The maximum degree of inactivation so far obtained has been 80 ± 5%, as measured in the standard assay.[3] To determine whether this represents a mixture of 20% of active chymotrypsin with 80% of totally inactive enzyme or an enzyme possessing 20% activity, samples of the inactivated enzyme were allowed to react with diisopropylfluorophosphate. Since it is known that during the complete inactivation of chymotrypsin by diisopropyl fluorophosphate,[7] one mole of diisopropyl phosphate per mole of enzyme[7] is bound to the serine hydroxyl group at the active site,[8] the phosphorus content of inhibited chymotrypsin, allowed to react with diisopropylfluorophosphate until completely inactive, is a measure of the proportion of enzyme molecules capable of reaction at the active site.[9] The phosphorylated proteins, which had less than 0.25% activity, contained[10] one mole of phosphorus per mole of enzyme (Table I).

Amino acid analyses[11] of chymotrypsin and of the irreversibly inactivated chymotrypsin show that one methionine residue in the latter is destroyed, and that the products (after hydrolysis) from it are the same as those obtained by Gundlach, Stein, and Moore (ref. 12, fig. 2c) with ribonuclease inactivated by iodoacetic acid at pH 2.8. In addition, roughly one mole of α-aminoisobutyric acid is found in the hydrolysate (Table II). No other change in amino acid composition has been detected.

TABLE II

METHIONINE, ITS DECOMPOSITION PRODUCTS AND α-AMINOISOBUTYRIC ACID IN HYDROLYSATES OF MODIFIED CHYMOTRYPSIN

Amino acid	Modified CT	CT	DIP-CT[a]
Methionine	1.19[b]	2.08[b]	2.10
S-Carboxymethylhomocysteine	0.5	...	*ca.* 0.01
Homoserine	0.1	...	0.00
Homoserine lactone	0.3	...	0.00
α-Aminoisobutyric acid	*ca.* 1.0[c]	...	trace

[a] The DIP (diisopropylphosphoryl)-CT, which had 0.25% activity, was allowed to react with 25 moles of I for 6 hr. [b] Average of three determinations. [c] This amino acid was difficult to determine with accuracy since it gives a low ninhydrin color yield and falls on one side of the cystine peak in chromatograms.[11]

We conclude that the activated bromine atom of the acyl enzyme alkylates a methionine residue in the vicinity of the active site, either while attached to the serine or shortly after hydrolysis of the acylserine bond. Since the liberated serine hydroxyl group is capable of reaction with diisopropylfluorophosphate, the active site is still capable of functioning, but much less efficient than it was before modification of the methionine residue near it. The inefficiency of the modified enzyme is attributable to a considerable increase (about 11-fold) in the Michaelis constant over that of native chymotrypsin.[13]

Our work corroborates the finding of Ray, *et al.*,[14] that destruction of methionine leads to the inactivation of chymotrypsin. It is unlikely that the sulfide group of the critical methionine behaves as a neighboring nucleophile at the active site of chymotrypsin (though this is entirely possible in the case of phosphoglucomutase[14]), since this activity should be abolished upon alkylation, which gives a ternary sulfonium salt,[12,15] or upon subsequent peptide cleavage.[15,16] The increase in the Michaelis constant indicates that modification of the critical methionine residue results in decreased affinity for substrate.

This general method for the modification of enzymes, as well as the complementary method of Baker, *et al.*,[17] should prove to be a good tool for the "mapping" of enzyme active sites.

Acknowledgments.—We are grateful to the National Institutes of Health for grants A-5299 Bio. and 2E-140 (C1) which helped to support this work.

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(10) "Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists," 6th Ed., A.O.A.C., Washington, D. C., 1945, p. 127.

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(13) Procedure of L. W. Cunningham, Jr., *ibid.*, **207**, 443 (1954).

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(15) W. B. Lawson, E. Gross, C. M. Foltz and B. Witkop, *ibid.*, **83**, 1509 (1961); E. Gross and B. Witkop, *ibid.*, **83**, 1510 (1961).

(16) Subsequent peptide cleavage at the carboxyl group of the methionine is likely to be slight under the conditions used (*cf.* ref. 15). This point, as well as the possibility of cleavage upon heating, is under investigation.

