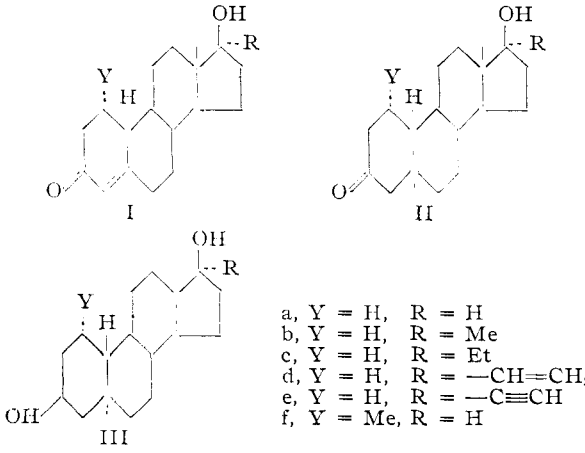
78.63; H, 10.71[8]); IIIe (m.p. 192–193°, $[\alpha]_D$ −15°. Found for $C_{20}H_{30}O_2$: C, 79.63; H, 9.84[8]).



a, Y = H, R = H
b, Y = H, R = Me
c, Y = H, R = Et
d, Y = H, R = —CH=CH$_2$
e, Y = H, R = —C≡CH
f, Y = Me, R = H

Similarly, 1α-methyl-19-nortestosterone (If)[9] was converted to the allo[7] compound, 1α-methyl-19-norandrostan-17β-ol-3-one (IIf) (m.p. 186–188°, $[\alpha]_D$ +46°. Found for $C_{19}H_{30}O_2$: C, 78.78; H, 9.94) and to the 3β,17β-diol (IIIf) (m.p. 204–206°, $[\alpha]_D$ +51°. Found for $C_{19}H_{32}O_2$: C, 77.55; H, 10.84).

This series of nordihydrotestosterone derivatives (II) and the diols (III) are potent anti-estrogens as measured by inhibition of the uterotrophic activity of estrone in the immature mouse.[10] Nordihydrotestosterone (IIa) exhibits about 8× the activity of 19-nortestosterone (Ia) in this assay (subcutaneous route) while the 17-alkyl compounds (*e.g.* IIb) are highly active by the oral as well as subcutaneous route. Clinical evaluation of some of these compounds in certain types of hormone dependent tumors has been initiated.

(9) H. J. Ringold, G. Rosenkranz and F. Sondheimer, THIS JOURNAL, **78**, 2477 (1956). For assignment of the 1α-configuration, see C. Djerassi, R. Riniker and B. Riniker, *ibid.*, **78**, 6377 (1956).

(10) Bioassays carried out at the Worcester Foundation for Experimental Biology under the direction of one of the authors (R. I. D.).

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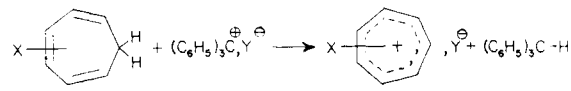
SYNTHESIS OF TROPENIUM (CYCLOHEPTATRIENYLIUM) SALTS BY HYDRIDE EXCHANGE[1]

Sir:

The previously reported[2] novel method of synthesis of tropenium[3] perchlorate by hydride abstraction from cycloheptatriene by trityl perchlorate in acetonitrile has been found to be versatile and adaptable to the preparation of a number of tropenium salts containing various anions and/or nuclear substituents, some previously unknown or unavailable by other methods.



