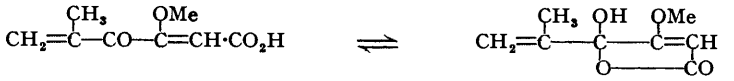
155. *Compounds related to Penicillic Acid. Part II. Synthesis of Dihydropenicillic Acid.*

By R. A. Raphael.

On the basis of ultra-violet light absorption measurements it is concluded that in solution penicillic acid possesses the lactol structure (II). The synthesis of dihydropenicillic acid (VIII) is described.

Penicillic acid, first isolated by Alsberg and Black (*U.S. Dept. Agric. Bureau Plant Ind. Bull.*, Nos. **199, 270**) from *Penicillium puberulum* Bainier, and later by Birkinshaw, Oxford, and Raistrick (*Biochem. J.*, 1936, **30**, 394) from *P. cyclopium* Westling, has been assigned the tautomeric structures (I) and (II) by the latter authors.



(I.) (II.)

The hypothesis of this tautomeric system to explain the reactions of penicillic acid has now been shown to be unnecessary. The ultra-violet absorption spectra of the acid in both acid and alkaline solutions have been found to be practically identical and in complete accord with the lactol structure (II); the spectrum of the *methyl* ester is very similar (Table I). The presence of even a trace of the more highly conjugated keto-compound (I), which would be expected to exhibit maximal absorption at about 2700 A., would be easily detectable. Again, the compound obtained by saturation of the exocyclic double bond, dihydropenicillic acid (VIII), exhibits light absorption very similar to that of penicillic acid itself.*

Table I.

	Solvent.	$\lambda_{max.}$, A.	$\epsilon_{max.}$
Penicillic acid	0·02 M-KOH	2210	12,500
,, ,,	0·02 M-HCl	2280	11,500
Methyl penicillate	Alcohol	2260	20,000
Dihydropenicillic acid	0·02 M-KOH	2170	9,000
,, ,,	0·02 M-HCl	2270	14,000

It is noteworthy that no ketonic derivatives corresponding to structure (I) have been prepared, all the reagents employed giving compounds derived from the breakdown product of penicillic acid, 3 : 4-diketo-2-methylpent-1-ene (III).

(III.) (IV.)

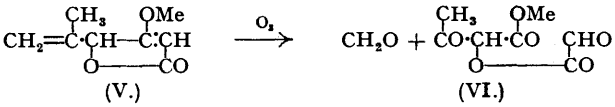
Further, the cyclic structure is compatible with the constitutions assigned to two other antibiotics, protoanemonin (Baer, Holden, and Seegal, *J. Biol. Chem.*, 1946, **162**, 65) and patulin (Gye *et al.*, *Lancet*, 1943, 625).

The starting material for the present synthetic investigation was technical methylallyl alcohol. This was oxidised by means of selenium dioxide to α-methylacraldehyde (Kautter, D.R.-P. 634,501), and the resulting aldehyde condensed with sodium acetylide in liquid ammonia to yield *iso*propenylethynylcarbinol (Heilbron, Jones, McCombie, and Weedon, *J.*, 1945, 87) in 73% yield. Carbonation of this *via* the disodium salt by the method of Zoss and Hennion (*J. Amer. Chem. Soc.*, 1941, **63**, 1151) produced a 68% yield of *3-hydroxy-4-methylpent-4-en-1-yne-1-carboxylic acid* (IV) [this yield is comparable with those recently obtained by Haynes and Jones (*J.*, 1946, 503) by carbonating the Grignard complexes of ethynylcarbinols]. The

* *Note added May 30th*, 1947.—After this paper had been submitted for publication, a communication appeared (Shaw, *J. Amer. Chem. Soc.*, 1946, **68**, 2510) in which the ultra-violet light absorption properties of penicillic acid were described. In aqueous solution the results were substantially in agreement with those reported above, but it was declared that, in 0·1N-sodium hydroxide, a new maximum at 2950 A. appeared; this was attributed to structure (I). In view of this, Dr. Braude has redetermined the light absorption of penicillic acid in 0·1N-sodium hydroxide. No sign of a maximum at 2950 A. was observed, the result being identical with that obtained above. Again, the American author himself reports that the light absorption properties of acetylacrylic acid, a compound structurally very similar to penicillic acid, are the same in both acid and alkaline solution, and are fully compatible with the lactol structure.

