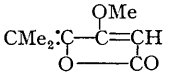
303. *Compounds related to Penicillic Acid. Part III. Synthesis of Penicillic Acid.*

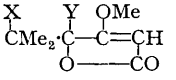
By R. A. Raphael.

The course of several attempted syntheses of penicillic acid (III; Y = OH) is detailed. The successful synthesis is described.

As a starting point for the synthesis of penicillic acid the readily obtainable lactone (I) (Raphael, *J.*, 1947, 805) was the logical choice. It would be reasonable to expect that addition to the exocyclic double bond, giving an adduct of type (II), followed by elimination of HX, would lead to a compound of type (III), a simple derivative of penicillic acid (III; Y = OH).



(I.)



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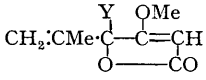
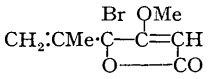


(III.)

The first addendum to be tried was bromine; this readily reacted with a glacial acetic acid solution of the lactone (I) with concomitant evolution of hydrogen bromide. The crystalline product obtained analysed for a *monobromo-lactone* for which the three structures (IV)—(VI) were possible. The first could arise by addition and elimination as suggested above, (V) could



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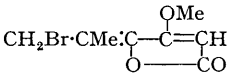

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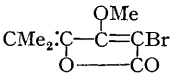
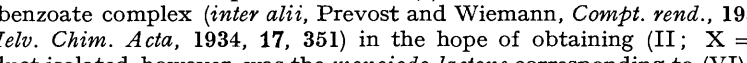


(VI.)

be formed by the same process followed by an anionotropic rearrangement, and a simple substitution could yield (VI). The ultra-violet light absorption of the compound showed a maximum at 2820 A., thus indicating the presence of a triply conjugated system; this at once eliminated (IV) which would be expected to exhibit maximal absorption at about 2200 A. Examination of the chemical properties of the compound revealed that the bromine atom was very firmly bound; for example, refluxing with potassium acetate in glacial acetic acid for 24 hours yielded only unchanged material, and boiling with alcoholic potash had to be continued for 15 minutes before formation of potassium bromide started. This property strongly favoured the constitution (VI), and confirmation was provided by ozonolysis, whereby acetone was obtained in 57% yield as its 2 : 4-dinitrophenylhydrazone. A bromo-lactone of this structure was of course unsuitable for further relevant synthetic use.

The next addition to be attempted on lactone (I) involved the use of the benzene-soluble iodine–silver benzoate complex (*inter alii*, Prevost and Wiemann, *Compt. rend.*, 1937, **204**, 989; Hershberg, *Helv. Chim. Acta*, 1934, **17**, 351) in the hope of obtaining (II; X = Y = OBz). The only product isolated, however, was the *monoiodo-lactone* corresponding to (VI) in structure.

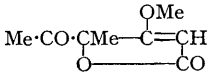
Recourse was then had to the methoxymercuration reaction (*Org. Synth.*, Vol. 20, pp. 81, 101; Romeyn and Wright, *J. Amer. Chem. Soc.*, 1947, **69**, 697). This consists in reaction of an ethylenic compound with a solution of mercuric acetate in methanol at room temperature and treating the resulting adduct as follows :



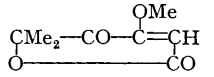
As is seen, the net effect is that of adding the elements of methyl hypobromite across the double bond. Application of this reagent to lactone (I) might be expected to yield (II; X = Br,

Y = OMe), from which methyl penicillate should be obtainable by elimination of hydrogen bromide. Unfortunately, mercuration occurred in the ring, and treatment of the mercury complex with potassium bromide and bromine yielded the same monobromo-lactone as had been produced by direct bromination.

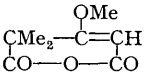
It was then found that the lactone (I) reacted smoothly with performic acid (Swern, Billen, Findlay, and Scanlon, *J. Amer. Chem. Soc.*, 1945, **67**, 1786) to yield the *dihydroxy*-compound (II; X = Y = OH). This constitution was confirmed by fission with periodic acid whereby acetone (estimated as its 2 : 4-dinitrophenylhydrazone) was furnished in 82% yield; further proof of the vicinal position of the hydroxyl groups was provided by the preparation of an iso*propylidene* derivative with acetone. It was to be expected that dehydration of the dihydroxy-compound would yield penicillic acid directly, but relatively mild dehydrating agents led to recovery of unchanged starting material (acetic anhydride gave the *diacetyl* compound) and vigorous methods produced a neutral *ketone* (2 : 4-*dinitrophenylhydrazone*) obviously derived from the dihydroxy-compound by means of a pinacolone rearrangement. There are three theoretical ways in which this could occur, yielding the products (VII)—(IX). Structure (IX)



(VII.)



