TABLE I

A COMPARISON OF THE ACYLATION AND DEACYLATION REACTIONS OF TRYPSIN AND α-CHYMOTRYPSIN USING N-*trans*-CINNAMOYLIMIDAZOLE AS SUBSTRATE AT 25.0°

Parameter	Trypsin[a]	α-Chymotrypsin[b]
$k_{acylation}$ at *p*H 5.2[d] (l./mole sec.)	63.4[c]	12×10^3
$k_{deacylation}$ at *p*H 5.2[e] (sec.$^{-1}$)	1.69×10^{-4}	1.4×10^{-4}
λ_{max} of cinnamoyl-enzyme (mμ)	296	292
ϵ_{max} of cinnamoyl-enzyme	19,300	17,700
k_{OH} of cinnamoyl-enzyme in 7.74 *M* urea (l./mole sec.)	4.5×10^{-2}	4.1×10^{-2}
pK_a' of deacylation of cinnamoyl-enzyme	7.3	7.15

[a] 1.6% acetonitrile–water, tris–acetic acid buffer. [b] 1.6% acetonitrile–water, acetate buffer. [c] Assuming molecular weight of trypsin is 24,000 and using protein absorbance (optical factor = 0.694) as a measure of concentration. [d] Determined by the disappearance of the substrate. [e] Determined by the disappearance of the cinnamoyl-enzyme and/or by the appearance of cinnamate ion.

77.7% of acyl-enzyme, 20.6% of reactant and 1.7% of product. The agreement between the two spectra was better than 2%. The difference spectrum of cinnamoyl-trypsin *vs.* trypsin based on the average of these two calculations is shown in Fig. 1.[4a]

The spectrophotometric and kinetic results of the trypsin-catalyzed hydrolysis of N-*trans*-cinnamoylimidazole give direct experimental proof of the two-step catalytic mechanism, and support the earlier indirect kinetic evidence of Schwert and Eisenberg[5] and of Stewart and Ouellet.[6a]

The acylation of trypsin by N-*trans*-cinnamoylimidazole is considerably slower than that of α-chymotrypsin. It is possible that the difference in (second-order) acylation constants results from a difference in the adsorptive equilibrium constants and not in the rate constants of acylation themselves.[6b]

The rate constants for the deacylation of cinnamoyl-trypsin and cinnamoyl-α-chymotrypsin are very similar in magnitude. The effects of pH on the deacylation rate constants are also similar.[7] The similarity of cinnamoyl-trypsin and cinnamoyl-α-chymotrypsin is further seen in a comparison of the spectra of these compounds, and a comparison of the alkaline hydrolytic rate constants of these compounds in 7.74 *M* urea. The spectra of these intermediates cannot be analyzed from a structural point of view because of our ignorance of the effect of the enzyme environment on the spectrum. However, superficially the two compounds are quite similar to each other. When the two intermediates are converted to ordinary esters by the denaturing solvent, 7.74 *M* urea, they act kinetically similar to each other and to the model compound O-cinnamoyl-N-acetylserinamide.[3] Thus it appears that cinnamoyl-trypsin, like cinnamoyl-α-chymotrypsin, is an ester of a serine moiety of the enzyme.[3]

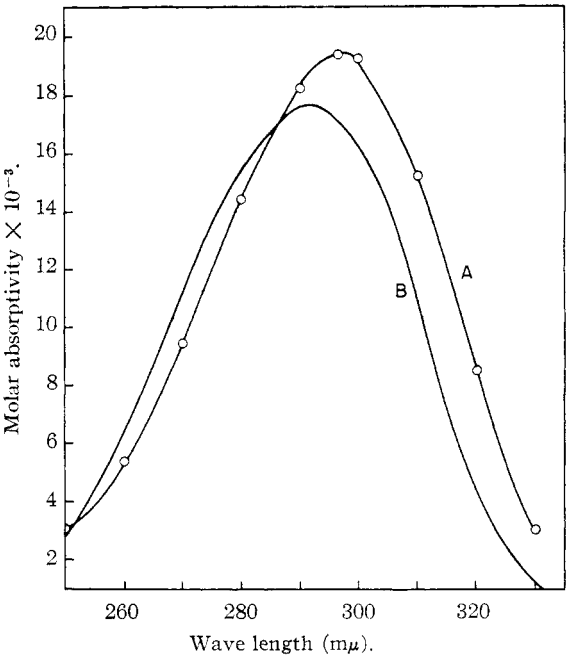

Fig. 1.—Difference spectra of cinnamoyl-trypsin, A, and cinnamoyl-α-chymotrypsin, B; see text for details.

