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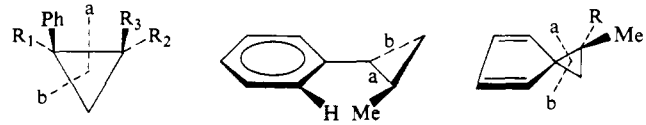
Steric and Electronic Factors in the Reductive Cleavage of Methyl-Substituted Phenylcyclopropanes and Spiro[2.4]hepta-4,6-dienes

Sir:

An interesting aspect of the general problem of cyclopropane conjugation,[1] *viz.*, the reductive cleavage of conjugated cyclopropane rings with alkali metals in liquid ammonia, has received an increasing amount of attention. Several authors have concluded that in various cyclopropyl ketones the bond which is cleaved is the one which best overlaps with an adjacent carbonyl[2] (or phenyl)[2c,d] orbital. The question of the importance of electronic or "inductive"[3] (as opposed to steric) effects has received somewhat less attention.[2d,4] Recently, however, it was reported that the cleavage of 1-methyl-2,2-diphenylcyclopropane (**1a**) by pathway a is favored over pathway b (to give 1,1-diphenylbutane (**2**) and 1,1-diphenyl-2-methylpropane (**3**), respectively) by a factor of 5.0–5.7.[5] It was stated that this result might be expected on the basis of the fact that a methyl group would be predicted to stabilize a radical-anion activated complex for pathway a relative to that for pathway b. However, only one canonical form of the activated complex was considered; in addition, steric effects in the activated complexes would be difficult to evaluate without data from simpler model compounds.[5]

We have employed methyl groups as probes of charge distribution in the activated complexes of several simple conjugated cyclopropyl systems. The reductive cleavages of *trans*- and *cis*-1-methyl-2-phenylcyclopropanes[6] by lithium in liquid ammonia at *ca.* −33° *have been found to be highly regioselective*[7] *reactions.*[8] The *trans* isomer **1b** is cleaved primarily *via* pathway b ($k_b/k_a = 360 \pm 20$) whereas the *cis* isomer **1c** is cleaved somewhat more slowly *in the opposite direction* (k_a/k_b = *ca.* 70). In addition, the cleavage of 1,1-dimethyl-2-phenylcyclopropane[9] was shown to have $k_a/k_b = 2.3 \pm 0.1$. In each case the products were analogous to **2** and **3**.[10]

The results for the conformationally mobile methylphenylcyclopropanes clearly demonstrate the importance of both electronic and steric factors. The high regioselectivity observed in the cleavage of the *trans* isomer **1b** (in which there is no steric bias for either pathway) shows that a methyl group exerts a large destabilizing "inductive" effect (relative to hydrogen). This is consistent with a description of the activated complex in which there is substantial negative charge on the cyclopropyl β-carbon of the bond undergoing cleavage. In the case of the *cis* isomer **1c** the conformation of maximum overlap for cleavage of bond b (**4**) possesses a substantial steric interaction between the methyl group and the *ortho* hydrogen on the phenyl ring. Therefore, cleavage of bond a is greatly favored in spite of the destabilizing effect of the methyl group. The nearly equal rates of cleavage of bonds a and b in 1,1-dimethyl-2-phenylcyclopropane (**1d**) represent a balancing of electronic and steric factors. On the basis of our results it can be stated that the cleavage of **1a** by pathway a is *hindered* (rather than accelerated)[5] by the "inductive" effect of the methyl group and that a steric effect similar to that discussed for **1c** is the dominant factor.



1a, R_1 = Ph; R_2 = H; R_3 = Me
b, $R_1 = R_3$ = H; R_2 = Me
c, $R_1 = R_2$ = H; R_3 = Me
d, R_1 = H; $R_2 = R_3$ = Me

4

5a, R = H
b, R = Me

The destabilizing effect of a methyl group appears to be reasonably general, as is shown by the results for the cleavage of 1-methylspiro[2.4]hepta-4,6-diene[11,12] (**5a**) by sodium in liquid ammonia at *ca.* −33°. This cleavage is instantaneous and exothermic; 1- and 2-*n*-propyl- and 1-

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(4) There is qualitative evidence which suggests that both steric and electronic factors are of importance: (a) H. E. Zimmerman, R. D. Rieke, and J. R. Scheffer, *ibid.*, **89**, 2033 (1967); (b) see also H. O. House and C. J. Blankley, *J. Org. Chem.*, **33**, 47 (1968).

(5) H. M. Walborsky and J. B. Pierce, *ibid.*, **33**, 4102 (1968); see also ref 2c and O. M. Nefedov, N. N. Novitskaya, and A. D. Petrov, *Dokl. Akad. Nauk SSSR*, **152**, 629 (1963), for related reactions.

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(8) The initial work on this problem was done by W. L. Maloy. Several compounds were prepared by A. Dorsky.

(9) G. L. Closs and R. A. Moss, *J. Am. Chem. Soc.*, **86**, 4042 (1964).

(10) The material balances in this work were essentially unity (by the internal standard method).

