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(13) On leave from CNRS during 1976–1977, supported in part by a U.S.–France exchange award of the NSF.

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Stereochemistry of Acrylonitrile Dimerization

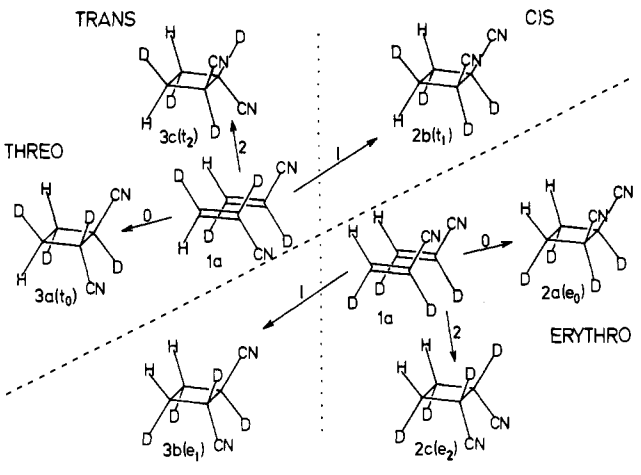
Sir:

Cycloaddition of two olefins and its reverse, cycloreversion or the cleavage of cyclobutane, have been the object of extensive theoretical[1] and experimental studies,[2,3] in which prediction and elucidation of stereochemistry have been the dominant theme. We now report the first stereochemically complete profile of a cycloaddition and the emergence of a simple statistical model as an adequate representation of the results.

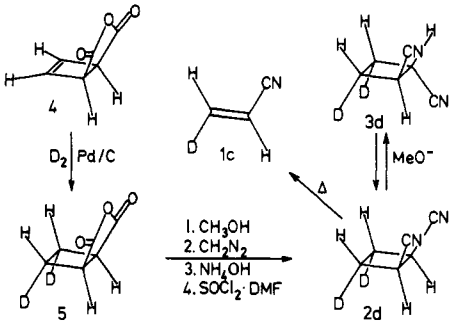
In the dimerization[4] of *cis*-1,2-dideuterioacrylonitrile (**1a**, Scheme I) two, not necessarily equal, modes of formation of the β,β' bond lead to a threo (t) and an erythro (e) set of three products each, which may be further characterized by the minimum number (indicated at each arrow) of 180° $[1 + 2n]$ rotations about the α,β and α',β' bonds required to generate the observed stereochemistry. These processes, formally involving zero, one, and two internal rotations (subscripts 0, 1, and 2), correspond in Woodward–Hoffmann notation to s + s, s + a, and a + a cycloadditions, respectively.

Deuterium-labeled acrylonitrile **1a** is synthesized by reduction of propiolamide-d_3 with lithium aluminum hydride,

Scheme I



Scheme II



with D_2O workup, and dehydration of the resulting acrylamide with P_2O_5 ($D_\alpha \geq 98\%$). The NMR spectrum (δ) of the purified product exhibits two 1:1:1 triplets at 6.14 (CCl_4) (5.18 (C_6D_6)) and 5.99 (CCl_4) (4.78 (C_6D_6)) in the ratio 94.3 (**1a**) to 5.7 (**1b**), respectively. Addition of Eu(fod)$_3$ causes the signal of the major component to shift downfield faster (1.00:0.66).[5] These data on chemical shifts,[6] coupling constants, and the lanthanide response are all consistent with assignment of the cis dideuterio configuration to the major isomer, **1a.**

