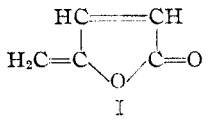
[CONTRIBUTION FROM THE DIVISION OF MEDICINAL CHEMISTRY, SQUIBB INSTITUTE FOR MEDICAL RESEARCH]

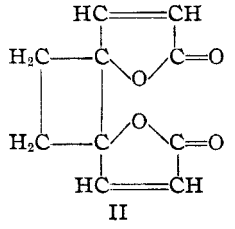
A Synthesis of Protoanemonin. The Tautomerism of Acetylacrylic Acid and of Penicillic Acid

BY ELLIOTT SHAW

The antibiotic agent present in extracts of buttercups and *Anemone pulsatilla*[1] has been shown[2] to be the lactone, protoanemonin (I), previously of interest[3] as the source of a crystalline dimer, anemonin (II), thought to be present in the plants.

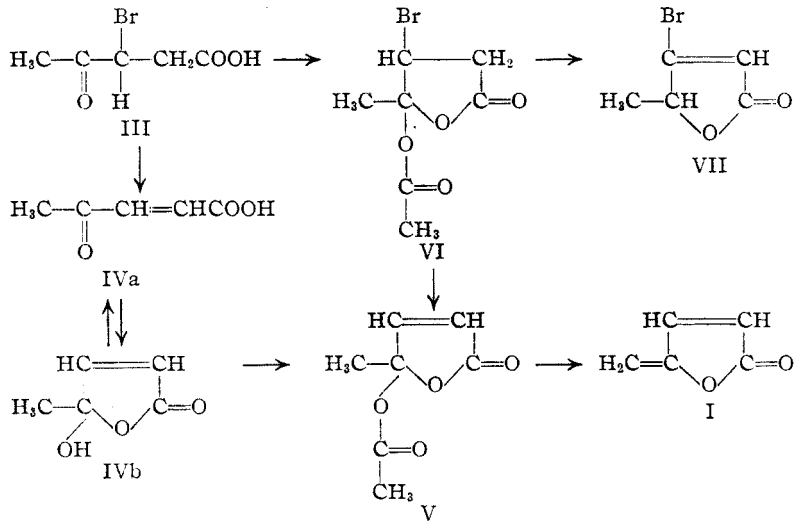


I



II

Protoanemonin has been secured in minute yields synthetically by pyrolysis of either 4-acetoxypentene-2-olide-1,4, (V)[3] or 4,5-dibromohexen-3-oic acid.[4,5] In view of the recently discovered antibacterial activity of protoanemonin, a suitable means of synthesis was sought.



On hydrolysis, protoanemonin undergoes ring opening with the formation[2] of acetylacrylic acid (IV). Since this acid is readily available from levulinic acid by bromination[6] and dehydrobromination,[7] a convenient method of forming a lactone between the carboxyl group and the enol of the keto group would complete a synthesis. This conversion was achieved through the acid-catalyzed action of acetic anhydride, apparently proceeding through the acetoxy lactone (V).

Both protoanemonin and acetylacrylic acid exhibit characteristic absorption maxima in the ultraviolet region of the spectrum as was anticipated. Since protoanemonin polymerizes rapidly on isolation as a liquid, the availability of a spectrophotometric means of assay was of great assistance, making isolation in most cases unnecessary. In aqueous solution, a purified sample of the antibiotic obtained from natural sources showed absorption at 260 mμ with a molecular extinction coefficient of 14,000 (Fig. 1).

In the case of acetylacrylic acid, tautomerism of the ring-chain type is possible between an open (IVa) and cyclic or pseudo-acid form (IVb) for which different characteristic absorption curves would be expected. An aqueous solution of acetylacrylic acid showed only a single band at 220 mμ (Fig. 2) indicating that in water the equilibrium lies completely in the direction of the pseudo-acid structure (IVb); absorption maxima in the region 210–220 mμ are characteristic of α,β-unsaturated lactones.[8] Apparently the ionic form of the acid is also derived from the cyclic structure since the aqueous solutions are strongly acidic and spectra of alkaline solutions show only a slight decrease of absorption at 220 mμ with production of a plateau in the region 270–290 mμ. Finally, the action of one mole of diazomethane on acetylacrylic acid *in ether* leads mainly to a methyl ester, m.p. 60°, which also has an absorption spectrum typical of α,β-unsaturated lactones and is probably, therefore, the methyl ester of the pseudo-acid form.

