

ORGANIC AND BIOLOGICAL CHEMISTRY

[CONTRIBUTION FROM THE BELL TELEPHONE LABORATORIES, INCORPORATED, MURRAY HILL, NEW JERSEY]

Chemical Topology¹

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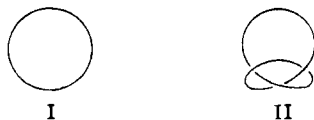
RECEIVED FEBRUARY 28, 1961

The concept of topological isomerism of cyclic molecules is introduced, *e.g.*, the isomerism between a knotted and unknotted loop and between interlocked and non-interlocked rings. The application to various chemical systems such as knots, chains and Möbius' strips is discussed. Calculations are given for the probability of formation and the stability of these materials.

The structure of a molecule is normally described by denoting (a) the order in which given numbers of specific atoms are joined, (b) the type of bonds which connect them and (c) the spatial arrangement around rigid centers such as asymmetric atoms and double bonds; in addition, one may consider the possibilities for rotational isomerism consistent with these characteristics. For most chemical systems such a description is unambiguous. For others it is not sufficient. Here we treat chemical entities in which topology must be considered.

For the purpose of molecular structure, topology is concerned with properties which remain invariant for the various spatial arrangements consistent with (a), (b) and (c). In the continuous transition from one arrangement to another, chemical bonds may not be broken or formed, even temporarily. Bonds are also not permitted to pass through one another, a process which is equivalent to a transient parting. However, bond lengths and angles can be distorted arbitrarily. The importance of adding topological distinctions to the above structural characteristics may be illustrated by two simple examples.

Examination of models indicates that a cycloparaffin of more than 50 carbon atoms can exist as a loop (I) or as a simple knot within a loop, a trefoil (II). A model of such a C_{54} system is depicted in Fig. 1. The structural features (a), (b) and (c) are the same for the two forms, yet they are clearly different species. Within the restriction given in the previous paragraph they cannot be converted into one another. Thus, I and II are topological isomers. Topological isomerism does not occur in



flexible linear systems. A knotted *n*-alkane is just another conformation of the unknotted paraffin. As with structural and stereoisomers, topological isomers may be expected to differ in some properties. For example, II is not identical to its mirror image and should be optically active while I must be inactive.

The second example occurs with cycloalkanes of more than 20 carbon atoms. These can assume a configuration which is sufficiently open to permit another saturated carbon chain to pass through it.

(1) A précis of this report has been published in "Papers presented at the New York Meeting," Division of Polymer Chemistry, September, 1960.

If the chain is closed two interlocking rings (III) are produced. Recently, an example of such a system has been prepared where each ring contains 34 members, a 34,34-catenane.² The catenane III is isomeric with the pair of non-interlocked rings (IV). Here topological isomerism involves systems of molecules. The system IV is clearly composed of two separate molecular entities. The number of molecules in III is a matter of definition. Although III is composed of two sets



of atoms, each set fulfilling the definition of a molecule and no atom of one set chemically bound to an atom of the other, the breaking of a bond is still required to separate the sets. The strength of the union between the two rings is the same as that of a linear molecule, *i.e.*, the strength of the weakest chemical bond. One may say that a "topological bond" exists between the two rings. We conjecture that molecular weight measurements will indicate III to be a single molecule, and we prefer to consider it as such.

Since some polymer systems are known to contain closed rings of large size,³ it is possible that not only II and III but also more complex examples involving knotted and interlocked rings have been prepared but have remained undetected. Below, we discuss some methods by which such systems may be prepared and characterized. Our discussion is confined to the simplest examples as the complexity increases rapidly when we leave the most elementary systems.

Some properties of these molecules may also be considered briefly. The more compact forms of II and III in comparison with their isomers should affect access by external reagents, aid transannular (and biannular) attack and influence the choice among possible transition states. However, only low molecular weight members of systems capable of exhibiting topological isomerism, such as the isomers of the 50-membered alkane ring,

(2) E. Wasserman, *J. Am. Chem. Soc.*, **82**, 4433 (1960).

(3) S. C. Ross, E. R. Coburn, W. A. Leach and W. B. Robinson, *J. Polymer Sci.*, **13**, 406 (1954); C. J. Brown, A. Hill and P. V. Youle, *Nature*, **177**, 128 (1956); H. Zahn and H. Spoor, *Chem. Ber.*, **92**, 1375 (1959); W. Patnode and D. F. Wilcock, *J. Am. Chem. Soc.*, **68**, 358 (1946); H. Jacobson, C. O. Beckmann and W. H. Stockmayer, *J. Chem. Phys.*, **18**, 1607 (1950); N. L. Paddock and H. T. Searle in H. J. Emeleus and A. G. Sharpe, ed., "Advances in Inorganic Chemistry and Radiochemistry," Vol. I, Academic Press, Inc., New York, N. Y., 1959, p. 349.