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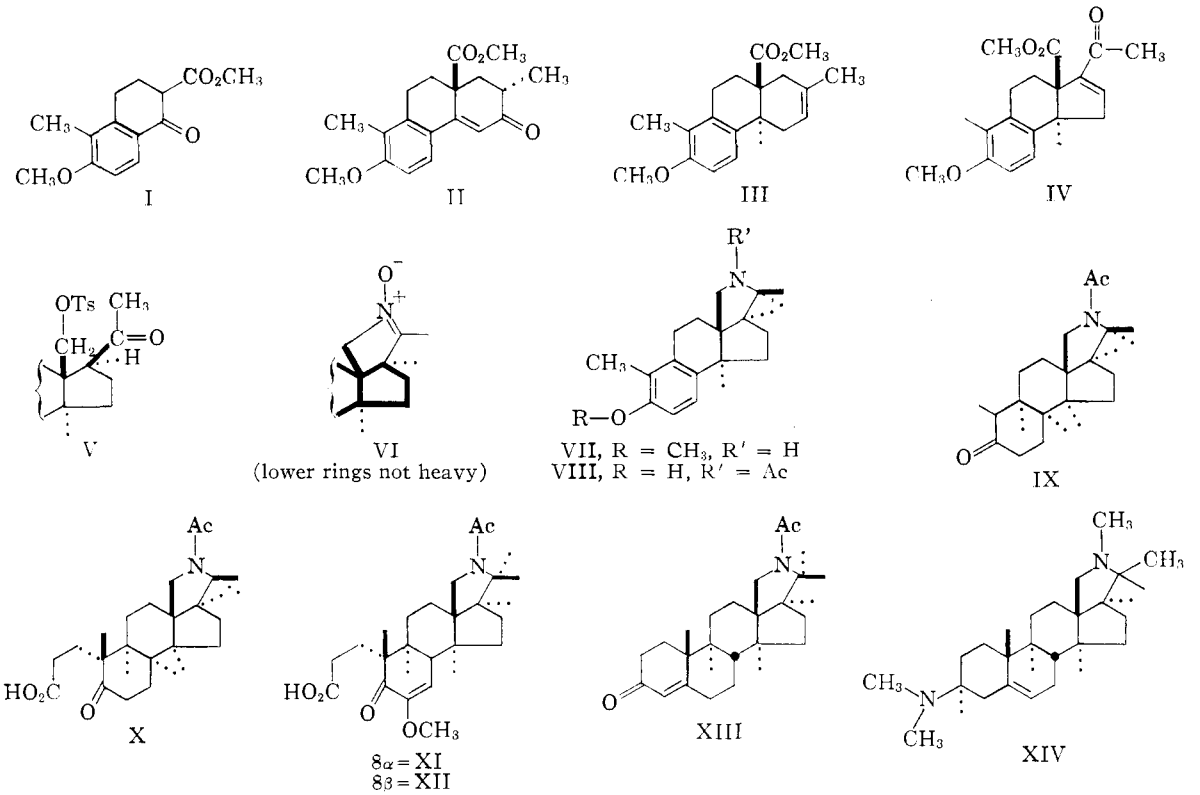
A STEREOSPECIFIC TOTAL SYNTHESIS OF 18-SUBSTITUTED STEROIDS. APPLICATION TO THE SYNTHESIS OF *dl*-CONESSINE

Sir:

This communication reports a total synthesis of 18-substituted steroids in which the B-C-D system is constructed following the lines laid in our previous steroid total synthesis,[1] but control of the C_{13} stereochemistry is achieved by the use of an 8-*iso* (*cis* B/C) structure which finally is inverted to the 8-normal (β) configuration. We illustrate the synthesis by building up the steroid alkaloid conessine,[2] but it will be obvious that a variety of 18-substituted 20-ketosteroids would be of considerably simpler access, *e.g.*, *via* IV.

(1) G. Stork, H. J. E. Loewenthal and P. C. Mukharji, *J. Am. Chem. Soc.*, **78**, 501 (1956).

(2) D. H. R. Barton and L. R. Morgan, *J. Chem. Soc.*, 622 (1962), have accomplished a partial synthesis of natural conessine from Δ⁵-pregnene-3β,20δ-diol.