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(12) The reductive cleavage of spiro[2.4]hepta-4,6-diene has been reported: K. Alder, H.-J. Ache, and F. H. Flock, *Chem. Ber.*, **93**, 1888 (1960). See also G. Schröder, *ibid.*, **97**, 3140 (1964), and W. Huckel, S. Gupte, and M. Wartini, *ibid.*, **99**, 1388 (1966), for related reductions.

and 2-isopropylcyclopentadienes were isolated as the only products of the reaction. Similarly, **5b**[13] gave 1- and 2-isobutyl- and 1- and 2-*t*-butylcyclopentadienes as the major products. Since the rigid spiro[2.4]hepta-4,6-diene structure provides no conformational advantage to either pathway a or b, the moderate preference for the latter ($k_b/k_a = 4.8 \pm 0.3$ for **5a** and 10.1 ± 0.4 for **5b**) indicates that only a small amount of excess negative charge accumulates on the methyl-substituted carbon in the activated complex for pathway b relative to the same carbon in pathway a.[14,15] This is consistent with a description of the activated complex in which the transition state occurs early on the reaction coordinate and the negative charge is largely localized on the incipient cyclopentadienyl ring.[16]

Both radical-anion[2e,5,14,17,18] and dianion[2b] mechanisms have been suggested for reductive cleavage reactions of this type, but there is very little evidence in support of either. Although we cannot distinguish between these mechanisms (or a combination thereof)[19] on the basis of the present data, it should be noted that the above conclusions are independent of this point.

Acknowledgment. We are pleased to acknowledge the support of this work by the donors of the Petroleum Research Fund, administered by the American Chemical Society.

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(14) A similarly small regioselectivity has recently been observed in the reductive cleavage of triptycene derivatives, for which a radical-anion mechanism has been suggested: T. D. Walsh and R. T. Ross, *Tetrahedron Lett.*, 3123 (1968).
(15) The cleavage of **5b** also produced *ca.* 1% of 1- and 2-methylallylcyclopentadiene, possibly *via* a radical-anion cleavage. These products were taken into account in the calculation of k_b/k_a.
(16) This interpretation is supported by the results of molecular orbital calculations.
(17) (a) N. A. LeBel and R. N. Liesemer, *J. Am. Chem. Soc.*, **87**, 4301 (1965); (b) R. Fraisse-Jullien, C. Frejaville, V. Toure, and M. Derieux, *Bull. Soc. Chim. France*, 3725 (1966).
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Modification of α-Chymotrypsin by Methyl *p*-Nitrobenzenesulfonate[1]

Sir:

We wish to report a new active-site modifying reagent, methyl *p*-nitrobenzenesulfonate, which methylates specifically histidine-57 of α-chymotrypsin. However, this methyl ester does not modify trypsin or subtilisin.

Previously L-1-tosylamido-2-phenylethyl chloromethyl ketone,[2] phenoxymethyl chloromethyl ketone,[3] and 2-phenyl-1,4-dibromoacetoin[4] have been reported as modifying reagents for this histidine of α-chymotrypsin. These compounds are structurally similar to the specific substrate for α-chymotrypsin; however, methyl *p*-nitrobenzenesulfonate has a structure analogous to nonspecific substrates for α-chymotrypsin. Therefore methyl *p*-nitrobenzenesulfonate is a member of a new class of active-site-specific reagents.

The methyl ester was prepared by the method of Morgan and Cretcher.[5] The product was identified by using ir, nmr, and elemental analyses.[6] *Anal.* Calcd for $C_7H_7O_5NS$: C, 38.7; H, 3.2; N, 6.4; S, 14.7. Found: C, 39.3; H, 3.4; N, 6.6; S, 14.4. A radioactive methyl ester was obtained by using methanol-^{14}C (2 mCi/mmol) in the preparation.

When this methyl ester was incubated with purified α-chymotrypsin in 0.1 *M* sodium phosphate buffer, pH 7.93, the enzyme activity decreased with incubation time. For this experiment, the activity of the enzyme was determined by titration with N-*trans*-cinnamoylimidazole[7a] and by rate assay with N-acetyl-L-tryptophan methyl ester.[7b] The results are shown in Figure 1. Inhibition of the enzymatic activity by the methyl ester was prevented by adding a competitive inhibitor, β-phenylpropionic acid. The pH profile of inhibition was a bell-shaped curve, and p*K* values were obtained by a graphical method.[8] The values are as follows: $pK_1 = 6.73$ and $pK_2 = 9.14$. These results indicate that methylation occurred at an active site of α-chymotrypsin.

The uv absorption spectra of native and modified α-chymotrypsin were almost identical (λ_{max} 281 mμ). There was no spectral shift after the modified enzyme had been denatured by 8 *M* urea.[9] This indicates that the *p*-nitrobenzenesulfonyl group is not involved in the modification.

By incubating the enzyme with methyl-^{14}C *p*-nitrobenzenesulfonate (sp act. 6.6×10^7 cpm/mol) in a sodium phosphate buffer solution, pH 7.9, methyl-^{14}C-α-chymotrypsin was prepared. The radioactivity was found almost stoichiometrically in the modified enzyme. In addition, this radioactivity was distributed principally in the B chain of α-chymotrypsin after the B and C chains had been separated by column chromatography.[10] A diagonal paper electropherogram[11] indicated that histidine-57 was modified, and amino acid analyses revealed that 1-amino-2-(1-methyl-4-imidazolyl)propanoic acid (3-methylhistidine) was formed with a loss of a corresponding amount of histidine in the modified enzyme.

As a model system, methyl *p*-nitrobenzenesulfonate was incubated with individual proposed functional amino groups of α-chymotrypsin to see the effects of these amino acid residues on the methyl ester in solution. To determine the effect of these amino acids, the disappearance of methyl *p*-nitrobenzenesulfonate was observed at 253 mμ at 25°. The results are shown in Table I. The spon-

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