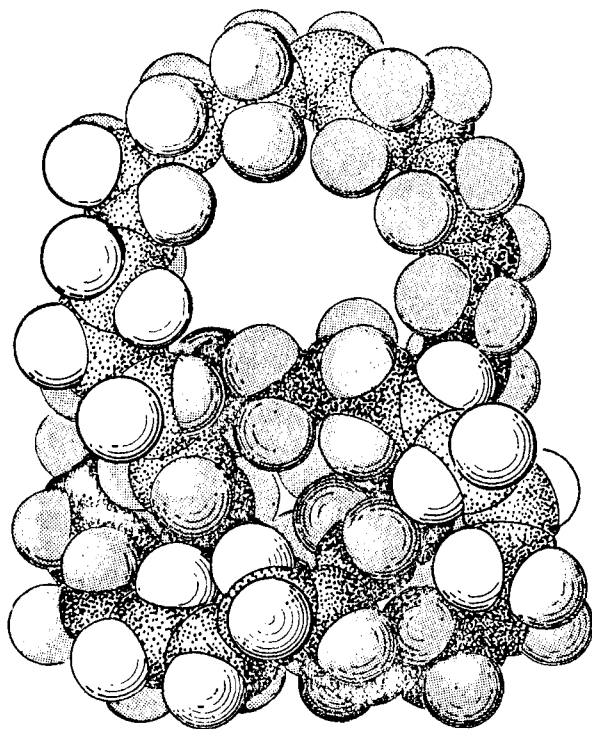


Fig. 1.—Model of a C_{64} trefoil.

I and II, would be expected to exhibit drastic differences of reactivity. Larger systems would retain enough flexibility to permit less dissimilar behavior for the isomers. Since the low molecular weight members are the most difficult to prepare, particularly by the statistical methods emphasized below, we feel that most examples of topological isomers will not differ very greatly in chemical properties.

Physical measurements, on the other hand, should demonstrate significant differences, e.g., the optical rotatory properties of I and II mentioned above. Other variations, e.g., viscosity and melting point, may also be expected. The reason for these large changes in physical properties in comparison with only small changes for chemical ones is the extended range involved in topological isomerism. These isomers differ in the structures of the entire molecules, not just a few atoms. Physical measurements which examine the properties of the whole molecule are thus more sensitive than chemical attack which confines itself to interacting with only a few active sites.

Some of the concepts considered, those of the simpler catenanes and Möbius strips, have undoubtedly been discussed informally in numerous chemical laboratories but have not appeared in print. We regret that this anonymity precludes adequate acknowledgment. To our knowledge the earliest reference to the possibility of interlocked rings in a specific chemical system is the suggestion of its existence in polysiloxanes.⁴ However, such a species had been considered fifty years ago. The late Professor R. Willstätter discussed interlocked rings in a seminar in Zürich prior to

(4) H. Frisch, I. Martin and H. Mark, *Monatsh.*, **84**, 250 (1953). A preliminary communication was given by H. Mark, *ibid.*, **83**, 545 (1952).

1912 (he was professor in Zürich between 1906–1912).⁵

Catenanes.—The formation of interlocking rings may be accomplished by the statistical threading of one ring by a linear molecule which is to be formed into the second ring.² Such a procedure utilizes the probability that the first ring, when sufficiently large, will take on a conformation which permits the precursor of the second to pass through. Alternatively, the two rings may be constructed about a central core, a procedure which should give rise to much higher yields of interlocking rings. The geometry around this foundation is chosen so that upon its cleavage from one or both rings, a catenane will result. In the yet-to-be-completed work of Kohler and Dieterich,⁶ a benzene ring is used as the core. One ring is built up on the 1,4-positions, another includes the ring and 2,5-positions. A particularly elegant application of a core is the use of a piperidine ring as employed by D. Lemal.⁷ He takes advantage of the bisecting of the nitrogen orbitals which are not in the ring by equatorial substituents in the 2,5-positions. The Möbius strip approach, mentioned below, is also an example of a core. The unsuccessful attempt to obtain a catenane by Lüttringhaus, Cramer, Prinzbach and Henglein⁸ exploits a combination of the two paths by having the first ring serve as a clathrating agent for the center of the linear molecule whose ends are free to cyclize.

The minimum number of atoms in a ring which will permit the existence of a catenane will, of course, depend upon the particular atoms which constitute the cycle and the degree of van der Waals compression that can be accepted. We have confined our study to an examination of Fisher-Hirschfelder models of saturated hydrocarbons as an indication of the possibility of the existence of various systems. We shall not discuss the many obvious extensions to different organic and inorganic chemical systems. Comparison of the size of the models with known intermolecular potential functions⁹ indicates that the former underestimates the repulsion between atoms. Although the 18-membered carbon rings can be made to interlock, we estimate that the addition of two additional carbon atoms to each ring would significantly reduce the repulsion between the loops. Thus we somewhat arbitrarily set a 20,20-catenane as the minimum size hydrocarbon possible. In the other estimates below we continue to add the two atoms to compensate for the inadequacies of the models.

