratio $[^3CH_2/^1CH_2]$ is predicted from a steady-state treatment

$$[^3CH_2/^1CH_2] = \frac{\alpha k_t k_i \omega}{k_s(k_t + \gamma\omega)(k_i + k_{fc} + \beta\omega)} \quad (1)$$

Equation 1 has the limiting value zero, for $\omega \rightarrow 0, \infty$. Also, $\omega_{max} = (k_t k_i)^{1/2}$ and

$$[^3CH_2/^1CH_2]_{max} = \frac{k_i k_t}{k_s(k_i^{1/2} + k_t^{1/2})^2} < (k_i/k_s), (k_t/k_s) \quad (2)$$

where, for simplicity, k_{fc} was omitted and α, β, and γ set at unity.

The present study was designed to test the two explanations. Experiments with ketene, diazomethane, and *cis*-butene-2 have been conducted at pressures above the highest previously used.[2,3] Since butene cannot be taken above 2 atm. at room temperature, ethylene was used in one experiment as an inert gas with respect to diazomethane. However, it is not an inert gas with respect to methylene and should not bring about collision-induced[10] intersystem crossing of the methylene itself, $^1CH_2 + M \not\rightarrow {}^3CH_2 + M$. Obviously, however, the noninterfering side products, cyclopropane and propylene were formed.

Some results for diazomethane are summarized in Figure 1. A maximum in proportion of $^3CH_2$ is achieved at ~1 atm. pressure; it drops markedly at higher pressures. Similar behavior was confirmed with ethylene diluent. The behaviors of diazomethane systems in the gas and liquid phases are thus to be reconciled by the second explanation. The temperature dependence of the triplet proportion, found both here and in the ketene system, merits further investigation.

On the basis of Scheme I, which is a reasonable, if not proven, basis of discussion, there can be no collision-induced intersystem crossing as originally tentatively suggested[2a,3]: if k_i is replaced or supplemented by a collision process, characterized by rate constant $\delta\omega$, then no maximum in the ratio $[^3CH_2/^1CH_2]$ can arise. In addition, both collision processes $\beta\omega$ and $\gamma\omega$ must be assumed to occur and to be dominant at high pressures.

The situation is somewhat different for ketene

(8) (a) G. B. Porter and B. T. Connelly, *J. Chem. Phys.*, **33**, 81 (1960); (b) G. A. Taylor and G. B. Porter, *ibid.*, **36**, 1353 (1962).

(9) Such a possibility is considered reasonable on the basis of existing information by Professor Porter (private communication). The internal conversion process, k_{fo}, is not definitely established; a phosphorescence process has not been seen for ketene.

(10) H. M. Frey, *J. Am. Chem. Soc.*, **82**, 5947 (1960).

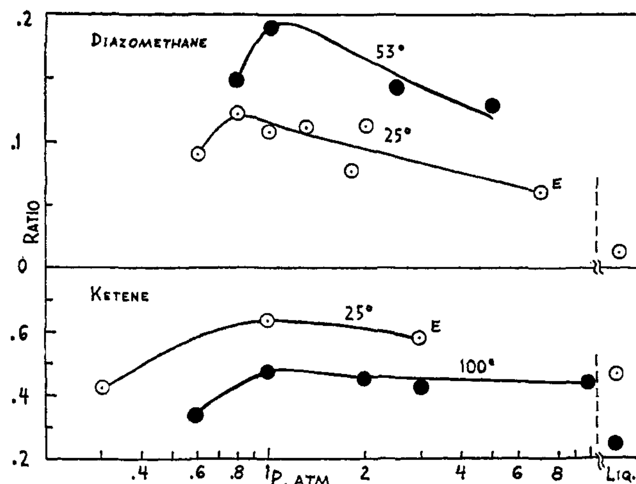


Figure 1. Plot of [*trans*-dimethylcyclopropane/*cis*-pentene-2] *vs.* pressure. The *trans* compound is the principal diagnostic product of $^3CH_2$ reaction. The use of *cis*-dimethylcyclopropane in the ratio, in place of *cis*-pentene-2, gives a curve of the same shape. Other diagnostic triplet products, 3-methylbutene-1 and *trans*-pentene-2, give similar, but inaccurate, trends. E stands for ethylene and Liq. for liquid.

