cooled, diluted with ether and extracted with dilute hydrochloric acid. The ether solution was washed with water, dried and concentrated. Addition of ethanol gave 19.6 g. of product, m.p. 101–103.5°. This was recrystallized from ethanol and gave 18.7 g. (73%) of yellow product, m.p. 102–104.5°. Chromatography on alumina gave a colorless product, 15.4 g., m.p. 104.4–105.5°, $[\alpha]^{25}D$ −37.1° (*c* 1.24, chloroform, 1 dm.).

Anal. Calcd. for $C_{32}H_{44}O_4$: C, 78.01; H, 9.00. Found: C, 78.13; H, 9.13.

3β-Acetoxy-Δ⁵-norcholenic Acid.—A suspension of 4.93 g. (0.01 mole) of benzyl 3β-acetoxy-Δ⁵-norcholenate in 200 ml. of 95% ethanol was shaken with pre-reduced palladium (from 200 mg. of oxide) under hydrogen. At the end of 13 hr. the uptake of hydrogen ceased at 0.01 mole. The solution was filtered and evaporated to dryness *in vacuo*. The crude product, m.p. 197.5–199°, was recrystallized from acetone–petroleum ether to give 3.7 g., m.p. 197.5–198.5°, $[\alpha]^{25}D$ −42° (*c* 1.14, chloroform, 1 dm.).

Anal. Calcd. for $C_{25}H_{38}O_4$: C, 74.59; H, 9.52: neut. equiv., 402.6. Found: C, 74.44; H, 9.37; neut. equiv., 405, 409.

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Studies on the Chemistry of Heterocyclics. XXVII.[1] α,β-Acetylenic Acids and their Esters in the Thiophene Series

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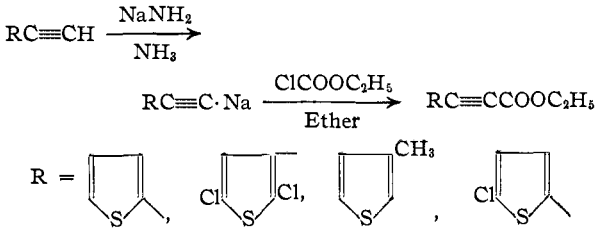
Continuing our studies on acetylenic derivatives in the thiophene series[3,4] we report in this paper the preparation of α,β-acetylenic acids and their esters. The intermediates (thienylacetylenes) were prepared according to the earlier procedure developed in this Laboratory.[5] We were able to improve the yields reported earlier. The yield for 2-thienylacetylene could be increased up to 80% while that for 2,5-dichloro-3-thienylacetylene amounted to 91%. The yield of 5-chloro-2-thienylacetylene was, however, low (28%).

The direct carbonation of the sodium salts of thienylacetylenes did not lead to any of the desired α,β-acetylenic acids. We observed that neither pressure nor different solvents had any effect on the products of the reaction. In all cases small amounts of 2-thenoic acids were isolated only. This phenomenon was observed earlier while studying γ-hydroxy-α,β-acetylenic acids. Perhaps the acetylenic acids once formed were hydrated to the keto acids. The latter on cleavage furnished the corresponding thenoic acids.

An alternate procedure *via* the α,β-acetylenic esters furnished the desired α,β-acetylenic acids in this series in satisfactory yields. The sodium salts of the thienylacetylenes were prepared by applying sodium amide in liquid ammonia as reported earlier.[4] The latter were treated with ethyl chlorocarbonate giving the corresponding esters as



The previously applied method for the preparation of γ-hydroxy-α,β-acetylenic esters in this series was modified by adding a large excess of ethyl or methyl chlorocarbonate to the reaction mixture and refluxing it for several hours. Complete separation of the acetylenic esters from the accompanying acetylenes by distillation was difficult, but separation could be effected by precipitation of the acetylene as the copper salt followed by distillation of the ester *in vacuo*.

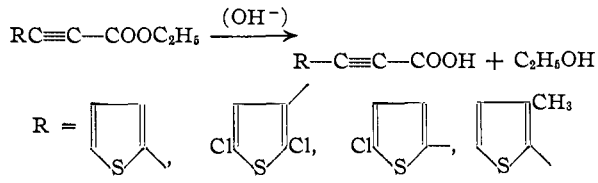
The properties and yields of the α,β-acetylenic esters are recorded in Table I.

