[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, BRANDEIS UNIVERSITY, WALTHAM 54, MASS.]

A New General Heterocycle Synthesis; Use of Acetylenedicarboxylic Esters[1]

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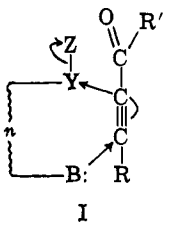
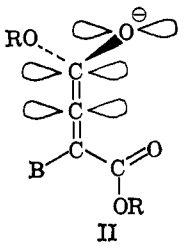
The reaction of acetylenedicarboxylic esters with a variety of nucleophiles selected by reference to a general scheme (I) affords a number of heterocyclic systems, including pyrroles, furans, thiophenes, quinolines, pyrrolinones, and thiazolinones among the cases investigated. The broad utility of the scheme is indicated as well as some evidence on the course of the reaction. The course of additions to the triple bond is determined by the presence of mobile protons in the reaction medium.

The present work was initiated to test the scope and practicality of a general approach to the syntheses of unsaturated heterocyclic compounds envisioned as a Michael addition of a nucleophilic element (B:) to an acetylenic carbonyl compound with cyclization, as symbolized in I. From a synthetic point of view the conception has the advantage that the triple bond survives as a double bond, required for the aromaticity of the product heterocycle, while the directionality of the Michael addition should assure a unique orientation in the coupling of the two fragments. Furthermore, the simplicity and potential breadth of applicability in synthesis interested us. Although this presentation (I, $n = 1$) bears a resemblance to 1,3-dipolar addition,[3] the Michael reactions of acetylenic carbonyls have been little studied.[4,5]



I

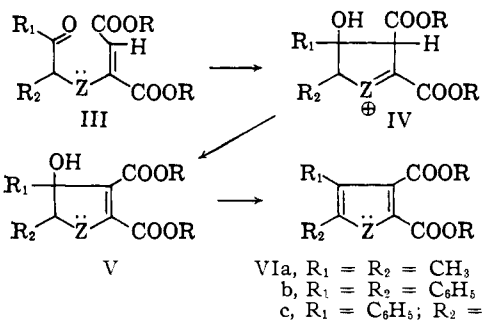


II

In order first to determine the general validity of the design we attempted reactions with the simpler acetylenedicarboxylic esters, which are uncomplicated by asymmetry and are known to undergo Michael additions with facility in basic media with such species as $RO^{\ominus}$,[5] $C_6H_5O^{\ominus}$,[5] $C_6H_5SO_2^{\ominus}$,[6] $C_6H_5SO_2^{\ominus}$,[7] $HSO_3^{\ominus}$,[8] and R_2NH[9] and in acid with H_2O and HX.[5] In the addition of bases (B:) the intermediate enolate II is formed; in the case of the addition of sodiomalonate in ether this enolate has in fact been isolated and identified by subsequent C-alkylation.[10] While the π-electrons on the central carbon of the enolate II can in principle overlap on either side with an electron-deficient species to yield *cis* or *trans* products, the preferred internal delivery of a coordinated proton by the ester carbonyl (as in II) and the development of the sterically favored fumarate (*trans*) product both conspire to produce predominantly *trans* addition in simple cases (*cf.* HB). In acid-catalyzed cases the same arguments, as well as the possibility of the common direct concerted *trans* addition to the multiple bond, rationalize the generality of the observance of *trans* products.

Even so, small yields of *cis* isomer have frequently been isolated and, of course, in the special cases of 1,3-dipolar addition (*cf.* B = $-O-\overset{\oplus}{N}\equiv C-C_6H_5$) *cis* addition is general because of internal collapse of the enolate to the positive center remaining in the added fragment B. These considerations encouraged us to examine the applicability of the scheme I.

Our first efforts were directed to the simple fully aromatic five-membered heterocycles, pyrroles, furans, and thiophenes. Examination of I in conjunction with the oxidation states of the desired products leads to the choice of a ketone for the group Y–Z. In particular, pyrroles should derive from α-amino ketones and the acetylenedicarboxylic ester. α-Amino ketones, however, dimerize readily to dihydropyrazines and are stable only as their salts.[11] Nevertheless, it was deemed reasonable that in the presence of a relatively weak base these salts would generate a concentration of free amino ketone low enough to hamper the bimolecular dimerization but suitable for Michael addition to the acetylenic ester in the medium. Accordingly, when equimolar amounts of α-aminoproprophenone hydrochloride, sodium acetate, and dimethyl acetylenedicarboxylate were boiled in methanol, sodium chloride was deposited over several minutes and, after 10 min., filtration and evaporation afforded a high yield of V (Z = NH, $R_1 = C_6H_5$, $R_2 = R = CH_3$), which could be easily transformed to VIc (Z = NH) by a trace of acid. Isolation of the intermediate V was not necessary and the three pyrroles VI (Z = NH) were prepared directly from acetylenedicarboxylic esters and the appropriate α-amino ketones. The analyses and infrared spectra attested to the identity of the products, compounds V showing both OH (2.80 μ) and NH (2.97 μ) bands, two ester bands (not conjugated with NH, 5.80 μ; conjugated at 5.92 μ), and a large double bond absorption at 6.2 μ. The true pyrroles (VI) exhibited the typical strong NH band at 2.9 μ and the same two ester bands but no double bond peak.