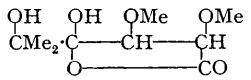
(VIII.)



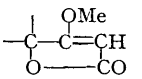
(IX.)

can be eliminated at once as it would not show ketonic properties. The discriminating factor between the other two was provided by the ultra-violet absorption properties of the compound, a maximum appearing at 2650 A.; the presence of a triply conjugated system as in (VIII) was thus indicated. A compound of structure (VII) would be expected to exhibit maximal absorption at about 2200 A. Confirmation was provided by alkaline hydrolysis of the ketone, whereby the dihydroxy-compound (II; X = Y = OH) was re-formed; this could only occur if the ketone possessed structure (VIII). The hydrolysis had to be carried out in an inert solvent, dioxan; when methanolic alkali was employed, addition of methanol occurred and the compound (X) was obtained.

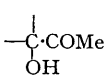
It might be thought that the iodoform reaction would serve as a diagnostic test between (VII) and (VIII), and in fact the ketone yielded iodoform readily with sodium hypoiodite in the cold. Unfortunately, however, it has been found that all compounds in this field containing the ring system (XI) [*e.g.*, the lactone (I), the dihydroxy-compound (II; X = Y = OH), penicillic acid, and dihydropenicillic acid] gave a strong positive reaction, thus depriving the test of its usual validity. It is thought that under the conditions of the test the ring system (XI) is hydrolysed and decarboxylated to the methyl ketone (XII) which, of course, would react readily to give iodoform.



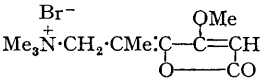
(X.)



(XI.)



(XII.)



(XIII.)

The successful synthesis was initiated by the observation that the lactone (I) reacted smoothly with *N*-bromosuccinimide in the presence of a trace of benzoyl peroxide (Schmid and Karrer, *Helv. Chim. Acta*, 1946, **29**, 573) to furnish a new *monobromo-lactone* possessing properties completely in harmony with structure (V). The compound obtained was a *cis–trans*-mixture, m. p. 74—95°, in which the bromine atom was very labile; it reacted exothermically with *cyclo*hexylamine (Ziegler, Späth, Schaaf, Schumann, and Winkelmann, *Annalen*, 1942, **551**, 80), and potassium acetate readily yielded the corresponding *acetyl* compound. Further verification was provided by ozonolysis, bromoacetone (estimated as 2 : 4-*dinitrophenylhydrazone*) being produced in 56% yield. This monobromo-lactone combined readily with trimethylamine in aqueous or ethereal solution to produce the *quaternary bromide* (XIII). On boiling an aqueous solution of (XIII) with excess of magnesium oxide, trimethylamine was evolved and, on acidification, penicillic acid was obtained in 87% yield.

The mechanism of the last step is formulated thus. The quaternary bromide (XIII) is first converted into the quaternary hydroxide, which then undergoes the rearrangement already encountered in this field (Raphael, *loc. cit.*); this has the net effect of adding the elements of water across the exocyclic double bond. Finally, occurrence of the familiar Hofmann reaction leads to penicillic acid.

The melting point of the synthetic acid was 86—87° undepressed on admixture with the

mould metabolic product; the acetyl derivative and the dibromide likewise gave no depression in melting point when mixed with the corresponding derivatives prepared from the natural product. The natural and the synthetic acid exhibited identical light absorption properties in the ultra-violet and infra-red.

Light absorptions determined in alcoholic solution.

	$\lambda_{max.}$, A.	$\varepsilon_{max.}$		$\lambda_{max.}$, A.	$\varepsilon_{max.}$
Lactone (I) [1]	2700	23,000	(II; X = Y = OH)	2240	13,500
Bromo-lactone (VI)	2820	18,500	(II; X = Y = OAc)	2300	12,500
Bromo-lactone (V)	2770	28,000	*iso*Propylidene compound of (II; X = Y = OH)	2370	11,500
Quaternary bromide (XIII)	2640	18,000	Penicillic acid (synthetic) ...	2255	10,500
Ketone (VIII)	2650	8,500	Penicillic acid (natural)	2255	10,500

[1] Raphael, *loc. cit.*

Experimental.

