To confirm the identity of α-DNP-lysine, paper electrophoresis in 1 N acetic acid, pH 2.3, was used. In the Spinco model R apparatus, with a potential difference of 400 volts, α-DNP-lysine migrated a distance of 7.9 cm. toward the cathode in 3 hr., whereas ϵ-DNP-lysine migrated a distance of 6.1 cm. To confirm the identity of DNP-leucine, the DNP amino acid was converted to the free amino acid with concentrated ammonium hydroxide at 105°,[14] and the latter was identified as leucine by paper chromatography in the t-amyl alcohol–phthalate buffer system.

Characterization of Band P-5(A+B).—A 0.063-μmole sample of this material was treated with 2,4-dinitrofluorobenzene, and the reaction mixture was extracted with ether and passed through a small column of Dowex 50. The effluent was concentrated and subjected to descending paper chromatography for 48 hr. with butanol–acetic acid–water (4:1:5). Two yellow, radioactive bands were detected at distances of 15.5 cm. (5A) and 17.5 cm. (5B) from the origin. Recovery of radioactivity in the eluates of these bands was 0.014 μmole (22%) and 0.026 μmole (42%), respectively. Each DNP-peptide was hydrolyzed with 5.7 N hydrochloric acid at 105° and the DNP amino acid (ether-soluble) was identified by paper chromatography and analyzed quantitatively. The results are shown in Table III.

Characterization of Peptide K-2.—A mixture of 0.038 μmole of peptide K-2 and 6×10^{-4} μmole of carboxypeptidase was adjusted to pH 8.0 and then incubated at 25° for 1 hr. The incubation mixture was treated with 2,4-dinitrofluorobenzene.[5] Analysis of the ether-soluble DNP amino acids showed 0.032 μmole of DNP-leucine and 0.034 μmole of DNP-valine. The aqueous layer was concentrated and subjected to paper electrophoresis at pH 2.3 for 3 hr. A major yellow, radioactive band was detected at a distance of 16.3 cm. toward the anode. This band was cut out, eluted with water, and the eluate was passed through a small column of Dowex 50. Recovery of radioactivity was 56% (0.021 μmole). The eluate was evaporated to dryness and the residue was heated with 5.7 N hydrochloric acid for 15 hr. at 105°. The hydrolysate was evaporated to dryness and the residue was subjected to paper chromatography in the t-amyl alcohol·phthalate buffer system together with authentic samples of lysine, valine, α-DNP-lysine and ϵ-DNP-lysine. α-DNP-lysine was detected on the chromatogram. Quantitative analysis of this spot showed 0.020 μmole. The chromatogram was then sprayed with a solution of 0.2% ninhydrin in water-saturated butanol, and the color was allowed to develop at room temperature in the dark for 3 days. A single ninhydrin-positive spot was detected, corresponding in R_f to valine. This spot was cut out, eluted with 50% ethanol in water, and its concentration estimated as 0.023 μmole on the basis of the absorbancy at 570 mμ.[7]

Characterization of Peptide K-3.—A 0.2-μmole sample of peptide K-3 was dissolved in 0.2 ml. of 5.7 N hydrochloric acid and the solution was allowed to stand in a sealed tube for 5 days at 37°. The hydrolysate was evaporated to dryness and the residue was treated with 2,4-dinitrofluorobenzene. The ether-soluble DNP derivatives were subjected to two-dimensional paper chromatography[18] with the toluene–chloroethanol–pyridine–aqueous ammonia system in the first dimension and 1.5 M phosphate buffer, pH 6, in the second dimension. A major yellow spot and six yellow spots of lesser intensity were detected. Each spot was cut out, eluted and then hydrolyzed with 5.7 N hydrochloric acid at 105°. The ether-soluble DNP amino acids and the water-soluble amino acids were identified by paper chromatography in the t-amyl alcohol–phthalate buffer system and the butanol–acetic acid–water (4:1:5) system, respectively. Five of the yellow spots of lesser intensity appeared to be DNP-glutamic acid, DNP-aspartic acid, DNP-threonine, DNP-valine and DNP-leucine. The sixth spot appeared to be a dipeptide possessing the structure DNP-Thr.Asp. The major yellow spot appeared to be a tripeptide possessing the structure DNP.Val.(Val,-Leu). Quantitative analysis of this peptide showed the following molar ratios: DNP-Val, 1.0; Val, 1.0; Leu, 1.2.