III IV

V

VIa, $R_1 = R_2 = CH_3$
b, $R_1 = R_2 = C_6H_5$
c, $R_1 = C_6H_5$; $R_2 = CH_3$

In an analogous reaction with basic catalysis (potassium carbonate) the α-hydroxy ketone benzoin yielded

(1) A preliminary communication appeared in *J. Am. Chem. Soc.*, **83**, 1250 (1961).

(2) Alfred P. Sloan Foundation Fellow.

(3) L. I. Smith, *Chem. Rev.*, **23**, 193 (1939); R. Huisgen and A. Eckell, *Tetrahedron Letters*, **12**, 5, 9 (1960).

(4) E. D. Bergmann, D. Ginsburg, and R. Pappo, *Org. Reactions*, **10**, 179 (1959).

(5) A. W. Johnson, "Chemistry of the Acetylenic Compounds," Vol. 11, Longmans Green, New York, N. Y., pp. 199–266.

(6) W. F. Truce and R. B. Kruse, *J. Am. Chem. Soc.*, **81**, 5372 (1959).

(7) E. 1. Grinblat and 1. Y. Postovsky, *Dokl. Akad. Nauk SSSR*, **133**, 847 (1960).

(8) H. J. Backer and A. E. Beute, *Rec. trav. chim.*, **54**, 200, 523 (1935); J. B. Hendrickson, *J. Am. Chem. Soc.*, **84**, 653 (1962).

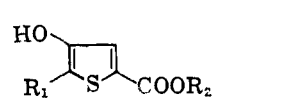
(9) S. Ruhemann and A. V. Cunnington, *J. Chem. Soc.*, **75**, 954 (1899); H. R. Snyder, H. Cohen, and W. J. Tapp, *J. Am. Chem. Soc.*, **61**, 3560 (1939).

(10) E. H. Farmer, S. C. Ghosal, and G. A. R. Kon, *J. Chem. Soc.*, 1804 (1936).

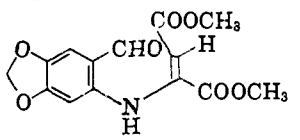
(11) Y. T. Pratt in "Heterocyclic Compounds," Vol. V1, R. C. Elderfield, Ed., John Wiley and Sons, Inc., New York, N. Y., 1957, p. 377 ff.

the hydrated furan V (Z = O, $R_1 = R_2 = C_6H_5$, R = CH_3) which dehydrated easily in methanolic acid to the furan VIb (Z = O). As to the course of these reactions, it was implicit in the preceding discussion that the initial enolate corresponding to II would collapse directly to V, thus closing both new ring bonds in a single fast sequence analogous to that for 1,3-dipolar addition. Alternatively, however, the enolate II could be protonated to a neutral intermediate, III, the product of simple *trans* addition of R–$\ddot{Z}$H to the triple bond. This could in turn cyclize, *via* IV, to V. Evidence bearing on this choice of routes derived from the experiments aimed at thiophene syntheses analogous to those above.

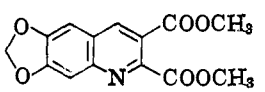
When diethyl acetylenedicarboxylate was boiled in ethanol with mercaptoacetone there was formed a crystalline enol, $C_9H_{10}O_4S$, with spectral properties corresponding to VIIa. When ethyl mercaptoacetate was treated with dimethyl acetylenedicarboxylate a crystalline enolic acid was obtained if the mixture was extracted with alkali, which brought about a very facile saponification of the methyl ester grouping. The presence of the ethyl ester grouping in the product was shown by the characteristic proton magnetic resonance of the ethyl group and, with the analysis and spectra, the product was taken to be VIIb. The ester VIIc had previously been prepared by Fieselmann and Schipprak,[12] who did not, however, distinguish between the possible formulations VIIc and the isomeric VI (Z = S, R_1 = OH, R_2 = H, R = CH_3) which would have been analogous to our pyrrole and furan cases. Use of the mixed esters in the present case allowed the distinction to be made, however. The esters VIIb and VIIc yield the same diacid on saponification.