Bromination of Lactone (I).—The lactone (1·78 g.) was dissolved in glacial acetic acid (10 c.c.), and a solution of bromine (1·8 g.; 1·95 atoms) in glacial acetic acid (10 c.c.) dropped in slowly with shaking (the slight excess of lactone is essential for good yields). The solution rapidly decolourised and hydrogen bromide was evolved. The mixture was evaporated over solid potassium hydroxide in a vacuum desiccator, and the residual crystalline mass was extracted with boiling light petroleum (b. p. 80—100°). The extract on cooling deposited the *bromo-lactone* (VI) as a mass of needles (1·7 g.; 63%). Recrystallisation from the same solvent and from aqueous methanol gave needles, m. p. 121—122° (Found: C, 41·3; H, 3·9; Br, 33·7; OMe, 13·4. $C_8H_9O_3Br$ requires C, 41·25; H, 3·9; Br, 34·3; OMe, 13·3%).

Ozonolysis.—The bromo-lactone (VI) (180 mg.) was dissolved in purified acetic acid (10 c.c.), and ozonised oxygen passed in until absorption ceased. The resulting solution was poured into water (10 c.c.) containing zinc dust (5 g.), and the mixture subjected to steam-distillation. The first four 5-c.c. portions of distillate were treated with excess of aqueous 2 : 4-dinitrophenylhydrazine sulphate and allowed to stand overnight. The yellow precipitate was filtered off and dried on porous tile (105 mg.; 57%); crystallisation from alcohol gave yellow needles, m. p. 125°, undepressed on admixture with acetone 2 : 4-dinitrophenylhydrazone.

Action of Iodine–Silver Benzoate Complex on Lactone (I).—The lactone (1·03 g.), iodine (1·7 g.), and silver benzoate (3·05 g.) were heated under reflux overnight in dry benzene (150 c.c.). The solution was filtered, and the benzene evaporated. The solid residue was crystallised twice from alcohol and finally from light petroleum (b. p. 100—120°), forming needles, m. p. 154—156° (560 mg.; 31%). The analysis corresponded to that of a *monoiodo-lactone* (Found: C, 34·6; 34·6; H, 3·6, 3·2; OMe, 11·25. $C_8H_9O_3I$ requires C, 34·3; H, 3·25; OMe, 11·05%).

Action of Methanolic Mercuric Acetate on Lactone (I).—A solution of mercuric acetate (2·1 g.) and the lactone (1 g.) in methanol (10 c.c.) was kept for 3 days at room temperature. A solution of potassium bromide (1 g.) in water (5 c.c.) was added rapidly with stirring. The crystalline precipitate formed was filtered off, washed with water, and dried on porous tile in a vacuum. The thoroughly dry solid was dissolved in dry chloroform, and bromine in chloroform (10%) added at 0° until a faint permanent colour persisted. The mercuric bromide was filtered off and the chloroform evaporated, leaving a viscous oil which soon solidified to a mass of needles (860 mg.; 57%). The product crystallised from light petroleum (b. p. 80—100°) in needles, m. p. 119—120°. The analysis corresponded to that of a monobromo-lactone and the compound gave no depression of m. p. on admixture with the monobromo-lactone (VI) described above (Found: C, 41·25; H, 3·65%).

Action of Performic Acid on Lactone (I).—The lactone (1·5 g.) was dissolved in anhydrous formic acid (5 c.c.), and hydrogen peroxide (100-vol.; 2 c.c.) added; a vigorous reaction set in after about 10 minutes. After two hours' standing the solution was steam-distilled to remove formic acid and hydrolyse any formic ester, and the distillate evaporated to dryness under reduced pressure. The residual viscous liquid slowly solidified. Crystallisation from ethyl acetate or nitroethane yielded colourless prisms of the *dihydroxy*-compound (II; X = Y = OH) (1·3 g.; 71%), m. p. 148—150° (Found: C, 51·2; H, 6·65; OMe, 16·3. $C_8H_{12}O_5$ requires C, 51·05; H, 6·45; OMe, 16·5%). The compound reacted as a monobasic acid and was very soluble in water and other hydroxylic solvents. The *diacetyl* derivative, prepared in the usual manner, crystallised from light petroleum (b. p. 80—100°) in prismatic needles, m. p. 107—108° (Found: C, 53·25; H, 5·95. $C_{12}H_{16}O_7$ requires C, 52·95; H, 5·95%). The iso*propylidene* derivative, prepared in 42% yield by refluxing an acetone solution of the compound (1 g.) with anhydrous potassium carbonate (2 g.), crystallised in long needles, m. p. 102—103°, from light petroleum (b. p. 60—80°) (Found: C, 57·6; H, 6·85; OMe, 13·5. $C_{11}H_{16}O_5$ requires C, 57·9; H, 7·05; OMe, 13·6%).

