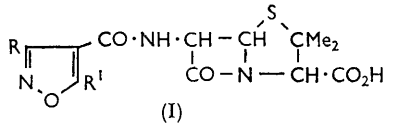
1121. *Derivatives of 6-Aminopenicillanic Acid. Part IX.*[1] *2,4-Di- and 2,4,5-Tri-substituted-3-furylpenicillins*

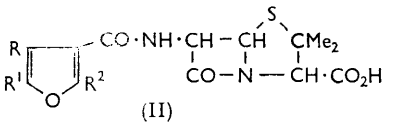
By J. C. Hanson, J. H. C. Nayler, T. Taylor, and (in part) P. H. Gore

Condensation of acylacetic esters with acylcarbinols or acyloins gave various 2,4-di- and 2,4,5-tri-substituted 3-furoic acids, respectively. Measurements of pK_a values and ultraviolet absorption for some of the acids are recorded. Reaction of the derived acid chlorides with 6-aminopenicillanic acid gave the correspondingly substituted 3-furylpenicillins, which had interesting antibacterial activity, and some of which were resistant to inactivation by penicillinase.

Acylation of 6-aminopenicillanic acid with the chlorides of sterically hindered carboxylic acids of various types gives penicillins which resist inactivation by penicillinase.[2] Opportunities for structural variation within this general class are particularly numerous when the side-chain is heterocyclic, and many penicillins have been prepared from heteroaromatic carboxylic acids carrying two *ortho*-substituents.[3] The most useful of these have proved to be 3,5-disubstituted 4-isoxazolylpenicillins (I), several of which have found clinical application in the treatment of infections due to Gram-positive bacteria including penicillinase-producing staphylococci.[1,2] The present Paper describes some intermediates for the synthesis of 2,4-di- and 2,4,5-tri-substituted 3-furylpenicillins (II), which may be regarded as analogues of the isoxazolylpenicillins (I) in which the azole nitrogen has been replaced by carbon.

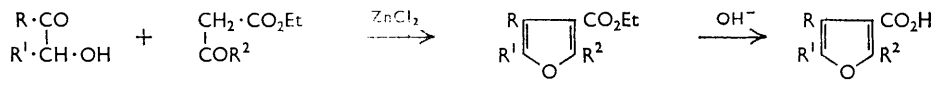


(I)



(II)

Although a number of 2,4-di- and 2,4,5-tri-substituted 3-furoic acids has been reported, the development of generally applicable methods for their synthesis has received relatively little attention. One simple route which appeared to merit further exploration is that of Gonzalez,[4] who prepared 2,4-dimethyl-3-furoic acid (V; $R = R^2 = Me$, $R^1 = H$) and 2,4,5-trimethyl-3-furoic acid (V; $R = R^1 = R^2 = Me$) by zinc chloride-catalysed condensation of ethyl acetoacetate with acetol (III; $R = Me$, $R^1 = H$) and acetoin (III; $R = R^1 = Me$), respectively. The potential versatility of the procedure is indicated by its successful use with various sugars as the carbonyl component (III).



(III) (IV) (V)

In view of the ready availability of acyloins (III; $R = R^1$) the synthesis of trisubstituted 3-furoic acids was examined first. Condensation of acetoin with slightly more than a molar equivalent of ethyl propionylacetate, ethyl isobutyrylacetate, or ethyl benzoylacetate was readily brought about by heating under reflux for a few hours with zinc chloride in ethanol. Alkaline hydrolysis of the resulting crude esters (IV) then gave fair yields of the appropriate trisubstituted 3-furoic acids (V; $R = R^1 = Me$, $R^2 = Et$, Pr^i, or Ph). Similar reactions with propionoin (III; $R = R^1 = Et$) gave the acids (V; $R = R^1 = Et$, $R^2 = Me$ or Ph), whilst the analogue (V; $R = R^1 = Pr^n$, $R^2 = Me$)

