257. *Investigations Relating to the Synthesis of Patulin.*

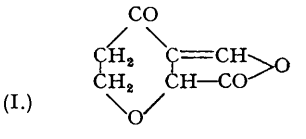
By ZOLTÁN FÖLDI, GÁBOR FODOR, and (the late) ISTVÁN DEMJÉN.

Attempts to synthesise patulin, investigations leading to an isomer of it, and the preparation of various intermediates and related compounds are described. (This work was completed in 1944 and relevant patent applications were filed in the same year.)

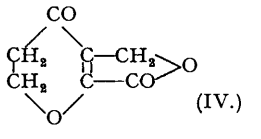
RAISTRICK and his co-workers (Raistrick, Birkinshaw, Bracken, and Michael, *Lancet*, 1943, ii, 625) recorded the isolation and investigation of a crystalline antibiotic, produced by *Penicillium patulum* and named patulin, to which they assigned the structure (I) or a tautomeric form of it.

Bergel, Morrison, Moss, and Rinderknecht (*J.*, 1944, 415) proved the identity of clavatin, a metabolite of *Aspergillus clavatus*, with patulin and accepted the structure (I) or one of several tautomeric forms, *e.g.*, (IV). We started our work on the synthesis of patulin before the latter paper had been published; our aim was to synthesise (IV) and, if this should not be identical

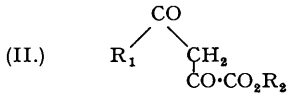
with patulin, to secure a double bond in the alternative position. We used a route very similar to that chosen by Puetzer, Nield, and Barry (*J. Amer. Chem. Soc.*, 1945, **67**, 832), and like them,



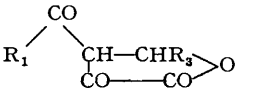
(I.)



(IV.)



(II.)



(III.)

	R_1.	R_3.
(*a*)	$MeO\cdot CH_2\cdot CH_2$	Me
(*b*)	$EtO\cdot CH_2\cdot CH_2$	Et
(*c*)	$CH_3\cdot CH(OH)\cdot CH_2$	,,

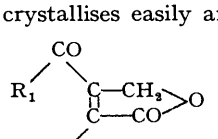
	R_1.	R_3.
(*a*)	$MeO\cdot CH_2\cdot CH_2$	H
(*b*)	,,	Me
(*c*)	,,	Ph
(*d*)	$EtO\cdot CH_2\cdot CH_2$	H
(*e*)	Me	,,
(*f*)	Et	,,
(*g*)	$EtO_2C\cdot CH_2\cdot CH_2$	,,
(*h*)	$CH_2Cl\cdot CH_2$	,,

found that (IV) was not identical with patulin. Attenburrow, Elks, Elliot, Hems, Harris, and Brodrick (*J.*, 1945, 571) and Borrows and Hems (*ibid.*, p. 577), in experiments on the synthesis of patulin, obtained certain intermediates identical with ours, but, owing to the prevailing conditions, we had been unable to publish our results. Now, therefore, we record only observations or findings supplementary to those of Puetzer *et al.* and of Hems *et al.*

Our route for the synthesis of (IV) was (II*a*) ⟶ (III*a*) ⟶ (IV). β-Hydroxypropionylpyruvic acid esters (II; $R_1 = CH_2\cdot CH_2\cdot OH$) could not be obtained from 1-hydroxybutan-3-one by Claisen condensation. From acetonylmethylcarbinol it was, however, possible to obtain on Claisen condensation *ethyl* β-*hydroxybutyrylpyruvate* (II*c*), though in small and varying yields and contaminated with various amounts of ethyl 6-methyl-5 : 6-dihydro-γ-pyrone-2-carboxylate, into which it changes rather easily. Acrylylpyruvic esters (II; $R_1 = CH_2{:}CH$) could not be obtained sufficiently pure from methyl vinyl ketone on Claisen condensation; they were always largely contaminated with β-alkoxypropionylpyruvic esters (*e.g.*, II*b*), owing to simultaneous addition of alcohol during the condensation, and sometimes these formed the main product, but only in moderate yield. These compounds could also be prepared, though in poor yield, from 1-alkoxybutan-3-one by the usual Claisen condensation.

