1,4 PARTICIPATION IN CYCLOHEXYL SYSTEMS

Sir:

We have observed that *trans*-4-methoxycyclohexyl *p*-toluenesulfonate undergoes abnormal solvolysis in acetic acid with retention of configuration.

The two isomeric 4-methoxycyclohexanols were separated *via* the acid phthalates and the *p*-toluenesulfonates and characterized by the following derivatives: *trans*-4-methoxycyclohexanol (I), acid phthalate, m.p. 184.6–149.0° (calcd. for $C_{15}H_{18}O_5$: C, 64.73; H, 6.52. Found: C, 64.71; H, 6.49); *p*-toluenesulfonate, m.p. 65.5–66.2° (calcd. for $C_{14}H_{20}O_4S$: C, 59.13; H, 7.09; S, 11.27. Found: C, 59.03; H, 7.13; S, 11.18); 3,5-dinitrobenzoate, m.p. 125.5–126.5° (calcd. for $C_{14}H_{16}O_7N_2$: C, 51.85; H, 4.95; N, 8.64. Found: C, 51.61; H, 4.93; N, 8.79); acetate (distinguishing infrared bands at 8.77, 10.20, 11.04 μ).

cis-4-Methoxycyclohexanol (II), acid phthalate, m.p. 61–65° (Found: C, 64.66; H, 6.45); *p*-toluenesulfonate, m.p. 87.8–88.2° (Found: C, 59.25; H, 7.18; S, 11.16); 3,5-dinitrobenzoate, m.p. 116.2–116.5° (Found: C, 52.03; H, 4.73; N, 8.65); acetate (distinguishing infrared bands at 8.70, 8.93, 10.45, 11.22 μ).

To establish the configuration of I, the known *trans*-4-hydroxycyclohexanol was converted by partial methylation with methyl iodide and silver oxide to I, characterized as the *p*-toluenesulfonate and the 3,5-dinitrobenzoate.

The rates of reaction observed for I-tosylate and II-tosylate under a variety of conditions are summarized in Table I.

TABLE I

RATES OF REACTION OF 4-METHOXYCYCLOHEXYL TOSYLATES AT 75.09°

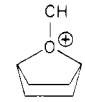
Ethanolysis[a] $k_1 \times 10^5$ sec.$^{-1}$	Acetolysis[a,b] $k_1 \times 10^5$ sec.$^{-1}$	Elimination[a] 0.06 *N* NaOEt $k_2 \times 10^5$ l. mole^{-1} sec.$^{-1}$
cis-4-Methoxycyclohexyl tosylate		
0.654 ± 0.006	0.766 ± 0.022	303 ± 15
trans-4-Methoxycyclohexyl tosylate		
2.48 ± 0.04	3.20 ± 0.11	59.1 ± 1.7

[a] Concentration of tosylates *ca.* 0.03 *M* in all cases. [b] In dry acetic acid, containing 0.06 g./l. of acetic anhydride and 0.06 *M* in sodium acetate.

The products resulting from the acetolysis of I-tosylate were separated by chromatography on alumina, using 5:1 and 5:2 pentane–ether as eluents. There were obtained 4-methoxycyclohexene (75% yield), infrared spectrum compared with an authentic sample, and *trans*-4-methoxycyclohexyl acetate (II) in 20% yield. The spectrum of III was identical with an authetic sample. Further, III was hydrolyzed to I, and converted to the 3,5-dinitrobenzoate, m.p. and m.m.p. 125.5–126.5°.

In like manner the products from the acetolysis of II-tosylate were separated, to afford 4-methoxycyclohexene (40%) and III (40%), likewise characterized by infrared spectrum and the preparation of the dinitrobenzoate.

The retention of configuration accompanying the solvolysis of I-tosylate, in conjunction with the demonstration of rate enhancement for its solvolysis[1] lead us to propose that the solvolysis of I-tosylate proceeds through an intermediate of structure IV, with two inversions accompanying solvolysis.



IV

The driving force associated with the formation of the 5-membered ring oxonium ion compensates for the energy required for the conversion of the stable chair to the boat conformation.

We wish to acknowledge the support of the National Science Foundation, Grant NSF-G2387.

