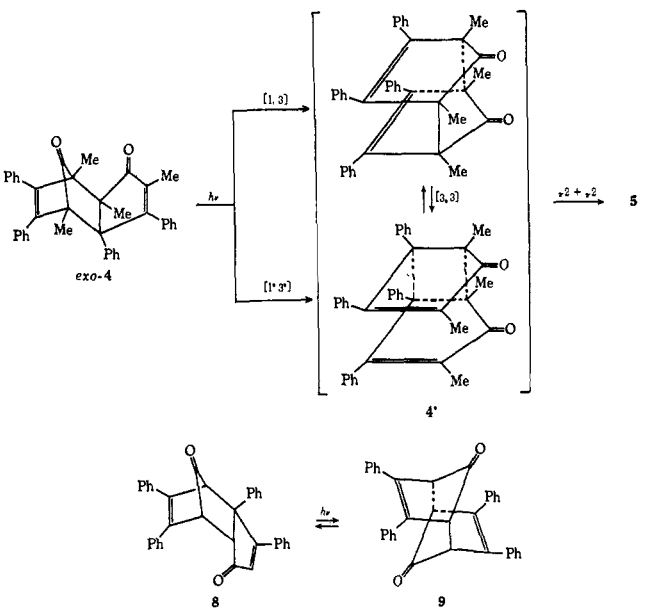
monomer–dimer structures in tetrasubstituted cyclopentadienones.[8,11,12]

The photochemical transformation of **4** to **5** can then be formulated as a [1,3] sigmatropic rearrangement, *exo*-**4** → **4'**, followed by intramolecular $_{\pi}2 + _{\pi}2$ cyclo-



addition **4'** → **5**. An alternative and, to this author's knowledge, unprecedented, completely concerted rearrangement, *exo*-**4** → **5**, cannot be excluded but is also difficult to prove. It would imply a six-center electron redistribution process with two conceivable bonding interactions between the two allylic counterparts, between the lowest bonding or highest antibonding orbitals. The latter would obtain in our case.

The small yields of **6** in the above photochemical experiment are tentatively attributed to internal cycloaddition of contaminating *endo*-**4** isomers in the starting material.

Turning to another instance, the tetraphenyl derivative **8**[13] (mp 206°; uv max 233 (ϵ 1.4 × 10^4), 287 nm (ϵ 2 × 10^4); ir (KBr) 1780, 1690 cm^{-1}; nmr ($CDCl_3$) τ 7 (m, 21), 5.6 (d, 1), 6.2 (dd, 1), 6.9 (d, 1); mass spectrum (70 eV) *m/e* 464 (M^+)) was irradiated at λ > 310 nm,[9] yielding (20%) exclusively **9** (mp 257°; uv max (dioxane) 238 (ϵ 4.4 × 10^4), 280 nm (ϵ 2.4 × 10^4); ir (KBr) 1770 cm^{-1}; mass spectrum (70 eV) *m/e* 464 (M^+)).[10]

Continual spectrophotometric scanning of the irradiation mixture revealed two isosbestic points at 268 and 318 nm.[9] Moreover, irradiation of pure **9** under identical conditions gave a similar mixture of **8** and **9**. The transformation is thus shown to be reversible and to involve no long-lived intermediates.

Sensitization and quenching experiments[9] were performed. Irradiation of **7** in the presence of acetophenone (E_T = 74 kcal/mol) almost entirely suppressed the formation of **9**; naphthalene (E_T = 61 kcal/mol) slightly enhanced its formation, whereas piperylene (E_T = 56 kcal/mol) reduced it appreciably. These preliminary results indicate the intermediacy of triplet excited states at least in the transformation **9** → **8** if not also in the reverse process. Should the latter situation obtain, one would be led to the conclusion that $E_T^9 > E_T^8$ Surprisingly, no internal cycloaddition product analogous to **2** was detected. In all other respects, however, the situation is similar to that now found for the unsubstituted dimer,[7,14] the process consisting apparently of a [1,3] sigmatropic transformation in an undissociating dimer **8**, of endo configuration.