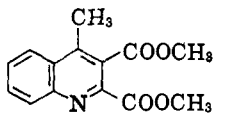
VIIa, R_1=$COCH_3$; R_2=C_2H_5
b, R_1=$COOC_2H_5$; R_2=H
c, R_1=$COOCH_2$; R_2=H



VIII



IX

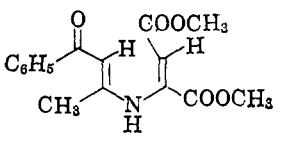


X

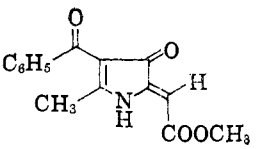
These results argue for the reaction course *via* III rather than direct cyclization of II to V, for, while cyclization of III to IV in the cases of Z = O and N is reasonable, it is not so with the sulfur analog owing to the presence of the unfavorable C=S double bond in IV, and conversely the alternative Dieckmann-type cyclization of III (Z = S) to VII is favored in the sulfur case by the extra stabilization afforded the enolate in such a cyclization by overlap with the d-orbitals of the adjacent sulfur atom. The German authors[12] in fact isolated III (Z = S, $R_1 = OC_2H_5$, R_2 = H, R = C_2H_5) simply by shaking the two components in benzene; they subsequently converted this to VIIc by treatment with alkali. In some of our subsequent experiments (*vide infra*) we also isolated the simple *trans* adducts of the Michael reaction, corresponding to III.

Turning our attention to six-membered heterocycles (*cf.* I, *n* = 2), we allowed 6-aminopiperonal to react with dimethyl acetylenedicarboxylate in hot methanol to form the simple adduct VIII; this was cyclized with methanolic acid, affording the quinoline IX in accord with the expectations of I. Under more vigorous conditions *o*-aminoacetophenone afforded the quinoline X directly. In an attempt to change the course of the initial addition by use of an aprotic solvent, *o*-aminopiperonal was heated with the acetylenic diester in dry benzene; the product, however, was the same (VIII), indicating that the mobile protons of the amino group are sufficient to protonate the initial enolate II faster than a direct internal cyclization of the enolate to the aldehyde, as implied in I. Similar reactions with salicylaldehyde or salicylic acid chloride, however, yielded only starting material.

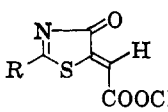
(12) H. Fieselmann and P. Schipprak, *Chem. Ber.*, **89**, 1897, 1902 (1956).



XI



XII



XIIIa, R = NH_2
b, R = $NHNH_2$
c, R = NHN = CHC_6H_5
d, R = C_6H_5

Monocyclic pyridines should arise from utilization of a β-amino-α,β-unsaturated ketone in I and indeed the initial reaction of benzoylacetone imine in hot methanol to produce the *trans* adduct XI went smoothly as before. The case is now, however, more complex than those previously considered in the variety of subsequent cyclizations available to the molecule XI when treated with methanolic acid and the probable *trans* orientation of the original enamine ketone, reflected in XI, renders the desired cyclization to a pyridine diester unlikely unless a facile *cis–trans* equilibrium is set up in this medium. In any case a single nonbasic product resulted from the acid-catalyzed cyclization and indicated on analysis loss of the elements of one mole of methanol rather than water. The product was accordingly formulated as XII, arising by cyclization of XI at the less conjugated (more reactive) ester group and corroborated by the infrared spectra (see Experimental) which did not show typical pyridone absorption.

A very similar reaction occurred with derivatives of thiourea or thiobenzamide, from which the thiazolinones XIII were prepared. The reactions in these cases proceeded rapidly in high yield directly to the completely cyclized products. The benzal derivative XIIIc of the thiazolinone (XIIIb) from thiosemicarbazide could be prepared either from XIIIb by reaction with benzaldehyde or from benzaldehyde thiosemicarbazone and dimethyl acetylenedicarboxylate, thus demonstrating that the hydrazine moiety of the semicarbazide is not involved in the reaction with the acetylenic ester.

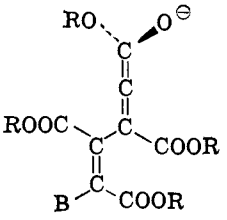
In summary, then, it may be said that the conception embodied in I is capable of providing syntheses of a wide variety of heterocycles; those reported in the present work certainly do not exhaust the possibilities. As a synthesis of pyrroles it has the peculiar advantage of affording pyrroles with esters in both the α- and β-positions; since these groups can be removed selectively for replacement by other functions,[13] these diester products have especially high potential as syn

(13) The α-ester can be saponified and removed in strong alkali without affecting the other, while warming in strong acid (*cf.* 85% phosphoric) readily hydrolyzes and decarboxylates the β-ester, leaving the α-group untouched. See A. H. Corwin in "Heterocyclic Compounds," Vol. 1, R. C. Elderfield, Ed., John Wiley and Sons, Inc., New York, N. Y., 1950, p. 27 ff., and H. Fischer and H. Orth, "Die Chemie des Pyrrols," Akad. Verlag Leipzig, 1934.