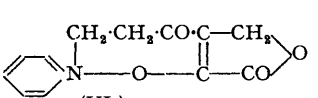
Ketoacylbutyrolactones of type (III) were obtained in the same way [*i.e.*, condensation of compounds (II) with formaldehyde in aqueous medium] and under almost the same experimental conditions as those used by Hems *et al.* (*loc. cit.*) to prepare α-keto-β-acetobutyrolactone; the amount of solvent used in our reaction mixtures was, however, kept to a minimum and paraformaldehyde was used as source of formaldehyde, and probably for these reasons our yields were almost double those of Hems *et al.* The same method proved to be equally useful in the preparation of the analogous lactones (III, *a*, *d*, *f*, and *g*) from the corresponding acylpyruvic esters and the appropriate aldehyde.

α-Keto-β-methoxypropionylbutyrolactone (III*a*) has been investigated in the greatest detail. Hydrogen chloride in cold ethereal or ethyl acetate solution eliminates the methoxy-group from (III*a*) and replaces it by chlorine, affording (III*h*). Acetyl chloride in the cold acts similarly. The *keto-lactone* (III*h*) is oily and difficult to purify, decomposing on distillation even in a high vacuum and forming (IV). Warm acetyl chloride not only replaces the methoxy-group by chlorine but also effects acetylation of the enolised α-keto-group. The resulting *compound* (V*b*) crystallises easily and can be obtained in excellent yield. Analogous treatment of the

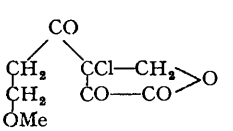


(*a*, $R_1 = Me$; *b*, $R_1 = CH_2Cl\cdot CH_2$.)

(V.)



(VI.)



(VII.)

keto-lactone (III*e*) affords, likewise, a well crystallised enol acetate, undoubtedly the α-enol acetate (V*a*). The action of pyridine, preferably in the cold, on (V*b*) eliminates both the chlorine

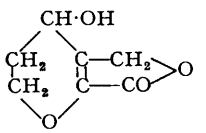
and the acetyl group, affording a quaternary *pyridinium* inner salt, which is soluble in water, gives a deep colouration with ferric chloride, loses pyridine in boiling alkaline solution, and reduces hot Tollens's reagent. The same compound results, but more slowly, from the action of pyridine, containing some pyridine hydrochloride, on (IV). This pyridinium salt is considered to have the structure (VI). The action of cold sulphuryl chloride on (III*a*) yields a crystalline, sensitive chlorinated *product* (VII), contaminated, however, with appreciable amounts of a secondary product resulting from the action of the evolved hydrogen chloride in replacing the methoxy-group by chlorine. Attempts to split off the β-chloro-atom as hydrogen chloride did not lead to well-characterised products.

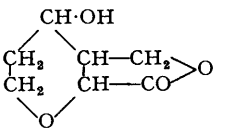
Cyclisation of (III*a*) to (IV) was effected by hydrogen chloride or by anhydrous formic acid. As mentioned above, (IV) was also produced on distillation of (III*h*). The action of hydrogen chloride on (III*a*) in ethyl acetate solution and on heating in a sealed tube afforded (IV) in yields up to 80%. Anhydrous formic acid at 100° effects cyclisation in about 65% yield.

The bacteriostatic action of (IV) on *Staphylococcus aureus*, assayed in the Florey test, was not detectable in dilutions higher than 1 : 1500 to 1 : 2000.

Hydrolysis of (IV) with 2N-sulphuric acid, in the same manner as used by Raistrick *et al.* (*loc. cit.*) for patulin, effected evolution of 0·2 mol. of carbon dioxide per mol. of (IV), and no formation of acids in the hydrolysis mixture could be detected. Alkaline hydrolysis first opens the dihydropyrone ring and then splits off oxalic acid.

Attempted acetylation of (IV), precisely as used by Raistrick *et al.* for patulin, left it unaltered.

(VIII.) 

 (IX.)

Attempts to rearrange (IV) into patulin, under various conditions, were unsuccessful, leading to the same conclusion as that of Puetzer *et al.* (*loc. cit.*), *viz.*, that (IV) cannot be a tautomeric form of patulin.