TABLE I

PROPERTIES AND YIELDS OF THE THIENYLPROPIOLIC ESTERS

Ethyl propiolate	B.p. (i mm.), °C.	Yield, %	Analyses, % Calcd. C	Calcd. H	Calcd. Cl	Found C	Found H	Found Cl
2-Thienyl-	95–98	91	60	4.45	...	59.8	4.2	..
5-Chloro-2-thienyl	56–58	45	50.35	3.26	16.5	50.5	3.5	17
2,5-Dichloro-3-thienyl	92–95	80	43.37	2.41	28.59	43.5	2.3	28.3
3-Methyl-2-thienyl	65–67	70	63.5	5.29	...	63.8	5.4	..

While applying our earlier procedure for the isolation of the free γ-hydroxy-α,β-acetylenic acids[4] the free 2- and 3-thienyl-α,β-acetylenic acids were obtained in satisfactory yields according to the reaction



The properties and yield of the α,β-acetylenic acids are recorded in Table II.

TABLE II

PROPERTIES AND YIELDS OF THE THIENYLPROPIOLIC ACIDS

Propiolic acid	M.p., °C.	Yield, %	Analyses, % Calcd. C	Calcd. H	Calcd. Cl	Found C	Found H	Found Cl
2-Thienyl-	130–133	85	55.26	2.63	...	55.0	2.8	..
2,5-Dichloro-3-thienyl	139–141	79	38.0	0.905	32.12	37.9	1.2	32.0
5-Chloro-2-thienyl	118–120	45	45.04	1.6	19.02	44.8	2.0	18.9
3-Methyl-2-thienyl	115–118	60	57.8	3.61	...	56.0	3.8	..

Polymerization took place to a greater or lesser extent in all the reactions carried out in this series. In the case of the 5-chloro compound the polymerization was increased so far that only small yields of the desired products could be obtained. The polymerization of the acetylenic compounds in this series probably is due to the mobility of the electrons of the hetero atom.

(1) For a comprehensive review of some recent developments in the field of thiophene see F. F. Nord, A. Vaitiekunas and L. J. Owen, *Fortschritte d. chem. Forschung*, **3**, 309 (1955).

(2) Condensed from a portion of the thesis of A. J. O. submitted to the Graduate School of Fordham University in fulfillment of the requirements of the M.Sc. degree.

(3) A. Vaitiekunas and F. F. Nord, THIS JOURNAL, **76**, 2733 (1954).

(4) A. Vaitiekunas and F. F. Nord, *ibid.*, **76**, 2737 (1954).

(5) A. Vaitiekunas and F. F. Nord, *J. Org. Chem.*, **19**, 902 (1954).

Experimental

Materials.—The thiophene used in this work was obtained through the courtesy of the Monsanto Chemical Co., Inc., St. Louis, Mo. The 2-chlorothiophene and 2,5-dichlorothiophene were obtained through the courtesy of the Jefferson Chemical Co., Inc., New York, N.Y. Ethyl chlorocarbonate, 3-methylthiophene and anhydrous ammonia were commercial products.

General Procedure for the Preparation of Ethyl Esters of Thienyl-α,β-acetylenic Acids.—To freshly prepared sodium amide (0.05 mole) in 350 ml. of liquid ammonia in a three-necked flask there was added 0.05 mole of the thienylacetylene or its derivatives dissolved in a threefold volume of absolute ether. After stirring for an additional one-half hour, the liquid ammonia was allowed to evaporate on a steam-bath, while replacing it with absolute ether. The contents of the flask were cooled to 0–5° and 0.3 of a mole of ethyl chlorocarbonate dissolved in 35 ml. of absolute ether was added slowly. The reaction mixture turned from red-brown to pale yellow. It was allowed to reach room temperature and was stirred for an additional four hours. The sodium chloride was filtered off, the filtrate washed with 5% sodium carbonate solution, finally twice with ice-water, dried over anhydrous sodium sulfate and rectified.

General Procedure for the Hydrolysis of Ethyl Ester of Thienyl-α,β-acetylenic Acids.—One gram of ethyl ester of thienyl-α,β-acetylenic acid dissolved in 15 ml. of benzene was added to a solution of 25 ml. of 1.5 *N* sodium hydroxide and the reaction mixture shaken at room temperature for 48 hours. The isolation of the acid was carried out in the usual manner. The yields and elemental analyses of α,β-acetylenic acids are recorded in Table II.