(1) The rate enhancement is about a factor of 5–7. The expected rates of solvolysis may be predicted with some confidence, A. Streitwieser, Jr., THIS JOURNAL, **78**, 4935 (1956), and D. S. Noyce and H. I. Weingarten, THIS JOURNAL, **79**, in press.

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THE ISOLATION OF A PANCREATIC INSULINASE

Sir:

We wish to report the isolation of a new pancreatic enzyme which is highly active in hydrolyzing insulin. The enzyme was detected as a component of the crystalline elastase recently isolated in these laboratories,[1] and was separated from the other constituents by ion-exchange chromatography on diethylaminoethyl (DEAE)-cellulose. It represents about 3% of the crystalline elastase, and behaves as a single substance in electrophoretic and ultracentrifugal studies.

The composition of crude crystalline porcine elastase has been examined by fractionation on DEAE-cellulose, and the insulinase isolated in this way. The ion-exchanger was prepared by the method of Ellis and Simpson,[2] and 20 g. of the modified cellulose was used to chromatograph 500 mg. of the elastase preparation. The starting material was dissolved in a sodium carbonate–hydrochloric acid buffer of *p*H 8.8 and $\Gamma/2$ of 0.1, dialyzed against buffer, and applied to a column prepared with the same buffer solution. The column was developed with an increasing salt gradient produced by addition of sodium chloride to the carbonate buffer, also as described by Ellis and Simpson.[2] The effluent was analyzed by measuring the absorption at 280 mμ.

Figure 1 represents a typical elution diagram. It may be seen that in addition to elastase (I), the crude crystalline preparation contained at least four other components, of which V represents pancreatic insulinase. Component II and the pro-

(1) U. J. Lewis, D. E. Williams and N. G. Brink, *J. Biol. Chem.*, **222**, 705 (1956).

(2) S. Ellis and M. E. Simpson, *ibid.*, **220**, 939 (1956).

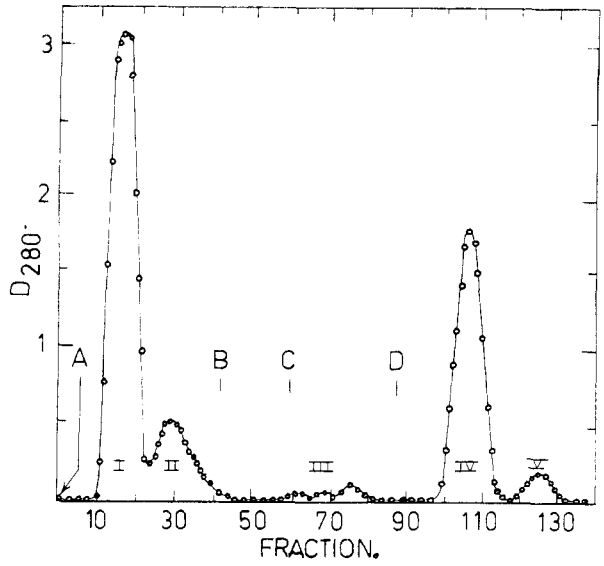

Fig. 1.—Elution diagram for crude crystalline elastase: buffers, A, *p*H 8.8, Na_2CO_3–HCl; B, *p*H 8.8, carbonate–0.15 *M* NaCl; C, *p*H 8.8, carbonate–0.2 *M* NaCl; D, *p*H 8.8, carbonate–0.23 *M* NaCl. See text for explanation of numerals I–V.

teins designated as III had no activity either on elastin or insulin; they disappeared on recrystallization of the crude material. Component IV showed no insulin-hydrolyzing activity, but did have a weak proteinase activity against albumin.

Pancreatic insulinase, isolated as indicated above, showed a single, symmetrical boundary during electrophoresis and ultracentrifugation. The electrophoretic mobilities at *p*H 8.8 and 4.0 were -4.9×10^{-5} and $+1.2 \times 10^{-5}$ cm.^{2}/sec./volt, respectively. The isoelectric point is therefore near *p*H 4.

Insulinase activity was measured, essentially according to the procedure of Mirsky, Perisutti and Dixon,[3] by following the degree of solubilization of radioactivity by enzyme treatment of I^{131}-labeled insulin. In a typical experiment with 200 μg. of insulin as substrate, 26 μg. of insulinase caused 50% hydrolysis of the insulin in 15 minutes. Carboxypeptidase is without action in the assay. Chymotrypsin produced a slight hydrolysis of insulin, but in rate and extent nowhere approached the activity of the new pancreatic insulinase.