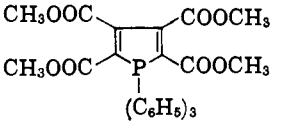
thetic intermediates. No attempt was made to optimize the yields by more detailed variation in reaction conditions, so that it is probable that the yields given can in many cases be substantially improved in practical use. Two experiments were conducted on another acetylenic ketone in preliminary exploration of the scope of the triple-bonded component; 4-phenyleth-3-yn-2-one (acetylphenylacetylene) afforded no reaction either with 6-aminopiperonal or desylamine under the conditions found successful with the acetylenic diesters and more vigorous conditions have not as yet been examined.

It is apparent that the reactions often do not proceed in the direct *cis* addition cyclization implied in diagram I. In fact, our experience, taken with that in the literature, strongly implies that, *whenever mobile protons are available, the first product of additions to acetylenedicarboxylic esters is the simple trans adduct.* The possibility that this adduct will take a subsequent course at variance with that of diagram I, as in several examples above, must be kept in mind in making practical synthetic use of this method.

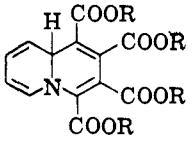
When no mobile protons are available the enolate II of the initial addition may subsequently add to an electron-deficient site in the addend B, as in 1,3-dipolar additions, *or attack a second molecule of the acetylenic ester* to form the enolate of a 1:2 adduct, XIV. The latter possibility has been the source of some interesting reactions, including the reaction of acetylenedicarboxylic esters with sulfur to yield tetracarbethoxythiophene,[5] with triphenylphosphine to yield tetracarbomethoxytriphenylphosphole (XV),[14] with pyridine to yield XVI,[15] and with N-methylpyrrole to yield XVII.[5]



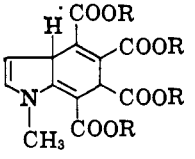
XIV



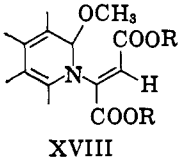
XV



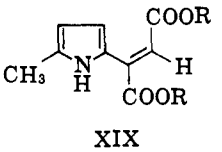
XVI



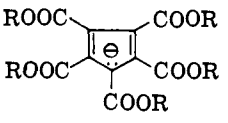
XVII



XVIII



XIX



XX

When protons are available the reaction course differs, pyridines in methanol going to the simple adducts XVIII[16] with added methanol while 2-methyl- differs strikingly from N-methylpyrrole in yielding XIX.[5]

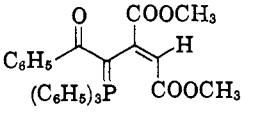
(14) A. W. Johnson and J. C. Tebby, *J. Chem. Soc.*, 2126 (1961); J. B. Hendrickson, R. E. Spenger, and J. J. Sims, *Tetrahedron*, **19**, 707 (1963).

(15) R. M. Acheson and G. A. Taylor, *J. Chem. Soc.*, 1691 (1960).

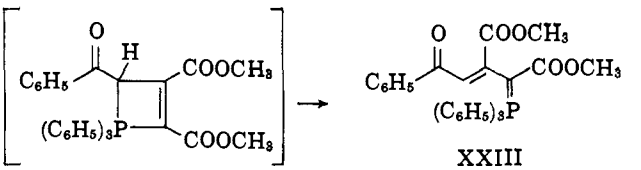
(16) R. M. Acheson and G. J. F. Bond, *ibid.*, 246 (1956).

Farmer, *et al.*,[10] found that in methanol, sodiomalonate added to the triple bond affording a white salt which they formulated, quite reasonably, as the sodium salt (at the α-carbon of the malonate residue) of the simple 1:1 adduct, while the same reaction in dry ether led to a yellow salt to which they assigned the simple 1:1 enolate anion formula II (B = $CH(COOC_2H_5)_2$). The yellow color and the recent experience of Cookson[17] in obtaining the anion XX by this route suggest that the yellow salt may be XIV (B = $CH(COOC_2H_5)_2$), the enolate of the 1:2 adduct.

Another case of the two courses of addition has been found[18] in the reaction of triphenylphosphorylidene-acetophenone with dimethyl acetylenedicarboxylate which affords the simple adduct XXI in methanol, but the isomer XXIII in dry ether; in the latter instance the initial enolate (*cf.* II) can attack the positive phosphorus, cyclizing to a pentacovalent phosphorane XXII which in turn collapses to the isomer XXIII.



XXI



XXII XXIII

Experimental

Melting points are corrected; analyses were performed by Miss Heather King of UCLA and Schwarzkopf Laboratories, Woodside 77, N. Y. Infrared spectra were determined in chloroform solution on a Perkin–Elmer Infracord, ultraviolet spectra on a Cary Model 14 spectrophotometer, and nuclear magnetic resonance spectra on a Varian HR-60 instrument.