Although the specificities of trypsin and α-chymotrypsin may differ from each other, the mechanism of their catalytic action appears to be the same. Trypsin and α-chymotrypsin are similar with respect to biological origin, molecular weight, types of substrates on which they act, the presence of a single active site per molecule, the presence of a DFP-inhibitable serine hydroxyl group in this active site, and a proton of the peptide sequence surrounding this active site. To these similarities can now be added similarities with respect to the stepwise catalytic sequence, pH dependence of the catalytic action and similarities in the spectral and kinetic behavior of the acyl-enzyme intermediate. The formation of an acyl-enzyme intermediate now has been demonstrated for two related serine proteinases[10]; it is not unreasonable to extrapolate this mechanism to all enzymes of this family.

(5) G. W. Schwert and M. A. Eisenberg, *J. Biol. Chem.*, **179**, 665 (1949).

(6) (a) J. A. Stewart and L. Ouellet, *Can. J. Chem.*, **37**, 751 (1959). (b) These authors found that the first-order acylation constants for the α-chymotrypsin- and trypsin-catalyzed hydrolyses of *p*-nitrophenyl acetate were similar.

(7) The trypsin-catalyzed hydrolysis of N-α-benzoyl-L-arginine ethyl ester depends on a group with an apparent pK_a of 6.25[8] or 6.02[9] in water at 25°. While the apparent pK_a found here does not quantitatively agree with these results, the findings are similar and indicate that the process being observed here is related to those observed with specific substrates.

(8) H. Gutfreund, *Trans. Faraday Soc.*, **51**, 441 (1955).

(9) T. Inagami and J. M. Sturtevant, *Biochim. et Biophys. Acta*, **38**, 64 (1960).

(10) B. S. Hartley, *Ann. Revs. Biochem.*, **29**, 45 (1960).

(11) Alfred P. Sloan Foundation Research Fellow.

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HEPTALENIUM ION

Sir:

We wish to report the synthesis of 1-heptalenium fluoroborate (I) by a four-step sequence from 1,5-naphthalenedicarboxylic acid utilizing the ring enlargement route developed by Nelson, Fassnacht and Piper[1] for the preparation of cycloheptatrienes.

(1) N. A. Nelson, J. H. Fassnacht and J. U. Piper, *J. Am. Chem. Soc.*, **83**, 206 (1961).

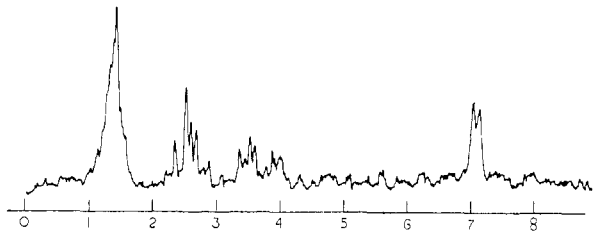
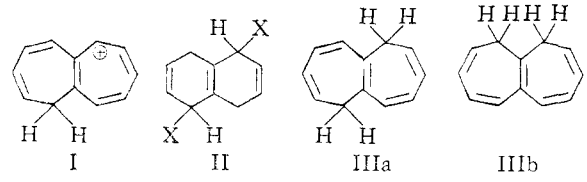


Fig. 1.—N.m.r. spectrum of 1-heptalenium fluoroborate.

Heptalenium ion is of interest because it constitutes not only the next higher cyclic vinylog of 1-azulenium ion but also the conjugate acid of the unknown and theoretically important nonalternate, $4n$ π-electron, non-classical aromatic hydrocarbon, heptalene.[2] Heptalenium ion represents a very desirable immediate precursor of heptalene; should heptalene be a stable, isolable compound, mild deprotonation conditions, utilizing excess base at low temperatures to circumvent electrophile-induced polymerization[3] of the product, could be used to effect the transformation, and should heptalene be capable of only transient existence, its presence might be indicated by deuteron exchange or Diels–Alder capture.