[1] Part VIII, J. C. Hanson, A. A. W. Long, J. H. C. Nayler, and E. R. Stove, preceding Paper.
[2] F. P. Doyle and J. H. C. Nayler, *Adv. Drug Research*, 1964, **1**, 50.
[3] F. P. Doyle and J. H. C. Nayler, B.P. 905,778/1962.
[4] F. G. Gonzalez, J. L. Aparicio, and F. Sanchez-Laulke, *Anales real Soc. españ. Fis. Quím.*, 1954, **50***B*, 407.

was prepared from butyroin and ethyl acetoacetate. Benzoin (III; R = R^1 = Ph) proved to be much less reactive than its aliphatic analogues, and even after reaction with ethyl acetoacetate had proceeded for 24 hr. the yield of 2-methyl-4,5-diphenyl-3-furoic acid (V; R = R^1 = Ph, R^2 = Me) was only 7%. This particular acid is therefore better prepared by Lacey's method,[5] which involves acid-catalysed rearrangement of 3-acetyl-2,5-dihydro-4,5-diphenylfuran-2-one.

The pK_a values in 50% aqueous ethanol for the various 2,4,5-trialkyl-3-furoic acids were all found to lie between 6·7 and 7·0. These acids were therefore weaker than 2,4,5-trimethyl-3-furoic acid (pK_a 6·62 in 50% aqueous ethanol or 4·99 in water), which in turn was weaker than 3-furoic acid and its 2-methyl and 2,5-dimethyl derivatives (pK_a in water 3·95, 4·53, and 4·64, respectively [6]). Evidently, introduction of alkyl substituents into any position of the ring of 3-furoic acid reduces the acid strength whereas, as a result of steric inhibition of resonance, benzoic acid is strengthened by alkylation in one or both *ortho*-positions.[7] Replacement of an alkyl substituent in a 2,4,5-trialkyl-3-furoic acid by phenyl, especially in the 2-position, was found to increase the acid strength.

Treatment of the trisubstituted 3-furoic acids with thionyl chloride gave the corresponding carboxylic acid chlorides, which were then treated with 6-amino-penicillanic acid in either aqueous or anhydrous medium to give 2,4,5-trisubstituted 3-furylpenicillins (II). Typical procedures for 2,4,5-trimethyl- and 4,5-dimethyl-2-phenyl-3-furylpenicillin have been described in a patent.[3] The penicillins were not purified completely, but were pure enough for evaluation of their antibacterial activity. They all showed fair activity *in vitro* against streptococci and non-penicillinase-producing strains of staphylococci, but only those containing the bulky phenyl group in the 2- or 4-position were sufficiently stable towards penicillinase to show good activity against " resistant " staphylococci.

We have previously reported precisely parallel conclusions on penicillinase susceptibility amongst 3,5-disubstituted 4-isoxazolylpenicillins (I), the best activity against penicillinase-producing staphylococci being found when one of the *ortho*-substituents was methyl and the other phenyl or chlorophenyl.[2] Turning our attention to 2,4-disubstituted 3-furylpenicillins (II; R^1 = H), we therefore determined to introduce similar pairs of *ortho*-substituents into the furan nucleus.

Condensation of acetol (III; R = Me, R^1 = H) with ethyl benzoylacetate in ethanolic zinc chloride gave, after saponification of the intermediate ester (IV), 4-methyl-2-phenyl-3-furoic acid (V; R = Me, R^1 = H, R^2 = Ph). A similar reaction with ethyl *p*-chlorobenzoylacetate gave the acid (V; R = Me, R^1 = H, R^2 = C_6H_4Cl-*p*), but in both cases the yield was small. The relatively slow reaction with aroylacetic esters probably permits more extensive decomposition of the acetol than can occur during condensation with the more reactive acetoacetic ester, which is reported [4] to give 2,4-dimethyl-3-furoic acid in high yield. An attempt to employ chloroacetone instead of acetol in the reaction with ethyl benzoylacetate gave none of the desired furoic acid.