Hydrogenation of (IV) by means of Raney nickel caused an uptake of 2 atoms of hydrogen, forming (VIII), and hydrogenation of this *lactone* by means of palladium–charcoal gave, with further uptake of 2 atoms of hydrogen, a water-soluble oil. If (IV) and patulin have identical carbon skeleton, this oil must have structure (IX) and be identical with the oily product obtained by Raistrick *et al.* from patulin with absorption of 4 atoms of hydrogen.

Bromination of (IV) gave a well crystallised *monobromo*-compound.

Experimental.

(M. p.s are uncorrected.)

Ethyl β-Hydroxybutyrylpyruvate (II*c*).—Sodium (0·5 g.), dissolved in anhydrous ethanol (5 c.c.), is mixed, under ice cooling, with ethyl oxalate (2·8 c.c.) and stirred until solution is complete. Acetonylmethylcarbinol (2·1 c.c.) is added, and the mixture kept overnight in an ice-box, then acidified with 5N-hydrochloric acid (4·4 c.c.) and extracted with ether. The residual oil obtained on evaporation of the dried ethereal solution is distilled, and the fraction distilling at 115—150° [bath temp.]/1·5 mm. is again fractionated; the *ester* (II*c*) distils at 59—61° [bath temp.]/10^{-4} mm. as a pale yellow oil (1 g.). It gives a deep red coloration with ferric chloride solution (Found: equiv., by titration, 202·2; by saponification, 88. $C_9H_{14}O_5$ requires equiv., 202·1 and 101, respectively).

Ethyl β-Ethoxypropionylpyruvate (II*b*).—To a solution of sodium (4·6 g.) in anhydrous ethanol (92 c.c.) a mixture of ethyl oxalate (29·2 g.) and methyl vinyl ketone (14 g.) is added dropwise, the temperature being kept between − 25° and − 20°. After standing for some time in ice–salt and then overnight in the ice-box, the mixture is acidified with 5N-hydrochloric acid (40 c.c.) and extracted with ether. The residue from the ethereal layer is taken up in benzene (100 c.c.) and washed several times with water to remove free oxalic acid. The residue from the benzene layer is fractionated; the ester distils at 90—94° [bath temp.]/10^{-3} mm.; yield, 3·5—4 g. (Found: OEt, 37·6; equiv., by titrn., 228; by saponification, 108. Calc. for $C_{10}H_{16}O_5$: OEt, 41·6%; equiv., 216·1 and 108, respectively).

Methyl β-Methoxypropionylpyruvate (II*a*).—To a solution of 1-methoxybutan-3-one (11·5 g.) and methyl oxalate (13·5 g.) in toluene (115 c.c.), sodium ribbon (2·3 g.) is added in portions, the mixture being cooled occasionally with ice. After all sodium has been consumed and after a further ½ hr.'s standing, ice (30 g.) and 10N-hydrochloric acid (10 c.c.) are added, the toluene layer separated, the toluene evaporated off, and the residual oil distilled, the ester distilling over at about 100° (bath temp.)/10^{-2} mm. as a pale yellow oil (11 g.). When cooled with ice, it solidifies and melts again at room temperature (Found: equiv., by titrn., 191. Calc. for $C_8H_{12}O_5$: equiv., 188·1).

α-Keto-β-acetylbutyrolactone (III*e*).—To a mixture of potassium carbonate (14 g.), water (25 c.c.), and paraformaldehyde (3·4 g.), ethyl acetopyruvate (15·8 g.) is added in portions. The mixture is stirred and occasionally cooled so as to be kept under 40°. After about 20 minutes 6N-hydrochloric acid (38 c.c.)

is added, the mixture cooled in ice-water, and the finely crystallised lactone collected by suction; yield, after recrystallisation from ether, 9—10 g.; m. p. 127—128°. The lactone can be distilled without decomposition at 0·4 mm. between 90° and 100° (bath temp.) (Found: C, 50·4; H, 4·2; equiv., by titrn., 141·5; by saponification, 72. Calc. for $C_6H_6O_4$: C, 50·6; H, 4·3%; equiv., 142 and 71, respectively).