As the sizes of the rings rise above 20 only the simple system III is possible for two rings until one ring is C_{30} and the other C_{37} or both are C_{33} . Then the system can exist as III or may possess

(5) V. Prelog, "relata refero."

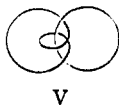
(6) H. Kohler and D. Dieterich, D. P. 1069617; see *Nach. Chem. und Technik*, **8**, 87 (1960); in *Angew. Chem.*, **72**, No. 6 (1960).

(7) Another interesting suggestion has been that of W. Closson to utilize the geometry of the ligands about a metal atom as a core. Both approaches are being investigated in Dr. Lemal's laboratory at the University of Wisconsin. (D. Lemal, private communication.)

(8) A. Lüttringhaus, F. Cramer, H. Prinzbach and F. M. Henglein, *Ann.*, **613**, 185 (1958). This report also discusses several alternative approaches to catenanes.

(9) E. A. Mason, *J. Chem. Phys.*, **23**, 49 (1955).

a doubly wound ring as in V. When the rings of V contain the same number of atoms, they are equivalent. Statistically, III is much more probable than V, but the latter could, in principle, be



isolated by some sensitive separation technique such as chromatography. Also, in contrast with III, V is not identical with its mirror image and is capable of exhibiting optical activity. Fre-

A possible experimental procedure is to cyclize a C_{30} chain to an acyloin in the presence of a C_{26} ring which contains an isotopic label. We would seek a C_{22} fraction which would also possess the isotope. This fraction could be subjected to partial resolution after reduction of the acyloin function to a ketone. Reduction to the hydrocarbon should then lead to an optically active system.

quently, saturated hydrocarbons exhibit small rotations; but this probably is due to the flexibility of the molecules near the asymmetric center. With greater rigidity the rotations can be substantial.¹⁰ In V the optical activity will be due to a fairly tight system which extends over an entire ring. We may expect that molecular rotations will be sufficient to permit observation of the activity with samples of low optical purity, thus aiding detection of V.¹¹

Two rings can be joined by a third when the latter contains a minimum of 26 carbon atoms. The linear chain VI can be obtained from two C_{20} rings at the ends and a C_{26} in the center. Even when the rings are all the same size, the two end cycles are not equivalent to the central one.

Experimentally, one would desire the two end rings to be non-identical as an aid in characterization. One ring could be a saturated, isotopically labeled, hydrocarbon, the other an olefin. Equal parts of each would serve as the solvent for a saturated acyloin closure of the middle ring. We then seek fractions containing the isotope, double bond and acyloin. Selective cleavage of the olefin would lead to a catenane. The yield of VI should be low as threading two rings is even less likely than the square of the probability that one will be threaded since the linear chain is restricted by the first cycle through which it passes.



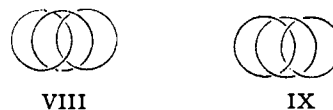
When all these rings are at least C_{30} they may be joined in a fashion which is topologically equivalent to a brewer's trademark VII.¹² Here the three rings are equivalent. All three but no two are locked together. The cleavage of any one of them separates the other two. Since the minimum size for the cycles of VII is greater than that for VI, the formation of VII is less likely.

(10) For example, poly-(S)-4-methyl-1-hexene and poly-(S)-3-methyl-1-pentene can have specific rotations of $+286^\circ$ and $+194^\circ$: P. Pino and G. P. Lorenzi, *J. Am. Chem. Soc.*, **82**, 4745 (1960).

(11) Observation of the rotatory power in the ultraviolet should increase the sensitivity of the measurement. C. Djerassi, "Optical Rotatory Dispersion," McGraw-Hill Book Co., New York, N. Y., 1960.

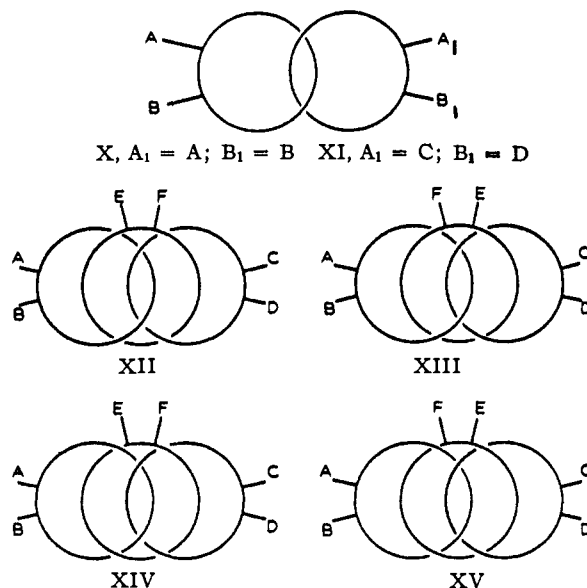
(12) P. Ballantine and Sons, Newark, New Jersey. A much more hindered system in which all three but no two rings are linked is shown in P. G. Tait, "Collected Works," Cambridge University Press, 1898, Vol. I, Plate IV, No. 18.

