The Synthesis of Malonimide Derivatives as Potential Penicillin Analogs

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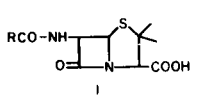
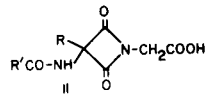
Malonimidoacetic acid derivatives (VIII, XIII) were synthesized as a potential penicillin analogs, but they failed to inhibit the growth of bacteria when tested *in vitro* against a range of Gram-positive and Gram-negative microorganisms.

It is assumed that penicillin (I) exerts its antibiotic action by interfering with the formation of certain cross-linking bonds necessary to complete and consolidate the cell wall of growing bacteria (1). The penicillin molecule may be regarded as an acyl dipeptide in which the peptide bond forming part of the fused β-lactam ring shows unusual chemical activity and is capable of performing acylation reactions. It has therefore been postulated that penicillin may act by acylating the active site of certain bacterial enzymes (2). Hypothetically suitable activated synthetic dipeptides might therefore act as antibacterial agents. The synthesis of a few activated dipeptides was reported (3a-c) but when compared with penicillin their antibacterial activity was very low. More pronounced antibacterial activity could, however, be expected from malonimide derivatives (II), which do possess a much more activated peptide bond.



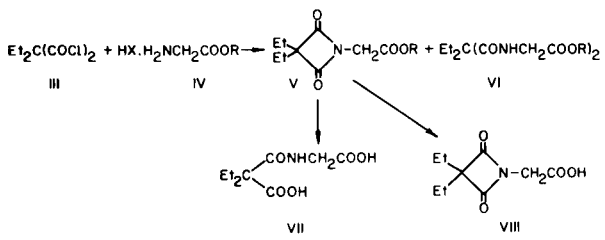
I



II

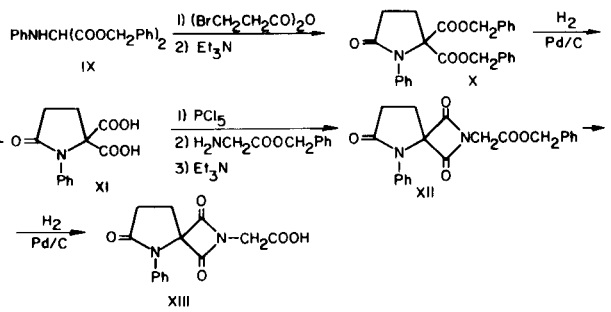
Three routes lead to the preparation of malonimides (4); (a) cycloaddition of ketenes to isocyanates (5,6); (b) ring closure of malonamidic acids (7); (c) condensation of malonyl chlorides with primary amines (5). It was felt that the condensation reaction between malonyl chloride and primary amines would constitute the most suitable method. A literature search revealed (5) that this reaction, when performed with aliphatic amines, led exclusively to the formation of malondiamides instead of malonimides. Therefore, suitable reaction conditions between malonyl chlorides and amino esters had first to be established.

Diethylmalonyl chloride (III) reacted with ethyl glycinate hydrochloride in the presence of more than 3 equivalents of triethylamine to give both malondiamide in 22% yield and malonimide in 12% yield. It had been reported (5) that surprisingly enough, the acidic treatment of a malonimide derivative bearing an acetyl amide group leads to the hydrolysis of this group without cleaving the imide ring. However, no reaction took place when ethyl 3,3-diethylmalonimidoacetate (V, R = Et) was treated with hydrochloric acid at 20° for 20 hours. When the reaction mixture was heated to 50° both ester and imide were hydrolized to give diethylmalonylglycine (VII). Benzyl diethylmalonimidoacetate (V, R = benzyl), was prepared by the same method, and then its hydrogenolysis over 5% palladium-on-charcoal at atmospheric pressure yielded the free acid (VIII).



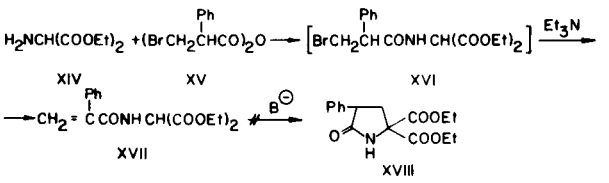
After establishing suitable reaction conditions for the preparation of malonimidoacetic acid derivatives, the synthesis of *N*-acyl aminomalonimidoacetic acid was successfully performed.

Dibenzyl *N*-phenylaminomalonate (IX) reacted with β-bromopropionic anhydride and then with triethylamine to give 1-phenyl-5,5-carbobenzoxy-2-pyrrolidone (X), which was catalytically debenzylated to the free malonic acid derivative (XI). The corresponding malonyl chloride was prepared by reacting the free acid with phosphorus pentachloride. The crude acid chloride was condensed with benzyl glycinate to give the malonimide derivative (XII) in 15% yield. The free acid (XIII) was obtained in 90% yield by catalytic hydrogenation.