When refluxed with acetic anhydride, acetylacrylic acid was largely unattacked, a small amount of a neutral substance forming for which the acetoxy lactone structure (V) is consistent with the chemical facts. When, however, acetylacrylic acid was warmed with acetic anhydride and acetic acid containing a small amount of concentrated sulfuric acid, a rapid change took place with the formation of protoanemonin. The conversion of acetylacrylic acid to protoanemonin could be conveniently followed spectro-

(1) Seegal and Holden, *Science*, **101**, 413 (1945).
(2) Baer, Holden and Seegal, *J. Biol. Chem.*, **162**, 65 (1946).
(3) Asahina and Fujita, *Acta phytochim. Japan*, **1**, 1 (1922).
(4) Muskat, Becker and Lowenstein, THIS JOURNAL, **52**, 326 (1930).
(5) Kipping, *J. Chem. Soc.*, 1145 (1935).
(6) Wolff, *Ber.*, **20**, 425 (1887).
(7) Wolff, *Ann.*, **264**, 246 (1891).
(8) Paist, Blout, Uhle and Elderfield, *J. Org. Chem.*, **6**, 280 (1941).

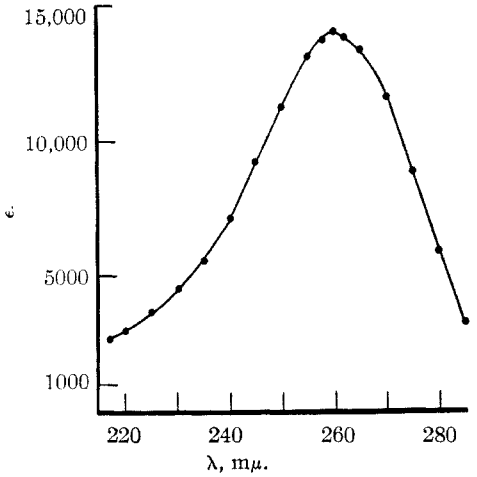


Fig. 1.—Ultraviolet absorption spectrum of protoanemonin (water).

scopically; samples removed periodically from the reaction mixture showed decreasing absorption at 220 mμ and increasing absorption at 260 mμ, reaching a constant maximum (Fig. 3). The neutral material isolated from the reaction mixture was identical with protoanemonin as shown by its dimerization to anemonin (II) and by the correlation of the intensity of the 260 mμ band with antibacterial activity *in vitro*. Solutions of both natural and synthetic protoanemonin, the concentrations of which had been determined spectrophotometrically, gave a minimal inhibiting concentration of about 1:150,000 (6.5 μg./ml.) against *S. aureus* Heatley strain.[9]

Since the action of acetic anhydride alone on acetylacrylic acid leads to the acetoxy lactone (V), it seems likely that the acid-catalyzed conversion of acetylacrylic acid to protoanemonin proceeds through this stage. Consequently, a sample of the acetoxy lactone was warmed with a small amount of sulfuric acid in acetic acid–acetic anhydride solution; protoanemonin was rapidly formed.

Ashina[3] has described a synthesis of the acetoxy lactone (V) by dehydrobromination of β-bromo-γ-methyl-γ-acetoxybutyrolactone (VI). It was not possible to obtain complete, or even appreciable, removal of the bromine by his method. A mixture of lactones was obtained from which the previously unreported 3-bromopentene-2-olide-1,4 (VII), m.p. 51–53°, was isolated. Several samples of the bromo-acetoxy lactone (VI) standing in stoppered flasks at room temperature deposited crystals of anemonin over long periods of time.