(actually, for other reasons, ketene-d_2 was used). Here, only a moderate maximum in the ratio $[^3CD_2/^1CD_2]$ and only a modest further drop in liquid butene were found. We know of no prior liquid phase results bearing on these matters for ketene. Insofar as a maximum ratio effect exists, it militates against the likelihood of a collisional intersystem crossing process[11] (even though such a process is compatible with a plateau in $^3CH_2$ proportion). Whether a plateau or a maximum in the ratio exists (a study of the effect of variation of λ would be of interest), it is necessary to invoke *at least* one of the collisional processes, $\beta\omega$ or $\gamma\omega$, in I. For a plateau, the limiting ratio, as $\omega \rightarrow \infty$, becomes either $<k_i/k_s$ or $<k_t/k_s$, depending on whether $\beta\omega$ or $\gamma\omega$ is retained. Porter[8] has given k_s as 2×10^9 sec.$^{-1}$, which places the present phenomena in the proper pressure region. Scheme I is not necessarily complete. For example, collisional excitation of T could be included; this would modify the above discussion regarding the optional roles of $\beta\omega$ and $\gamma\omega$ in an obvious way.

The present type[2a] of chemical evidence is a powerful complement to quantitative spectroscopic–photochemical studies on these systems. Further details and extension will be submitted shortly.

(11) C. S. Parmenter, *J. Chem. Phys.*, **41**, 658 (1964), has found no evidence for such collisional processes with other carbonyl-containing compounds, which supports, but does not prove, our present conclusion with respect to ketene.

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Molecular Weight and Molecular Weight Distribution of Unfractionated Yeast Transfer Ribonucleic Acid[1]

Sir:

The marked functional similarities of the different amino acid specific s-RNA's[2] suggest that this class of

(1) This investigation was supported by grants from the National Science Foundation (GB-492), the American Heart Association, and the National Institutes of Health (GM-07654).

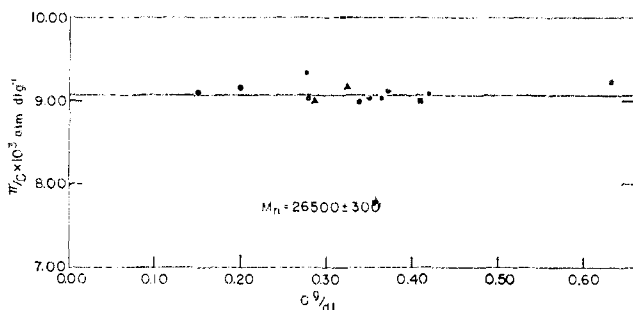

Figure 1. Reduced osmotic pressure, π/c, of yeast s-RNA as a function of concentration, c, at 20 ± 0.01°. A modified Fuoss–Mead block-type osmometer was used,[12] together with Visking cellophane membranes. Equilibrium was attained within 12 hr. and equilibrium positions remained constant over a 3-day period, reflecting membrane impermeability to the s-RNA as well as sample stability. Circles, sample 1; triangles, sample 2; square, sample 3. The value of M_n is for s-RNA essentially lacking the adenosine terminal residue.

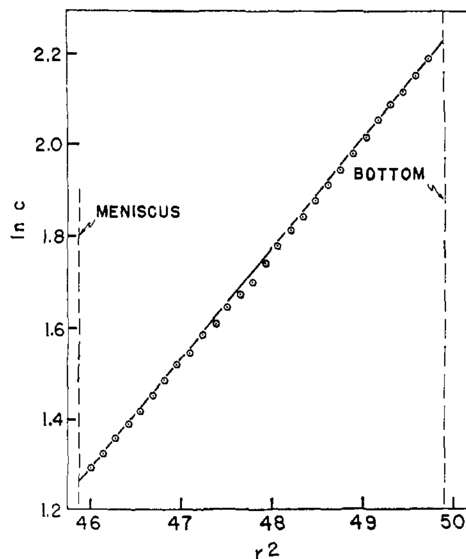

Figure 2. Sedimentation equilibrium data on yeast s-RNA, obtained with ultraviolet optics of a Spinco Model E ultracentrifuge, plotted as ln c *vs.* r^2, where c is concentration and r the distance from the center of rotation. Experimental details: rotor, AN-J; r.p.m., 9341; column height, 0.3 cm.; temperature, 20°; time, 20 hr.

molecules may share, at least within a species, certain size and shape characteristics. Consistent with this expectation, we wish to report the molecular weight parameters of unfractionated s-RNA from yeast and the finding that the molecular weight distribution of this mixed population is exceedingly narrow.