Phenacyl alcohol (III; R = Ph, R^1 = H) underwent condensation with ethyl acetoacetate in the usual way to give, after saponification of the intermediate ester, 2-methyl-4-phenyl-3-furoic acid (V; R = Ph, R^1 = H, R^2 = Me). In a similar preparation of the *p*-chloro-derivative (V; R = C_6H_4Cl-*p*, R^1 = H, R^2 = Me) it proved more convenient to use *p*-chlorophenacyl acetate rather than the alcohol, whilst the yield was improved by increasing both the reaction time and the proportion of ethyl acetoacetate.

Treatment of *o*-chlorobenzoyl chloride with ethereal diazomethane, and subsequent hydrolysis with hot dilute sulphuric acid, gave a mixture of *o*-chlorophenacyl alcohol (III; R = C_6H_4Cl-*o*, R^1 = H) and *o*-chlorophenacyl chloride, which proved difficult to separate. The crude mixture was therefore condensed with ethyl acetoacetate in the presence of zinc

[5] R. N. Lacey, *J.*, 1954, 822.
[6] W. E. Catlin, *Iowa State Coll. J. Sci.*, 1935, **10**, 65.
[7] H. C. Brown, D. H. McDaniel, and O. Hafliger in " Determination of Organic Structures by Physical Methods," ed. E. A. Braude and F. C. Nachod, Academic Press, New York, 1955, p. 603.

chloride, and the resulting furoic ester was separated by distillation from unchanged *o*-chlorophenacyl chloride. Reaction of the latter with sodium acetate gave *o*-chlorophenacyl acetate, which was then condensed with ethyl acetoacetate to give a further quantity of furoic ester. Saponification of the ester gave unexpectedly a mixture of two isomeric acids, which were separated, with some difficulty, by fractional crystallisation. The ultraviolet spectrum of the more abundant constituent was consistent with the expected structure (V; R = C_6H_4Cl-*o*, R^1 = H, R^2 = Me), but the second component absorbed at longer wavelength and was formulated as 5-*o*-chlorophenyl-2-methyl-3-furoic acid (V; R = H, R^1 = C_6H_4Cl-*o*, R^2 = Me). The second isomer presumably arose as a result of partial rearrangement of *o*-chlorophenacyl alcohol (III; R = C_6H_4Cl-*o*, R^1 = H) to *o*-chlorophenylglycolaldehyde (III; R = H, R^1 = C_6H_4Cl-*o*), possibly by way of the common enol, during heating with zinc chloride.

The new disubstituted 3-furoic acids were converted into acid chlorides by treatment with thionyl chloride, temperatures in excess of 50—60° being avoided when the sensitive 5-position was unsubstituted. Subsequent reaction with 6-aminopenicillanic acid as described for the trisubstituted analogues gave the corresponding disubstituted 3-furylpenicillins. The penicillin derived from the relatively unhindered 5-*o*-chlorophenyl-2-methyl-3-furoic acid differed from the others in being susceptible to hydrolysis by staphylococcal penicillinase, thus providing confirmatory evidence for the structure of the parent acid. By contrast, all five 2,4-disubstituted 3-furylpenicillins inhibited penicillinase-producing staphylococci *in vitro* at concentrations less than 1 μg./ml., so that they were nearly as active as their immediate analogues in the isoxazole series.

