[CONTRIBUTION FROM THE BUREAU OF ENTOMOLOGY AND PLANT QUARANTINE, AGRICULTURAL RESEARCH ADMINISTRATION, UNITED STATES DEPARTMENT OF AGRICULTURE]

# Alkaloids from *Tripterygium wilfordii* Hook: Wilforgine and Wilfortrine[1]

BY MORTON BEROZA

After wilforine and wilfordine had been isolated from the methanol-insoluble fraction there remained two main alkaloidal fractions. From these fractions two new alkaloids, designated "wilforgine" and "wilfortrine," were isolated by partition chromatography employing ultraviolet absorbancy ratios. The purity of the alkaloids was established by countercurrent distribution. The compounds are insecticidally active ester alkaloids, which upon saponification yield 1 mole of 3-furoic acid, 5 moles of acetic acid and 2 moles of steam non-volatile acid per mole of compound. Ultraviolet and infrared absorption spectra of the alkaloids are given.

The isolation of wilfordine, an insecticidal alkaloid from the roots of *Tripterygium wilfordii* Hook, has been reported by Acree and Haller.[2] It was subsequently shown by countercurrent distribution that their wilfordine was a mixture of alkaloids.[3] Two new alkaloids, designated "wilforine" and "wilfordine," were isolated from the mixture by partition chromatography employing ultraviolet absorbancy ratios. The countercurrent distribution pattern of these alkaloids[4] indicated that the compounds were pure. Upon saponification wilforine and wilfordine yielded 1 mole of benzoic acid, 5 moles of acetic acid and 2 moles of steam non-volatile acid per mole of compound.

In the isolation of larger quantities of wilforine and wilfordine, which were needed for structure studies on these compounds, there remained two main fractions. From one of the fractions the new alkaloid wilforgine was isolated. Wilforgine follows wilforine off the partition column, but separates incompletely from it under the conditions used in this investigation. Since the absorbancy ratio of wilforgine at 270 and 255 mμ is 0.96 as compared with 1.38 for wilforine, it was possible to separate the two alkaloids in pure form by following the absorbancy ratios of the eluted fractions. Figures 4, 5 and 6 of reference 3 demonstrate how the alkaloids may be separated. In these figures C-1 is wilforine and the impurity is wilforgine.

In a like manner the new alkaloid wilfortrine was isolated from the other main fraction. Wilfortrine follows wilfordine off the partition column and separates incompletely from it under the conditions used in this investigation. The absorbancy ratio of wilfortrine at 270 and 255 mμ is 0.96 as compared with about 1.41 for wilfordine.

To test the purity of wilforgine, it was subjected to countercurrent distribution between benzene–hexane and hydrochloric acid. The distribution pattern, given in Fig. 1, shows excellent agreement with the theoretical.[5] Likewise, the purity of wilfortrine was established by countercurrent distribution of this alkaloid between benzene and hydrochloric acid. The results, which are plotted in Fig. 2, are in excellent agreement with the theoretical curve.[5]

The formulas of wilforgine and wilfortrine have been calculated from molecular weight, carbon, hydrogen and nitrogen determinations to be $C_{41}H_{47}O_{19}N$ and $C_{41}H_{47}O_{20}N$, respectively. The compounds melted at 211 and 238°, respectively, which is considerably higher than for wilforine and wilfordine. The alkaloids did not contain any methoxy, methylenedioxy, or alkimide groups, but the infrared spectra gave evidence that they contained hydroxyl groups. Active hydrogen determinations, to be reported in a later communication, have confirmed the presence of these hydroxyl groups in both compounds, one more being present in wilfortrine than in wilforgine.