Reduction of 1,5-naphthalenedicarboxylic acid (dimethyl ester, m.p. 110–112°), prepared by acid hydrolysis of the 1,5-dinitrile derived from the 1,5-dibromide[4] by cuprous cyanide in N,N-dimethylformamide[5a] or N-methylpyrrolidone,[5b] by sodium–ethanol in ammonia at −78° for 8 hr. furnished 55–60% 1,4,5,8-tetrahydronaphthalene-1,5-dicarboxylic acid (II, X = COOH) (m.p. > 220° (dec.); ultraviolet (HOH), only end absorption; calcd. for $C_{12}H_{12}O_4$: C, 65.45; H, 5.49; neut. equiv., 110.2; found: C, 65.30; H, 5.74; neut. equiv., 109.9, 110.4). Further reduction of the tetrahydrodiacid (II, X = COOH) by lithium aluminum hydride in ether using Soxhlet extraction technique yielded 1,5-di-(hydroxymethyl)-1,4,5,8-tetrahydronaphthalene (II, X = CH_2OH) (46% recrystallized yield; white needles (ether), m.p. 73–75°; ultraviolet (HOH): only end absorption; calcd. for $C_{12}H_{12}O_2$: C, 74.96; H, 8.38; found: C, 75.08; H, 8.31), which was better converted without isolation by treatment with *p*-toluenesulfonyl chloride and pyridine in chloroform–benzene at 5° for 48 hr. to 1,5-di-(hydroxymethyl)-1,4,5,8-tetrahydronaphthalene ditosylate (II, X = CH_2OTs) (51–59% over-all yield; white needles (abs. EtOH); m.p. 114–117° (dec.); ultraviolet (EtOH): 223 mμ (1750); calcd. for $C_{26}H_{28}O_6S_2$: C, 62.37; H, 5.64; S, 12.81; found: C, 62.13; H, 5.54; S, 12.66).

Solvolytic rearrangement of the tetrahydroditosylate (II, X = CH_2OTs) in pure acetic acid containing sodium dihydrogen phosphate[1] and deoxygenated nitrogen at 90° for 24 hr. gave a product mixture shown by vapor phase chromatography (Ucon polar, 190°) to contain yields of 76.5% hydrocarbon and 4.3–5.3% ester products from which the hydrocarbon products could be separated readily by chromatography (neutral alumina, hexane); a high resolution n.m.r. spectrum in carbon tetrachloride clearly showed the hydrocarbon fraction to be devoid of possible dimethylnaphthalene and methylbenzocycloheptatriene products, and by only doublet methylene proton resonances to consist of a 2:3 (or 3:2) mixture of the isomeric 1,5- and 1,10-dihydroheptalenes (IIIa and b) (light yellow liquid; polymerizes on exposure to air unless inhibitor added; ultraviolet (hexane): 252 (7860), 308 mμ (3680); calcd. for $C_{12}H_{12}$: C, 92.25; H, 7.75; mol. wt., 156.2; double bonds, 5.0; found: C, 92.19, 92.18; H, 7.75, 7.74; mol. wt. (mass spec.), 156; double bonds (PtO_2, EtOH), 4.98). Triphenylcarbonium fluoroborate[6] in methylene chloride effected rapid hydride ion abstraction from the isomeric dihydroheptalenes (IIIa and b) and formed 85% triphenylmethane and 88% 1-heptalenium fluoroborate (I) (bright yellow cubes (acetone–ether), moderately stable in air except for gradual green coloration (faster when impure), and stable in water at pH 7; ultraviolet (96% H_2SO_4): 223 (17,800), 251 (16,150), 298 (9930), 416 mμ (5300); infrared (KBr): 3.28 m, 3.37 w, 6.18 w, 6.82 s, 6.92 m, 7.49 w, 7.72 w, 8.5–9.7 s, 12.25 w, 12.80 w, 13.19 w, 13.33 w, 14.30 w; n.m.r. (80% D_2SO_4–20% H_2SO_4; τ(Me_4Si) = 10.00 p.p.m.) see Fig. 1; calcd. for $C_{12}H_{11}F_4B$: C, 59.54; H, 4.58; found: C, 59.41, 59.28; H, 4.42, 4.50).

The close similarity of the n.m.r. spectrum of 1-heptalenium ion with that of 1-azulenium ion[7] clearly confirms its structure, and the nearness of the proton absorptions of its aromatic ring to those of tropenium ion, and of its non-aromatic ring to those of dihydroheptalene indicates, as in azulenium ion,[7] relatively little charge delocalization from the aromatic ring in heptalenium ion. Consequently, the greater stability of 1-heptalenium ion ($pK_a \geq +7$) than of 1-azulenium ion ($pK_a -1$)[8] probably is not attributable to appreciably different resonance energies of these ions, but very likely is due to considerably greater basicity, and lesser resonance energy, of heptalene than azulene.