The above results permit the formulation of a unified picture of light-induced sigmatropic rearrangements[4,15] in cyclopentadienone dimers. The outcome depends upon the configuration of the tricyclic starting material; endo dimers undergo internal cycloaddition to type **2** cages and/or rearrangements to diketones of type **3** whereas exo dimers photoisomerize to cages of type **5** possibly *via* intramolecular cycloaddition in unsaturated rearranged intermediates. To be sure, these are not the only available modes for photoisomerization and, therefore, the scope and generality of these processes as well as their driving forces are problems still demanding elucidation.[16]

Quantitative photochemical measurements, further transformations of the photoproducts, and additional instances of light-induced isomerizations in these systems are now under investigation.

Acknowledgments. Mrs. Sarah Weinman and Mr. Shimon Hauptman rendered skillful technical assistance. The mass spectrometric measurements were kindly performed at the Chemistry Departments of Bar-Ilan University, Ramat Gan, and/or Israel Institute of Technology, Haifa, courtesy of Drs. B. Sklarcz and A. Mandelbaum, respectively.

(11) M. A. Ogliaruso, M. G. Romanelli, and E. I. Becker, *Chem. Rev.*, **65**, 261 (1965).
(12) For pertinent discussion and references on this subject, *cf.* ref 3e.
(13) C. F. H. Allen and J. W. Gates, Jr., *J. Amer. Chem. Soc.*, **64**, 2120, 2123 (1942).
(14) J. Gauthier, K. Schaffner, M. Pasternak, and B. Fuchs, in preparation.
(15) The general photochemical [1,3] sigmatropic suprafacial rearrangement is well documented;[4] *cf.* also D. I. Schuster and D. H. Sussman, *Tetrahedron Lett.*, 1661, 1657 (1970), and references therein.
(16) The significance of these findings is, in this author's opinion, well beyond the specific cases treated here. Indeed, a variety of related literature cases, the results of which appear equivocal if not unfounded, demand reinterpretation or further investigation. Some such cases are now being investigated in this laboratory and will be reported in due course.

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Magnetic Circular Dichroism Studies. XII.[1] The Determination of Tryptophan in Proteins

Sir:

In spite of extensive studies[2–7] the quantitative and in several cases[8] even qualitative determination of tryptophan in proteins remains difficult. Because of the

(1) For part XI see: D. L. Elder, E. Bunnenberg, C. Djerassi, M. Ikehara, and W. Voelter, *Tetrahedron Lett.*, 727 (1970).
(2) J. R. Spies and D. C. Chambers, *Anal. Chem.*, **21**, 1949 (1949).
(3) T. W. Goodwin and R. A. Morton, *Biochem. J.*, **40**, 628 (1946).
(4) F. W. J. Teale, *ibid.*, **76**, 381 (1960).
(5) T. F. Spande and B. Witkop, *Methods Enzymol.*, **11**, 498 (1967).
(6) H. Edelhoch, *Biochemistry*, **6**, 1948 (1967)
(7) M. K. Gaitonde and T. Dovey, *Biochem. J.*, 117, 907 (1970).
(8) U. Henning, D. R. Helinski, F. C. Chao, and C. Yanofsky, *J. Biol. Chem.*, **237**, 1523 (1962).