In connection with the spectroscopic data obtained on the tautomerism of acetylacrylic acid,

(9) I am indebted to Dr. Geoffrey Rake and Dr. Dorothy Hamre of of the Division of Microbiology of The Squibb Institute for Medical Research for these measurements. The solutions were sterilized by Seitz filtration and assayed in a standard 2-ml. test for penicillin, containing about 1000 staphylococci per ml. The tubes were read after eighteen hours incubation at 37°.

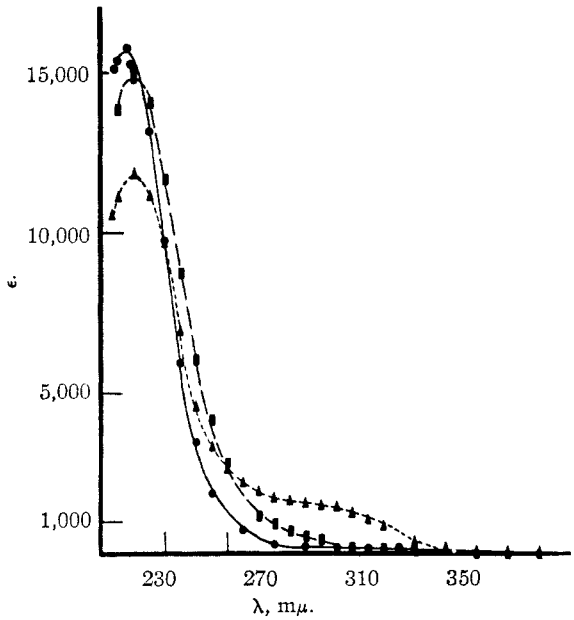


Fig. 2.—Ultraviolet absorption spectra of acetylacrylic acid: -■-, acetylacrylic acid (water); -▲-, acetylacrylic acid (0.1 *N* NaOH); -●-, acetylacrylic acid, methyl ester (ether).

it was of interest to make similar measurements on the antibiotic, penicillic acid, for which chemical studies[10] had shown the existence of a tauto-

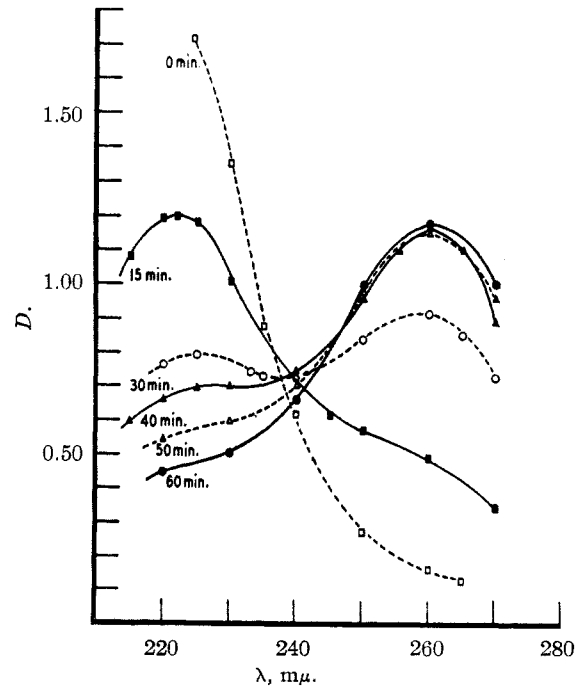
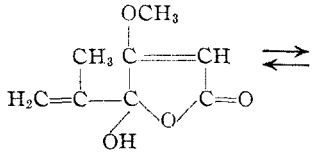


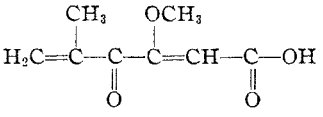
Fig. 3.—Conversion of acetylacrylic acid to protoanemonin as shown by ultraviolet absorption spectra.

(10) Birkinshaw, Oxford and Raistrick, *Biochem. J.*, **30**, 394 (1936).

meric system (VIIIa, b) like that in acetylacrylic acid and probably general for γ-keto acids.