Wilforgine and wilfortrine are ester alkaloids and appear to be very similar to wilforine and wilfordine. Upon saponification they yielded 6 equivalents of steam volatile acids and 2 equivalents of steam non-volatile acid per mole of alkaloid. By chromatographic analysis the steam volatile acids were found to be composed of 5 equivalents of acetic acid and 1 equivalent of another acid. Although it had the same threshold volume as benzoic acid in the chromatographic separation, the unknown acid was definitely not benzoic acid. This fact was recognized when the attempt was made to crystallize the new acid from water. The acid was so much more soluble in water than benzoic acid, that only a few small crystals separated from a saponified 30-mg. lot of alkaloid. The crystals melted unsharply or sublimed about 112°. Identification of this acid was complicated by the fact that only a few hundred mg. of the alkaloids were available and only about one-eighth of this quantity was the desired acid. It was therefore desirable to identify the acid before isolation, if possible, so as to avoid wasting any of the compound.

Since wilforgine and wilfortrine are so similar to wilforine and wilfordine, respectively, the assumption was made that the only difference between the two sets of alkaloids was that the former contained the unknown acid and the latter benzoic acid. The empirical formulas of the former contained one oxygen more and two carbons and two hydrogens less than the latter; therefore, the unknown acid should have the same elemental difference from benzoic acid, or its formula should be $C_5H_4O_3$. The ultraviolet absorption spectrum of the unknown acid contained no evidence of a benzenoid structure but instead had a peak at 237 mμ. This result indicated the presence of a conjugated double-bond structure. The data fitted 2-furoic acid except that the ultraviolet spectrum of this compounds was different. Its peak was about 245 mμ. Another possibility remained, and that was

(1) Report of a study made under the Research and Marketing Act of 1946.

(2) F. Acree and H. L. Haller, THIS JOURNAL, **72**, 1608 (1950).

(3) M. Beroza, *Anal. Chem.*, **22**, 1507 (1950).

(4) M. Beroza, THIS JOURNAL, **73**, 3656 (1951).

(5) B. Williamson and L. C. Craig, *J. Biol. Chem.*, **168**, 687 (1947).

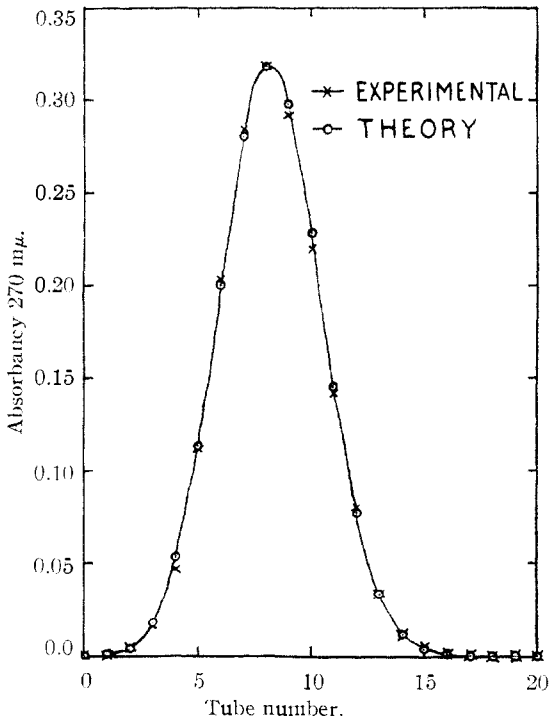


Fig. 1.—Countercurrent distribution of wilforgine, 55% benzene in hexane (v./v.)–2% HCl.