NMR analysis of the dimerization products **2** and **3** is based upon the nonequivalence of protons cis or trans to a vicinal cyano group. For example, the spectrum of **2** in benzene-d_6 exhibits a singlet for **2a,** a different singlet for **2c,** and an AB pattern for **2b,** resolved only upon deuterium decoupling[7] (similarly for **3**). Assignments are based upon the lanthanide shift response of independently synthesized deuterated cyclobutanes (Scheme II). Exo deuteration (≥96%) of the anhydride **4**[8] is confirmed by a lanthanide shift study of unlabeled **5,** in which it is assumed that those β protons with the larger response are endo. The NMR spectrum ($CDCl_3$) of **2d** exhibits two doublets at 3.52 and 2.55. Base-catalyzed deuteration of **2d** affords a mixture of **2a, 2c,** and **3b.** In the NMR of the **2a** + **2c** mixture the assignments 2.55 ($CDCl_3$) (1.48 (C_6D_6)) to **2a** and 2.48 ($CDCl_3$) (1.01 (C_6D_6)) to **2c** are confirmed upon showing that the **2a** signal is indeed shifted downfield faster (1.00:0.71) by addition of Eu(fod)$_3$. A lanthanide shift study in benzene-d_6 on the dideuterio trans isomer **3d** permits assignment of the faster moving β signal (1.00:0.73) to the protons cis to the vicinal cyano group (as in **3a**).

Dimerization is effected by heating 100–200-mg samples in the liquid phase in silanized Pyrex ampules at 209.5 °C for 15 h or at 246.4 °C for 2 h. (Under these conditions decomposition of the cyclobutane products is negligible.) With diphenylamine as inhibitor, conversion to cyclobutanes is 9.3% at 209.5 and 10.0% at 246.4 °C, with acrylonitrile being recovered in 65.5 and 57.2% of theory, respectively.

Recovered acrylonitrile contains 19.0% at 209.5 and 20.3% at 246.4 °C of the trans dideuterio isomer, **1b,** corresponding to 15 and 16.5% isomerization, respectively. When heated at 208 °C for 24 h at 225 mm, **1a** is recovered unchanged.

Dimerization products **2** and **3** are separated by GC for

Table I. Dimerization of *cis*-1,2-Dideuterioacrylonitrile at 209.5 and 246.4 °C

		209.5 °C				246.4 °C			
Product	Process[a]	% (exptl)[b]	% (cor)[c,d]	k_{rel}	% (stat)[e]	% (expt)	% (cor)	k_{rel}	% (stat)
2a	e_0	19.8	22.3 (21.4)	1.00	22.1	18.4	20.4	1.00	20.3
3b	e_1	28.1	28.9 (28.5)	0.65	27.0	28.0	29.0	0.71	28.2
2c	e_2	6.9	6.2 (7.1)	0.28	8.3	9.0	8.7	0.43	9.8
3a	t_0	17.5	18.3 (17.1)	1.00	18.6	16.4	17.0	1.00	17.1
2b	t_1	20.1	18.4 (18.5)	0.50	19.1	20.5	18.7	0.55	19.3
3c	t_2	7.6	6.0 (7.4)	0.33	4.9	7.6	6.0	0.35	5.4

[a] Erythro and threo processes are denoted by e and t; subscripts denote the minimum number of rotations. [b] Total dimers from a single experiment. [c] Corrected to 100% **1a** at zero time. [d] The values in parentheses are the result of combining two experiments, one at 205 ± 4 °C for 20 h, the second at 210 ± 4 °C for 13 h. [e] Predicted by statistical model (see text).

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NMR analysis. Uncorrected percentage yields (Table I) are determined by cutting and weighing five copies of each spectrum and combining this information with the cis/trans ratio determined by GC. Since the small amount (5.7%) of **1b** in starting material increases to ~20% by the end of the reaction, the "uncorrected" data are corrected to zero time and 100% **1a** as starting material, to reflect participation of this "wrong" isomer in the dimerization. Note that in calculating the rate constants for e_1 and t_1 processes, the percentage values must first be divided by two, the statistical degeneracy of two equivalent rotations.