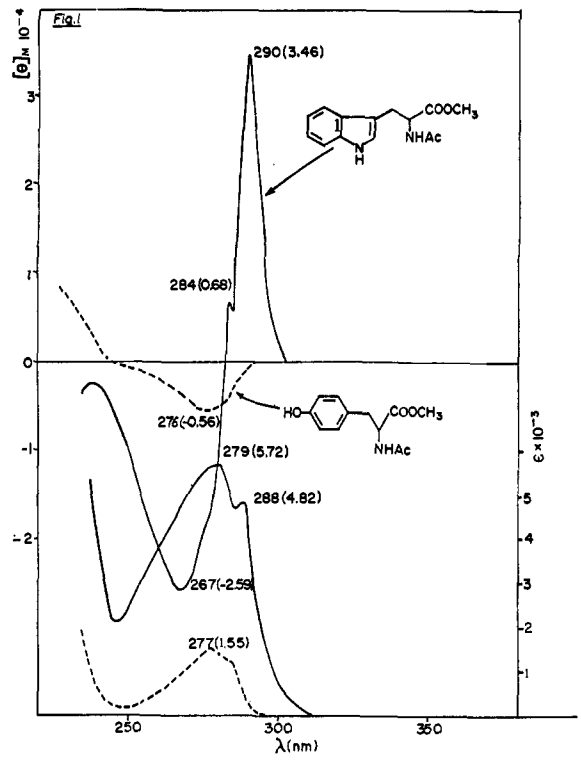

Figure 1. MCD[9] (upper curves) and uv (lower curves) spectra of *N*-acetyl-L-tryptophan methyl ester (—) and *N*-acetyl-L-tyrosine methyl ester (- - -) in 0.15 *M* phosphate buffer solution (pH 7.07).

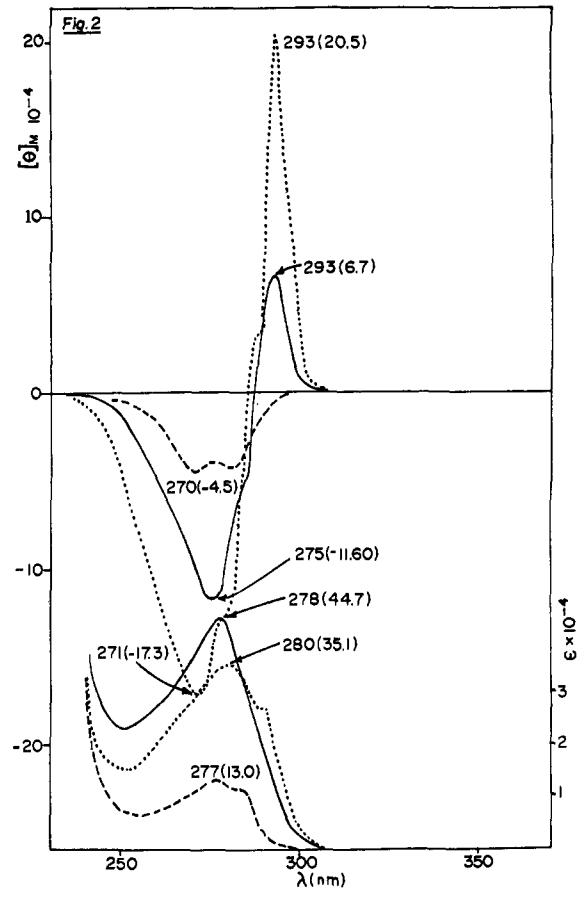

Figure 2. MCD[9] (upper curves) and uv (lower curves) of albumin (bovine) (—), lysozyme (hen egg white) (· · ·), and tryptophan synthetase α chain (*E. coli*) (- - -) in 0.15 *M* phosphate buffer solution (pH 7.07).

extreme lability of tryptophan toward acid hydrolytic conditions,[2] spectrophotometric determination[3,6] using the *intact* protein has been the method of choice. This approach is based on the fact that tryptophan and tyrosine show overlapping absorption bands between 250 and 300 nm. Neglecting the small absorption contributions resulting from phenylalanine and cystine, measurement at two distinct wavelengths (288 and 280 nm)[6] allows the simultaneous determination of both amino acids. Although convenient, this method suffers from severe limitations in those cases where the tyrosine–tryptophan ratio is high and where corrections for end absorption and cystine content have to be introduced. Colorimetric methods[5,7] suffer primarily from the possible difference in reactivity of the tryptophan residue with respect to the location in the macromolecule.

Our recent observation that the magnetic circular dichroism spectra of natural amino acids absorbing in the 250–300-nm region are significantly different in intensity and sign of their MCD bands created the possibility of selectively determining tryptophan in intact proteins by this technique.