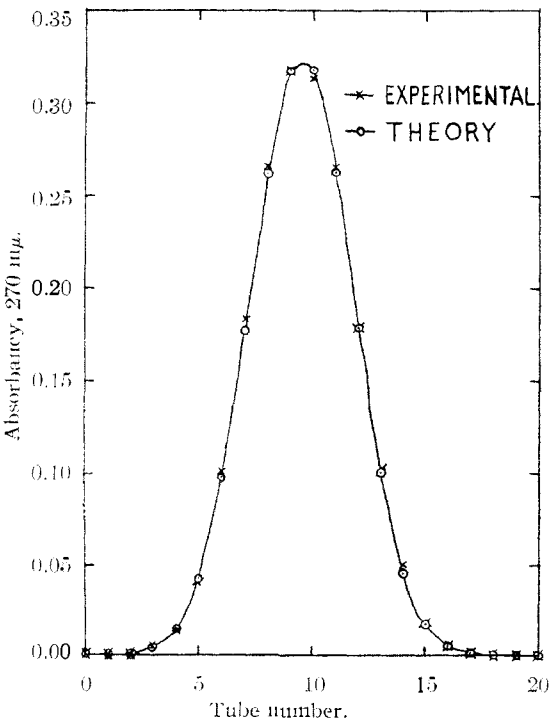


Fig. 2.—Countercurrent distribution of wilfortrine, benzene-1.8% HCl.

the rarely found 3-furoic acid.[6] The author is grateful to Dr. Henry Gilman for kindly supplying him with several mg. of this acid. The ultraviolet absorption spectra of 3-furoic acid and its sodium salt were identical with that of the unknown acid and its sodium salt, respectively. In subsequent experiments some of the pure 3-furoic acid was isolated from both wilforgine and wilfortrine. The melting points were the same as that of the authentic sample and were not depressed in admixture with it.

The two new alkaloids have been tested for toxicity to young European corn borer larvae and found to be toxic.[7]

## Experimental[8]

**Absorption Spectra.**—The ultraviolet spectrophotometric determinations were carried out with a Beckman quartz spectrophotometer, Model DU, Serial No. 757.

The infrared spectra were kindly run by S. P. Sadtler and Son, Inc., Philadelphia 3, Penna., on a Baird double-beam infrared spectrophotometer.

**Isolation of Wilforgine.**—The preparation and operation of the partition chromatographic columns have been described.[4]

After a number of batches of wilforine has been separated and purified by chromatography, there remained the fractions that followed wilforine and preceded wilfordine off the column. These fractions, which contained the wilforgine, were combined and chromatographed with 1.75% hydrochloric acid as the immobile solvent. The eluate was separated into three fractions according to the following absorbancy ratios at 270 and 255 mμ: I, 1.35–1.38; II, 1.00–1.34; and III, 0.96–0.99.

I contained almost pure wilforine, II contained wilforine plus wilforgine, and III contained almost pure wilforgine. III was chromatographed two more times with 1.75% hydrochloric acid as the immobile solvent. In the last chromatographing the material possessed a constant ultraviolet absorbancy ratio of 0.96 and was found to be pure by countercurrent distribution (Fig. 1). It was dissolved in a minimum of acetone and, upon the addition of absolute methanol, crystallized as rectangular plates with two corners cut off at an angle, m.p. 211°, $[\alpha]^{25}D$ + 25 (acetone). The compound was dried under high vacuum at 56° for analysis.

*Anal.* Calcd. for $C_{41}H_{47}O_{19}N$: C, 57.40; H, 5.52; N, 1.6; mol. wt., 858. Found: C, 57.61, 57.24; H, 5.69, 5.79; N, 1.8; mol. wt.,[9] 863.

**Isolation of Wilfortrine.**—Wilfortrine was collected from the fractions that followed wilfordine off the column. These fractions were combined and chromatographed with 0.6% hydrochloric acid as the immobile solvent and separated into three fractions according to the following ultraviolet absorbancy ratios at 270 and 255 mμ: I, 1.37–1.41; II, 1.00-1.36; and III, 0.96–0.99.

I contained almost pure wilfordine, II contained wilfordine plus wilfortrine, and III contained almost pure wilfortrine. III was chromatographed two more times with 0.6% hydrochloric acid as the immobile solvent. In the last chromatographing the material possessed a constant ultraviolet absorbancy ratio of 0.96 and was found to be pure by countercurrent distribution (Fig. 2). It was dissolved in a minimum of acetone and, upon the addition of absolute methanol, crystallized as thin triangular plates, m.p. 237.5–238°, $[\alpha]^{25}D$ +10 (acetone). For analysis the alkaloid was dried at 56° under high vacuum.