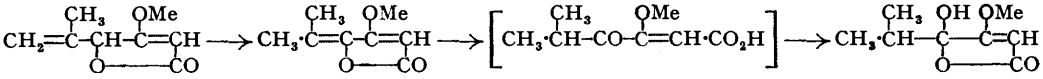
constitution of the acid was confirmed by complete hydrogenation to the lactone of 3-hydroxy-4-methylpentane-1-carboxylic acid, which was identified by its physical properties and by its conversion into the solid 3-bromo-4-methylpentane-1-carboxylic acid. Further characterisation was provided by the preparation of an *amide* and an S-*benzylisothiuronium* salt. The acid was easily convertible into its *methyl* ester by refluxing with methanolic sulphuric acid; both compounds had practically identical absorption spectra, thus showing that no anionotropic rearrangement had taken place during the esterification.

The methyl ester reacted vigorously with methanol in the presence of the boron trifluoride–mercuric oxide–trichloroacetic acid catalyst of Killian, Hennion, and Nieuwland (*inter alia*, *J. Amer. Chem. Soc.*, **1936**, **58**, 80) to give a good yield of a neutral isomeric compound, m. p. 42—43°. Unlike the starting material it contained no active hydrogen and was unaffected by chromium trioxide solution even on warming; ready absorption of bromine in the cold showed that an unsaturated centre was present. These properties suggested that it was the *lactone* of 3-hydroxy-2-methoxy-4-methylpenta-1 : 4-diene-1-carboxylic acid (V), produced by addition of methyl alcohol to the triple bond of the acetylenic ester and subsequent elimination of the elements of methyl alcohol between the carbomethoxyl and the hydroxyl group. In confirmation of this structure ozonolysis of the compound yielded formaldehyde (identified as its dimedone derivative) and the ketone (VI) (characterised as its 2 : 4-*dinitrophenylhydrazone*).



(V.) (VI.)

Treatment of the lactone (V) with cold, dilute sodium hydroxide produced dihydropenicillic acid (VIII), identical with a specimen made by hydrogenation of natural penicillic acid. The production of dihydropenicillic acid is thought to be due to a prototropic change followed by fission of the lactone ring :



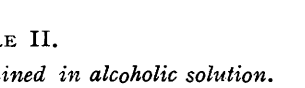
(VII.) (VIII.)

This mechanism is confirmed by the fact that use of a weaker base, ammonia, led to the formation of the *lactone* of 3-hydroxy-2-methoxy-4-methylpenta-1 : 3-diene-1-carboxylic acid (VII), which also yielded dihydropenicillic acid on hydrolysis. Ozonolysis of this lactone furnished a good yield of acetone 2 : 4-dinitrophenylhydrazone.

The correctness of the constitutions assigned to the two lactones is borne out by their ultra-violet absorption spectra (Table II).

TABLE II.

Absorption spectra determined in alcoholic solution.

	$\lambda_{max.}$, A.	$\epsilon_{max.}$		$\lambda_{max.}$, A.	$\epsilon_{max.}$
Acid (IV)	2150	4000	Lactone (V)	2210	13,000
Methyl ester of (IV)	2150	4000	 [2]	2210	15,500
Pr·CH(OH)·C:C·CO_2H [1]	2150	4500	Lactone (VII)	2700	23,000

[1] Haynes and Jones, *loc. cit.* [2] Jones and Whiting, private communication.

Further work along these lines, aiming at the ultimate synthesis of penicillic acid, is being continued.

EXPERIMENTAL.

3-*Hydroxy*-4-*methylpent*-4-*en*-1-*yne*-1-*carboxylic Acid* (IV).—To a solution of sodamide (from sodium, 11·5 g.) in liquid ammonia (1 l.) was added *iso*propenylethynylcarbinol (24 g.) in dry ether (20 c.c.). The ammonia was allowed to evaporate overnight; more dry ether was introduced and dry nitrogen passed through to remove all traces of ammonia. A large excess of solid carbon dioxide was then added. When the reaction mixture had warmed to room temperature, dilute sulphuric acid was run in to decompose the sodium salt, and the ethereal layer was removed and extracted with dilute sodium carbonate solution. The sodium carbonate was acidified and the precipitated acid extracted with ether; the ethereal extract was washed with water and dried ($MgSO_4$). Evaporation of the ether yielded a viscous oil which soon solidified, crystallisation from benzene yielding the required *acid* (23·8 g.; 68%) in nacreous plates, m. p. 80—81° (Found: C, 61·2; H, 5·5; equiv., 142. $C_7H_8O_3$ requires C, 60·0; H, 5·8%; equiv.,

140). The S-*benzylisothiuronium* salt crystallised from a small volume of alcohol in needles, m. p. 156—157° (decomp.) (Found : N, 9·0. $C_{15}H_{18}O_3N_2S$ requires N, 9·1%).