2,4,5-Trisubstituted 3-furoic acids (V)

No.	R = R^1	R^2	Reflux time (hr.)	Yield (%)	M. p.	Found (%) C	Found (%) H	Formula	Reqd. (%) C	Reqd. (%) H	pK_a *	$\lambda_{max.}$ (mμ) (ε in parentheses)
1	Me	Et	4	33	84—85°	64·2	7·2	$C_9H_{12}O_3$	64·3	7·2	6·76 ± 0·04	259 (3930)
2	Me	Pr^i	6	53	139—140	66·3	7·7	$C_{10}H_{14}O_3$	65·9	7·7	6·89 ± 0·03	259 (4240)
3	Me	Ph	2·5	26	154—156	72·2	5·8	$C_{13}H_{12}O_3$	72·2	5·6	5·70 ± 0·02	297 (17,820)
4 †	Et	Me	4	50	107	65·8	8·0	$C_{10}H_{14}O_3$	65·9	7·7	6·84 ± 0·06	259 (3500)
5	Et	Ph	4	13	97—98	73·6	6·6	$C_{15}H_{16}O_3$	73·8	6·6	5·85 ± 0·01	297 (18,300)
6 †	Pr	Me	4	38	62—63	68·8	8·9	$C_{12}H_{18}O_3$	68·6	8·6	6·96 ± 0·03	259 (3570)
7 †	Ph	Me	24	7	213—214	77·6	5·1	$C_{18}H_{14}O_3$	77·7	5·1	6·36 ± 0·03	284 (17,720)

* In 50% aqueous ethanol. † Previously prepared by a different method.[5]

Experimental

2,4,5-*Trisubstituted* 3-*Furoic Acids*.—The compounds listed in the Table were prepared by the following general procedure. The appropriate acyloin (0·4 mole) and β-oxo-acid ethyl ester (0·44—0·56 mole) in 95% ethanol (60 ml.) containing powdered anhydrous zinc chloride (40 g.) was heated under reflux for the period indicated, and the solution was cooled and poured into water (300 ml.). In one case (No. 7 in the Table) the crude furoic ester separated as a solid and was collected, thoroughly washed with water, and hydrolysed directly. In the remaining experiments the mixture was extracted with benzene (150 ml., then 2 × 50 ml.), and the combined benzene extracts were washed successively with water, 30% sodium hydrogen sulphite solution, 5% sodium hydroxide, dilute hydrochloric acid, and finally water again. The solution was dried ($MgSO_4$) and distilled under reduced pressure to give the crude furoic ester, which was collected over a broad boiling range. The crude ester was treated with 40% aqueous sodium hydroxide (60 to 100 ml.) and sufficient ethanol to give a clear solution after brief boiling. The mixture was heated under reflux for 3—4 hr., concentrated to remove ethanol, and acidified with concentrated hydrochloric acid. The furoic acid was collected, washed with water, and recrystallised from ethanol (No. 7), aqueous ethanol (Nos. 1, 2, 3, and 5), or aqueous methanol (Nos. 4 and 6). The yields and melting points given in the Table refer to recrystallised acids. No attempt was made to establish optimal reaction conditions for individual compounds, and it is likely that in some cases (*e.g.*, Nos. 3 and 5) an extended reaction period would improve the yield.

Ionisation constants of the acids were determined by the method of Albert and Serjeant [8] in 50% aqueous ethanol at 25°, the concentration at half-neutralisation being 0·01M for Nos. 1—6 and 0·001M for the sparingly soluble No. 7. Ultraviolet absorption spectra were determined in methanolic solution.

2,4,5-*Trisubstituted* 3-*Furamides*.—(*a*) 4,5-Dimethyl-2-phenyl-3-furoic acid (6·25 g.) was covered with thionyl chloride (20 ml.), and 1 drop of pyridine was added to initiate reaction. The mixture was heated on the steam-bath under reflux for 90 min., then distilled to give the acid chloride (4·42 g.), b. p. 105—113°/0·02 mm. A portion was then shaken with concentrated aqueous ammonia for 2 hr. to give 4,5-*dimethyl*-2-*phenyl*-3-*furamide*, which crystallised from benzene in colourless iridescent platelets, m. p. 192—193° (Found: N, 6·4. $C_{13}H_{13}NO_2$ requires N, 6·5%).