To arrive at these observations, a procedure was designed for obtaining large quantities of s-RNA in which all members of the natural population are present, but from which extraneous macromolecules are excluded; two independent methods, osmotic pressure and sedimentation equilibrium, were employed to determine M_n,[2] M_w,[2] and M_z.[2]

s-RNA was obtained from fresh bakers' yeast by phenol extraction[3] and further purified by DEAE-cellulose chromatography,[4] methoxyethanol extraction,[3] ammonium sulfate fractionation,[5] and dialysis against EDTA,[2] NaCl, and distilled water. All purification steps allowed essentially quantitative recovery of amino acid acceptor activity, and the later steps enabled similar recovery of the A_{260}.[2] Samples 1 and 2 were extracted from the same batch of cells but purified at different times, while sample 3 was obtained from another cell batch. These preparations (sodium salt) had a protein content[6] of $<0.5\%$, DNA[7] $<0.1\%$, and phosphorus[8] 9.0%, and $A_{258}^{1\%} = 215$ (ϵ_P[2] 7400) at 22° in 0.2 M Na^+, pH 6.85. After exhaustive hydrolysis by pancreatic RNAase,[2] the only nucleosides observed (thin layer chromatography) were cytidine and traces of adenosine.[9] Typical acceptor activities[10] (moles of amino acid/moles of s-RNA) were 0.022 for valine, 0.017 for alanine, 0.016 for tyrosine, and 0.022 for serine. Samples kept at 20° for 48 hr. lost no biological activity.

The solvent for physical measurements contained 0.20 M NaCl, 0.01 M phosphate (Na^+), and 0.0005 M EDTA, pH 6.85, at 20°. s-RNA solutions were dialyzed against excess solvent. Such dialysis renders solutions of different polyelectrolyte concentrations equivalent with respect to solvent, which is critical for valid measurements of osmotic pressure and partial specific volume.[11] s-RNA concentrations were determined spectrophotometrically.

Figure 1 shows the reduced osmotic pressure as a function of concentration for the three samples. The data, indistinguishable on the basis of sample, describe a line with no apparent slope, *i.e.*, no second virial coefficient. The ordinate intercept gives $M_n = 26{,}500 \pm 300$ (D.P.$_n$ = 77; a residue weight of 345 is assumed throughout).

Partial specific volume measurements were made in a 10-ml. pycnometer at 20 ± 0.01°, in the concentration range 0–4 g./dl. $\bar{V}$ was found to be 0.531 ± 0.002 ml./g.[2]

Both schlieren and ultraviolet optics were used in sedimentation equilibrium measurements. M_w and M_z values were calculated by methods I and II of Van Holde and Baldwin[13]; M_z values were also obtained according to Lansing and Kraemer.[14] The data at finite concentrations (schlieren optics, c = 0.2–1.0 g./dl.) revealed a strong concentration dependence and were extrapolated to infinite dilution. This leads to $M_w = 26{,}000 \pm 300$ (D.P.$_w$ = 75) and $M_z = 29{,}800 \pm 600$ (D.P.$_z$ = 86). Again, the data for the three samples were indistinguishable.

(2) Abbreviations used: s-RNA = transfer ribonucleic acid; DNA = deoxyribonucleic acid; M_n = number average molecular weight; M_w = weight average molecular weight; M_z = Z-average molecular weight; EDTA = ethylenediaminetetraacetate; A_{260} = absorbance at 260 mμ; ϵ_P = molar extinction coefficient based on phosphorus; RNAase = ribonuclease; $\bar{V}$ = partial specific volume; D.P. = degree of polymerization.