*Anal.* Calcd. for $C_{41}H_{47}O_{20}N$: C, 56.35; H, 5.42; N, 1.6; mol. wt., 874. Found: C, 56.17, 56.67; H, 5.58, 5.59; N, 1.7; mol. wt.,[9] 875.

The similarity of the infrared spectra in both carbon tetrachloride and Nujol mulls (Fig. 3) reflects the similarity in structure of the two alkaloids. The peaks at 2.86 microns in the carbon tetrachloride spectra indicate the presence of hydroxyl groups in both compounds. In the Nujol mull spectrum wilfortrine has a peak at 2.83 microns, which is either much smaller or absent in the corresponding spectrum of wilforgine. This peak is undoubtedly due to an

(6) Only two references to the isolation of this compound from natural sources were found: H. Rogerson, *J. Chem. Soc.*, **101**, 1044 (1912), and A. H. Salway and F. B. Power, *Pharm. J.*, **90**, 550 (1913).

(7) These tests were carried out by D. D. Questel of the Bureau of Entomology and Plant Quarantine.

(8) M.p. determinations were made in capillary tubes. All m.ps. are corrected. Determinations of carbon, hydrogen and nitrogen were made by the Oakwold Laboratory, Alexandria, Va.

(9) Method of E. P. Clarke, *Ind. Eng. Chem., Anal. Ed.*, **13**, 820 (1941).

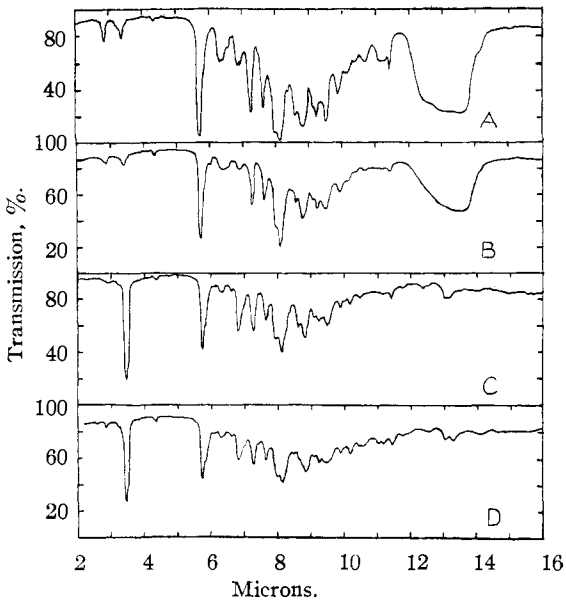


Fig. 3.—Infrared absorption curves of wilforgine and wilfortrine: A, wilforgine, 10% solution in $CCl_4$; B, wilfortrine, saturated solution in $CCl_4$; C, wilforgine as Nujol mull; D, wilfortrine as Nujol mull.

extra hydroxyl group in the wilfortrine, which contains one more active hydrogen than wilforgine. This hydroxyl group may be the only difference between the two alkaloids.

A comparison of the infrared spectra of wilforine and wilfordine with those of wilforgine and wilfortrine[10] shows a great deal of similarity and indicates that the four alkaloids are very similar in chemical structure.

Wilforgine and wilfortrine also have similar ultraviolet absorption spectra in absolute ethanol and in 1% hydrochloric acid (Fig. 4), although these spectra are quite different from the corresponding spectra of wilforine and wilfordine.[11] The difference in spectra of the alkaloids reflects the spectral differences between 3-furoic acid and benzoic acid.