Hydride ion abstraction from 1-heptalenium ion by triphenylcarbonium ion has failed thus far to produce any evidence of the double cationic species

(2) For cogent discussion and relevant references, see (a) D. P. Craig, (b) E. D. Bergmann, chapters in "Nonbenzenoid Aromatic Compounds" (D. Ginsburg, Editor), Interscience Publishers, Inc., New York, N. Y., 1959.

(3) Heptafulvalene is readily polymerized by proton acids (J. R. Mayer (with W. v.E. Doering), Ph.D. Thesis, Yale University, 1955) and by the weakly Lewis-acidic tropenium ion (present work). Since heptafulvalene and heptalene have comparable large and favorable nucleophilic localization energies (A_1^e = 0.473β, 0.340β, respectively), heptalene might be expected to show analogous, facile, electrophile-induced polymerization.

(4) Yu. S. Zalkind and S. B. Faerman, *J. Russ. Phys.-Chem. Soc.*, **62**, 1021 (1930).

(5) (a) L. Friedman and H. Shechter, *J. Org. Chem.*, **26**, 2522 (1961); (b) M. S. Newman and H. Boden, *ibid.*, **26**, 2525 (1961).

(6) H. J. Dauben, L. R. Honnen and K. M. Harmon, *ibid.*, **25**, 1442 (1960).

(7) S. S. Danyluk and W. G. Schneider, *J. Am. Chem. Soc.*, **82**, 997 (1960); in 80% D_2SO_4–20% H_2SO_4, the four absorptions are centered at 1.08, 2.11, 2.38 and 5.88 τ (W. F. Harrison (with A. G. Anderson), Ph.D. Thesis, University of Washington, 1960).

(8) P. A. Plattner, E. Heilbronner and S. Weber, *Helv. Chim. Acta*, **32**, 574 (1949).

($C_{12}H_{10}^{++}$, $4n + 2$ π-electrons), but proton abstraction has resulted in the synthesis of heptalene ($C_{12}H_{10}$, $4n$ π-electrons), as described in another Communication.

(9) Partial support of this work by the U. S. Army Research Office is gratefully acknowledged; D. J. B. is indebted to the Standard Oil Company (California) for a fellowship held during a portion of this research.

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HEPTALENE

Sir:

One of the most intriguing unsolved problems in non-classical aromatic chemistry involves the synthesis, properties, and theoretical understanding of the simple members of the group of bicyclic, nonalternant, $4n$ π-electron hydrocarbons, pentalene and heptalene. Even though the simple valence-bond and molecular orbital methods are concordant in their predictions of appreciable delocalization energies (pentalene: 1.09 α (32.7 kcal./mole), 2.456 β (40.5); 1.087γ (35.9); heptalene: 3.618β (59.7), 1.465γ (48.3))[1,2] for compounds of this type, considerable doubt has arisen about the reliability of their predictions because these semi-empirical methods are discordant in their predictions of such a fundamental property as the symmetries of their ground states and some of their excited states.[3] Craig[3a] has argued on the basis of non-empirical considerations that only deductions about these compounds made by a valence-bond approach have a sound theoretical basis, and that pentalene, heptalene, and other compounds for which this method predicts a non-totally symmetrical ground state ("pseudoaromatics") should show greatly decreased π-electron resonance interaction and increased bond length alternation. Numerous attempts have been made to synthesize pentalene[3b] and heptalene[3b] and their only known derivatives[4] contain additional fused rings that obscure their most relevant properties.[3d] We wish to report the synthesis of heptalene (I) and some of its properties, which when augmented by results from additional studies now in progress should provide an experimental answer to this theoretical enigma.

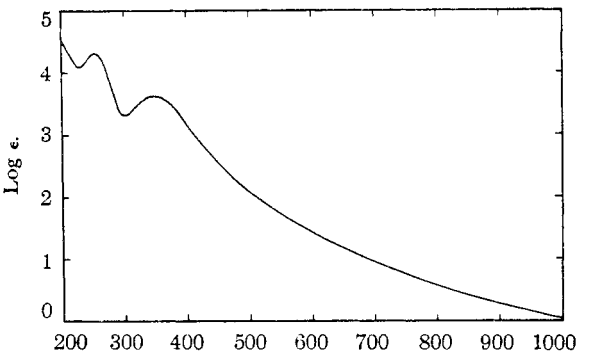
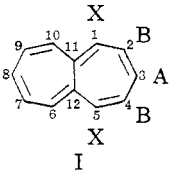


Fig. 1.—Ultraviolet–visible absorption spectrum of heptalene.