(3) R. Monier, M. L. Stephenson, and P. C. Zamecnik, *Biochim. Biophys. Acta*, **43**, 1 (1960).

(4) R. W. Holley, *Biochem. Biophys. Res. Commun.*, **10**, 186 (1963).

(5) T. Lindahl and J. R. Fresco, unpublished results.

(6) A. G. Gornall, C. T. Bardawell, and M. M. David, *J. Biol. Chem.* 177, 751 (1949).

(7) K. Burton, *Biochem. J.*, **62**, 315 (1956).

(8) B. N. Ames and D. T. Dubin, *J. Biol. Chem.*, **235**, 769 (1960).

(9) The terminal adenosine of commercial yeast s-RNA has previously been found essentially lacking: *cf.* V. M. Ingram and J. A. Sjöquist, *Cold Spring Harbor Symp. Quant. Biol.*, **28**, 133 (1963).

(10) S. Nishimura and G. D. Novelli, *Biochim. Biophys. Acta*, **80**, 574 (1964).

(11) E. F. Casassa and H. Eisenberg, *Advan. Protein Chem.*, **19**, 287 (1964).

(12) H. Vink, *Arkiv Kemi*, **13**, 193 (1958); S. Claesson and G. Palm, unpublished.

(13) K. E. Van Holde and R. L. Baldwin, *J. Phys. Chem.*, **62**, 734 (1958).

(14) W. D. Lansing and E. O. Kraemer, *J. Am. Chem. Soc.*, **57**, 1369 (1935).

The molecular weights obtained with ultraviolet optics on solutions that are effectively infinitely dilute (c = 0.0025 g./dl.) are M_w = 26,300 ± 300 (D.P.$_w$ = 76) and M_z = 27,400 ± 600 (D.P.$_z$ = 79). Figure 2 shows the data from an ultraviolet optics experiment plotted as ln c *vs.* r^2. The resulting line shows a very slight but distinct curvature from the meniscus to the cell bottom, where the point M_w values are 25,600 ± 300 (D.P.$_w$ = 74) and 26,800 ± 300 (D.P.$_w$ = 78), respectively. A similar direct analysis of polydispersity from the equilibrium distribution plots derived from schlieren optics experiments was not possible because of the strong concentration dependence of equilibrium distribution.

The determined M_n and M_w values (Table I) are indistinguishable, and when corrected for the missing terminal adenosine residue are seen to be coincident with the known molecular weight of one member of the population, an alanyl s-RNA for which M = 26,600 (D.P. = 77).[15] These findings cannot, however, be taken as valid evidence that all the s-RNA's contain the same number of nucleotides. M_n and M_w values, identical within experimental error, could result from a variety of narrow molecular weight distributions which would also lead to the observed M_z. Furthermore, the slight concavity in Figure 2 and the M_w values at the extremes of the curve indicate a real, but very small heterogeneity in the s-RNA samples examined. Of course, the possibility cannot now be discounted that the molecular weight distribution is even narrower than that suggested by Figure 2, since the true distribution might have superimposed on it a contribution from trace ultraviolet-absorbing contaminants. Differences in nucleotide composition among the different s-RNA molecules could only account for a part (<1 nucleotide) of the observed heterogeneity. The close proximity of the M_n, M_w, and M_z values shows that dimers or higher aggregates of s-RNA molecules, if at all present, account for <0.2% of the total material. It should be noted that the higher M_z value from schlieren optics indicates minor contamination (<1%) with nonnucleic acid material, not observable with the selective ultraviolet optics.

Table I. Molecular Weight Data on Yeast s-RNA[a]

	Osmotic pressure	Sedimentation equilibrium: Schlieren optics	Sedimentation equilibrium: Ultraviolet optics
M_n	26,500 ± 300	...	...
M_w	...	26,000 ± 300	26,300 ± 300
M_z	...	29,800 ± 600	27,400 ± 600

[a] These data are for s-RNA essentially devoid of the terminal adenosine residue.

The foregoing observations make it appear that the vast majority of s-RNA species from yeast differ by no more than a very few nucleotides from 77. This narrow molecular weight distribution suggests that the function of this class of RNA molecules has placed a severe restriction on their evolutionary development.