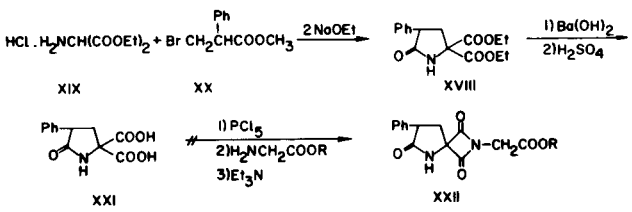


Both malonimidoacetic acids (VIII, XIII) failed to inhibit the growth of bacteria when tested *in vitro* against a range of Gram-positive and Gram-negative microorganisms. It should, however, be kept in mind that there is a marked decrease in antibacterial activity of penicillin when the nitrogen atom of the amido side chain becomes tertiary. In order to attain closer structural relationship to penicillin G (I, R = benzyl), while constituting a secondary amide, it seemed reasonable to synthesize an isomer of the acyl amido malonimide derivative (XIII) in which the phenyl group is at position 3 of the pyrrolidone ring (XXII, R = H).

The route which had led to the successful synthesis of 1-phenyl-5,5-dicarbobenzoxy-2-pyrrolidone (X) was first chosen for the preparation of 3-phenyl-5,5-carboethoxy-2-pyrrolidone (XVIII). Diethyl aminomalonate (XIV) reacted with β-bromo-α-phenylpropionic anhydride (XV) to give the intermediate (XVI) which after treatment with triethylamine did not yield the expected γ-lactam (XVIII) but the open chain compound (XVII). The phenyl group seems to facilitate the abstraction of the proton from a position α to the amide rather than α to the two carboethoxy groups. No Michael addition took place when XVII was refluxed with strong base ("triton B" or sodium ethoxide).



When diethyl aminomalonate hydrochloride (XIX) reacted with 2 moles of sodium ethoxide and afterwards with methyl β-bromo-α-phenylpropionate (XX), *C*-alkylation occurred and subsequently the desired γ-lactam (XVIII) was formed in 40% yield. 3-Phenyl-5,5-dicarboxy-2-pyrrolidone (XXI) was obtained by hydrolysis of the diester (XVIII) in hot barium hydroxide solution (8). When the malonic acid derivative (XXI) was subjected to reaction conditions favoring the formation of malonimides, no imide (XXII) could be isolated. The amido hydrogen probably participates in the reaction thus preventing the formation of the desired acylaminomalonimidoacetic acid derivative (XXII).



EXPERIMENTAL

All melting points are uncorrected. The nmr spectra were determined on a Varian A-60 instrument with TMS as internal standard. Chemical shifts are given in δ (ppm), and J in cps. Silica gel HF_{254} (Merck) was used for column chromatography.

Ethyl Diethylmalonimidoacetate (V).

A solution of triethylamine (9 g., 0.09 mole) in dry dioxane (25 ml.) was added dropwise during 1.5 hours to a well-stirred mixture of diethyl malonyl chloride III (4.925 g., 0.025 mole) and ethyl glycinate hydrochloride IV (3.475 g., 0.025 mole) in dry dioxane (50 ml.). The mixture was subsequently refluxed for 2.5 hours, cooled, filtered from triethylamine hydrochloride and evaporated to dryness. The residue was chromatographed on a silica gel column, using mixtures of ethyl acetate and benzene as eluant. The first fraction to be eluted was ethyl diethylmalonimidoacetate V (1 ethyl acetate-4 benzene) which was purified by distillation to yield 0.7 g. (12%), b.p. 104-106° (0.3 mm); ir 1890 cm^{-1} (malonimide); nmr (deuteriochloroform): δ 4.21 (q, J = 7, 2H $-OCH_2CH_3$), 3.98 (s, 2H $-NCH_2CO-$), 1.77 (q, J = 7, 4H 2-CH_2CH_3), 1.30 (t, J = 7, 3H $-OCH_2CH_3$), 1.03 (t, J = 7, 6H 2 $-CH_2CH_3$).

Anal. Calcd. for $C_{11}H_{17}NO_4$: C, 58.14; H, 7.54; N, 6.16. Found: C, 58.28; H, 7.85; N, 6.06.

The second fraction to be eluted (3 ethyl acetate-1 benzene) was diethyl diethylmalonyldiglycinate VI (R = ethyl) which was recrystallized from benzene-petroleum ether (60-80°) to yield 1.8 g. (22%), m.p. 109-110°; nmr (deuteriochloroform): δ 8.03 (broad t, J = 5.5, 2H 2-$NHCH_2$- which disappeared when treated with deuterium oxide and deuterium hydrochloride), 4.21 (q, J = 7, 2-OCH_2CH_3), 4.04 (d, J = 5, 5, 2-$NHCH_2$- which turned to s when treated with deuterium oxide and deuterium hydrochloride), 1.95 (q, J = 7, 4H 2-CH_2CH_3), 1.23 (t, J = 7, 6H 2-OCH_2CH_3), 0.90 (t, J = 7, 2-CH_2CH_3).

Anal. Calcd. for $C_{15}H_{26}N_2O_6$: C, 54.53; H, 7.93; N, 8.48. Found: C, 54.36; H, 7.99; N, 8.22.