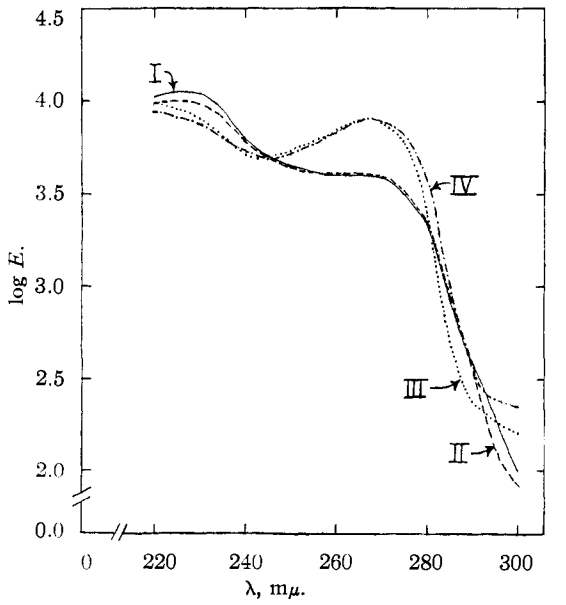


Fig. 4.—Ultraviolet absorption curves: I, wilforgine in absolute ethanol; II, wilfortrine in absolute ethanol; III, wilforgine in 1% HCl; IV, wilfortrine in 1% HCl.

(10) Figure 5 of reference 4.

(11) Figure 4 of reference 4.

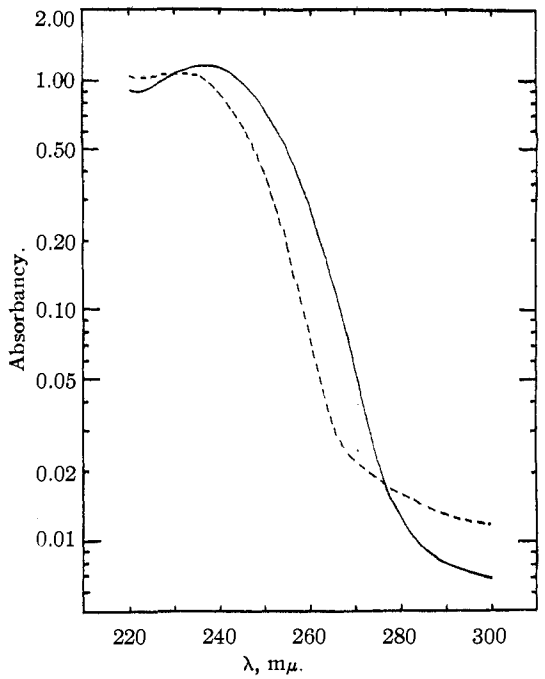


Fig. 5.—Ultraviolet absorption curves in water (0.000446 *M*); solid line, 3-furoic acid; broken line, sodium 3-furoate.

Both wilforgine and wilfortrine are soluble in chloroform, acetone, benzene, carbon tetrachloride and hydrochloric acid; less soluble in ether, carbon disulfide, methyl and ethyl alcohol; and practically insoluble in petroleum ether and water.

**Countercurrent Distribution.**—Distributions were carried out manually as described in reference 4, with a special transfer syringe.[12] Solvents were selected to give a partition coefficient close to 1. A solution containing 55% benzene in hexane (v./v.) and 2% hydrochloric acid were used to distribute wilforgine (Fig. 1). The previous experience in distributing wilforine between benzene and 10% acid had resulted in some decomposition of that alkaloid. Consequently the benzene–hexane solution was used to avoid possible decomposition due to too high a concentration of hydrochloric acid as one of the solvents.

Wilfortrine was distributed between benzene and 1.8% hydrochloric acid (Fig. 2).

**Saponification of Wilforgine.**—30.57 mg. of wilforgine was saponified with diethylene glycol–potassium hydroxide reagent.[13] The saponification equivalent was determined to be 108.8 (7.88 equivalents of acid per mole of alkaloid). The titrated solution was made acid to congo red with sulfuric acid and steam distilled exhaustively. The distillate required 1.70 ml. of 0.1242 *N* sodium hydroxide (5.93 equivalents of steam volatile acid per mole). By difference there were 1.95 equivalents of steam non-volatile acid per mole.