Three rings can also exist in two other arrangements, VIII and IX, each of which exists as a pair of enantiomorphs. With VIII the minimum size is C_{26} and with IX, C_{31} . In both instances all three rings are equivalent to one another. The cleavage of any ring leaves the other two united.



Experimentally, III, containing one olefin and one saturated, isotopically tagged ring, would serve as solvent for the closure of a third ring with an acyloin linkage. Out of this reaction we would expect VI as the major interlocked product, VIII next abundant and IX in smallest quantity. Cleavage of either the olefin or acyloin would yield only a catenane with the last two. With VI one would obtain a mixture of catenane and separate rings since two forms of VI are possible. In one the acyloin loops through the olefin ring; in the other it passes through the saturated hydrocarbon. Separation of these two is conceivable since the olefin grouping when in the center ring should be less readily accessible to an adsorbent in chromatography.

Distinguishing between VIII and IX would be most facile by the relative quantities of the two. However, it is possible to utilize optically active derivatives for differentiation. A catenane where each ring contains two substituents exists as a pair of enantiomorphs (W. Closson, private communication). The system, X, may then be considered as analogous to an active allene. If the substituents are to be attached to different centers (as in X), double-bonded substituents such as keto, thioketo and exocyclic methylene are to be preferred to avoid the introduction of asymmetric carbon atoms.



In the preparation of VIII and IX, the solvent could be a catenane XI in optically active form, with C and D able to be converted to A and B, respectively. The third ring when formed would contain E and F which could be converted into A and B so that all three rings could be identical. Out of the reaction mixture would come two diastereomers derived from VIII: XII and XIII, and two from IX: XIV and XV. After separation, say by chromatography, two separate ring cleavages are performed on each fraction. With XII, cleavage of the C,D ring followed by conversion of E and F to A and B gives the same enantiomorph as cleavage of the A, B ring and conversion of E and C to A and F and D to B. With XIII, the two cleavages and conversions also give rise to the same enantiomorph, which, however, is the mirror image of the one obtained from XII. With both XIV and XV, the two cleavages give rise to the

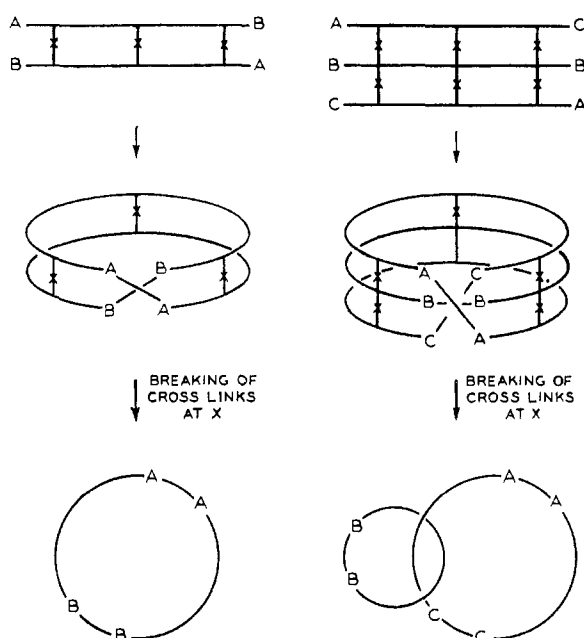


Fig. 2.—Möbius strips.

two different enantiomorphs. Thus, this involved procedure can differentiate VIII and IX.

Higher ring systems can be obtained by taking the next lower member of the series as solvent for another ring closure, as III can serve as a precursor for VI. However, after a very few rings we may expect the experimental difficulties to become enormous when statistical methods are used, and we do not feel it fruitful to discuss them further.

One final point concerns the postulation of extended interlocked ring systems in polysiloxanes⁴ and polymeric phosphonitrile chloride.¹³ Chains containing 50 to 100 rings with the order of 100 atoms per ring were postulated as arising statistically and constituting a significant fraction of the polymer. Considering the small fraction of III observed experimentally, about 1% of the acyloin rings are catenanes,² we consider such chains unlikely.

Knots.—The trefoil, II, is the simplest example of a knot.¹⁴ When the carbon chain surpasses fifty atoms we expect that knots would be of greater importance than interlocking rings in many systems since the formation of catenanes is first order in the performed rings and first order in the cyclizing chain. In polymeric systems the concentration of large rings is usually too low to permit significant formation of catenanes.³ The formation of knots is first order only in the cyclizing chain. We guess that for a C₆₀ chain, the probability that the formed ring would exist as a knot is about 10⁻³–10⁻².

Detection could be made by resolution employing the acyloin group as a handle. Reduction to the hydrocarbon should yield an optically active material. Alternatively one could convert the acyloin to an alcohol or ketone and

(13) F. Patat and P. Derst, *Angew. Chem.*, **71**, 105 (1959).