VIIIIa



VIIIIb

An aqueous solution of penicillic acid[11] showed only a single intense maximum at 225 mμ (ε = 12,750) indicating that, like acetylacrylic acid, penicillic acid exists completely in the pseudo-acid form (VIIIa) in aqueous solution (Fig. 4), the slight shift of the maximum toward longer wave lengths being attributable to the β-methoxy substitution. In 0.1 *N* alkali, however, a major shift occurs to a new maximum at 295 mμ; the ionic form must be largely derived from the open structure (VIIIb). It may be of significance with regard to the mechanism of biological action of penicillic acid that at a physiological *p*H it exists in the cyclic form.

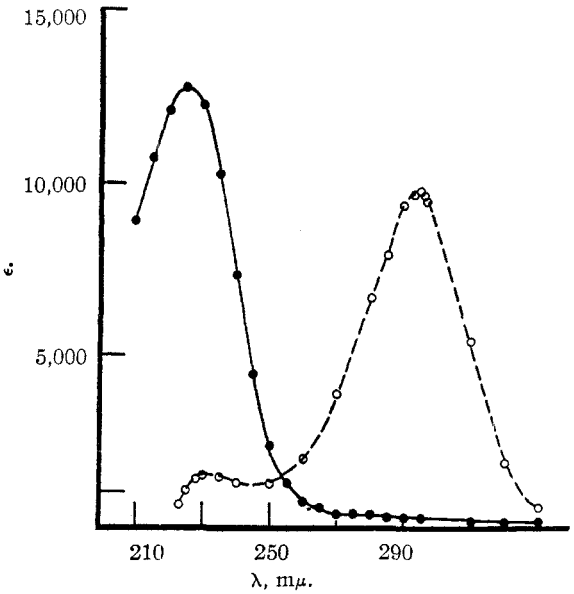


Fig. 4.—Ultraviolet absorption spectra of penicillic acid: -●-, in water; -○-, in *N*/10 sodium hydroxide.

Experimental[12]

Protoanemonin.—Steam distillates[2] from *A. pulsatilla* were extracted with ethylene dichloride. The organic layers, dried over sodium sulfate, were concentrated at 15 mm. from a 50-ml. flask to which the ethylene dichloride solution could be added continuously as the concentration proceeded. A water-bath at 30° was used. The few cc. of amber oil that remained gave a distillate at 1.5 mm., b. p. 45°.

Anal. Calcd. for $C_5H_4O_2$: C, 62.50, H, 4.19. Found: C, 62.51; H, 4.98.

The ultraviolet absorption spectrum of this sample is given in Fig. 1. A fraction of the distillate left at room temperature turned to a hard polymer which, when ground and extracted with boiling ethyl acetate, yielded anemonin, m. p. 149–150° The variations in melting point recorded for this compound[2,3,4,5] are probably due to a rather large effect of differences in the rate of heating.

Anal. Calcd. for $C_{10}H_8O_4$: C, 62.50; H, 4.19. Found: C, 62.30; H, 4.09.

In the ultraviolet region, this sample in ethanol showed high absorption, ε = 14,300, at 220 mμ, the shortest wave length to which the measurement was extended.

Stability of Protoanemonin.—The main source of loss of potency of protoanemonin is polymerization, which can be inhibited by addition of small amounts of hydroquinone. Protoanemonin is comparatively stable to hydrolysis; solutions in the *p*H range 4.5 to 8 retained 80% of their potency after standing three weeks at room temperature.

Acetylacrylic Acid.—Prepared by the method of Wolff,[7] a sample of this acid was purified for spectroscopic measurements by recrystallization alternately from benzene and ethyl acetate to m. p. 122–125°.

Anal. Calcd. for $C_5H_6O_3$: C, 52.63; H, 5.26. Found: C, 52.20; H, 5.71.

A 0.1 *M* solution of acetylacrylic acid, like crotonic acid, gave a *p*H of 2.3.

Methyl Ester of the Cyclic Form of Acetylacrylic Acid.—When ethereal diazomethane was added to an equivalent of acetylacrylic acid in ether, reaction was very rapid. The ether solution was washed with aqueous sodium bicarbonate, dried over anhydrous sodium sulfate, and concentrated to a partially crystalline residue. The crystals were washed with a mixture of ether and hexane (1:1) and dried in a vacuum desiccator, m. p. 59–60°.