Acknowledgment. We are indebted to Miss Alice Adams for assistance in this work and to Professor S. Claesson and Dr. F. Friedberg for loans of the osmometers.

(15) R. W. Holley, J. Apgar, G. A. Everett, J. T. Madison, M. Marquisee, S. H. Merrill, J. R. Penswick, and A. Zamir, *Science*, 147, 1462 (1965).

(16) Research Fellow of the Helen Hay Whitney Foundation.

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Structures of Leurocristine (Vincristine) and Vincaleukoblastine.[1] X-Ray Analysis of Leurocristine Methiodide

Sir:

Leurocristine (LCR), also known as vincristine (VCR), and vincaleukoblastine (VLB) are alkaloids which have been isolated from *Vinca rosea* Linn (Madagascar periwinkle) and which are known to have antitumor activity.[2] Molecular structures for LCR and VLB have been proposed,[3] along with some tentative assignments of a few of the steric arrangements at asymmetric centers. We wish to report the complete molecular structure, stereochemistry, and absolute configuration from an X-ray diffraction study of single crystals of leurocristine methiodide. The structures of VLB and LCR follow from the known relationships[4] among these molecules.

Crystals of leurocristine methiodide, $(C_{47}H_{59}O_{10}N_4)^+I^-$, hydrate are monoclinic in the space group $P2_1$. There are two molecules in the unit cell, which has parameters a = 10.96 Å., b = 21.89 Å., c = 12.68 Å., and β = 124° 53′. Because of slow decomposition of the material in the X-ray beam, the data (about 800 reflections/crystal) were taken from five different single crystals. Anomalous dispersion pairs were recorded with the use of the Buerger automated diffractometer, and data from the crystals were correlated after the usual corrections were made for Lorentz and polarization factors. The structure was solved from a Fourier synthesis based partly upon phases for the I atoms and upon phases as given by the differences ($\Delta|F|^2$) between anomalous dispersion pairs.[5] This synthesis, which showed as a clearly recognizable feature only one of the two indole rings, was then compared with the weighted (by b) sum function, $P_c + bP_s$,[6] which combines the mirror plane of P_c with the antimirror plane of P_s. This comparison yielded 36 peaks which formed the starting point for several Fourier and least-squares cycles of refinement. The value of $R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$ is 0.12 for the 1378 observed reflections.

The molecular structure, stereochemistry, and absolute configuration, which was preserved in the X-ray

(1) American Medical Association approved generic names are Vincristine (VCR) for leurocristine and vinblastine (VLB) for vincaleukoblastine. VLB is supplied as Velban® and VCR as Oncovin® (Lilly).

(2) (a) R. L. Noble, *Canadian Cancer Conf.*, 4, 333 (1961); (b) I. S. Johnson, J. Vlantic, B. Mattus, and H. F. Wright, *ibid.*, 4 339 (1961); (c) C. T. Beer, *ibid.*, 4, 355 (1961); (d) J. H. Cutts, *ibid.*, 4, 363 (1961); (e) R. Hertz, *ibid.*, 4, 383 (1961); (f) M. E. Hodes, R. J. Rohn, and W. H. Bond, *ibid.*, 4, 389 (1961); (g) I. S. Johnson, H. F. Wright, G. H. Svoboda, and J. Vlantis, *Cancer Res.*, 20, 1016 (1960); (h) also see the symposium in *Lloydia*, 27, 275 (1964).

(3) N. Neuss, M. Gorman, W. Hargrove, N. J. Cone, K. Biemann, G. Büchi, and R. E. Manning, *J. Am. Chem. Soc.*, 86, 1440 (1964).

(4) N. Neuss, M. Gorman, H. E. Boaz, and N. J. Cone, *J. Am. Chem. Soc.*, 84, 1509 (1962).

(5) (a) A. F. Peerdeman and J. M. Bijvoet, *Acta Cryst.*, 9, 1012 (1956); (b) G. N. Ramachandran and S. Raman, *Current Sci* (India), 25, 348 (1956).

(6) W. N. Lipscomb, *J. Chem. Phys.*, 26, 713 (1957).