(14) An excellent introductory discussion to the mathematical theory of knots is P. G. Tait, ref. 11, Vol. I, p. 273. A more concise treatment is K. Reidemeister, "Knotentheorie," Chelsea Publishing Co., New York.

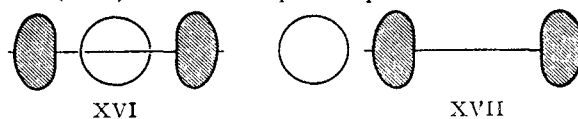
then resolve. In this case there is no complication from an additional asymmetric center, and a direct observation of the material after resolution could indicate the presence of activity due to the knot. Cleavage of the ring after this observation should cause disappearance of the activity and generation of the same open chain compound as could be obtained from the unknotted cycle. Some separation of the trefoil might be accomplished by chromatographic techniques. Possibly the much more compact structure of II will lead to adsorption properties significantly different from I.

More complex knots can exist, but their opportunity for formation from the shorter linear precursors is considerably less than that of II. Their characterization is also expected to be more difficult than that of the trefoil. One property which many of the higher knots have in common is the potentiality for optical activity.¹⁴ The symmetry required for identity with the mirror image becomes less likely as the number of random crossings increases. The usefulness of rotatory power as an aid to characterization decreases when we are forced to compare two active systems.

We conjecture that the probability that a cyclizing chain will produce a knot (moreover an optically active knot) approaches unity as the length of the chain increases without limit. However, since there does not appear to be any unambiguous example of rings containing many hundreds of atoms, we regard a test of this guess as something for the far future.

Möbius' Strips.¹⁵—In principle the Möbius' strips are a general means of obtaining some catenanes and knots. The simple strip results from taking a narrow band, giving it a half-twist about its long axis and fastening its short edges together. The resulting figure has one surface and one edge. If cut in half, parallel to the edge, a large ring results. If cut on a line one-third of the width from the edge two interlocked rings result as in Fig. 2. If one of the interlocked rings, which is a strip with four half-twists, is subjected to two additional half-cuts, a trefoil will result.^{16,17} Other possibilities for twisted strips exist: e.g., a strip with two half-twists, severed in half, will yield two interlocking rings. The strip with two twists and that with none are topological isomers.

In closing these descriptive sections, we wish to mention some isomeric systems which superficially resemble topological isomers but which actually owe their existence to the limited flexibility, extension and compression possible with molecular entities. For example, the ring which is threaded by a chain with bulky groups on either end (XVI) does not require rupture of a bond for



(15) Recently, N. van Gulick has given an extensive discussion of the possibilities of Möbius strips and braids in chemical systems. We should like to thank Dr. van Gulick for making a preprint of his work available.

(16) Listing, "Vorstudien zur Topologie" (1847) cited in Tait, ref. 12, Vol. II, p. 83.

(17) In the synthesis one must take care that cross-links are sufficiently closely spaced so that a free end (A, B or C) cannot pass through a closed cycle in the center of the band. Otherwise a unique product need not be obtained.

separation into its components (XVII). With suitably large end groups and small ring the stability of XVI may be as great as II or III. However, within the definition of a topological transition given in the second paragraph, the two parts of X may be separated. Another example of this type of isomer is the pair of "cut inner tubes" shown in Fig. 3.

Quantitative Considerations.—In the statistical procedure used to form catenanes a relatively small number N_2 of straight chains consisting of n_2 "statistical" segments (n_2 may be taken to be the number of C atoms in first approximation) are cyclized in solution of total volume V containing a relatively large number N_1 of dissolved (single) closed rings consisting of n_1 "statistical" segments. The exact calculation of the expected number $\langle N_{12} \rangle$ of n_1, n_2 -catenanes formed presents formidable difficulties. An order of magnitude estimate can be carried out if we make certain assumptions (which will be numbered by Roman numerals in what follows).

(I) The simple rings and straight chains exist in solution as (more or less) flexible (gaussian¹⁸) random coils, *i.e.*, they exist as essentially spherically symmetric distributions of connected segments, whose radii are $R_1 = R_1(n_1)$ and $R_2 = R_2(n_2)$, respectively, ($n_1, n_2 > 20$).