α-Enol acetate (V*a*). (III*e*) (1 g.) is heated with acetyl chloride (4 c.c.) in a sealed tube for an hour on the steam-bath, the acetyl chloride removed in a vacuum, and the residue taken up in benzene, washed with sodium carbonate solution, and evaporated to dryness. The crystalline residue is recrystallised from ether; yield, 0·7 g.; m. p. 75—78°. It is neutral to litmus (Found: equiv., by saponification, 60·6. Calc. for $C_8H_8O_5$: equiv., 61·4).

α-Keto-β-propionylbutyrolactone (III*f*).—This *lactone* was prepared as for (III*e*); m. p. 128—129° (Found: equiv., by titrn., 158. $C_7H_8O_4$ requires equiv., 156·1).

α-Keto-β-(β′-methoxypropionyl)butyrolactone (III*a*).—This lactone too was prepared as for (III*e*); m. p. 122—124° (Found: OMe, 16·6; equiv., by titrn., 186·5; by saponification, 85·5. Calc. for $C_8H_{10}O_5$: OMe, 16·7%; equiv., 186·1 and 93, respectively). It can also be prepared directly from 1-methoxybutan-3-one in satisfactory overall yield as follows: the ketone (23 g.) and methyl oxalate (27 g.) are dissolved in toluene–benzene (200 c.c.; 1 : 1) and sodium ribbon (4·6 g.) is added in portions, the mixture being cooled with ice. When solution is complete, ice (60 g.) and water (30 c.c.) are added, and the aqueous layer is separated and mixed with 35% formaldehyde solution (19 c.c.). After standing for about 25 minutes, the mixture is acidified with concentrated hydrochloric acid (20 c.c.). The lactone crystallises as a thick crystalline pulp which after standing in ice is separated by suction and washed; yield, 20 g., m. p. 122—123°.

α-Keto-β-(β′-ethoxypropionyl)butyrolactone (III*d*).—Obtained from (II*b*) in analogous manner to that described for (III*e*), this *lactone* has m. p. 89—93° (Found: equiv. by titrn., 215; by saponification, 93. $C_9H_{12}O_5$ requires equiv., 200·1 and 100, respectively). It is easily soluble in most organic solvents except light petroleum.

α-Keto-β-(β′-carbethoxypropionyl)butyrolactone (III*g*).—From 2·44 g. of diethyl αγ-diketopimelate (cf. Wislicenus and Münzesheimer, *Ber.*, 1898, **31**, 624) in the usual manner, 2·07 g. (90%) of the *lactone* were obtained; m. p. 75—77° (Found: equiv., 225. $C_{10}H_{12}O_6$ requires equiv., 228·1).

α-Keto-β-(β′-methoxypropionyl)-γ-valerolactone (III*b*).—This *lactone* was obtained in the usual manner from (II*a*) (3·76 g.) and acetaldehyde (1·1 g.); m. p. 102—103° (Found: equiv., by titrn., 205; by saponification, 102. $C_9H_{12}O_5$ requires equiv., 200·1 and 100, respectively).

α-Keto-β-(β′-methoxypropionyl)-γ-phenylbutyrolactone (III*c*).—Similarly, (II*a*) (1·8 g.) and benzaldehyde (1·06 g.) by the usual process afforded this *lactone*; m. p. 129° (Found: equiv., by titrn., 259; by saponification, 136. $C_{14}H_{14}O_5$ requires equiv., 262·1 and 131, respectively).

α-Keto-β-(β′-chloropropionyl)butyrolactone (III*h*).—The lactone (III*a*) (1 g.) is allowed to stand with an ethereal solution of hydrogen chloride (8·5%; 6 c.c.) and dissolves during 24 hours. The almost colourless solution is evaporated to dryness under diminished pressure. The pale oil is the crude *lactone* (III*h*); yield, 0·97 g., containing some unchanged (III*a*) (Found: OMe, 1·9%). It gives a dark red precipitate with ferric chloride solution, and is soluble in sodium carbonate solution (Found: Cl, 18·6; equiv., 200. $C_7H_7O_4Cl$ requires Cl, 18·6%; equiv., 190·5).

This lactone also results from the action of acetyl chloride (10 c.c.) on (III*a*) (3·7 g.) in the cold. When the mixture is kept in a sealed tube overnight, a pale solution results which is evaporated to dryness under diminished pressure and at room temperature. The oil cannot be induced to crystallise (Found: OMe, 0·3; Cl, 17·7%). Distillation at 110° (bath temp.)/10^{-3} mm. affords 1·67 g. of distillate which solidifies and yields, after being washed with ether, 1·4 g. of (IV), m. p. 85—87°.