Anal. Calcd. for $C_6H_8O_3$: C, 56.25; H, 6.25. Found: C, 56.49; H, 6.63.

The absorption spectrum of the crystalline ester is shown in Fig. 2; the spectrum of the total neutral fraction was also measured and indicated the presence of about 65% of the form isolated in the crystalline state. A secondary peak at 305 mμ, ε = 2400, was observed.

4-Acetoxypenten-2-olide-1,4 (V).—Acetylacrylic acid (20 g.) was refluxed with acetic anhydride (25 g.) for one hour and then concentrated at 2 mm. on a steam-bath. Since unreacted acetylacrylic acid (2 g.) crystallized out at this point, the residue was dissolved in chloroform and shaken with cold sodium carbonate solution. The dried organic layer, when fractionated, yielded 1.8 g., b. p. 102–104° (4 mm.), m. p. 18–21°.

Anal. Calcd. for $C_7H_8O_4$: C, 53.82; H, 5.13. Found: C, 53.69; H, 5.05.

The product is insoluble in water but dissolves in warm alkali. From such a solution on acidification and extraction with ether, an acid was obtained which, after recrystallization, did not depress the m. p. of acetylacrylic acid. Unexpectedly, the ultraviolet absorption spectrum of this lactone showed no maximum; at 216 mμ, ε = 2750 in hexane. When warmed in acetic acid and acetic anhydride, the lactone was rapidly converted to protoanemonin detected spectroscopically by the development of an intense peak at 260 mμ.

Synthesis of Protoanemonin.—The points plotted in Fig. 3 were obtained by heating acetylacrylic acid (5 g.), acetic anhydride (10 ml.), glacial acetic acid (20 ml.) and concentrated sulfuric acid (7 drops) in an Erlenmeyer flask on a steam-bath. At the time intervals indicated, 1-ml. samples were removed and diluted 1:10^4 with distilled water. For the solvent cell of the Beckman spectrophotometer, a similar solution containing all components but acetylacrylic acid was diluted to the same concentration. For preparative purposes, a solution such

(11) Kindly furnished by Dr. Walton Geiger of the N. J. Agr. Exp. Sta., New Brunswick, N. J.

(12) All melting points are uncorrected.

as that described was heated three-quarters of an hour and then poured into 200 ml. of water. Sodium bicarbonate was added to excess. After reaction ceased, the solution was extracted twice with chloroform and the combined, dried extracts were concentrated under reduced pressure to a residual oil which was dissolved without delay in about 70–100 ml. of water and assayed spectrophotometrically. Under the conditions described, 30% was a typical yield. Addition of a few crystals of hydroquinone is advisable. Solutions without antioxidant deposit anemonin in various degrees of purity quite rapidly. The mixed m. p. of a purified sample with anemonin from natural protanemonin was not depressed.

Variations in the amount of sulfuric acid used affected the rate at which the maximum yield of protoanemonin was reached, but not the yield itself. The conversion of acetylacrylic acid to protoanemonin was also achieved in solvents other than acetic acid, such as dioxane (peroxide-free), and with other catalysts, such as *p*-toluene sulfonic acid.

3-Bromopentene-2-olide-1,4 (VII).—The attempted dehydrobromination of the bromoacetoxy lactone (VI) with anhydrous sodium acetate in dry ether[3] gave a neutral fraction which, even after fifteen hours of reflux, contained a very high bromine content. Fractionation gave material b. p. 60–64° (1 mm.) which crystallized in a Dry Ice–alcohol-bath. Rapid filtration left a colorless solid, m. p. 49–53°, which, after recrystallization from ethyl acetate, reached a constant m. p. 51–53°.

Anal. Calcd. for $C_5H_5O_2Br$: C, 33.91; H, 2.83; Br, 45.24. Found: C, 33.25; H, 3.08; Br, 44.73.