As model compounds, *N*-acetyl-L-tryptophan methyl ester and *N*-acetyl-L-tyrosine methyl ester were chosen. As can be seen from Figure 1, *N*-acetyl-L-tryptophan methyl ester shows two oppositely signed MCD bands, a positive one at 290 nm and a negative one at 267 nm of almost equal intensity. The tyrosine derivative on the other hand shows only a negative MCD band of much lower intensity. The contributions of phenylalanine and histidine are negligibly small and cystine shows only a weak negative MCD band ($[\theta]_M = 0.035 \times 10^4$)[9] at 254 nm. From this comparison it is obvious that the positive MCD band at 290 nm of tryptophan is well separated from the other bands and since no other amino acid shows a positive MCD band at this wavelength, *its existence is an unambiguous indication of the presence of tryptophan.* Thus the tryptophan-containing proteins lysozyme and albumin give (see Figure 2) positive peaks at 293 nm, whereas the α chain of tryptophan synthetase shows (Figure 2) no positive band in this region, in agreement with the known absence[8] of tryptophan in this enzyme.

In contrast to natural circular dichroism which is the consequence of the internal molecular dissymmetry, magnetic circular dichroism results from the external dissymmetry induced by the magnetic field[10,11] and therefore will be to a first approximation insensitive to conformational changes. On this basis it can be expected that the contributions from different tryptophan units within the polypeptide chain will be equal and additive and not dependent on the location and kind of secondary and tertiary structure. Consequently, measurement of the intensity of the 290-nm MCD band should lead to quantitative tryptophan determinations, and this proved to be the case, as is demonstrated in Table I.

(9) MCD $[\theta]_M$ values (corrected for natural CD) are expressed in deg cm^2 dmol^{-1} at a magnetic field of 49.5 kG.

(10) C. Djerassi, E. Bunnenberg, and D. L. Elder, *Pure Appl. Chem.*, in press.

(11) P. N. Schatz and A. J. McCaffery, *Quart. Rev., Chem. Soc.*, in press.

Table I. Tryptophan Content of Representative Proteins

	Mol wt	No. of tryptophan residues per molecule Lit.	Detd by MCD	No. of tyrosine residues per molecule
Lysozyme (hen egg white)	14,300	6[c]	5.92	3
Albumin (bovine)	67,000[a,b]	1.85[b]	1.93	17.9
Tryptophan synthetase α chain (*E. coli*)	28,900	0[c,d]	0	7

[a] Molecular weight as indicated by the supplier Mann Research Laboratories as compared to 64,000 given by footnote *b*. [b] G. R. Tristam and R. H. Smith, *Advan. Protein Chem.*, **18**, 227 (1963). [c] M. O. Dayhoff and R. V. Eck, "Atlas of Protein Sequence and Structure," Vol. 4, The National Biochemistry Research Foundation, Silver Spring, Md, 1969. [d] Reference 8.

Finally it should be pointed out that the sensitivity of our method is comparable to the spectrophotometric technique, and depending on the tryptophan content protein concentrations of 0.05–0.5 mg/ml have been used in our measurements. The lower detection limit for tryptophan by the MCD method is approximately 10^{-5} mol/l.

Further applications and a delineation of the scope of this method will be presented subsequently in a full publication.

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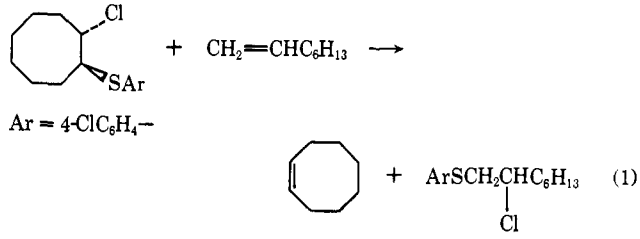
The Exchange of 4-Chlorobenzenesulfenyl Chloride between Olefins[1]

Sir:

We report that the addition product of 4-chlorobenzenesulfenyl chloride with a number of cyclic and acyclic olefins exchanges 4-chlorobenzenesulfenyl chloride with 1-octene when heated in *sym*-tetrachloroethane. Thus, 2-chlorocyclooctyl 4-chlorophenyl sulfide, obtained from the addition of 4-chlorobenzenesulfenyl chloride to *cis*-cyclooctene, gives *cis*-cyclooctene and 2-chlorooctyl 4-chlorophenyl sulfide in quantitative yield after heating for 24 hr in *sym*-tetrachloroethane in the presence of an 8.6 *M* excess of 1-octene (eq 1).

