carbonate, and extracted with four 75-ml. portions of ethyl acetate. The extract was dried and evaporated in vacuum to a sirup which crystallized after cooling and standing. The yield was 5.5 g. (35% based on 4-aminomethylthiazole) of ethyl α-acetamino-α-carbethoxy-β-(4-thiazole)-propionate. A sample was recrystallized from an ether–petroleum ether mixture; m. p. 103–104°.

Anal. Calcd. for $C_{13}H_{18}N_2O_5S$: C, 49.67; H, 5.77; N, 8.91. Found: C, 49.84; H, 5.88; N, 8.91.

The above crude product, 5 g., in 25 ml. of concentrated hydrochloric acid was heated on the steam-bath for four hours, and then the solution was evaporated in a vacuum. The residual sirup was taken up in 100 ml. of water, and the solution was freed of chloride ion with silver carbonate in the usual way. Evaporation of the aqueous solution until crystallization began, and then addition of absolute alcohol gave 1.3 g. of finely divided, white crystalline, 4-thiazolealanine (47% yield or 16.8% over-all yield); m. p. 227–230°. It was readily soluble in water, very sparingly soluble in absolute alcohol. A sample recrystallized from a large volume of 95% alcohol melted at 237–238° (dec.) uncor.

Anal. Calcd. for $C_6H_8N_2O_2S$: C, 41.85; H, 4.68; N, 16.27. Found: C, 42.01; H, 5.35; N, 16.23.

2-Thiazolealanine.—This was prepared starting from 2-aminomethylthiazole in the same manner as described for the preparation of the 4-isomer. The intermediate ethyl α-acetamino-α-carbethoxy-β-(2-thiazole)-propionate was not isolated in a crystalline form but was hydrolyzed directly. The over-all yield was 28.5%; m. p. 197–198° (dec.) uncor.

Anal. Calcd. or $C_6H_8N_2O_2S$: C, 41.85; H, 4.68. Found: C, 41.47; H, 4.35.

Acknowledgment.—The authors express their thanks to W. L. Brown, H. L. Hunter and W. J. Schenck for the microanalyses reported here.

Summary

The preparations of the following alkylaminothiazole compounds are described: 2-aminomethylthiazole, 4-aminomethylthiazole, 2-β-aminoethylthiazole, 4-β-aminoethylthiazole, 2-methyl-4-β-aminoethylthiazole, 4-phenyl-2-β-aminoethylthiazole, and bis-2,4-(β-aminoethyl)-thiazole.

The preparations of 2-thiazolealanine and 4-thiazolealanine are described.

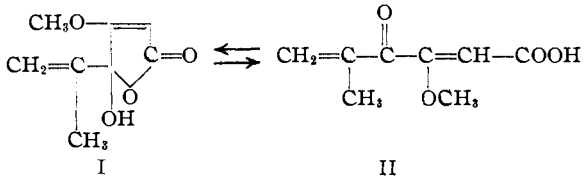
INDIANAPOLIS, INDIANA RECEIVED FEBRUARY 23, 1950

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE UPJOHN COMPANY]

The Structure of Penicillic Acid

BY JARED H. FORD, AGATHA R. JOHNSON AND J. W. HINMAN

In 1936, Birkinshaw, Oxford and Raistrick[1] proposed the following structure for the antibiotic penicillic acid, in which the lactol form (I) was in



I II

equilibrium with the keto acid form (II). Recently, Munday[2] has reported infrared spectra which, according to his interpretation, indicated the presence of both of the tautomeric forms in the solid state. Furthermore, it was stated that the crystals of the keto acid form (II) were converted to the lactol form (I) by mulling with Nujol in accordance with a widely used method[3] for obtaining random orientation of the crystals in the absorption cells. In the present investigation, it has been found that the infrared absorption spectrum of Munday's "keto acid" is identical with that of the monohydrate[1] of penicillic acid (see Fig. 1). Thus it appears that the reported "tautomerism" was probably the conversion of the monohydrate to anhydrous penicillic acid (I).

We have found that penicillic acid and its monohydrate give identical infrared spectra in chloroform solution. These spectra show hydroxyl absorption at 3335 cm.$^{-1}$, γ-lactol carbonyl at 1757 cm.$^{-1}$, carbon–carbon unsaturation at 1645 cm.$^{-1}$, and lack of the absorption which is characteristic of conjugated ester and ketone (near 1720 cm.$^{-1}$ and 1685 cm.$^{-1}$, respectively). Munday[2] failed to report the hydroxyl region of the spectrum which we have found to be critical in distinguishing lactols from the corresponding open chain keto acids.