Dimethyl 4-Phenyl-5-methylpyrrole-2,3-dicarboxylate (VIc, Z = NH).—α-Aminopropiophenone hydrochloride[19] (185 mg., 1 mmole), 142 mg. (1 mmole) of dimethyl acetylenedicarboxylate (Matheson Coleman and Bell, redistilled), and 82 mg. (1 mmole) of dry sodium acetate were dissolved in 5 ml. of methanol and refluxed for 10 min., sodium chloride being deposited from the initially clear solution in the first 2 min. The mixture was poured onto ice and the white crystals filtered and dried: 220 mg. (80%), m.p. 108–110°. The infrared spectrum showed bands at 2.80, 2.97, 5.80, 5.92, and 6.22 μ; on recrystallization from ethanol and drying for analysis, the crystals showed a melting range of 110–175° and marked diminution of the large band at 6.2 μ.

Anal. Calcd. for $C_{15}H_{17}NO_5$: C, 61.85; H, 5.88. Calcd. for $C_{15}H_{15}NO_4$: C, 65.92; H, 5.53. Found: C, 63.64; H, 5.62.

Recrystallization from methanol containing a trace of hydrochloric acid yielded white needles, m.p. 182–183°, with infrared bands at 2.88, 5.80, 5.92 μ.

Anal. Calcd. for $C_{15}H_{15}NO_4$: C, 65.92; H, 5.53 N, 5.13. Found: C, 65.88; H, 5.41; N, 5.38.

Dimethyl 4,5-Diphenylpyrrole-2,3-dicarboxylate (VIb, Z = NH).—Desylamine hydrochloride[20] (123 mg., 0.5 mmole), 72 mg. (0.5 mmole) of dimethyl acetylenedicarboxylate, and 41 mg. (0.5 mmole) of dry sodium acetate were refluxed for 1 hr., then 2 drops of hydrochloric acid was added and the solution (with sodium chloride deposited as before) boiled 15 min. more. The mixture was poured into ice and extracted with methylene chloride. Drying and evaporating solvent afforded 147 mg. (44%) of crystals, recrystallized from methanol with no change in infrared spectrum to m.p. 185–187°; infrared bands at 2.85, 5.78, 5.90 μ.

(17) R. C. Cookson, J. Hudec, and B. Whitear, *Proc. Chem. Soc.*, 117 (1961).

(18) J. B. Hendrickson, R. Rees, C. Hall, and J. F. Templeton, in preparation for *J. Org. Chem.*

(19) L. Behr-Bregowski, *Ber.*, **30**, 1521 (1897).

(20) R. Pschorr and F. Bruggemann, *ibid.*, **35**, 2740 (1902).

Anal. Calcd. for $C_{20}H_{17}NO_4$: C, 71.63; H, 5.11; N, 4.18. Found: C, 71.69; H, 5.26; N, 4.11.

Dimethyl 4,5-Dimethylpyrrole-2,3-dicarboxylate (VIa, Z = NH).—3-Amino-2-butanone hydrochloride[21] (615 mg., 5 mmoles), 710 mg. (5 mmoles) of dimethyl acetylenedicarboxylate, and 410 mg. (5 mmoles) of dry sodium acetate were refluxed in 15 ml. of methanol for 1 hr., cooled, poured on ice, and extracted with methylene chloride. Drying and evaporating solvent yielded 630 mg. of dark nonbasic oil; no basic fraction was found by extraction of the aqueous phase made alkaline. Crystallization of the oil from ether–pentane yielded 200 mg. (20%) of fine colorless needles, m.p. 146–147°; infrared bands at 2.88, 5.81, 5.93 μ. Treatment of the mother liquors with methanolic acid yielded no further crystalline product.

Anal. Calcd. for $C_{10}H_{13}NO_4$: C, 56.86; H, 6.20; N, 6.64. Found: C, 57.01; H, 6.26; N, 6.89.

In a similar experiment with diethyl acetylenedicarboxylate the corresponding diethyl ester was formed, m.p. 112–113° after recrystallization from ether–pentane or alcohol–water (lit.[22] m.p. 110°).

Dimethyl 4,5-Diphenyl-4-hydroxy-Δ^2-dihydrofuran-2,3-dicarboxylate (V, R = CH_3, $R_1 = R_2 = C_6H_5$, Z = O).—Benzoin (1.00 g., 4.7 mmoles) and 0.80 g. (5.6 mmoles) of dimethyl acetylenedicarboxylate were refluxed for 20 hr. in 25 ml. of acetone containing 0.65 g. of potassium carbonate. The mixture was cooled, poured onto ice, and extracted with ether; drying and evaporating the solvent yielded an oil which congealed crystalline and was recrystallized from methanol: 0.72 g. (44%) of white crystals, m.p. 116–117°; infrared bands at 2.75, 5.72, 5.85, 6.10 μ.