The molecular weight in camphor was 365 in comparison with a calculated 177 for the monomeric lactone. Dimerization may have taken place during the heating in camphor. The volatility (b. p. 80° (5 mm.)), molecular extinction of 12,000 at 220 mμ (ether), and ease of formation directly from β-bromolevulinic acid with acetic anhydride are best explained by the simple structure given. The bromine atom is inert to boiling alcoholic silver nitrate. The lactone is probably formed by isomerization of an intermediate Δ^3-lactone.

Acknowledgment.—The author is indebted to Mr. W. A. Lott for his interest and encouragement. The assistance of Dr. Nettie H. Coy of the Biological Laboratories, E. R. Squibb & Sons, in the spectroscopic work is gratefully acknowledged. Microanalyses were performed by Mr. J. F. Alicino.

Summary

Protoanemonin is formed by the action of acetic anhydride on acetylacrylic acid in the presence of an acid catalyst.

Spectroscopic data on the ring-chain tautomerism of acetylacrylic acid and of penicillic acid are given.

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[CONTRIBUTION FROM THE MORLEY CHEMICAL LABORATORY OF WESTERN RESERVE UNIVERSITY]

The Preparation and Identification of Alkylcyclopropanes: 1,1,2-Trimethylcyclopropane and 1,2-Dimethyl-3-ethylcyclopropane

BY JOHN D. BARTLESON, ROBERT E. BURK[1] AND HERMAN P. LANKELMA

The Freund reaction[1a,2] for the preparation of cyclopropane by the action of zinc on 1,3-dibromopropane has been employed for the preparation of several of its homologs. Although the reaction appears to be generally applicable to the preparation of hydrocarbons of this type the yields obtained have in certain cases been unsatisfactory due largely to the formation of olefins as the principal product. Shortridge and Boord[3] have pointed out that the type of dibromide employed determines the yield of cyclopropane hydrocarbon obtained. They state that, in general, primary-primary 1,3-dibromides give high yields, primary-secondary dibromides give good yields. Secondary-secondary dibromides give fair yields but all condensations involving a tertiary bromide give products containing an olefin as the principal or sole product.

The present work deals with the preparation by the Freund reaction of 1,1,2-trimethylcyclopropane from 2-methyl-2,4-dibromopentane and 1,2-dimethyl-3-ethylcyclopropane from 3-methyl-2,4-dibromohexane, secondary-tertiary and secondary-secondary dibromides, respectively. Both hydrocarbons were obtained in excellent yield and in a high degree of purity. Infrared absorption spectra were applied as a guide to purity and to the identification of by-product hydrocarbons formed in the ring closure reaction.

Results

1,1,2-Trimethylcyclopropane has been prepared by the Freund reaction from 2-methyl-2,4-dibromopentane by Zelinsky and Zelikow,[4] by Oestling,[5] by Whitmore and Carney[6] and by Shortridge and Boord.[3] It has also been prepared by Kishner[7] by the pyrolysis of 3,5,5-trimethylpyrazoline and by Whitmore and Carney[6] by the action of sodium on 1-chloro-2,2-dimethylbutane. Zelinsky and Zelikow carried out the ring closure reaction at the temperature of a steam-bath. The product was purified by distillation and by prolonged treatment with aqueous potassium permanganate. Oestling did not describe the reaction condition employed in his work. Although the physical constants of his product checked quite closely with those reported by Zelinsky and Zeli-

(1) Present address: E. I. du Pont de Nemours and Co., Wilmington, Del.

(1a) Freund, *J. prakt. Chem.*, [2] **26**, 367 (1882).

(2) Gustavson, *ibid.*, [2] **36**, 300 (1887).

(3) Shortridge and Boord, "The Preparation of Three 1,1-Dialkylcyclopropanes," Cleveland Meeting of Am. Chem. Soc., April, 1944.

(4) Zelinsky and Zelikow, *Ber.*, **34**, 2656 (1901).

(5) Oestling, *J. Chem. Soc.*, **101**, 457 (1912).

(6) Whitmore and Carney, THIS JOURNAL, **63**, 2633 (1941).

(7) Kishner, *J. Russ. Phys.-Chem. Soc.*, **44**, 165 (1912).