Reaction of 5-methyl-6-methoxy-α-tetralone with dimethyl carbonate (sodium hydride–benzene) gave the β-ketoester I, m.p. 104–106° (C, 67.64; H, 6.58). Addition of methyl isopropenyl ketone (potassium *t*-butoxide, *t*-butyl alcohol) and then cyclization (toluenesulfonic acid in benzene) gave the tricyclic ketone II, m.p. 184–184.5° (C, 72.79; H, 6.91). Reduction with palladium on charcoal in dioxane–ethanol yielded the dihydro alcohol, m.p. 167.5–168.5°, which led (phosphorus oxychloride–pyridine at 85°) to the corresponding chloro compound, m.p. 100–102° (C, 67.90; H, 7.52) and, after refluxing the latter with methanolic sodium methoxide, to the unsaturated ester III, m.p. 121–123° (C, 76.08; H, 7.99).[3]

Transformation of ring D was carried out by ozonolysis of III (methylene chloride–pyridine at −75°) to the keto aldehyde, m.p. 139–140° (C, 69.06; H, 7.29) which was cyclized by heating in acetic acid containing hydrogen chloride and methanol to IV, m.p. 170–172° (C, 73.13; H, 7.05). Reduction (lithium aluminum hydride) of the carbomethoxy group, after protection of the acetyl function as its dioxolane, led to the unsaturated carbinol, m.p. 162–163.5°, which was converted to its tosylate, m.p. 157–158° (C, 68.17; H, 6.45) and then (palladium on charcoal, ethyl acetate) to the saturated ketotosylate V, m.p. 162.4–162.7° (C, 68.12; H, 6.63). The latter, upon being heated with hydroxylamine hydrochloride in pyridine gave, after neutralization, the nitrone VI, m.p. 247.4–250.4° (C, 76.06; H, 8.16). Hydrogenation of VI with rhodium on charcoal established the pyrrolidine ring in the proper stereochemical sense and gave VII, m.p. 131.8–132.6° (C, 79.63; H, 9.17).

Cleavage of the phenolic ether (hydrobromic–acetic acid) and acetylation (acetic anhydride–potassium hydroxide) gave the acetamidophenol VIII, m.p. 282–284.5° (C, 76.18; H, 8.52) which, on hydrogenation (ruthenium oxide in alcohol, 100°, 2,000 p.s.i.) and oxidation (chromic acid–acetone–sulfuric acid), furnished the ketone IX, m.p. 134.8–136.1° (C, 75.28; H, 9.79).

Construction of ring A then was begun with the addition of acrylonitrile (Triton B in dioxane) to IX, thus leading to the cyanoethyl derivative, m.p. 212–213° (C, 73.84; H, 8.90) and to the corresponding acid X, m.p. 282–283° dec. (C, 69.97; H, 8.46).

Transformation of the 8α skeleton to the required 8β epimer was the next objective. Bromination, then aqueous base hydrolysis to the α-ketol and oxidation with Benedict solution gave the enolic α-diketone which was converted (dimethyl sulfate, methanolic potassium hydroxide) into the enol ether XI. The latter had m.p. 277–278.5° (C, 68.34; H, 8.11 λ_{max}^{EtOH} 267.5 mμ) and could be converted into the desired 8β isomer XII, m.p. 265–267° dec. (C, 68.45; H, 8.39; λ_{max}^{EtOH} 262 mμ) by refluxing for seventeen hours with 10% aqueous sodium hydroxide.

Removal of the double bond and of the methoxyl then could be achieved by hydrogenation (palladium on strontium carbonate in ethanol, trace of base) followed by treatment with calcium in liquid ammonia, reacetylation, and oxidation of the resulting saturated lactone (as the hydroxyacid sodium salt) with chromic acid–pyridine. The 8β epimer of X thus obtained was converted (acetic

(3) This ester was first synthesized in This Laboratory by Dr. Peter Quitt.

anhydride–sodium acetate) into the corresponding enol lactone m.p. 196–197° (C, 73.55; H, 8.45) which readily gave the pentacyclic enone XIII, m.p. 142–143° (150°) (C, 77.45; H, 9.20 infrared identical with XIII *ex* natural conessine) on treatment with ethereal methylmagnesium iodide. Formation of the N,N-dimethylenamine with methanolic dimethylamine and *p*-toluenesulfonic acid was followed by its reduction with sodium borohydride–diglyme in a mixture of aqueous ethanol, acetic acid and sodium acetate,[4] and the resulting 3β-dimethylamino compound was then deacetylated (calcium in liquid ammonia) and methylated by the usual formic acid-formaldehyde procedure to give *dl*-conessine (XIV), m.p. 127.5–128.5° (Kofler). The identity of the infrared spectrum (CCl_4) with that of an authentic sample of the natural material establishes the suggested stereochemical course of the various synthetic steps.[5]

(4) This procedure is adapted from that first described by W. S. Johnson, *et al.* (*Tetrahedron Letters*, No. 2, 72 (1961)).