Addition of a large excess of trimethylamine in chloroform to an ice-cooled *ca.* 0.005 *M* solution of 1-heptalenium fluoroborate[5] in chloroform produced immediate orange coloration that darkened to red during the reaction period (20 min.); filtration yielded 82% trimethylammonium fluoroborate and a red filtrate that was concentrated, chromatographed (neutral alumina, activity II; CCl_4 or cyclohexane) to give *ca.* 41% yield, evaporatively distilled (*ca.* 25° (0.4 mm.)) onto a Dry Ice-cooled condenser, rechromatographed, and concentrated to give presumably pure heptalene (I) (dark yellowish or reddish brown viscous liquid; crystallization of pure or diluted product not yet successful even at −78°; readily polymerized by oxygen or by mild warming (*ca.* 50°), moderately stable when oxygen-free either neat at −78° or in dilute cyclohexane or carbon disulfide solutions at 25°; ultraviolet (cyclohexane): 256 mμ (21,400), 352 mμ (4,140), and long tail throughout the visible region, see Fig. 1; infrared (2–7.5 μ region; CS_2 or CCl_4): 3.33 s, 3.44 s, 3.52 m, 5.19 w, 5.70 w, 5.76 w, 6.08 w, 6.28 m, 6.95 m, 7.23 m; n.m.r. (CCl_4; $\tau(Me_4Si)$ = 10.00 p.p.m.), see Fig. 2; mol. wt.: calcd. for $C_{12}H_{10}$, 154.2; found (mass spec.): 154; sensitivity to oxygen has precluded accurate weighings needed for carbon and hydrogen analyses.

The heptalene structure for the product is clearly delineated by the observations: (i) derivation from 1-heptalenium ion of established structure by a simple deprotonation reaction under mild conditions, (ii) reconversion to 1-heptalenium ion and no apparent other product by extraction from its cyclohexane solution by 96% sulfuric acid (method used to determine heptalene concentration in ultraviolet spectral determination), or to insoluble, crystalline 1-heptalenium hexachloroantimonate on treatment with excess hexachloroantimonic acid, (iii) rapid

(1) For source references, see: B. Pullman and A. Pullman, "Les Theories Electroniques de la Chimie Organique," Masson et Cie., Paris, 1952, pp. 226–227.

(2) Evaluated using the parameter values (α = 30, β = 16.5, γ = 33 kcal./mole) that best fit the experimental resonance energies of twelve classical benzenoid aromatic hydrocarbons; with these values only the LCAO-MO-with-overlap method, after correction for σ-bond skeletal strain energies, gives satisfactory predictions (±6 kcal./mole) of the resonance energies of the nonclassical aromatic hydrocarbons, azulene, dimethylfulvene, fulvalene, heptafulvalene (H. J. Dauben, Jr., unpublished results). Estimated strain energies of pentalene (32.0 kcal./mole) and heptalene (21.1 kcal./mole) should be subtracted from the calculated delocalization energies to give their predicted resonance energies.

(3) For cogent discussion and relevant references, see (a) D. P. Craig, and (b) E. D. Bergmann chapters in "Nonbenzenoid Aromatic Compounds" (D. Ginsburg, editor), Interscience Publishers, New York, N. Y., 1959; (c) H. C. Longuet-Higgins in "Theoretical Organic Chemistry, The Kekule Symposium," Butterworths, London, 1958; (d) M. Asgar Ali and C. A. Coulson, *Molec. Physics*, **4**, 65 (1961).

(4) (a) Dibenzo[a,e]pentalene: C. T. Blood and R. P. Linstead, *J. Chem. Soc.*, 2263 (1952); *cf.* K. Brand, *Ber.*, **45**, 3071 (1912); (b) 3,5-dimethylcyclohepta[c,d]pentalene and 3,5-dimethylcyclopenta[e,f]heptalene: K. Hafner and J. Schneider, *Ann.*, **624**, 37 (1959).

(5) H. J. Dauben, Jr., and D. J. Bertelli, *J. Am. Chem. Soc.*, **83**, 4657 (1961).