In order that threading of a particular ring by a given chain can occur, their spherically symmetric segment distribution must overlap in the total volume V . If the ends of this open chain are then connected by the cyclization reaction, whose extent is, say, α , there exists a conditional probability $\bar{\beta}$ (averaged over all possible configurations of chain and ring) that the resultant configuration corresponds to a stable catenane. $\bar{\beta}$ can in principle consist of two (not necessarily independent) factors: (a) an energetic activation factor for the threading and (b) a geometrical factor that a "soap film" surface enclosed by the single ring is crossed by the chain an odd number of times more often in the direction of the outward drawn normal than in the direction of the inward drawn normal to the "soap film" surface. On the basis of some trials with simple models we assume: (II) the geometric factor is about $1/2$ and (III) the activation factor we take to be unity as long as sufficient space is available in the ring to just permit threading by the chain, $\bar{\beta}$ in our final formulas can be set equal to about $1/2$. The probability p_{12} that a given ring and a now cyclized but initially open chain form a catenane is thus $\bar{\beta}$ times the probability of overlap of their segment distributions, *i.e.*, the ratio of their spherical covolume $\frac{4}{3}\pi(R_1 + R_2)^3$ to the total volume V

$$p_{12} = \bar{\beta} \frac{4}{3} \pi (R_1 + R_2)^3 / V \quad (1)$$

As long as $N_1 p_{12} < 1$, the threading of rings by the chains is effectively an independent process, so that neglecting certain effects, *e.g.*, the formation of catenanes from more than two rings, etc., we can write for $\langle N_{12} \rangle$

$$\langle N_{12} \rangle = N_1 \cdot \alpha N_2 \cdot p_{12} = \alpha \bar{\beta} N_1 N_2 \frac{4}{3} \pi (R_1 + R_2)^3 / V \quad (2)$$

(18) We neglect here the "polymer" excluded volume effect.

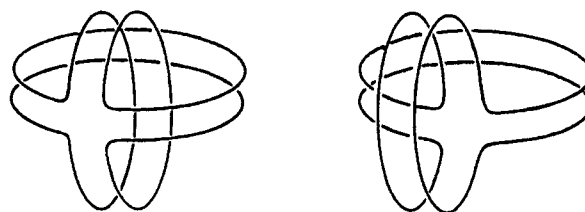


Fig. 3.—"Cut inner tubes."

Indeed the distribution of N_{12} should approximate, under our assumptions, a Poisson distribution with a mean given by the above formula. The relation between the radii and segment numbers follows from assumption I as¹⁹

$$R_i = \frac{1}{2} (n_i/2)^{1/2} b_i, \quad i = 1, 2 \quad (3)$$

with b_i the effective length of the statistical segment.

For rings composed of not too large a number of segments (2) is in error due to the actual volume occupied by the segments themselves

$$\omega = \frac{1}{2} (v_1 n_1 + v_2 n_2) \quad (4)$$

where v_i is the volume of a segment. To correct for this effect we subtract ω from the covolume and obtain thus our final estimate of $\langle N_{12} \rangle$

$$\langle N_{12} \rangle = \alpha N_1 \bar{\beta} \left\{ \frac{4\pi}{3} \left[\frac{1}{2} \left(\frac{n_1}{2} \right)^{1/2} b_1 + \frac{1}{2} \left(\frac{n_2}{2} \right)^{1/2} b_2 \right]^3 - \frac{1}{2} (v_1 n_1 + v_2 n_2) \right\} \left(\frac{N_1}{V} \right) \quad (5)$$

The yield of catenanes per cyclized chain is

$$F = \langle N_{12} \rangle / \alpha N_2 \quad (6)$$

Because the bracket of (5) involves a small difference between two large terms, $\langle N_{12} \rangle$ is quite sensitive to the values chosen for b_i and v_i . Using the density of linear polyethylene extrapolated to absolute zero, 1.08 g./cc.,²⁰ the volume of a methylene group is 21.4×10^{-24} cc./molecule. Although the individual segment length is 1.54 Å, the restriction of tetrahedral bonds about the carbon atoms reduces this value to 1.26 Å, when the chain is in its most extended form. We arbitrarily take the arithmetic mean of these limits, 1.40 Å, as b . With such parameters the minimum ring size for catenane formation as obtained from (5) is 29 methylene groups, considerably more than the value of 20 obtained from models.

For a 34,34-catenane we have $N_1/V = 10^{21}$ rings/cc. (density of liquid rings taken as 0.8 g./cc.) and $\bar{\beta} \sim 1/2$. Then $F \approx 4\%$ as compared with the experimental value of about 1%.² More than an order of magnitude agreement cannot be expected since within the limits of b mentioned above the value for F can be -7% (*i.e.*, vanish) or up to 18%. Also the assumption of random coils is likely to break down for small rings. Another difficulty with the application of (5) is that above 100 methylene groups the probability of catenane formation becomes greater than unity. However, one might expect that $N_1 p_{12} > 1$ in systems with

(19) See, *e.g.*, J. J. Weidmann, H. Kuhn and W. Kuhn, *J. chim. phys.*, **50**, 226 (1953).

(20) P. R. Swan, *J. Polymer Sci.*, **42**, 525 (1960).

such size rings so that (2) no longer is valid. (An approximate extension of (2) valid for $p_{12} > 1/N$ can easily be found but this is not warranted by present applications.)