Attempts to Prepare the Thienyl-α,β-acetylenic Acids *via* the Direct Carbonation of the Sodium Salts of Thienylacetylenes.—Sodium acetylide was formed in the usual manner using 0.2 g. of iron nitrate plus 0.42 g. of sodium and 200 ml. of liquid ammonia. Ten grams of pure 2-thienylacetylene was added dropwise over a period of one hour. The solution was then stirred ten minutes longer. The three-necked flask was then placed on a steam-bath and the ammonia driven off, using a stirrer and reflux condenser. Then 40 ml. of dry benzene were added from a dropping funnel to be used as solvent in the carbonation reaction. This solution containing the solvent and the sodium acetylide salt was placed in a bomb and carbonated with 200 g. of Dry Ice for 24 hours. The reaction product was acidified with 5 *N* sulfuric acid at 0° and twice extracted with ethyl ether. The combined organic layers were extracted again with 10% sodium carbonate and the aqueous layer cooled to 0° and acidified with 3 *N* sulfuric acid giving the free acid. The product, if recrystallized from carbon tetrachloride, was identified as 2-thenoic acid only.

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Evidence from Titration Curves on the "Acyl Shift" in Proteins

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A recent paper by Lumry and Eyring[1] has drawn attention to the "acyl shift" which may occur at peptide bonds adjacent to serine or threonine residues in proteins. This intramolecular rearrangement appears to explain satisfactorily the lability, during acid hydrolysis, of peptide bonds adjacent to serine or threonine residues. Lumry and Eyring make the further suggestion that the acyl shift may be important under conditions much milder than those of acid hydrolysis and that, therefore, "acid titration experiments will almost universally need re-evaluation in light of this finding."

The purpose of this note is to show that such re-evaluation of titration data is not necessary. In fact, titration data show that the acyl shift does not occur, at least in proteins so far carefully examined, in the relatively mild acidities (down to *p*H 2) reached in titration studies.

The connection between the acyl shift and titration curves lies in the fact that an amino group is released during the rearrangement.[1] Each serine or threonine residue capable of undergoing the acyl shift would therefore be expected to contribute a cationic group to the protein molecule. The total number of cationic groups would then be considerably greater than the number calculated from amino acid analysis on the basis that only arginine, lysine, histidine and terminal α-amino residues contribute to the number of cationic groups.

The total number of cationic groups of a protein molecule, present at the acid end-point of a titration curve, is equivalent to the number of hydrogen ions bound in going from the isoionic point to the acid end-point.[2] This number can therefore be accurately determined for those proteins for which the isoionic point can be established. For proteins soluble in water at the isoionic point, this point may be established by direct measurement on deionized solutions. In some other proteins, where salt binding is not important, it may be established as the point of intersection of titration curves at different ionic strengths.

The pertinent data are available for five different proteins and the total numbers of cationic groups in these proteins, as determined from titration, are listed in Table I. The figures are compared with analytical data. It is seen that in every case the agreement between analytical and titration data is within experimental error.[3] The titration curves do not show any excess cationic groups due to acyl shift. If all serine and threonine residues were capable of undergoing an acyl shift alkaline to *p*H 2, the number of excess cationic groups would have been 26 for ribonuclease, 16 for lysozyme, 32 for β-lactoglobulin, 50 for ovalbumin, and 46 for serum albumin.

To the five proteins of Table I should be added insulin.[7] In this protein the isoionic point has

(1) R. Lumry and H. Eyring, *J. Phys. Chem.*, **58**, 110 (1954).

(2) E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids and Peptides," Reinhold Publishing Corp., New York, N. Y., 1943, p. 446.

(3) For the sake of uniformity, all analytical data have been taken from the same compilation (Tristram, ref. 4). To indicate the probable error involved one may examine the data for lysozyme. Here Tristram's compilation gives the analysis of Lewis, *et al.*, (ref. 5). Another analysis by Fromageot and de Garilhe (ref. 6) would lead to a total of 19.8 rather than 17.8 cationic groups. The average of these would correspond exactly to the number found by titration.

(4) G. R. Tristram in H. Neurath and K. Bailey, ed., "The Proteins," Vol. 1, Part A, Academic Press, Inc., New York, N. Y., 1953, p. 181.

(5) J. C. Lewis, N. S. Snell, D. J. Hirschmann and H. Fraenkel-Conrat, *J. Biol. Chem.*, **186**, 23 (1950).

(6) C. Fromageot and M. P. de Garilhe, *Biochim. Biophys. Acta*, **4**, 509 (1950).

(7) C. Tanford and J. Epstein, THIS JOURNAL, **76**, 2163 (1954).