(*b*) 2,4,5-Triphenyl-3-furoic acid [5] was similarly converted *via* 2,4,5-*triphenyl*-3-*furoyl chloride*, needles from light petroleum, m. p. 97—98° (Found: C, 77·1; H, 4·4; Cl, 10·0. $C_{23}H_{15}ClO_2$ requires C, 77·0; H, 4·2; Cl, 9·9%), into 2,4,5-*triphenyl*-3-*furamide*, which after recrystallisation from benzene and then from ethanol formed colourless prisms, m. p. 209—210° (Found: C, 81·0; H, 5·3; N, 4·2. $C_{23}H_{17}NO_2$ requires C, 81·4; H, 5·0; N, 4·1%).

(*c*) 2,4,5-Trimethyl-3-furoic acid [4] with thionyl chloride alone gave 2,4,5-*trimethyl*-3-*furoyl chloride*, b. p. 39—41°/0·02 mm. (Found: Cl, 20·3. $C_8H_9ClO_2$ requires Cl, 20·5%) which with concentrated aqueous ammonia (water cooling) gave 2,4,5-*trimethyl*-3-*furamide*, colourless needles from hot water, m. p. 176—177° (Found: C, 62·5; H, 7·1; N, 9·0. $C_8H_{11}NO_2$ requires C, 62·7; H, 7·2; N, 9·1%).

4-*Methyl*-2-*phenyl*-3-*furoic Acid*.—Acetol (31 g.), ethyl benzoylacetate (90 g.), zinc chloride (60 g.), and ethanol (70 ml.) were heated under reflux on the steam-bath for 4 hr., then the solvent was removed *in vacuo*. The product was taken up in ether, washed successively with water, 2N-sodium hydroxide, and water again, dried, and distilled. A mixture of the furoic ester and unreacted β-oxo-ester was collected at 100—115°/0·1 mm. and hydrolysed with sodium hydroxide (20 g.), water (40 ml.), and ethanol (120 ml.) under reflux for 2 hr. After concentration to remove ethanol the mixture was diluted with water, washed with ether, and acidified. The precipitate of mixed acids was crystallised twice from ethanol to remove benzoic acid and give 4-*methyl*-2-*phenyl*-3-*furoic acid* (7·8 g.), m. p. 161°, $\lambda_{max.}$ 291 mμ (ε 12,900). (Found: C, 71·5; H, 5·1. $C_{12}H_{10}O_3$ requires C, 71·3; H, 5·0%).

2-p-*Chlorophenyl*-4-*methyl*-3-*furoic Acid*.—Acetol (26·9 g.), ethyl *p*-chlorobenzoylacetate (22·7 g.), zinc chloride (27 g.), and ethanol (30 ml.) were heated under reflux on the steam-bath for 6 hr., then evaporated *in vacuo*. The residue was freed from zinc salts with water, and most of the residual β-oxo-ester was destroyed by treating with potassium hydroxide (5·6 g.) in aqueous methanol for 24 hr. After removing the methanol the residual ester was extracted into ether and distilled, b. p. 114—118°/0·1 mm. This product (5·2 g.) was heated under reflux for 3 hr. with sodium hydroxide in aqueous ethanol, cooled, and acidified. The precipitate was collected and crystallised from chloroform to give a first crop (0·3 g.) of *p*-chlorobenzoic acid. The mother liquor was evaporated and the residue recrystallised twice from ethanol to give 2-p-*chlorophenyl*-4-*methyl*-3-*furoic acid*, m. p. 151—153°, $\lambda_{max.}$ 298 mμ (ε 17,000) (Found: C, 60·8; H, 4·0; Cl, 15·4. $C_{12}H_9ClO_3$ requires C, 60·9; H, 3·8; Cl, 15·0%).