Anal. Calcd. for $C_{20}H_{18}O_6$: C, 67.79; H, 5.12. Found: C, 67.63; H, 4.87.

Sublimation of these crystals afforded crystals of benzoin, m.p. 129–132°, mixture m.p. 130–133°.

Dimethyl 4,5-Diphenylfuran-2,3-dicarboxylate (VIb, Z = O).—Dihydrofuran (122 mg.) from above was refluxed for 1 hr. in 5 ml. of methanol containing a drop of sulfuric acid and the mixture poured onto ice and extracted with ether, yielding 104 mg. of white crystals; after crystallization from methanol, m.p. 87–90°, infrared absorption at 5.76 μ, the other peaks characteristic of the hydro derivative (above) having disappeared.

Anal. Calcd. for $C_{20}H_{16}O_5$: C, 71.42; H, 4.80. Found: C, 71.58; H, 4.98.

Ethyl 4-Hydroxy-5-acetylthiophene-2-carboxylate (**VIIa**).—Mercaptoacetone[23] (180 mg., 2 mmoles), 340 mg. (2 mmoles) of diethyl acetylenedicarboxylate, and 160 mg. (2 mmoles) of dry sodium acetate were dissolved in 5 ml. of ethanol and boiled under reflux for 0.5 hr. Diluting with water and extracting with ether afforded an oil from which 50 mg. (23%) of white crystals, m.p. 87–88°, was obtained on crystallization from ethanol–water. The compound gave a dark color with ferric chloride solutions and showed scalloped hydrogen-bonded –OH absorption from 2.8 to 3.5 μ in the infrared as well as major bands at 5.82 and 6.12 μ; ultraviolet absorption (λ_{max} and log ϵ in 95% EtOH): 287 (4.08), 335 mμ (3.74); in NaOH: 292 (4.12), 365 mμ (3.84).

Anal. Calcd. for $C_9H_{10}O_4S$: C, 50.47; S, 14.90; H, 4.67. Found: C, 50.53; H, 4.80; S, 14.75.

4-Hydroxy-5-carbethoxythiophene-2-carboxylic Acid (**VIIb**).—Ethyl mercaptoacetate (5 ml.) and 5 ml. of dimethyl acetylenedicarboxylate were refluxed for 20 hr. in 100 ml. of methanol, cooled, diluted with 150 ml. of ether, and extracted with 1 *N* NaOH. Acidification of the aqueous phase and extraction with ether afforded 5.1 g. of crude acid fraction, crystallized from benzene to yield 2 g., m.p. 155–160°. Several recrystallizations afforded colorless crystals, m.p. 169–171°, soluble in bicarbonate and yielding a deep red color in ferric chloride solution (1% anhydrous $FeCl_3$ in pyridine); major infrared bands (in KBr) at 3.0 and 5.97 μ; ultraviolet absorption (λ_{max} and log ϵ in 95% EtOH): 273 (4.11), 310 mμ (3.83); in NaOH: 277 (3.98), 355 mμ (3.83). The proton magnetic resonance spectrum: singlets at 2.81 and 4.89 τ (aromatic H and enolic OH), methylene quartet centered at 5.50 τ, and methyl triplet centered at 8.34 τ (ethyl ester, J 8 c.p.s.).

Anal. Calcd. for $C_8H_8SO_5$: C, 44.45; H, 3.73; S, 14.80. Found: C, 44.27; H, 3.85; S, 14.97.

Various attempts to prepare the mixed methyl–ethyl ester directly from the reactants above were unsuccessful owing chiefly to the ready solvolysis of the esters. In one such preparation transesterification occurred when KOH in methanol was used as the reaction medium[12] and the known[12] dimethyl ester, m.p. 70° (lit.[12] 69°), was formed instead of the mixed ester.

Saponification of the half-ester was carried out on 216 mg. in 3 ml. of 4 *N* sodium hydroxide at room temperature overnight. Acidification at 0° yielded a white precipitate which was filtered, dissolved in hot methanol, and precipitated slowly by addition of water (198 mg.). The melting behavior of this acid paralleled the literature description[12] of the diacid dihydrate purified the same way, *i.e.*, initial partial melting and decomposition at about 190° to form the monoacid which solidified and remelted at 207–208°.

Formation of VIII from 6-Aminopiperonal.—When a mixture of 165 mg. (1 mmole) of 6-aminopiperonal[24] and 142 mg. (1 mmole) of dimethyl acetylenedicarboxylate was boiled in 3 ml. of methanol until completely dissolved and then cooled to 0°, colorless crystals of the adduct VIII slowly separated as yellow needles (240 mg., 78%), m.p. 169–172° after recrystallization from methanol. Infrared absorption at 3.0 μ (NH), 5.75 μ (ester not conjugated with NH), 5.91 μ (ester conjugated with NH), 6.00 μ (aldehyde), 6.20 μ (double bond), and 9.62 μ (methylenedioxy).