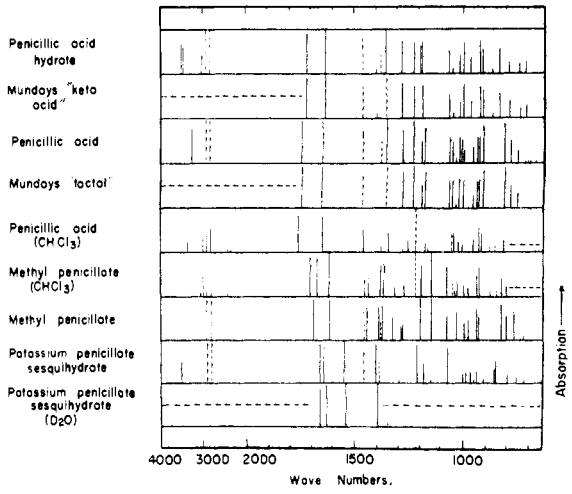


Fig. 1.

The cyclic structure has previously been assigned to penicillic acid and also to its methyl es-

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

(2) Munday, *Nature*, **163**, 443 (1949).

(3) Barnes, Gore, Williams, Linsley and Petersen, *Ind. Eng. Chem., Anal. Ed.*, **19**, 620 (1947).

ter on the basis of ultraviolet absorption spectra.[4,5] These spectra have been redetermined in the present investigation (see Fig. 2) and found to be in good agreement with those reported by Shaw[4] and Raphael.[5] However, we have found that the

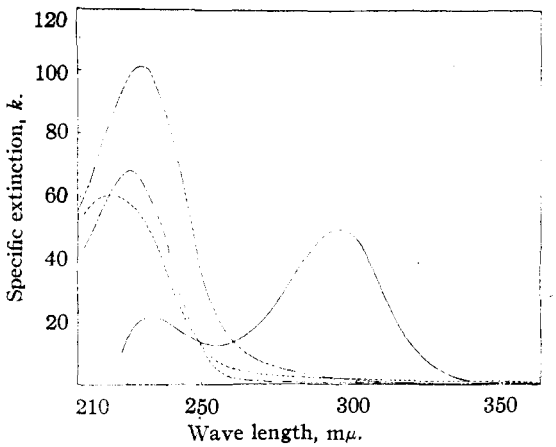
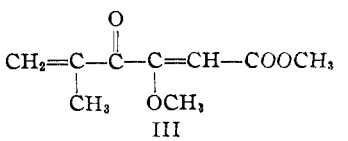


Fig. 2.— ——-Penicillic acid in water; - - - - - - - - - - potassium penicillate sesquihydrate in water; —— penicillic acid in 0.1 *N* sodium hydroxide after standing two hours; — - - — methyl penicillate in 2% ethanol.

infrared spectrum of methyl penicillate differs markedly from that of penicillic acid (see Fig. 1) and it appears to be that of the open chain keto ester (III).



III

Instead of the single carbonyl band near 1760 cm.$^{-1}$ which is characteristic of conjugated γ-lactones and γ-lactols, the spectrum of a chloroform solution of methyl penicillate contains two carbonyl bands at 1718 cm.$^{-1}$ and 1686 cm.$^{-1}$ which are characteristic of conjugated ester and conjugated keto groups, respectively. The frequency range of each of these three types of carbonyl groups has been found to be rather narrow and they do not overlap.[6,7,8] The absorption at 1625 cm.$^{-1}$ arises from a C=C vibration.

As can be seen in Fig. 1, bond association in the crystalline samples affects many of the absorption frequencies. For this reason it is not desirable to base interpretations on the positions of bands in crystalline samples. The lactol carbonyl band of penicillic acid appears at a frequency noticeably below its normal range and the ester and ketone bands of methyl penicillate are not resolved in the crystalline samples.

(4) Shaw, THIS JOURNAL, **68**, 2510 (1946).
(5) Raphael, *J. Chem. Soc.*, 805 (1947).
(6) Hartwell, Richards and Thompson, *ibid.*, 1436 (1948).
(7) Thompson and Torkington, *ibid.*, 640 (1945).
(8) Randall, Fowler, Fuson and Dangl, "Infrared Determination of Organic Structures," D. Van Nostrand Co., New York, N. Y., 1949.