**Identification of Volatile Acids in Wilforgine.**—The titrated volatile acids were analyzed according to the method of Marvel and Rands[14] on a 10-g. column, 1.8 cm. in diameter. The acids were extracted into chloroform solution for placement on the column according to the method of Ramsey and Patterson.[15] Ten-ml. fractions were collected and titrated with 0.0512 *N* sodium hydroxide. The Dreft solution was not used in the titrations to avoid contamination. The first zone (10–30 ml.) was eluted with chloroform and required 0.675 ml. of alkali (0.97 equivalent of acid per mole of alkaloid). After 50 ml. of chloroform had passed through the column, the mobile solvent was changed to 5% butanol in chloroform. The second zone (80–110 ml.) required 3.34 ml. of alkali (4.80 equivalents of acid per mole of alkaloid). No other acid was eluted from the column.

(12) M. Beroza, *Anal. Chem.*, **23**, 1055 (1951).

(13) F. Schneider, "Qualitative Organic Microanalysis," John Wiley and Sons, Inc., New York, N. Y., 1946, p. 161.

(14) C. S. Marvel and R. D. Rands, THIS JOURNAL, **72**, 2642 (1950).

(15) L. L. Ramsey and W. I. Patterson, *J. Assoc. Offic. Agr. Chemists*, **28**, 644 (1945).

Acetic acid, run on a column under the identical conditions, had the same threshold volume as the acid in the second zone. This acid was positively identified as acetic by Duclaux numbers; known acetic acid—6.45, 7.1, 7.2; acetic acid from wilforgine—6.5, 7.1, 7.3.

The water layer of the first zone was evaporated to a small volume and then acidified with a drop of concentrated hydrochloric acid, but there separated only a few small crystals which either melted or sublimed at 112° (hot stage). The ultraviolet spectrum of the remaining mother liquor was identical with that of 3-furoic acid (Fig. 5).

In a subsequent experiment 0.2984 g. of wilforgine was saponified. Its saponification equivalent was 107.6 (7.97 equivalents of acid per mole). After repetition of the procedure described above 10.8 mg. of pure 3-furoic acid was isolated from the first zone by repeated recrystallizations from water. An additional 13.6 mg. of less pure material was recovered from the mother liquors. The isolated acid and the authentic sample melted at 120–120.5° (in sealed tube to prevent sublimation), and the mixed melting point showed no depression. The ultraviolet spectrum in water was identical with that of the authentic sample (Fig. 5). The ultraviolet spectra of the sodium salts, formed by the addition of the theoretical quantity of alkali, were also identical (Fig. 5). After drying for several days over phosphorus pentoxide, the acid was analyzed.

*Anal.* Calcd. for $C_5H_4O_3$: C, 53.57; H, 3.60; neut. equiv., 112. Found: C, 53.40; H, 3.97; neut. equiv., 109.

**Saponification of Wilfortrine.**—32.50 mg. of wilfortrine was saponified as above. The saponification equivalent was determined to be 108.9 (8.02 equivalents of acid per mole). After acidification to congo red with sulfuric acid, the solution was steam distilled exhaustively. The distillate required 1.785 ml. of 0.1242 *N* sodium hydroxide (5.96 equivalents of steam volatile acid per mole). By difference there were 2.06 equivalents of steam non-volatile acid per mole.

**Identification of Volatile Acids in Wilfortrine.**—The titrated volatile acids were separated and analyzed as described above for wilforgine. The first zone required 0.735 ml. of 0.0512 *N* alkali (1.01 equivalents of acid per mole). The second zone required 3.505 ml. of 0.0512 *N* alkali (4.82 equivalents of acid per mole). The acid in the second zone was identified as acetic in the same manner as described above; Duclaux numbers—6.6, 7.1, 7.3.

On another lot of 0.1545 g. of wilfortrine the saponification equivalent was determined to be 108.7 (8.04 equivalents of acid per mole). From this lot 12.2 mg. of crude 3-furoic acid was isolated, which was purified and identified as above.