α-Acetoxy-β-(β′-chloropropionyl)crotonolactone (V*b*).—When (III*a*) (10 g.) and acetyl chloride (40 c.c.) are heated for an hour in a sealed tube in a water-bath, the former gradually dissolves; excess of acetyl chloride is then removed under reduced pressure, and the crystalline residue washed with anhydrous ether, affording the *lactone* (8·7 g.) as stout, rhombic crystals; m. p. 87—88° (Found: Cl, 15·3; equiv., by saponification, 59·5. $C_9H_9O_5Cl$ requires Cl, 15·3%; equiv., 59·5). It is insoluble in sodium carbonate solution and does not give a coloration with ferric chloride solution.

Action of Pyridine on (V*b*).—2 G. of (V*b*) are dissolved in ice-cold pyridine (16 c.c.) and kept overnight in an ice-box. The brownish orange-red solution is filled with small spherical rosettes which are collected by suction and washed with ice-cold pyridine, affording the *compound* (VI) (1·2 g.) as pale yellowish crystals, m. p. 136—137° (decomp.) (Found: N, 5·7; equiv., by saponification, 121. $C_{12}H_{11}O_4N$ requires N, 6·0%; equiv., 116·6). It is soluble in water, sparingly soluble in methanol, insoluble in ether. It reduces hot Tollens's reagent and gives a red-brown coloration with ferric chloride. On boiling with dilute sodium hydroxide pyridine is evolved.

The substance (VI) can also be obtained from (IV): 0·05 g. of (IV) is dissolved in pyridine (0·18 c.c.) containing pyridine hydrochloride (0·02 g.). After 48 hours in an ice-box spherical rosettes are formed (0·015 g.); m. p. 136—137° (decomp.).

β-Chloro-α-keto-β-(β′-methoxypropionyl)butyrolactone (VII).—When (III*a*) (3·7 g.) is mixed with sulphuryl chloride (2·2 c.c.), it gradually dissolves with evolution of hydrogen chloride and sulphur dioxide. After about 5 minutes the mixture rapidly turns to an almost solid crystalline pulp. Excess of sulphuryl chloride is removed in a vacuum without any heating, affording 4·6 g. of white crystals. This crude product (Found: OMe, 8·2; Cl, 22·8%) does not show any coloration with ferric chloride, but reduces hot Tollens's reagent; it is insoluble in water, but easily soluble in most organic solvents, except carbon tetrachloride and light petroleum. It is rather unstable and changes in some days into a syrup. The crude product was partly purified by trituration with small amounts of chloroform; white crystals, m. p. 96—99° (Found: OMe, 11·4; Cl, 19·1. Calc. for $C_8H_9O_5Cl$: OMe, 14·1; Cl, 16·1%).

3-Hydroxymethyl-5 : 6-dihydro-γ-pyrone-2-carboxylic Acid Lactone (IV).—The lactone (III*a*) (15 g.) is heated with ethyl acetate (50 c.c.) containing 10% of dry hydrogen chloride in a sealed tube for 25 minutes in the water-bath. The residual oil left after evaporation under reduced pressure yields on

addition of ether 6·2 g. of (IV), m. p. 85—87°. The residue of the ethereal mother-liquor yields, on distillation in the vacuum of the mercury pump (bath temp. 100—120°) and trituration of the distillate with ether, an additional amount (3·9 g.) of (IV), m. p. 88—89° (total yield > 80%). The compound is moderately soluble in water or in ether, easily soluble in cold ethyl acetate and hot benzene. It is neutral to litmus, does not give a coloration with ferric chloride, and reduces hot Tollens's reagent (Found : C, 54·4; H, 4·0. Calc. for $C_7H_6O_4$: C, 54·5; H, 3·9%). It affords an orange-yellow 2 : 4-dinitrophenylhydrazone which decomposes, with blackening, at 265—267°.