Periodic Acid Fission of Dihydroxy-compound (II; X = Y = OH).—The dihydroxy-compound (470 mg.) was dissolved in water (5 c.c.), and a solution of periodic acid (570 mg.) in water (5 c.c.) added in one portion with cooling. After 16 hours the solution was steam-distilled and the first 30 c.c. of distillate were treated with excess of aqueous 2 : 4-dinitrophenylhydrazine sulphate. The yellow precipitate was filtered off, washed with water and dried on a porous tile (595 mg.; 82%). Crystallisation from alcohol gave needles, m. p. 126°, undepressed on admixture with acetone 2 : 4-dinitrophenylhydrazone.

Attempted Dehydration of Dihydroxy-compound (II; X = Y = OH).—Use of relatively mild dehydrating agents (iodine, toluene-*p*-sulphonic acid, magnesium sulphate, thionyl chloride in pyridine) led only to the recovery of unchanged starting material. More drastic methods (hot anhydrous formic

acid, anhydrous oxalic acid, phosphoric oxide, potassium pyrosulphate) yielded the ketone (VIII). As the method involving the last-named reagent produced the best yield, it alone will be described.

The dihydroxy-compound (1 g.) was intimately mixed with powdered potassium pyrosulphate (2 g.) and heated to 180° for 1 hour in an oil-bath. The cooled reaction mixture was treated with water to dissolve the potassium salt; the residual flocculent solid was filtered off, washed with water, and dried on porous tile. Crystallisation from light petroleum (b. p. 80—100°) furnished needles of the *ketone* (VIII), m. p. 96—97° (470 mg.; 52%) (Found: C, 56·85; H, 5·9; OMe, 18·2. $C_8H_{10}O_4$ requires C, 56·45; H, 5·9; OMe, 18·25%). The 2 : 4-*dinitrophenylhydrazone*, prepared by treating the ketone with alcoholic 2 : 4-dinitrophenylhydrazine sulphate, crystallised from *iso*amyl alcohol in orange plates, m. p. 228—229° (Kofler block) (Found: N, 15·65. $C_{14}H_{14}O_7N_4$ requires N, 16·0%).

Hydrolysis.—(a) A solution of the ketone (VIII) (366 mg.) in dioxan (5 c.c.) was treated with N-sodium hydroxide (1 mol.) and kept for 24 hours. N-Sulphuric acid (1 mol.) was added, and the solution evaporated to dryness at room temperature under reduced pressure. The residual solid was extracted with boiling ethyl acetate; the extract was evaporated to 2 c.c. and allowed to crystallise. The crystalline solid obtained (210 mg.; 51%) had m. p. 148—149° undepressed on admixture with the dihydroxy-compound (II; X = Y = OH).

(b) A solution of the ketone (VIII) (245 mg.) in methanol (3 c.c.) was treated exactly as in (a). The ethyl acetate extract was evaporated to dryness and the residual crystalline solid crystallised from benzene in needles (185 mg.; 58%), m. p. 98—99° depressed to 70—78° on admixture with the starting material. The *compound* (X) was acidic and showed no selective absorption in the ultra-violet (Found: C, 49·3; H, 7·35. $C_9H_{16}O_6$ requires C, 49·1; H, 7·3%).

Action of N-*Bromosuccinimide on Lactone* (I).—The lactone (5·8 g.) was dissolved in carbon tetrachloride (30 c.c.), *N*-bromosuccinimide (6·7 g.) and benzoyl peroxide (50 mg.) were added, and the mixture refluxed until the formation of succinimide was complete (*ca.* 2 hours). The mixture was cooled in ice, filtered, and the filtrate evaporated under reduced pressure; the pale yellow, residual oil slowly solidified to a mass of needles. Crystallisation from light petroleum (b. p. 100—120°) furnished the *bromo-lactone* (V) as plates, m. p. 74—95° (8·1 g.; 91%) (Found: C, 41·4; H, 4·1. $C_8H_9O_3Br$ requires C, 41·25; H, 3·9%). The compound possessed a marked irritant effect on the skin and mucous membrane. Mixing the substance with an equal weight of *cyclo*hexylamine resulted in a spontaneous rise in temperature to 190°. The corresponding *acetyl* compound, prepared by treatment with potassium acetate in glacial acetic acid, crystallised from light petroleum (b. p. 60—80°) in needles, m. p. 72—84° (Found: C, 56·55; H, 5·75. $C_{10}H_{12}O_5$ requires C, 56·6; H, 5·7%).