Anal. Calcd. for $C_{14}H_{13}NO_7$: C, 54.74; H, 4.23; N, 4.55. Found: C, 54.72; H, 4.40; N, 4.61.

The same reaction carried out in dry benzene afforded 72% of the same crystalline product but no basic fraction.

Dimethyl 6,7-Methylenedioxyquinoline-2,3-dicarboxylate (**IX**).—The above adduct VIII (300 mg.) was dissolved in 30 ml. of 7:3 methanol–chloroform and 6 drops of concentrated hydrochloric acid added. The solution, which immediately turned orange, was refluxed for 1 hr. and cooled. Addition of concentrated ammonia caused crystallization of 180 mg. of yellow needles, m.p. 165–174°. Charcoaling and recrystallization from acetone–ether afforded white needles, m.p. 178–179°, infrared absorption at 5.80 and 9.62 μ.

Anal. Calcd. for $C_{14}H_{11}NO_6$: C, 58.13; H, 3.83; N 4.84. Found: C, 58.15; H 3.97; N, 5.03.

Reaction of 1-Phenyl-3-amino-2-buten-1-one[25] **with Dimethyl Acetylenedicarboxylate** (**XI**).—Equimolar amounts (2 mmoles each) of the two components were dissolved in 5 ml. of methanol and refluxed for 0.5 hr. Evaporation of the methanol and crystallization from the ether–pentane afforded 370 mg. (61%) of yellowish needles, m.p. 155–160°; infrared bands at 2.80, 5.80, and 6.25 μ; insoluble in 5% HCl.

Anal. Calcd. for $C_{16}H_{17}NO_5$: C, 63.36; H, 5.65, N, 4.62. Found: C, 63.53; H, 5.84; N, 4.34.

Cyclization of XI to 2-Methyl-3-benzoyl-5-carbomethoxymethylidene-Δ^2-pyrrolinone-3 (**XII**).—The above product (XI, 150 mg.) was refluxed for 0.5 hr. in 5 ml. of methanol containing 3 drops of concentrated hydrochloric acid. The solvent was evaporated under vacuum, ether added and washed with water, and the ether layer dried and evaporated. The residue was crystallized from acetone–ether–pentane affording 50 mg. (37%) of yellow needles, m.p. 163–165° dec.; infrared bands at 2.80, 5.80, 6.00 μ and a weaker pair at 6.25, 6.30 μ.

Anal. Calcd. for $C_{15}H_{13}NO_4$: C, 66.41; H, 9.83; N, 5.16. Found: C, 66.23; H, 4.77; N, 5.31.

2-Amino-5-carbomethoxymethylidenethiazolinone-4 (**XIIIa**).—A slurry of 1.0 g. of thiourea (13.2 mmoles) in 10 ml. of methanol warmed and went clear on addition of 1.85 g. (13.0 mmoles) of dimethyl acetylenedicarboxylate; white crystals (2.19 g., 91%) were shortly deposited and recrystallized from dimethylformamide–water; m.p. 270–275°; infrared bands (in Nujol) at 2.97, 5.80, 6.00, 6.22 (w), and 6.47 μ.

Anal. Calcd for $C_6H_6N_2O_3S$: C, 38.72; H, 3.25; S, 17.21. Found: C, 38.78; H, 3.43; S, 17.38.

2-Hydrazino-5-carbomethoxymethylidenethiazolinone-4 (**XIIIb**).—The same reaction as above using 1.0 g. of thiosemicarbazide yielded 1.4 g. (64%) of yellowish crystals, m.p. 117–188°. Recrystallization from methanol did not significantly change the color or raise the melting point; infrared bands (Nujol): 2.92, 5.75, 5.90, 6.05, and 6.22 μ.

Anal. Calcd. for $C_6H_7N_3O_3S$: C, 35.83; H, 3.51. Found: C, 35.75; H, 3.43.

2-Benzalhydrazino-5-carbomethoxymethylidenethiazolinone-4 (**XIIIc**).—The same reaction as above on 358 mg. (2.0 mmoles) of benzaldehyde thiosemicarbazone and 284 mg. (2.0 mmoles) of dimethyl acetylenedicarboxylate afforded 416 mg. (72%) of yellowish crystals, m.p. 274–276°; infrared bands (Nujol): 5.80, 5.90, 6.10, 6.15, and 6.30 (w) μ.

Anal. Calcd. for $C_{13}H_{11}N_3O_3S$: C, 53.98; H, 3.83; N, 14.53. Found: C, 53.90; H, 3.98; N, 14.34.

The same compound was produced on boiling XIIIb (above) with benzaldehyde in methanol containing some pyridine; m.p. 273–275°, mixture melting point with XIIIc as prepared above, 273–276°.