Hydride exchange reactions between cycloheptatrienes and trityl salts occur rapidly and quantitatively only in solvents which effect dissociation of these salts. In consonance with conductivity studies, trityl halides function satisfactorily as hydride abstractors in liquid sulfur dioxide but trityl perchlorate or fluoborate gives equally good results in acetonitrile or sulfur dioxide. Most tropenium salts may be prepared by the following general procedure (all reactants and conditions anhydrous): equivalent quantities of trityl salt and the cycloheptatriene[4] in acetonitrile (minimum amount) at room temperature or sulfur dioxide (30–50 ml./g. triene) at −20° or lower are allowed to react for a few minutes, all solvent then evaporated (acetonitrile *in vacuo;* sulfur dioxide by warming, finally *in vacuo*), triphenylmethane removed by trituration and extraction with ether from the tropenium salt product; pure perchlorate and fluoborate salts obtained by recrystallization from acetonitrile–ethyl acetate, halide salts by sublimation at 80–100° (1 mm.); yields of crude salts almost quantitative, of pure salts 60–90%. Tropenium salts prepared (λ_{max} and ϵ_{max} in concd. sulfuric acid): (i) X = H, Y = ClO_4,[2] white, m.p. >300°, 217 mμ (41,000) and 273.5 mμ (4350)(ii, iii, iv, v show same spectrum), 44.03% C, 3.71% H; (ii) X = H, Y = BF_4,[5c] white, m.p. dec. slowly above 210°, 47.17% C, 3.59% H; (iii) X = H, Y = Cl,[5] white, 66.61% C, 5.56% H; (iv) X = H, Y = Br,[2,5] yellow, m.p. 203°; (v) X = H, Y = I,[5] red, m.p. 127°, readily transformed into tropenium triiodide, deep red-black, m.p. 127°, 292 mμ (39,800) and 361 mμ (22,100) in acetonitrile, 18.06% C, 1.51% H; (vi) X = Cl, Y = ClO_4, white, m.p. 164°, 237 mμ (29,800) and 310 mμ (8220), 37.37%, C 2.77% H, converted to tropone by water or ethanol[5b] (analogous preparation of bromide or iodide salts gives mixed halotropenium halides due to halogen interchange); (vii) X = Br, Y = ClO_4, white, m.p. 149.5°, 247 mμ (22,750) and 323 mμ (8900), 31.27% C, 1.89% H, converted to tropone by water or ethanol[5b]; (viii) X = MeO, Y = ClO_4, white, m.p. 107°, 234 mμ (32,100) and 315 mμ (10,050), 43.38% C, 4.34% H (similarly prepared bromide or iodide salts yield tropone and methyl bromide or iodide on warming); (ix) X = Me, Y = ClO_4,[5c] white, m.p. 109°, 226 mμ (37,100) and 288 mμ (3500), 46.89% C, 4.73% H (yellow bromide salt preparable in sulfur dioxide at −70° but decomposes on warming). Perchlorate and fluoborate salts, due to greater stability and non-hygroscopicity, are easier to handle than chloride (very hy-

(1) Supported in part by the Office of Ordnance Research, U. S. Army, Contracts DA-04-200-ORD-235 and 601.

(2) H. J. Dauben, Jr., and D. L. Pearson, Abstracts, 126th Meeting, American Chemical Society, New York, N. Y., Sept. 13, 1954, p. 18-O.

(3) Tropenium seems preferable to tropylium as the name for the $C_7H_7^{\oplus}$ ion because of its representation of all structural features of the ion and its consistency with benzenium, azulenium, etc., names for related carbonium ions.

(4) Substituted cycloheptatrienes prepared by the method of W. v. E. Doering and L. H. Knox, THIS JOURNAL, **75**, 297 (1953).

(5) Previously reported by: (a) Doering and Knox, *ibid.*, **76**, 3203 (1954); (b) W. v. E. Doering and H. Krauch, *Angew. Chem.*, **68**, 661 (1956); (c) M. J. S. Dewar and R. Pettit, *J. Chem. Soc.*, 2021, 2026 (1956); the color, stability and spectrum reported for their methyltropenium bromide monohydrate are inconsistent with this structural assignment.

groscopic), bromide (hygroscopic) or iodide (non-hygroscopic but unstable) salts. The allylically substituted 7-methoxy-1,3,5-cycloheptatriene,[5a] by giving with trityl bromide in sulfur dioxide only tropenium bromide and trityl methyl ether, shows methoxide instead of hydride exchange.

With more complete spectral data now available, it is evident that both predicted bands (4.33 ev, weak; 6.37 ev, intense)[6] for tropenium ion agree reasonably well with observed values (4.54 ev, 4350; 5.73 ev, 41,000). Results to date indicate that substituent effects on both $^1E_{1u}$ and $^1E_{3u}$ bands of substituted tropenium ions are in accord with those on $^1E_{1u}$[7] and $^1B_{1u}$, but not $^1B_{2u}$, bands of monosubstituted benzenes and are interpretable in terms of highly polar excited states for the ions.

Additional hydride exchange studies on tropenium and other systems for preparative and theoretical purposes are in progress.

(6) J. N. Murrell and H. C. Longuet-Higgins, *J. Chem. Phys.*, **23**, 2347 (1955).

(7) H. B. Klevens and J. R. Platt, Technical Report, Part 1, 1953-4, Laboratory of Molecular Structure and Spectra, University of Chicago, p. 145.

(8) (a) National Science Foundation Predoctoral Fellow, 1956-7; (b) Allied Chemical and Dye Co. Fellow, 1953-4.

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AN INTERMEDIATE IN THE DEACETYLATION OF MONO-ACETYL-δ-CHYMOTRYPSIN HAVING THE PROPERTIES OF ACETYL-IMIDAZOLYL[1]

Sir:

In a previous communication,[2] evidence was presented which indicated that upon acetylation of δ-chymotrypsin by *p*-nitrophenylacetate, there was no detectable change in the spectral characteristics of the enzyme in the region of 245 mμ. This region was studied carefully since a postulated intermediate in the reaction, acetyl-imidazolyl-,[3,4] possesses a characteristic absorption maximum at this wave length.[4] Recently, a mechanism of reaction of δ-chymotrypsin with NPA has been proposed[5] which accounts for this observation and several others: (1) The stability of monoacetyl-δ-chymotrypsin at low *p*H[6] (2) a difference of 0.7–0.8 *pK* unit for acetylation and deacetylation of the enzyme[6] (3) the reversible sensitivity of the acetylation and deacetylation reactions to denaturation by urea,[2] (4) the reversible loss of reactivity of the acetyl with hydroxylamine in urea.[2] This scheme is presented in Fig. 1 with certain modifications, the evidence for which will be presented below.