Ozonolysis.—The bromo-lactone (V) (500 mg.) was dissolved in carbon tetrachloride (25 c.c.), and ozonised oxygen bubbled through until no more gummy ozonide was precipitated. The solvent was carefully evaporated under reduced pressure, water (5 c.c.) added, and the mixture steam-distilled until the distillate remained clear. The turbid, strongly lachrymatory distillate was treated with excess of aqueous-alcoholic 2 : 4-dinitrophenylhydrazine sulphate; after 24 hours the yellow precipitate (383 mg.; 56%) was filtered off, washed with water, and dried on porous tile. Crystallisation from alcohol gave yellow needles, m. p. 124—125°, undepressed on admixture with authentic *bromoacetone* 2 : 4-*dinitrophenylhydrazone* (Found: N, 17·7. $C_9H_9O_4N_4Br$ requires N, 17·6%), but depressed by 15° when mixed with acetone 2 : 4-dinitrophenylhydrazone.

Quaternary Bromide (XIII).—(a) The bromo-lactone (V) (5·8 g.) was warmed gently by steam with aqueous trimethylamine (33%; 15 c.c.) until the solution became homogeneous. Evaporation to dryness under reduced pressure yielded a crystalline solid. The mass was triturated with dry acetone, filtered off, and washed well with more acetone. The hygroscopic *quaternary bromide* (5·3 g.; 73%) crystallised in clumps of needles from nitroethane and in plates from alcohol–ether; it had m. p. 145—148° (decomp.) (Found: C, 42·9; H, 6·4; N, 4·45; Br, 26·8. $C_{11}H_{18}O_3NBr$ requires C, 45·2; H, 6·2; N, 4·8; Br, 27·4%).

(b) The bromo-lactone (V) (2 g.) was dissolved in dry ether (20 c.c.), and a solution of trimethylamine (2 g.) in dry ether (20 c.c.) added. The mixture immediately became turbid and heat was evolved. After standing at room temperature for 24 hours the crystalline slurry was filtered off and the practically pure quaternary salt washed with dry ether (2·1 g.; 82%).

Penicillic Acid (III; Y = OH).—The quaternary bromide (XIII) (750 mg.) was dissolved in water (10 c.c.), magnesium oxide (750 mg.) added, and the suspension boiled in a stream of nitrogen until no more trimethylamine was evolved (90 mins.). The base was absorbed in a known volume of standard acid and back-titrated with standard alkali; the yield of trimethylamine was 92%. The reaction mixture was strongly acidified with 2N-sulphuric acid and extracted four times with ether. Drying ($MgSO_4$) and evaporation furnished an oil which partly solidified. Extraction with boiling *cyclo*hexane yielded penicillic acid (140 mg.; 32%), m. p. 86—87° after recrystallisation from the same solvent.

As it was noticed that most of the trimethylamine was evolved during the first ten minutes of boiling, the preparation was repeated employing this reaction time. Under these conditions the residual oil solidified completely and one crystallisation from *cyclo*hexane yielded pure penicillic acid (380 mg.; 87%) as needles, m. p. 86—87° (Found: C, 56·35, 56·15; H, 5·9, 5·7; OMe, 17·9, 17·5. Calc. for $C_8H_{10}O_4$: C, 56·45; H, 5·9; OMe, 18·25%). The synthetic acid gave no depression in m. p. when mixed with the natural product and exhibited the characteristic purple colouration on standing with concentrated ammonia (Birkinshaw, Oxford, and Raistrick, *Biochem. J.*, 1936, **30**, 394). Addition of bromine produced the dibromide, crystallising from benzene–light petroleum (b. p. 60—80°) in clumps of needles, m. p. 153—154°, undepressed on admixture with authentic penicillic acid dibromide (Birkinshaw, Oxford, and Raistrick, *loc. cit.*). Acetylation furnished the acetyl compound, which crystallised from light petroleum (b. p. 60—80°) in needles, m. p. 72—73° undepressed on admixture with authentic acetylpenicillic acid (Birkinshaw, Oxford, and Raistrick, *loc. cit.*).

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