Hydrogenation.—The above acid was fully hydrogenated in ethyl acetate solution using platinic oxide as catalyst. After removal of catalyst and solvent the residual lactone of 3-hydroxy-4-methylpentane-1-carboxylic acid had b. p. 106—108°/20 mm., $n_D^{20°}$ 1·4390 (Linstead and Rydon, *J.*, 1933, 584, give b. p. 98°/15 mm., $n_D^{20°}$ 1·4410). Reaction of the lactone with hydrogen bromide yielded 3-bromo-4-methylpentane-1-carboxylic acid, which crystallised from light petroleum (b. p. 40—60°) in plates, m. p. 40—41° (Boorman, Linstead, and Rydon, *J.*, 1933, 576, give m. p. 41°).

Methyl 3-hydroxy-4-methylpent-4-en-1-yne-1-carboxylate. The acid (9 g.) was refluxed overnight with dry methanol (35 c.c.) and sulphuric acid (*d* 1·84; 0·4 g.). The cooled solution was poured into water and the product isolated by means of ether. Distillation furnished the *ester* as a mobile oil, b. p. 125°/9 mm., $n_D^{17°}$ 1·4820 (Found : C, 61·7, 61·8; H, 6·6, 6·5; OMe, 20·4. $C_8H_{10}O_3$ requires C, 62·2; H, 6·6; OMe, 20·1%).

3-Hydroxy-4-methylpent-4-en-1-yne-1-carboxyamide. The above ester (5 g.) was shaken with aqueous ammonia (*d* 0·88; 20 c.c.) until the mixture became homogeneous. Next day the solution was evaporated to dryness under reduced pressure and the residue extracted with boiling toluene; much glassy material remained undissolved. On cooling the toluene solution, needles of the *amide* (1·8 g.), m. p. 105—106°, separated (Found : C, 60·2; H, 6·4; N, 10·4. $C_7H_9O_2N$ requires C, 60·4; H, 6·5; N, 10·1%)

Lactone of 3-Hydroxy-2-methoxy-4-methylpenta-1 : 4-diene-1-carboxylic Acid (V).—The acetylenic ester (5 g.) was slowly dropped into dry methanol (15 c.c.) containing red mercuric oxide (0·1 g.), boron trifluoride–ether complex (0·1 c.c.), and trichloroacetic acid (10 mg.). After the initial exothermic reaction had subsided, the mixture was kept for 3 hours and then poured into sodium hydrogen carbonate solution, and the precipitated oil was extracted with ether. Drying and evaporation of the ether yielded a pale yellow liquid, b. p. 140°/9 mm., which solidified to a crystalline mass, m. p. 42—43° (3·2 g.; 64%) (Found : C, 62·1, 61·8; H, 6·5, 6·6; OMe, 20·2; *M* [ebull. in acetone], 149. $C_8H_{10}O_3$ requires C, 62·2; H, 6·6; OMe, 20·1%; *M*, 154). The *compound* showed no active hydrogen (Zerewitinoff). It absorbed bromine rapidly in the cold but was unaffected by chromium trioxide solution even on warming.

Ozonolysis. The lactone (V) (1 g.) was dissolved in purified acetic acid (10 c.c.) and ozonised oxygen passed through until absorption ceased (6 hours), the issuing gases being bubbled through water to trap volatile products. The wash water was poured into the acetic acid solution, zinc dust (5 g.) added, and the mixture heated to boiling in a stream of nitrogen, the issuing gases being passed into a saturated aqueous alcoholic solution of dimedone. By next day the solution had deposited needles of the dimedone compound of formaldehyde (680 mg.; 36%), m. p. 190—191° after crystallisation from aqueous alcohol, undepressed on admixture with an authentic specimen,

The original solution was filtered from zinc dust and treated with alcoholic 2 : 4-dinitrophenylhydrazine sulphate. The heavy yellow precipitate (1·3 g.; 54%) was filtered off, dried, and crystallised from nitrobenzene; yellow needles, m. p. 232—233° (decomp.) (Found : N, 15·4; OMe, 8·5. $C_{13}H_{12}O_9N_4$ requires N, 15·2; OMe, 8·4%). The analysis corresponds to that of the 2 : 4-*dinitrophenylhydrazone* of the ketone (VI).