The aqueous layer from the 2,4-dinitrofluorobenzene treatment was concentrated and subjected to paper chromatography for 68 hr. in the butanol–acetic acid–water (4:1:5) system. A major and two minor radioactive bands were detected at distances of 14.5, 11.5 and 16.5 cm., respectively, from the origin. The major band was eluted and the eluate was passed through a column of Dowex 50. Recovery of radioactivity in the effluent was 33%. A single, yellow, radioactive component was detected by paper electrophoresis at pH 2.3. Quantitative analysis of this peptide showed the following molar ratios: DNP-Thr, 0.97; Asp, 1.1; Lys, 1.0. This peptide therefore appears to possess the structure DNP-Thr.Asp.ϵ-DSO-Lys.

Acknowledgments.—We are indebted to Dr. Masahiko Koike and Mrs. Elizabeth Thompson for the preparations of α-keto dehydrogenation complexes containing bound lipoic acid $-S_2^{35}$.

---

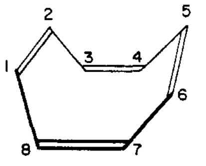
[14] A. G. Lowther, *Nature*, **167**, 767 (1951).

COMMUNICATIONS TO THE EDITOR

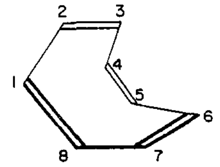
THE RATE OF BOND CHANGE IN CYCLOÖCTATETRAENE

Sir:

Cycloöctatetraene(I) is known[1] to have alternate single and double bonds in a tub-shaped (D_{2d}) non-aromatic structure (Ia). Structure Ib is equivalent to Ia in the absence of labelling[2]; pairs of carbon atoms which are doubly bonded in Ia are singly bonded in Ib and *vice versa*. The bond change from Ia to Ib almost certainly involves a strained planar intermediate or transition state with limited resonance stabilization, as a result of the unfavorable[3] number (8) of π electrons in I. Thus, an appreciable energy barrier would be expected to separate Ia from Ib.



Ia



Ib

---

[1] I. L. Karle, *J. Chem. Phys.*, **20**, 65 (1952); W. B. Person, G. C. Pimentel and K. S. Pitzer, *J. Am. Chem. Soc.*, **74**, 3437 (1952).

[2] In a monosubstituted cycloöctatetraene, Ia and Ib are enantiomers. Attempts to resolve such compounds have been unsuccessful (A. C. Cope, M. Burg and S. W. Fenton, *ibid.*, **74**, 173 (1952), A. C. Cope and M. R. Kinter, *ibid.*, **73**, 3424 (1951). The present work shows that the rate of racemization would be much too high to allow resolution. It is proposed to examine the n.m.r. spectra of such compounds at low temperature.

[3] See D. P. Craig in "Non-benzenoid Aromatic Compounds," edited by David Ginsburg, Interscience Publishers, Inc., New York, N. Y., 1959, p. 1.

We now report the first measurement of the rate constant (k) of the interconversion of Ia and Ib. This has been achieved by observing the C^{13} satellites[4] of the main line[5] of the high-resolution proton nuclear magnetic resonance (n.m.r.) spectrum of I over temperatures ranging from −55° to room temperature (Fig. 1).[6]

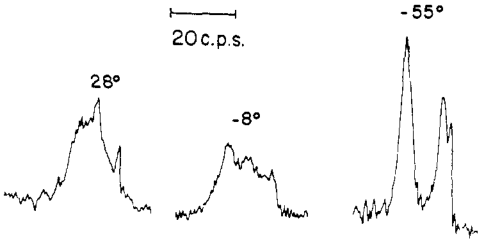


Fig. 1. High-field C^{13} satellite of cycloöctatetraene in carbon disulfide solution (2:1) at various temperatures. The low-field satellite was always a mirror image of the high field one. The spectra were measured on a 60 Mc./sec. Varian spectrometer. The magnetic field increases from left to right.

At low temperatures, when k is very small, Ia and Ib will be observable[7] as separate species. The proton on a C^{13} atom (say H1 of Ia) would be expected to be coupled to H2 by about 12 cps. (*i.e.*, normal *cis*-coupling[8] on a double bond). Although H2 is not chemically shifted from H3 it is not expected to be appreciably coupled to H3 because[9] the dihedral angle between the planes defined by H2,C2,C3 and H3,C3,C2 is about 90°. For the same reason H1 should not be coupled to H8. Therefore, H1 should give a doublet with a separation of about 12 cps., and H2, H3, *etc.*, whether in Ia or Ib should also give the same type of doublet when attached to C^{13} atoms. The C^{13} satellites of I at −55° (Fig. 1) are indeed doublets with a separation of 11.8 cps. The components of the doublet show additional but small splittings which can be ascribed to long-range coupling and/or not quite zero coupling the H1, H8 type.