The fact that the probability of possible overlap of segment distributions is a likely upper bound on p_{12} is substantiated by considering catenane formation from rigid plane circles of say radius R . Thus let the ring be a plane circle of radius R and the open chain two hinged plane circular arcs of curvature R^{-1} (lying in one plane) of $\pi + \epsilon$ and $\pi - \epsilon$ radians. We shall call the latter a "bracelet," whose center is the center of the larger circular arc. In order that interlinking of the original circle and the hinged circle, *i.e.*, the "bracelet," occur: (1) the center of the "bracelet" must lie in a limiting torus of volume $2\pi^2 R^3$ obtained by moving the center of a circle of radius R along the circumference $2\pi R$ of a circle lying in a plane normal to that of the first circle, and (2) the unit normal vector of the plane in which the bracelet lies, for every location of the center of the bracelet, must be restricted to lie within a certain cone defined by a solid angle Ω . Thus again we find (assuming the energetic factor is unity)

$$p_{12} = \bar{\beta} \cdot (2\pi^2 R^3) / V \quad (7)$$

with $\bar{\beta} = (\bar{\Omega}/4\pi)$, $\bar{\beta} \leq 1$, where $\bar{\Omega}$ is the average of Ω over all locations of the center of the bracelet. We note that, in the hypothetical model in which the hinge and lock of the bracelet are such that locking together of the arcs of the bracelet to form the plane circle occurs only if the plane of the bracelet intersects the plane circular ring along a radius of that ring, the upper bound is indeed obtained and

$$p_{12} = 2\pi^2 R^3 / V \quad (8)$$

For a chain of N linearly connected rigid rings of radius R , the probability density function $W_N(R_N)$ where R_N is the asymptotic head to tail distance can be shown to be (by Markov's method for random flights)

$$W_N(R) = \left[\frac{256}{15} \pi^3 N^3 R_0^8 \right]^{-1/2} \exp \left\{ -\frac{15}{64} \frac{R^2}{NR_0^2} \right\} \quad (9)$$

with

$$\langle R^2 \rangle_N = \frac{32}{5} NR_0^2 \quad (10)$$

For a partially flexible deformable ring $\langle R^2 \rangle_N$ may be either larger or smaller than the above depending on the degree of flexibility of the chain composing the ring. In any case $\langle R^2 \rangle_N$ is still proportional to the total molecular weight. This has some bearing on an elementary theory of the rubber-like elasticity of (somewhat cross linked) materials containing structures such as these, (or those similar to it), since the elastic modulus at vanishing strain is roughly inversely proportional to $\langle R^2 \rangle_N$.

Attempts to estimate analytically the order of magnitude of the probability of trefoil formation in a random walk (or flight) of t segments is a considerably more difficult problem. Direct estimates attempting to obtain this probability by first forming a loop of l segments which is subsequently "threaded" by $t - l$ segments, once and only once, neglect the possibility of a more complex

knot formation in the course of the random walk and must involve estimates of the shape and area of the loop formed by the l segments. Unfortunately, even assuming this area in some suitable projection to be plane, the conjecture that it is of the order of l may be false.²¹ This explains why we did not choose to estimate the probability of catenane formation using such a procedure. Using a result from the theory of subexponential functions, Hammersley²² has devised a plausibility argument that suggests that the probability that a closed random walk (taking into account excluded volume) is not knotted is an exponentially decreasing function of the number of steps of the random walk. Unfortunately, this argument is based on a topological assumption which, while very plausible, appears exceedingly difficult to demonstrate. Finally numerical Monte Carlo computations of the probability of knot formation are hindered by the fact that there exists no means for determining whether a given space curve, composed of polygonal segments, is knotted or not.

A final problem remains concerning the stability of two interpenetrating closed loops. Quantum mechanical tunneling of a portion of one loop through the other could cause separation. (An analogous question can be asked in the case of a knot.) Let the effective mass be m and the mean total energy (essentially thermal energy) E of the portion of the loop passing through the other. The transmission coefficient through the potential energy barrier need be calculated only in the semiclassical limit (WKB approximation) and the barrier can be replaced by a rectangular potential of width δ and height V_0 (we set the momentum quantum number equal to zero). The first-order rate constant for the tunneling reaction is then given by²³

$$k = \nu \exp \left[-\frac{2}{\hbar} \sqrt{2m(V_0 - E)}\delta \right] \quad (11)$$

where ν is the mean thermal vibration frequency of the first loop when interpenetrating the second (*i.e.*, $\nu \sim$ r.m.s. thermal velocity of a loop/effective diameter of a loop). The mass is taken as 14 hydrogenic units, the reduced mass for the square transition state for two perpendicular bonds, each containing two masses of 14 units, which are passing through one another. The distance (δ) is of the order 1 Å. ($V_0 - E$) is taken for an alkane to be at a reasonable guess of the order of 2 e.v. and $\nu \lesssim 10^{+12}$ seconds⁻¹. Then k turns out to be very small, *i.e.*, of the order of 10^{-89} seconds⁻¹. Thus catenanes are certainly indefinitely stable to decomposition by this process.