Dihydropenicillic Acid (VIII).—The lactone (V) (1·5 g.) was dissolved in methanol (10 c.c.), N-sodium hydroxide (11 c.c.; 1·1 mols.) added, and the mixture left overnight. The methanol was evaporated under reduced pressure and the solution acidified with dilute sulphuric acid. Isolation by means of ether furnished an acidic solid (800 mg.) which did not decolourise bromine and crystallised in needles, m. p. 85—86°, from light petroleum (b. p. 60—80°) containing a few drops of benzene (Found : C, 55·5; H, 7·0; OMe, 18·25. Calc. for $C_8H_{12}O_4$: C, 55·75; H, 7·0; OMe, 18·0%). Dihydropenicillic acid, prepared from natural penicillic acid by hydrogenation (Birkinshaw, Oxford, and Raistrick, *loc. cit.*), crystallised from the same solvent in needles, m. p. 84—85°. A mixture of the two products showed no depression in m. p.

The action of diazomethane on the synthetic compound, followed by alcoholic hydrazine hydrate, gave, after 8 days, clumps of the orthodiazine, m. p. 210—211° after crystallisation from benzene. This m. p. was undepressed on admixture with the compound prepared in a similar way from the natural acid (Birkinshaw, Oxford, and Raistrick, *loc. cit.*).

Lactone of 3-Hydroxy-2-methoxy-4-methylpenta-1 : 3-diene-1-carboxylic Acid (VII).—The lactone (V) (1 g.) was dissolved in methanol (3 c.c.), and aqueous ammonia (*d* 0·880; 0·5 c.c.) added. Next day the light brown liquid was poured into water; the precipitated oil quickly solidified, and the crystalline mass (800 mg.) was filtered off, washed with water, and dried. The *lactone* crystallised from light petroleum (b. p. 60—80°) in prisms or needles, m. p. 76—78° (Found : C, 62·3; H, 6·4; OMe, 20·2. $C_8H_{10}O_3$ requires C, 62·2; H, 6·6; OMe, 20·1%). The compound showed no active hydrogen (Zerewitinoff).

Hydrolysis with sodium hydroxide as described for the lactone (V) again furnished dihydropenicillic acid.

Ozonolysis. Lactone (VII) was treated as described for (V). The issuing gases from the decomposition of the ozonide were led into excess of alcoholic 2 : 4-dinitrophenylhydrazine sulphate. Next day the orange precipitate (960 mg.; 66%) was filtered off; after crystallisation from alcohol the derivative had m. p. 124—126° undepressed on admixture with an authentic specimen of acetone 2 : 4-dinitrophenylhydrazone.

Methyl Penicillate (prepared by Dr. J. O. Harris).—A mixture of penicillic acid (1 g.), methyl iodide (2 c.c.), anhydrous potassium carbonate (1 g.), and dry acetone (10 c.c.) was refluxed for 4 hours; the cooled solution was filtered and evaporated to dryness, and the residue extracted with ether. Evaporation of the ether furnished the *ester* as a pale yellow oil (800 mg.; 73%), b. p. 100—110° (bath temp.)/0·05 mm., $n_D^{25°}$ 1·4853. It solidified to colourless prisms, m. p. 35° (Found : C, 58·6; H, 6·3; OMe, 31·3. $C_9H_{12}O_4$ requires C, 58·65; H, 6·55; OMe, 33·6%).

The author thanks Dr. E. A. Braude for the absorption data and Mr. S. Bance, B.Sc., A.R.I.C., for the semimicroanalyses. He is also indebted to Dr. A. J. Ewins, F.R.S., Professor H. Raistrick, F.R.S.,

Dr. J. N. Ashley, and Dr. E. R. H. Jones for their interest in this work and to the Directors of Messrs. May & Baker Ltd. for permission to publish the results.

The penicillic acid employed in the above investigations was provided by courtesy of Professor Raistrick.

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[*Received, October 3rd*, 1946.]