We are indebted to Dr. N. G. Brink and Dr. G. E. Boxer for their many valuable suggestions, and we wish to thank Dr. D. E. Williams for the electrophoretic and ultracentrifuge studies.

(3) I. A. Mirsky, G. Perisutti and P. J. Dixon, *J. Biol. Chem.*, **214**, 397 (1955).

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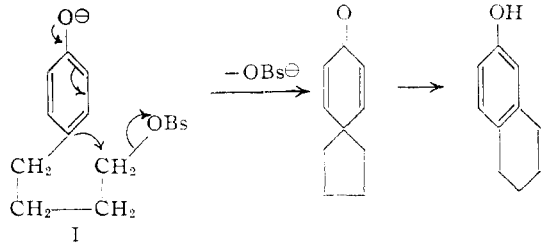
THE FORMATION OF DIENONES THROUGH Ar_1-PARTICIPATION

Sir:

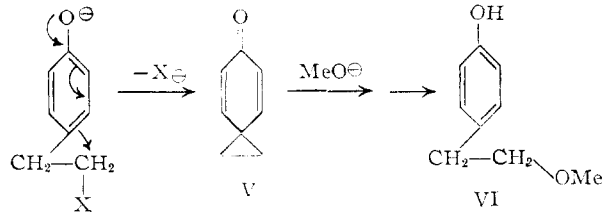
By proper selection of structure and reaction conditions, it is possible to arrange for the formation of dienones through Ar_1-participation[1] of a neighboring phenoxide-ion group.

Thus, Ar_1-5 assisted reaction may be made to dominate over anchimerically unassisted reactions by treatment of 4-*p*-hydroxyphenyl-1-butyl *p*-bromobenzenesulfonate (0.03 *M*) with a slight excess of potassium *t*-butoxide in anhydrous *t*-butyl alcohol. Under these conditions a first order rate of reaction of the ion I is observed, the first order rate constant being $4.85 \pm 0.08 \times 10^{-4}$ sec.$^{-1}$ at 50.00°. From this reaction was isolated a ketone, m.p. 34–35°, with a λmax. in the ultraviolet at 242 mμ and an ϵ of 16,000 in methanol, and with a strong band in the infrared for a conjugated carbonyl. That the ketone is *spiro*-(4:5)-deca-1,4-diene-3-one (II) is clear from the spectral evidence,[2] elementary analysis, quantitative hydrogenation (2.04 moles hydrogen absorbed), and quantitative dienone-phenol rearrangement[3] to 5,6,7,8-tetrahydro-2-naphthol (III), m.p. 60.5–61.0°.



The reaction is of value for the preparation of the dienone II, since the latter was obtained in a yield of greater than 50% on a small scale, without further exploration for optimal conditions.

With 2-*p*-hydroxyphenyl-1-ethyl derivatives it is relatively easy to arrange for essentially complete control of reaction by Ar_1-3 participation. Thus 0.03 *M* 2-*p*-hydroxyphenyl-1-ethyl bromide reacts by way of the ion IVa in 0.13 *N* sodium methoxide in absolute methanol at a rate which is initially faster than that of 2-*p*-anisyl-1-ethyl bromide by practically 10^3. Evidently the dienone, *spiro*-[2:5]octa-1,4-diene-3-one (V) is formed, but it reacts rapidly with methoxide ion and aryloxide ion. With sufficiently high methoxide ion concentration the product from IVa is predominantly 2-*p*-hydroxyphenyl-1-ethyl methyl ether (VI), m.p. 42–43°; the latter was isolated in 82% yield after ten half-lives of the ion IVa in 1 *N* methanolic sodium methoxide. With lower methoxide ion concentrations competition of aryloxide ion for the dienone



(1) S. Winstein R. Heck S. Lapporte and R. Baird, *Experientia*, **12**, 138 (1956).

(2) (a) R. H. Burnell and W. I. Taylor *J. Chem. Soc.*, 3486 (1954); (b) E. A. Braude and E. R. H. Jones *ibid.*, 498 (1945).

(3) A. L. Wilds and C. Djerassi, THIS JOURNAL, **68**, 1715 (1946).