(5) We thank the National Science Foundation and the National Institutes of Health for their support of this work.

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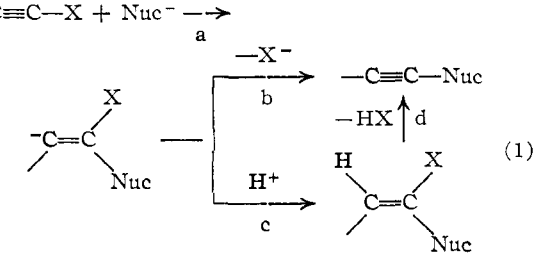
NUCLEOPHILIC SUBSTITUTION AT AN ACETYLENIC CARBON

Sir:

Analogs of the ordinary displacement reaction are now known in the benzene[1] and ethylene series.[2] Based on several negative reports, it is generally considered that haloalkynes are inert to nucleophilic displacement.[3,4] Otherwise, the formation of —C≡C—Nuc in the presence of a proton donor often has been the over-all result of the now familiar addition elimination sequence, a–c–d in eq. 1[2,5]; indeed, the presence of a proton donor often renders the substitution mechanism uncertain.[6] Ott and Dittus do describe what appears to be close to an authentic displacement, *e.g.*, dichloroethyne with diethyl sodioethylmalonate in ether.[4,7]



(1)

We have observed that sodium thiolates react with haloalkynes in dimethylformamide (DMF) according to a–b in eq. 1. Thus we have obtained 1-phenyl-2-phenylthioethyne, 1-phenyl-2-pentachlorophenylthioethyne, and 1-phenyl-2-*p*-*tert*-butylphenylthioethyne. Of course, all of the products mentioned in this paper have given satisfactory elemental analyses. Of these, only the 1-phenyl-2-phenylthioethyne appears to be known; its properties and those of the derived sulfone check with those previously reported.[3b] Provided that the parent acetylene is available, the displacement path compares favorably with standard routes to thioethers.[8]

Typical of these preparations was that of 1-phenyl-2-*p*-toluenethioethyne. Sodium toluenethiolate was prepared by treatment of the thiol with sodium in refluxing toluene. The salt was filtered, washed with toluene and vacuum dried. A solution of the sodium toluenethiolate and phenylbromacetylene in DMF was stirred for 1 hr. and then worked up. Chromatography over alumina yielded white crystals, m.p. 45.5–46.5°, in 65% yield (*Anal.* Calcd. for $C_{15}H_{12}S$: C, 80.31; H, 5.39. Found: C, 80.46; H, 5.50). The sulfone was prepared by oxidation of the thioether with hydrogen peroxide to give a solid, m.p. 80–81° (*Anal.* Calcd. for $C_{15}H_{12}SO_2$: C, 70.29; H, 4.72. Found: C, 70.0; H, 4.78). The infrared triple bond absorption in the thioether and in the sulfone was at 4.72 μ and 4.74 μ, respectively, in agreement with data for similar compounds.

The proposed mechanism of eq. 1 is of some interest. Nucleophilic displacements at other unsaturated centers, *e.g.*, aromatic,[1] ethylenic,[2] and carbonyl[9] presumably have similar intermediates. Such carbanions have been suggested for base-catalyzed alcohol additions to alkynes,[10] and presumably occur in base-catalyzed deuteration of alkenes.[11]

Arens, *et al.*, use scheme 1 as a matter of course in the reactions of haloalkynes with nucleophiles in liquid ammonia.[6a] While this mechanism appears to be plausible and useful, it can only be considered as tentative.

The preceding examples taken together with our experience with other nucleophiles, *e.g.*, cyanide and amines, as well as with comparative kinetic data[12] suggest that any preconceived notions of the low nucleophilic reactivity at an acetylenic carbon must be revised. It is true that nucleophilic attack on haloalkynes, even under forcing conditions, has

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