Cyclisation of (III*a*) *to* (IV) *by Formic Acid.*—(III*a*) (40 g.) and anhydrous formic acid (200 c.c.) are heated for an hour in a sealed tube on the water-bath. The residue left after evaporation under reduced pressure is taken up in ethyl acetate and shaken with anhydrous sodium carbonate. The filtrate yields, on evaporation to dryness, 22 g. of (IV), m. p. 87—88°, which gives a phenylhydrazone, m. p. 150—154°.

Alkaline Hydrolysis of (IV).—(IV) (0·5 g.) was mixed with 0·4N-baryta (17·5 c.c.) and kept in ice and occasionally shaken. Soon a yellow solution resulted. After about 20 minutes the mixture began to become turbid. After an additional hour the resulting precipitate was centrifuged, washed, and dried; yield, 0·78 g. of a yellowish powder [Found : Ba, 51·8; $(CO_2H)_2$, 34·7. Calc. for $C_2O_4Ba,2H_2O$: Ba, 52·6; $(CO_2H)_2$, 34·5%].

Acid Hydrolysis of (IV).—0·2 G. of (IV), dissolved in 2N-sulphuric acid (10 c.c.), was refluxed for 6 hours in a stream of hydrogen, which was bubbled through 5 c.c. of 0·4N-baryta. The hydrolysis finished, the baryta was filtered from the precipitate and titrated with 0·1N-hydrochloric acid, 15 c.c. being required; this corresponds to 11 mg. of carbon dioxide. The hydrolysis solution required 19·2 c.c. of N-sodium hydroxide for neutralisation.

Catalytic Hydrogenation of (IV).—Raney nickel (1 g.), suspended in water (10 c.c.), was saturated with hydrogen, a solution of (IV) (1 g.) in methanol (20 c.c.) added, and hydrogenation allowed to proceed : 170 c.c. of hydrogen (corr.) were absorbed. The filtrate, faintly coloured by nickel complexes, was evaporated to dryness under reduced pressure, and the crystalline residue distilled at 10^{-3} mm. After a little forerun, the hydrogenation *product* (VIII) distilled over at 170—175° (bath temp.), crystallising instantly on cooling; yield, 0·75 g., m. p. 95—96°, mixed m. p. with (IV), 60° (Found : equiv., by saponification, 156. $C_7H_8O_4$ requires equiv., 156·1). Unlike (IV), it does not reduce hot Tollens's reagent. Only a very small yield could be obtained of a 2 : 4-dinitrophenylhydrazone (decomp. 240°). In a Zerewitinoff estimation 0·096 g. gave 13·5 c.c. of methane (corr.), corresponding to 0·98 active hydrogen atom per molecule. The product does not give a precipitate with ice-cold 0·4N-baryta. It rapidly decolorises aqueous potassium permanganate solution.

Hydrogenation of (VIII) *to* (IX).—0·2 G. of palladium–charcoal (containing 30 mg. Pd), suspended in methanol (5 c.c.), was saturated with hydrogen, and (VIII) (0·5 g.) in methanol (10 c.c.) was added. 71 C.c. of hydrogen (corr.) were absorbed and the uptake then ceased completely. The filtrate was evaporated to dryness in a vacuum and left an almost colourless, thick oil which did not crystallise (Found : equiv. by saponification, 165. Calc. for $C_7H_{10}O_4$: equiv., 158·1). It was easily soluble in water and decolorised aqueous potassium permanganate solution.

Bromination of (IV).—To a solution of (IV) (308 mg.) in chloroform (2 c.c.), a 5% solution of bromine in chloroform (2 c.c.) was added. After about ½ hr., the solvent was removed under vacuum; a thick oil remained which crystallised on addition of ether and was then obtained as a faintly pink powder (440 mg.), m. p. 108° (Found : Br, 34·1. $C_7H_5O_4Br$ requires Br, 34·4%). Recrystallisation from water afforded white crystals, m. p. 112°, of the *monobromo*-compound.

The authors wish to thank Mr. J. Kollonics, Dr. K. Kovács, and Mr. J. Dory for assistance in some parts of this work, Mr. J. Bagossy for preparing some of the starting materials, and the staff of the Analytical Department of Chinoin Works Ltd. for carrying out most of the analyses.

RESEARCH LABORATORIES, CHINOIN CHEMICAL AND PHARMACEUTICAL WORKS LTD.,
UJPEST, HUNGARY. [*Received, July 8th,* 1947.]