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# NOTES

## Acetylenic Compounds from Fungi

BY MARJORIE ANCHEL

During the past few years, there have been isolated in this Laboratory, from culture liquids of different species of *Basidiomycetes*, several antibiotic substances which have in common an ultraviolet absorption spectrum of striking and unusual form. The spectra show a series of five to seven sharp maxima in the region of 205 to 360 mμ, giving a characteristic "finger" effect when plotted.

The first compounds of this type isolated and studied spectrophotometrically were obtained from the culture liquids of three different species of *Basidiomycetes: Poria tenuis*, *P. corticola* and an unidentified species.[1,2] These compounds are remarkable not only because of their striking absorption spectra, but also because of their extreme instability.[3] At that time the only naturally occurring compounds described in the literature as having both similar spectra and similar behavior were the polyene fatty acids.[5]

In 1950,[6] a most interesting series of papers from the laboratory of N. A. Sörensen appeared, in which the isolation of polyacetylenic compounds from several genera of *Compositae* was reported, and their ultraviolet absorption spectra presented.[8] The spectra of these compounds resemble closely those of the compounds isolated from *Basidiomycetes*. In correspondence with Dr. Sörensen, we mentioned this similarity and sent him data on the ultraviolet spectra of our compounds. From these spectra, Dr. Sörensen identified our compounds as polyacetylenes. He pointed out to us that according to the rule of Hausser, Kuhn and Seitz[9] concerning the frequency differences of absorption maxima of polyenes and polyacetylenes (about 44 and about 63 f., respectively), our compounds must belong to the latter class. He further made detailed comparisons of the maxima of our compounds with those of acetylenic compounds isolated in his laboratory, as well as some synthesized in the laboratory of E. R. H. Jones,[10] in Manchester. From the near-identity of these, Dr. Sörensen has suggested the presence in our compounds of several specific groupings to which the spectra might be attributed.

In the following tables, the wave lengths of the maxima of the compounds from the *Basidiomycetes* are compared with those of the acetylenic compounds from the laboratories of Dr. Sörensen and Dr. Jones, and the responsible groupings suggested

(1) M. Anchel, J. Polatnick and F. Kavanagh, *Arch. Biochem.*, **25**, 208 (1950).

(2) F. Kavanagh, A. Hervey and W. J. Robbins, *Proc. Nat. Acad. Sci.*, **36**, 1, 102 (1950).

(3) Biformin[4] also, in view of its behavior (instability and silver salt formation) and of the ultraviolet absorption spectrum[1,2] of a crude preparation, probably belongs to this class of compounds.

(4) W. J. Robbins, F. Kavanagh and A. Hervey, *Proc. Nat. Acad. Sci.*, **33**, 176 (1947).

(5) K. S. Markley, "Fatty Acids," Interscience Publishers, Inc., New York, N. Y., Chapter 5.

(6) The first paper in this series was published in 1941.[7] It described the isolation of a highly unsaturated ester to which an acetylenic structure was assigned, tentatively. Due to lack of facilities, spectrophotometric studies were not made.

(7) N. A. Sörensen and J. Steue, *Ann.*, **549**, 80 (1941).

(8) (a) R. T. Holman and N. A. Sörensen, *Acta Chem. Scand.*, **4**, 416 (1950); (b) T. Bruun, C. M. Haug and N. A. Sörensen, *ibid.*, **4**, 850 (1950); (c) N. A. Sörensen and K. Stavholt, *ibid.*, **4**, 1080 (1950); (d) K. Stavholt and N. A. Sörensen, *ibid.*, **4**, 1567 (1950); (e) N. A. Sörensen and K. Stavholt, *ibid.*, **4**, 1575 (1950).

(9) K. W. Hausser, R. Kuhn and G. Seitz, *Z. physik. Chem.*, **29B**, 391 (1935).

(10) E. R. H. Jones, unpublished data.