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2-Phenyl-5-carbomethoxymethylidenethiazolinone-4 (**XIIId**). —The same reaction was also performed using 1.00 g. (7.3 mmoles) of thiobenzamide and 1.05 g. (7.4 mmoles) of dimethyl acetylenedicarboxylate and resulted in 1.40 g. (77%) of yellowish crystals, m.p. 152–154°, sublimed or recrystallized from benzene–cyclohexane without significant change; infrared band (Nujol): no NH, 5.82, 6.22 (w), and 6.56 μ.

Anal. Calcd. for $C_{12}H_9NO_3S$: C, 58.30; H, 3.67; S, 13.01. Found: C, 58.07; H, 3.77; S, 13.41.

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, STANFORD UNIVERSITY MEDICAL CENTER, PALO ALTO, CALIF.]

Chemical Synthesis of a Homolog of Deoxyribonucleoside-5′ Triphosphates

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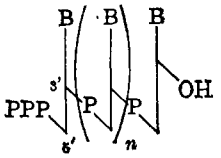
The synthesis of deoxyadenylyl-(5′ → 3′)-thymidine-5′ triphosphate (d-pppTpA) is reported. Material labeled with ^{32}P at the 5′-terminal ester phosphate does not serve as a substrate for the enzymatic synthesis of deoxyadenylate-thymidylate copolymer (dAT), a DNA-like polymer.

The absolute requirement for deoxyribomononucleoside-5′ triphosphates in the *in vitro* synthesis of DNA-like polymers[1] and related macromolecules[2,3] by enzymes from several sources has been clearly established.[4] There has been some speculation[5] that dinucleotides or higher homologs may be DNA precursors. This idea could be tested in the *in vitro* systems available with such "activated" species as



(where B designates purine or pyrimidine bases native to DNA).[6]

If such a biosynthetic pathway exists, it might be a method for the *in vivo* salvage of naturally occurring oligonucleotide sequences by their incorporation into DNA *via* activation by the pyrophosphate moiety. Furthermore, such structures might be models for the possible natural occurrence of polymeric pppXpY. . .pZ species in biosynthesis. The relative ease with which nucleoside triphosphates can be prepared chemically,[7] and especially the improvements and detailed experimental procedures now available from the work of Moffatt,[8] prompted us to attempt the chemical synthesis of a model triphosphate of the type described and to examine its behavior with the DNA polymerase from *Escherichia coli*.

We chose the structure deoxyadenylyl-(5′ → 3′)-thymidine-5′ triphosphate(d-pppTpA) as a convenient prototype of the general class, since it represents the ideal substrate in an enzymatic synthesis under the priming and template direction of dAT,[2] a copolymer of deoxyadenylate and thymidylate in an alternating sequence. The well-known base-matching requirements of such reactions, the technical advantages of solubility in the reaction media, and availability of precursors make this system especially favorable. The starting material for the triphosphate in question, deoxyadenylyl-5′ → 3′)-thymidylic-5′ acid (IV, Chart I), has already been prepared chemically,[9] and it only remained to be seen whether the pyrophosphorylation methods applicable to mononucleotides would also be useful in this instance.

The conversion of d-pTpA (IV, Chart I) into the desired 5′-terminal triphosphate (VI) would conveniently proceed by way of an activated intermediate, and the phosphomorpholidate (V) was selected. For synthesis of the precursor dinucleotide, a slight modification of the very recently reported[10] procedure of Schaller, *et al.*, was employed, because of its simplicity as compared to the older method[9] and its adaptability to the synthetic sequence where the 5′-phosphate in thymidylic acid is labeled with ^{32}P. The protected thymidylic acid (II) was condensed with $N^6,O^{3'}$-diacetyldeoxyadenylic-5′ acid (III),[11,12] stripped of its protecting groups by saponification, and the desired dinucleotide IV isolated, in moderate yield, by gradient dilution ion-exchange chromatography on DEAE-cellulose. It was characterized by its paper chromatographic migration characteristics, ultraviolet spectrum, hypochromicity,[13] and by the products of the action of snake venom phosphodiesterase[16] (see Experimental).

The next step involved activation of the 5′-terminal phosphate *via* its morpholidate. This is usually accomplished by the slow dropwise addition of dicyclohexylcarbodiimide in *t*-butyl alcohol to a refluxing solution of the nucleotide in aqueous *t*-butyl alcohol containing the reagent. Thus, there is always an excess of nucleotide present, and phosphomorpholidate synthesis takes place at the expense of the competing formation of the strongly basic 4-morpholine-N,N^1-dicyclohexylcarboxamidine,[14] the presence of which strongly inhibits the desired primary event. This procedure was not practical for our purpose because of the small quantities with which we had to work, especially for the preparation of radioactive species. As indicated in the Experimental section, the amidation was carried out in a sealed tube; we were encouraged

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