The possibility that two bonds will pass through one another by thermal activation over the potential barrier is unlikely. It has been estimated that the formation of the likely transition state is energetically less favorable than bond cleavage.²⁴

In conclusion we hope that some of the sugges-

(21) See, *e.g.*, J. M. Hammersley, *Proc. Camb. Phil. Soc.*, **52**, 78 (1956).

(22) J. M. Hammersley, private communication.

(23) See, *e.g.*, L. D. Landau and E. M. Lifshitz, "Quantum Mechanics," Pergamon Press, London, 1958, p. 171 ff.

(24) V. Griffing and J. S. Dooling, *J. Phys. Chem.*, **61**, 11 (1957).

tions made here will lead to additional experimental investigation of this new type of isomerism. One of the most interesting and feasible should be the preparation of the trefoil, II.

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Mass Spectra of Organic Molecules. I. Ethyl Esters of Amino Acids¹

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RECEIVED APRIL 27, 1961

The spectra of the ethyl esters of twenty-four amino acids have been determined and interpreted in terms of their structure. The results permit not only the identification of amino acids through their mass spectrum but also the determination of the structure of new compounds of this type. A detailed discussion of these mass spectra is given and serves as an illustration of some of the more important fragmentation reactions which complex organic molecules undergo on electron impact.

In the characterization or determination of the structure of amino acids, mobilities in chromatographic systems combined with color reactions and chemical degradation are still most commonly used. The spectroscopic methods, applied so successfully for such problems in other areas, are not generally utilized in this field. Two factors are mainly responsible for this situation. First, the variations among amino acids are not so much due to the presence of different functional groups as to their placement within the molecule, and often the only differences are size and structure of the side chain. Secondly, amino acids are primarily obtained by extraction of plant or animal tissues, in the metabolism of lower forms of life, or by degradation of proteins, which makes it difficult to obtain considerable amounts of the material in pure form. The increasing number of new amino acids found in plants and microorganisms makes it increasingly more imperative to search for additional means for their identification or for the determination of their structure.

Mass spectrometry seemed to us a method particularly suited to this field because it gives specific information about the arrangement of groups within a given molecule. In addition, the amount of sample required, a few tenths of a milligram, is well within the range which can be easily isolated from most sources.

There is one obstacle to the use of mass spectrometry for the characterization of amino acids: The sample has to be present in the vapor state in the ion-source of the instrument under conditions which do not lead to appreciable decomposition, if a spectrum representative of the original compound is to be obtained. Free amino acids, because of their zwitterion character, have a very low vapor pressure and frequently decompose if heated to the temperatures required for vaporization. It is therefore necessary to convert the free acids to more volatile derivatives which still retain all the structural features. Furthermore, it is important that the reaction employed is simple and can be done on a very small scale. To yield only one product the selected reaction should, if possible,

be quantitative and, for general use, applicable to polyfunctional amino acids.

For this purpose, either the carboxyl group, the amino group or both have to be removed. A number of reactions come to mind: (a) esterification, reduction to an amino alcohol, decarboxylation, degradation to the next lower aldehyde or a Dakin-West reaction would remove the free carboxyl group, or (b) acylation or deamination with nitrous acid would dispose of the basic amino group. The first-mentioned reaction, esterification, was chosen for our purpose. It was expected to proceed smoothly and to apply to all carboxyl groups, not only to the one α to the amino group. Next, the alcohol moiety and the method of esterification had to be selected. Diazomethane is most frequently chosen for small-scale esterification of carboxyl groups. However, this reagent is unsuitable because it is known to methylate amino acids on nitrogen, which cannot be tolerated for our purpose: the amino acids would in part be converted to isomers of their next higher homologs and finally to non-volatile quaternary ammonium compounds. Furthermore, the methyl esters of α -amino acids dimerize most easily to diketopiperazines, a reaction which has to be avoided. As the best compromise between the tendency of dimerization, boiling points and molecular size, the ethyl esters were chosen as the most suitable derivatives and Fischer-esterification for the method of preparation. The latter leads first to the hydrochlorides from which the free ester has to be prepared. This is done on a large scale in the conventional way: extraction with a suitable solvent from an alkaline solution, followed by distillation. Almost all spectra discussed in this paper were obtained from such distilled samples. For work on a very small scale, particularly if quantitative results on mixtures are required, we prefer the conversion of the hydrochloride into the free ester in homogeneous solution (dichloromethane) with dry ammonia. The low boiling point of the solvent permits its removal before the sample is introduced into the mass spectrometer using the technique described in the Experimental part.

Discussion of the Mass Spectra

The peaks comprising the spectra of ethyl esters of amino acids are due to fragmentation of the molecule by preferred cleavage of those bonds

(1) A preliminary account of part of this investigation was presented at the 135th Meeting of The American Chemical Society, Boston, Mass., April, 1959, and was published in *Biochem. Biophys. Res. Comm.*, **1**, 307 (1959). This work was supported by a research grant (RG-5472) from the National Institutes of Health, Public Health Service.