Product distribution within each series, erythro or threo, is predicted quite accurately (Table I) by a simple statistical model, the basic assumption behind which is total independence of α,β, and α',β' bond rotations. This assumption is antithetical to the theory of orbital symmetry control in concerted reactions. If x is the probability of observable rotation (and $1 - x$ the probability of no rotation) about one of the two identical bonds, the statistical distribution of products from zero, one, and two rotations is $(1 - x)^2{:}2x(1 - x){:}x^2$, respectively. Best values of x are 0.38 at 209.5 and 0.41 at 246.4 °C in the erythro series, 0.34 and 0.36 in the threo series, respectively (in a hypothetical freely rotating diradical $x = 0.50$). The higher values of x at 246.4 °C correspond to more rotation, consistent with an activation energy barrier. The slightly lower values of x in the threo series and formation of trans products in amounts slightly higher than predicted indicate a kinetic preference for trans. The origin of the slight favoring of erythro processes is unclear to us.

Cycloreversion of the cyclobutane **2d** has been examined in the gas phase from 238.8 to 290 °C. At 257 °C, where cleavage is approximately twice as fast as geometrical isomerization, acrylonitrile recovered at early points comprises 60.2% **1c** (Scheme II), corresponding to an excess of the s + s process. Excess retention of configuration is consistent with most other studies of cyclobutane pyrolysis.[3] Simple reversal of those dimerization processes ($e_0 + e_2 + t_1$) leading to cis products, when corrected to 257 °C, would predict 61.3% retention.

Our findings are inconsistent with a stereorandom process and unsupportive of even a vestige of concerted $[_\pi 2_s + _\pi 2_a]$ reaction. They are compatible with a statistically controlled process in which nonrotation is favored over rotation by factors of 1.44–1.94. If the results were to be discussed in the terms of the widely current diradical model,[2] these factors would correspond to ratios of rates of closure to rotation of 0.88–1.88. Yet unresolved is the question of whether hypothetical intermediary diradicals have antiperiplanar conformations and fates then determined by relative rates of cleavage, rotation about the α,β and α',β' bonds, and rotation about the β,β' bond to continuous diradicals.

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A Deuterium-Labeling Method for the Assignment of Histidine Nuclear Magnetic Resonance Peaks of Proteins

Sir:

A series of extensive NMR studies by Jardetzky and coworkers has demonstrated that ^{1}H NMR peaks of histidines give invaluable information about the structure of proteins in solution once each individual resonance can be assigned to a particular histidine residue in the amino acid sequence.[1] Differential deuterium exchange has been used in combination with enzymatic modification for the assignment of the histidine C-2 H peaks of ribonuclease A.[2-4] Markley and Kato[5] have developed a differential deuterium exchange technique, and used it for the assignment of the C-2 H peaks of the two histidine residues in soybean trypsin inhibitor. To determine the level of exchange at histidines in definite residue positions, they used cyanogen bromide to cleave the differentially deuterated protein into two large fragments each of which contains a single histidine residue. With large peptide fragments such as used in this method, however, the histidine C-2 H proton signals are very broad, and the C-4 H proton signals which would be used as an appropriate standard of intensity measurements are obscured by the envelope of a large number of aromatic proton signals; moreover, resonances from slowly exchangeable hydrogens may exist in the same spectral region, making the intensity measurements less reliable. Therefore, it appears that a more general method is needed, particularly to deal with larger proteins.

It has been shown that a tritium-labeling method, which is a combination of Matsuo and Narita's method[6] involving differential tritium exchange at the C-2 H position of histidines and ^{1}H NMR of differentially deuterated proteins, can be a general method for the assignment of the histidine NMR peaks.[7,8] This communication reports a deuterium-labeling method which is a modification of the tritium-labeling method. In this modification, tritium is replaced by deuterium for the analysis of the level of exchange at histidines in definite residue positions. Thus, the use of tritium becomes unnecessary, and at the same time any ambiguity which may result from the isotope effect in the tritium-labeling method is cleared away. In the deuterium-labeling method, differentially deuterated proteins are cleaved by trypsin into smaller peptides each containing a single histidine residue, which are separated in a conventional way using paper electrophoresis and chromatography, and finally extracted from the filter paper. Smaller histidine peptides thus obtained give sharp and well-resolved C-2 as well as C-4 H proton NMR peaks. Therefore, even in the case of large proteins such as used in the present experiment, ^{1}H NMR can be used very effectively to determine ac-

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