Although the infrared spectra on Nujol mulls and chloroform solutions of penicillic acid indicated a predominance of the lactol form (I), they did not furnish enough evidence to determine whether or not the equilibrium between the two forms that was postulated by Birkinshaw, Oxford and Raistrick could exist in aqueous solution. We have prepared the crystalline potassium salt of penicillic acid as a sesquihydrate, and have determined its infrared spectrum, both in the solid state and in deuterium oxide solution.[9] The spectrum of the deuterium oxide solution was essentially the same as that of the Nujol mull (see Fig. 1) and it indicated that the potassium salt was predominantly in the open chain form. Thus it appears that the equilibrium between the two forms does exist in aqueous solution and that the position of the equilibrium depends upon the *p*H.

In view of the fact that penicillic acid, on the basis of infrared spectra, appears to have the cyclic structure I, while its methyl ester and potassium salt appear to have open chain structures, the pronounced similarity of their ultraviolet spectra (see Fig. 2) was unexpected. In order to eliminate the possibility that a solvent effect was involved, the ultraviolet spectra of penicillic acid and methyl penicillate were determined in chloroform as well as in water, but the change in solvents was found to have very little effect (see Table I). Thus it appears that ultraviolet absorption spectra are of little value in determining whether or not compounds of this type, *e.g.*, acetylacrylic acid,[4,5] exist in the cyclic or open chain form.

TABLE I

ULTRAVIOLET ABSORPTION SPECTRA

Compound	Solvent	$\lambda_{max.}$, mμ	E_m
Penicillic acid	Water	227	11,700
Penicillic acid	Chloroform	226	8,770
Methyl penicillate	2% aqueous ethanol	230	18,600
Methyl penicillate	Chloroform	227	14,800
Potassium penicillate (sesquihydrate)	Water	221	14,200

Differing reports in the literature regarding the ultraviolet spectrum of penicillic acid in alkaline solution[4,5] led us to reinvestigate this problem. We have found that freshly prepared solutions of penicillic acid in 0.1 *N* sodium hydroxide give spectra which differ only slightly from that of a 95% alcohol solution of the free acid. This is an agreement with Raphael's observation. After standing for two hours at room temperature, the solutions are found to give the absorption maximum at 295 mμ which was reported by Shaw (see Fig. 2). It appears likely that the 295 mμ absorption is that of a degradation product.

Acknowledgments.—The authors wish to express thanks to Dr. J. C. Sylvester of Abbott

(9) Deuterium oxide was used instead of water to reduce solvent interference.

Laboratories and Dr. T. E. Eble of these Laboratories for the penicillic acid, to Mr. George C. Prescott for preparation of the methyl penicillate, and to Mr. Lambertus Scholten and Dr. George Pish for determination of the ultraviolet absorption spectra.

Experimental

Penicillic Acid.—Recrystallization from hot water with subsequent drying in moist air gave the monohydrate, m. p. 62–63° (lit.,[1] 64–65°). The monohydrate was found to lose water on standing in a desiccator over calcium chloride at room temperature. The melting point of the anhydrous acid was found to be 85–86° (lit.,[1] 87°).

Methyl Penicillate.—This compound was prepared by the potassium carbonate–methyl iodide method according to Raphael,[6] and was recrystallized from dilute methanol; m. p. 35–36° (lit.,[5] 35°).

Potassium Penicillate.—An aqueous solution of penicillic acid was titrated to *p*H 8 with dilute potassium hydroxide and lyophilized. The resulting solid was dissolved in 90% acetone and four volumes of anhydrous acetone were added. The salt crystallized rapidly in the form of very slender needles; m. p. 195–196°. The analytical sample was dried *in vacuo* over phosphorus pentoxide at 58°.

Anal.[10] Calcd. for $C_8H_9O_4K \cdot 1.5H_2O$: C, 40.84; H, 5.14. Found (average of four determinations): C, 40.87; H, 5.10.

Attempts to prepare the anhydrous salt were abandoned when it was found that drying for 18 hours over phosphorus pentoxide at 100° and 40–50 μ pressure removed only about one-half of the water of crystallization.

Infrared Absorption Spectra.—A Perkin–Elmer (model 12B) recording infrared spectrophotometer with a sodium chloride prism was used. Unless otherwise indicated, the spectra given in Fig. 1 were obtained on Nujol mulls which were prepared by the usual method.[3] An 0.05-mm. sodium chloride cell was used for the chloroform solutions and an 0.05-mm. calcium fluoride cell was used for the deuterium oxide solution of potassium penicillate.