Other chloroalkyl 4-chlorophenyl sulfides that undergo this reaction are listed in Table I along with the time necessary for one-half of the starting material to form the olefin as determined by nmr and vpc analysis of the reaction mixture.

(1) Reactions of Sulfenyl Chlorides and Their Derivatives. IV. Part III: G. H. Schmid, *Can. J. Chem.*, **46**, 3757 (1968).



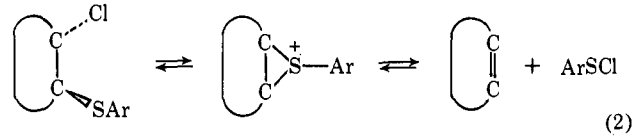
Exchange between 2-chlorooctyl 4-chlorophenyl sulfide and excess cyclooctene (the reverse of eq 1) is not observed.

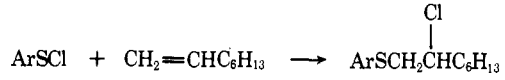
Table I. Elimination of 4-Chlorobenzenesulfenyl Chloride from Chloroalkyl 4-Chlorophenyl Sulfides, RSC_6H_4Cl

—R—	Time,[a] hr
2-Chlorocyclononyl	1.3
2-Chlorocyclooctyl	1.5
2-Chlorocycloheptyl	40.0
2-Chlorocyclohexyl	$>2.0 \times 10^3$
2-Chlorocyclopentyl	400.0
erythro-2-(1-Phenyl-1-chloropropyl) (**1**)	5.4
erythro-3-Chloro-2-butyl	7.0×10^2
erythro-2-Chloro-1,2-diphenylethyl	1.8
threo-2-Chloro-1,2-diphenylethyl	$>4 \times 10^2$

[a] Time necessary for one-half of the starting material to form olefin.

Rearrangements[2] and solvolysis reactions[3] of aryl sulfides have been postulated to occur by means of a mechanism involving a cyclic episulfonium ion which undergoes nucleophilic attack at the carbon atoms. A similar mechanism involving an episulfonium ion can be postulated for this exchange with the additional feature that the nucleophilic attack occurs also at sulfur. The 4-chlorobenzenesulfenyl chloride so formed then adds to the excess 1-octene to complete the reaction (eq 2).





This mechanism is consistent with the work of Helmkamp[4] who found that the reaction of cyclooctene-*S*-methylepisulfonium 2,4,6-trinitrobenzenesulfonate with a variety of nucleophiles occurs more often by attack at sulfur than at carbon. The major difference between our results and those of Helmkamp seems to be in the relative amounts of nucleophilic attack on sulfur compared to carbon. Nucleophilic attack on the episulfonium ion formed by participation of sulfur of the 2-chloroalkyl aryl sulfide occurs predominantly at carbon. This is evident from two results. (1) The solvolysis of 2-chlorocyclooctyl 4-chlorophenyl sulfide in 80% dioxane–water gives as products

(2) G. M. Beverly, D. R. Hogg, and J. H. Smith, *Chem. Ind. (London)*, 1403 (1968); W. A. Thaler, W. H. Mueller, and P. Butler, *J. Amer. Chem. Soc.*, 90, 2069 (1968).
(3) H. L. Goering and K. L. Howe, *ibid.*, 79, 6542 (1957).
(4) D. C. Owsley, G. K. Helmkamp, and S. N. Spurlock, *ibid.*, **91**, 3606 (1969).