2-*Methyl*-4-*phenyl*-3-*furoic Acid*.—Phenacyl alcohol (38·9 g.), ethyl acetoacetate (43 g.), zinc chloride (43 g.) and ethanol (50 ml.) were heated under reflux on the steam-bath for 4 hr., and the resulting crude ester (b. p. 85—110°/0·1 mm.) was hydrolysed as described for the 4-methyl-2-phenyl isomer to give 2-*methyl*-4-*phenyl*-3-*furoic acid* (9 g.), m. p. 171—172° (from ethanol), $\lambda_{max.}$ 246 mμ (ε 6800) (Found: C, 71·3; H, 5·2. $C_{12}H_{10}O_3$ requires C, 71·3; H, 5·0%).

4-p-*Chlorophenyl*-2-*methyl*-3-*furoic Acid*.—*p*-Chlorophenacyl acetate (50 g.), ethyl acetoacetate (61·2 g.), zinc chloride (64 g.), and ethanol (75 ml.) were heated under reflux on the steam-bath for 11 hr., and the mixture was worked up as described for the 2-*p*-chlorophenyl-4-methyl isomer. The resulting 4-p-*chlorophenyl*-2-*methyl*-3-*furoic acid* (23 g.), crystallised from benzene, had m. p. 191—192°, $\lambda_{max.}$ 214 mμ (ε 17,600), 249 mμ (ε 9650) (Found: C, 61·0; H, 3·9; Cl, 15·5. $C_{12}H_9ClO_3$ requires C, 60·9; H, 3·8; Cl, 15·0%).

4- *and* 5-o-*Chlorophenyl*-2-*methyl*-3-*furoic Acid*.—*o*-Chlorobenzoyl chloride (78 g.) was added to an excess of diazomethane in ice-cold ether. Next morning the solvent and excess of reagent were removed, and the residue was heated on the steam-bath with N-sulphuric acid

[8] A. Albert and E. P. Serjeant, "Ionisation Constants of Acids and Bases," Methuen, London, 1962.

(1 l.) until gas evolution ceased. The oil was dissolved in ether, washed with sodium hydrogen carbonate solution, dried, and distilled. The resulting mixture of *o*-chlorophenacyl alcohol and *o*-chlorophenacyl chloride (39 g., b. p. 100—140°/12 mm.) was heated under reflux with ethyl acetoacetate (60 g.), zinc chloride (63 g.), and ethanol (75 ml.) for 12 hr. After removing solvent and zinc salts the residue was distilled to give a first fraction (b. p. 74—82°/0·2 mm.) of *o*-chlorophenacyl chloride (this was then heated under reflux with sodium acetate in ethanol to give *o*-chlorophenacyl acetate, which in turn was condensed with further ethyl acetoacetate) and a second fraction (b. p. 100—110°/0·2 mm.) containing two isomeric furoic esters. The mixed esters were subjected to partial hydrolysis with potassium hydroxide (1 equiv.) in aqueous methanol at room temperature for 24 hr. The methanol was evaporated, and the unhydrolysed ester was extracted into ether. Acidification of the aqueous phase gave a small quantity of acid which crystallised from chloroform as needles of 5-o-*chlorophenyl*-2-*methyl*-3-*furoic acid*, m. p. 217°, $\lambda_{max.}$ 281 mμ (ε 23,000) (Found: C, 61·1; H, 3·8; Cl, 15·2. $C_{12}H_9ClO_3$ requires C, 60·9; H, 3·8; Cl, 15·0%).

Evaporation of the ether extracts gave an oil which was subjected to more vigorous hydrolysis with an excess of ethanolic potassium hydroxide under reflux for 3 hr. Fractional crystallisation of the acidic product from chloroform gave a further quantity of 5-*o*-chlorophenyl-2-methyl-3-furoic acid as the more soluble component, but the major product separated as square plates of 4-o-*chlorophenyl*-2-*methyl*-3-*furoic acid*, m. p. 207—208°, $\lambda_{max.}$ 245 mμ (ε 6100) (Found: C, 60·9; H, 3·8; Cl, 15·1. $C_{12}H_9ClO_3$ requires C, 60·9; H, 3·8; Cl, 15·0%).

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