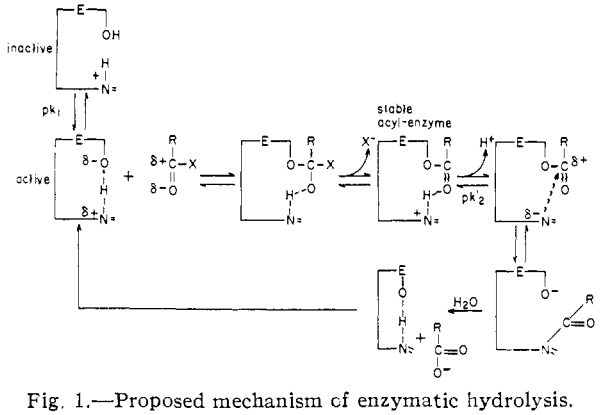

Fig. 1.—Proposed mechanism of enzymatic hydrolysis.

A solution of monoacetyl-δ-chymotropysin (1.95 mgm./ml.) at *p*H 3.5 was allowed to deacetylate at *p*H 9.0 in a Beckman DK-1 spectrophotometer which was set at 245 mμ. The resulting record of ΔE_{245} with time is seen in Fig. 2, curve 1; a control

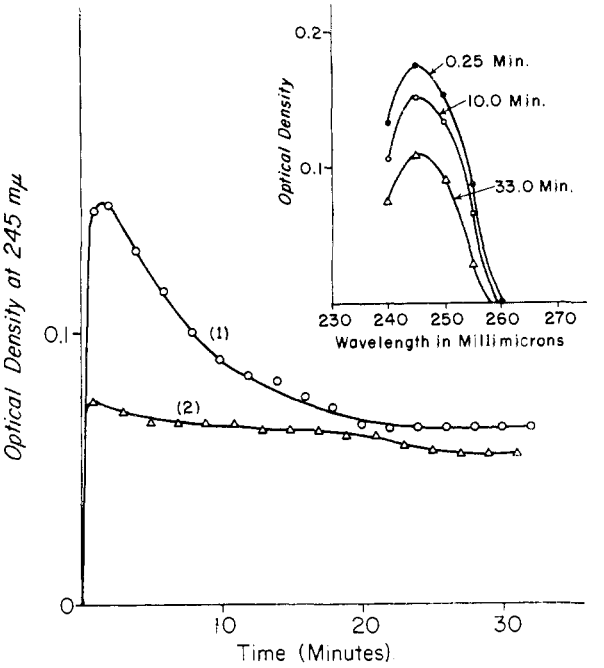

Fig. 2.—Both sample and reference cuvettes in a Beckman DK-1 spectrophotometer contained 3.0 ml. of the same solution of acetyl-δ-chymotrypsin[7] at *p*H 3.5; the temperature was 10.0 ± 0.2°. The *p*H of the sample was raised to 8.9 ± 0.1 by adding a small aliquot of a triethylammonium-ammonium acetate buffer on a plunger type rapid mixer.

of non-acetylated δ-chymotrypsin is seen in curve 2. Curve 2 was also followed when a portion of the acetyl-δ-chymotrypsin was allowed to deacetylate at *p*H 7.2 for 15 minutes at room temperature and then adjusted to *p*H 3.5 with acid before the reaction.

It is clear that only in the case of acetyl-δ-chymotrypsin is there the rapid formation of a species absorbing at 245 mμ which decreases slowly in concentration with time. The control curve indicates that part of the initial increase at 245 mμ is due to a non-specific, *p*H dependent, spectral change in the enzyme.

(1) The authors wish to acknowledge the financial support of grant No. RG-4617 from the National Institutes of Health, U. S. Public Health Service.

(2) G. H. Dixon, W. J. Dreyer and H. Neurath, THIS JOURNAL, **78**, 4810 (1956).

(3) H. Gutfreund, *Trans. Faraday Soc.*, **51**, 441 (1955); B. J. Jandorf, H. O. Michel, N. K. Schaffer, R. Egan and W. H. Summerson, *Faraday Soc. Discussions*, **20**, 134 (1955).

(4) E. Stadtman in "Mechanism of Enzyme Action," Johns Hopkins Press, Baltimore, Md., 1954, p. 581.

(5) L. W. Cunningham, *Science*, in press.

(6) G. H. Dixon and H. Neurath, *Fed. Proc.*, **16**, 173 (1957); G. H. Dixon and H. Neurath, *J. Biol. Chem.*, **223**, 1049 (1957).

(7) A. K. Balls and H. N. Wood, *J. Biol. Chem.* **219**, 245(1956).