At higher temperatures, when k is very large, only the average of Ia and Ib will be observed.[7] The spectrum (say when C1 is a C^{13} atom) would now be expected to be very complicated. The protons in the pairs (H1,H2), (H2,H3), (H3,H4), *etc.*, should be coupled together by about 6 cps. (*i.e.*, the average of 12 and zero), but since there is zero chemical shift between H2,H3 . . . H8, to be completely correct, the spectrum has to be analyzed as an eight-spin system. If H2 and H8 only are taken into account, then H1 would be expected to be a 1:2:1 triplet with a spacing of about 6 cps., but this is undoubtedly a crude approximation. With the inclusion of H3 and H7 and only 1,2 coupling assumed, H1 forms the X part of two separate ABX systems and is calculated to be approximately a 1:3:4:3:1 quintet with a spacing of about 3 cps. The true spectrum will contain still more lines, which probably will result in an unresolved broad band, although some fine structure may remain. The shape of the C^{13} satellite of I at room temperature (Fig. 1) fits very well with these expectations.

As the temperature is raised from −55°, little change in the C^{13} satellites is observed until about −25°, when both components of the doublet begin to broaden rapidly. At about −10° only a single flat band (Fig. 1) is obtained and by about +5° the band shape has become constant and is the same as observed at room temperature. The rate constant at about −10° is[7] of the order of magnitude of the averaging effect on the doublet found at low temperatures. This gives a value of about 26 $sec.^{-1}$ for k, and about 13.7 kcal./mole for the free energy of activation at −10°.

Unfortunately, the spectra are not suitable for directly obtaining accurate activation energies. If the entropy of activation is assumed to be zero, then ΔH is about 13.7 kcal./mole. This is probably a maximum value for the difference in energy between the planar and tub forms of I. Although it is difficult to estimate the strain energy in the planar form, a value[10] of 20–30 kcal./mole would seem reasonable. Thus the resonance energy in the planar form amounts to not more than 5–15 kcal./mole, in agreement with the theoretical deductions.[3]

[4] A. D. Cohen, N. Sheppard and J. J. Turner, *Proc. Chem. Soc.*, 118 (1958).

[5] Since all the protons of Ia and Ib have the same chemical shift, the main spectrum of I is a single line which does not change with temperature, and therefore cannot give any information about k.

[6] After submission of this paper, Dr. J. D. Roberts informed us that similar observations of the effect of temperature on the C^{13} satellites of cycloöctatetraene have been made in independent, unpublished research by G. M. Whitesides and J. D. Roberts at the California Institute of Technology.

[7] J. A. Pople, W. G. Schneider and H. J. Bernstein, "High Resolution Nuclear Magnetic Resonance," McGraw-Hill Book Company, New York, N. Y., 1959, p. 218.

[8] L. M. Jackman, "Applications of Nuclear Magnetic Resonance Spectroscopy," Pergamon Press, New York, N. Y., 1959, p. 85.

[9] M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959).

[10] See F. H. Westheimer in "Steric Effects in Organic Chemistry," edited by M. S. Newman, John Wiley and Sons, Inc., New York, N. Y. p. 533.

[11] This work was supported by a grant from the Ontario Research Foundation.

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF OTTAWA F. A. L. ANET[11]
OTTAWA, ONT., CANADA

RECEIVED NOVEMBER 18, 1961

EVIDENCE FOR PHOTOCHEMICAL SPACE INTERMITTENCY EFFECT

Sir:

If a solution is illuminated with a pattern of light and dark areas and if photochemically produced fragments are destroyed in pairs, then the average steady-state concentration of fragments is dependent upon the size and shape of illuminated regions as well as upon the total area illuminated. The theory of this photochemical space intermittency effect has been examined previously.[1] We have now obtained an experimental demonstration of its existence.

The solution contained 1.0×10^{-4} M iodine in hexane illuminated at 25° with the 4358 Å. mercury line. Illuminating light fell on the solution

[1] R. M. Noyes, *J. Am. Chem. Soc.*, **81**, 566 (1959).