Ultraviolet Absorption Spectra.—A Cary (model 11) recording ultraviolet spectrophotometer was used. A 1-cm. cell was used, except in the case of chloroform solutions, in which an 0.05-mm. cell was used.

(10) Analyses by Clark Microanalytical Laboratory, Urbana, Illinois, and by our Microanalytical Laboratory under the supervision of Mr. William A. Struck.

Summary

1. Infrared absorption spectra indicate that the equilibrium between the cyclic and open chain forms of penicillic acid exists in aqueous solutions but not in the solid state.

2. Methyl penicillate has the open chain structure (III).

3. Ultraviolet absorption spectra are of doubtful value in determining whether compounds of this type exist in the cyclic or open chain form.

KALAMAZOO, MICHIGAN RECEIVED MARCH 31, 1950

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF SYNTEX, S. A.]

Steroids. VI.[1] The Wohl–Ziegler Bromination of Steroidal 1,4-Dien-3-ones. Partial Synthesis of Δ^6-Dehydroestrone and Equilenin

BY ST. KAUFMANN, J. PATAKI, G. ROSENKRANZ, J. ROMO AND CARL DJERASSI

The reaction of N-bromosuccinimide (Wohl–Ziegler bromination) with cross-conjugated doubly-unsaturated ketones does not appear to have been studied[2] until very recently. In 1949, Martens[3] reported the isolation of 6-bromo-$\Delta^{1,4}$-cholestadien-3-one (IIc) from the Wohl–Ziegler bromination of $\Delta^{1,4}$-cholestadien-3-one (Ic), but subsequent dehydrobromination led to an oil, which formed an unstable and analytically impure semicarbazone.[3a] An independent investigation of this reaction with certain androstane derivatives yielded well-crystallized derivatives, which served as starting materials for the first partial synthesis of Δ^6-dehydroestrone (Δ^6-isoequilin) (IV) and equilenin (VI) from non-aromatic steroids.

$\Delta^{1,4}$-Androstadiene-3,17-dione (Ia)[4,5] readily reacted with N-bromosuccinimide in carbon tetrachloride solution in the presence of benzoyl peroxide to afford in 89% yield the corresponding 6-bromo derivative IIa, which was dehydrobrominated on short boiling with γ-collidine in 62% yield to $\Delta^{1,4,6}$-androstatriene-3,17-dione (IIIa); similar reactions with $\Delta^{1,4}$-androstadien-17-ol-3-one 17-acetate[5] led to $\Delta^{1,4,6}$-androstatrien-17-ol-3-one 17-acetate (IIIb). These triply unsaturated steroid ketones proved to be identical with material prepared by another method[6] and exhibited a characteristic ultraviolet absorption spectrum (Fig. 1) with maxima at 222, 256 and 298 mμ.

When the trienone IIIa in mineral oil solution[7] was passed through a glass tube, packed with helices and heated to 600°, 40% of an alkali-soluble phenol, m. p. 261–263°, $[\alpha]^{20}D$ −127° (dioxane), was obtained which exhibited an ultraviolet absorption spectrum (Fig. 1) typical of a phenol with an additional conjugated double bond. The substance possessed approximately one-third the biological activity of estrone (rats) and formed an acetate (m. p. 140.5°) and a benzoate (m. p.

(1) Paper V, Rosenkranz, Pataki, Kaufmann, Berlin and Djerassi, THIS JOURNAL, **72**, 4081 (1950).

(2) Djerassi, *Chem. Revs.*, **43**, 271 (1948).

(3) Martens, *Ann.*, **563**, 131 (1949).

(3a) See however Romo, Djerassi and Rosenkranz, *J. Org. Chem.*, **15**, 896 (1950).

(4) Djerassi and Scholz, *J. Org. Chem.*, **13**, 697 (1948).

(5) Inhoffen, Zuehlsdorff and Huang-Minlon, *Ber.*, **73**, 451 (1940).

(6) Djerassi, Rosenkranz, Romo, Kaufmann and Pataki, THIS JOURNAL, **72**, 4534 (1950).

(7) This superior modification of the conventional tetralin vapor phase aromatization of steroidal, 1,4-dien-3-ones (I) (Inhoffen in Fiat Report No. 996, London, 1947, H. M. Stationery Office; Djerassi and Scholz, THIS JOURNAL, **71**, 3962 (1949)) is due to Hershberg, Rubin and Schwenk, *J. Org. Chem.*, **15**, 292 (1950).