A more convenient procedure to purify V (R = ethyl) consists in distilling the residue before the chromatography, and to chromatograph the fraction boiling at 100-140° 0.1 mm over silica gel, using benzene as eluant.

Diethylmalonylglycine (VII).

Ethyl diethylmalonimidoacetate V (R = ethyl) (0.45 g., 0.002 mole) and 3*N* hydrochloric acid (8 ml.) were stirred at 50° for 20 hours until almost all the organic layer was dissolved. The mixture was washed with chloroform and evaporated to dryness.

The residue was recrystallized from ethyl acetate-benzene mixture to yield 0.15 g. (35%), m.p. 168-169°; nmr (deuterioacetonitrile) shows the pattern of -CONHCH$_2$CO- group; δ 7.8-7.2 (broad peak -*NH*CH$_2$- which disappeared when treated with deuterium oxide and deuterium hydrochloride), 6.00 (d, J = 5.5, 2H -HNC*H*$_2$- turned to s with deuterium oxide and deuterium hydrochloride).

Anal. Calcd. for $C_9H_{15}NO_5$: C, 49.76; H, 6.96; N, 6.45. Found: C, 49.92; H, 6.97; N, 6.42.

Benzyl Diethylmalonimidoacetate (V) (R = Benzyl).

The reaction was carried out as described for V (R = ethyl), using benzyl glycinate-*p*-toluenesulfonate as one of the reactants. The filtered solution was evaporated and the residue distilled. The fraction collected at 120-170° at 0.1 mm was chromatographed on a silica gel column, using benzene as eluant, to yield 0.85 g. (15%) of V (R = benzyl): m.p. 56-57° (petroleum ether 40-60°); ir (nujol) 1880 cm^{-1} (malonimide); nmr (deuteriochloroform): δ 7.31 (s, 5H -CH$_2$*Ph*), 5.15 (s, 2H -OC*H*$_2$Ph), 4.07 (s, 2H -NCH$_2$CO-), 1.82 (q, J = 7, 4H 2-C*H*$_2$CH$_3$), 0.99 (t, J = 7, 6H 2-CH$_2$C*H*$_3$).

Anal. Calcd. for $C_{16}H_{19}NO_4$: C, 66.42; H, 6.62; N, 4.84. Found: C, 66.31; H, 6.58; N, 4.73.

Diethylmalonimidoacetic Acid (VIII).

Hydrogenolysis of V (R = benzyl) (0.578 g., 0.002 mole) in ethyl acetate (15 ml.) was carried out for 30 minutes at room temperature and atmospheric pressure using prehydrogenated 5% palladium on charcoal (0.3 g.) as a catalyst. The filtered solvent from catalyst was evaporated and the residue recrystallized from methyl cyclohexane-benzene to yield 0.3 g. (75%), m.p. 79-81°; ir (nujol) 1890 cm^{-1}; nmr (deuteriochloroform): δ 10.45 (s, 1H -COOH disappeared with deuterium oxide), 4.17 (s, 2H -NCH$_2$CO-), 1.82 (q, J = 7.5, 4H 2-C*H*$_2$CH$_3$), 1.02 (t, J = 7.5, 6H 2-CH$_2$C*H*$_3$).

Anal. Calcd. for $C_9H_{13}NO_4$: C, 54.26; H, 6.58; N, 7.03. Found: C, 54.27; H, 6.70; N, 7.06.

1-Phenyl-5,5-carbobenzoxy-2-pyrrolidone (X).

A mixture of dibenzyl anilinomalonate (9) IX (5.65 g., 0.015 mole) and β-bromopropionic anhydride (5.76 g., 0.02 mole) was stirred at 80° for 2 hours, then cooled and treated with water and benzene. The organic layer was washed with water, dried over magnesium sulfate and evaporated. The oily residue was dissolved in dry benzene (120 ml.) and treated with triethylamine (4 ml.). Triethylamine hydrobromide precipitated at once and the reaction mixture was allowed to stand for 2 days. The filtered solution was washed successively with 5% sodium carbonate, water, 1*N* hydrochloric acid, water and dried over magnesium sulfate and evaporated to dryness. The oily residue was triturated with methylcyclohexane and then recrystallized from methylcyclohexane-benzene to yield 4.2 g. (66%), m.p. 99-100°; nmr (deuteriochloroform): δ 7.40-6.96 (m, 15H 3-Ph), 5.05 (s, 4H 2-OC*H*$_2$Ph), 2.63 (m, 4H -COCH$_2$CH$_2$C-).

Anal. Calcd. for $C_{26}H_{23}NO_5$: C, 72.71; H, 5.40; N, 3.26. Found: C, 72.54; H, 5.41; N, 3.02.

1-Phenyl-5,5-carboxy-2-pyrrolidone (XI).

Hydrogenolysis of X (3.87 g., 0.09 mole) in ethyl acetate (50 ml.) was carried out for 1 hour at room temperature and atmospheric pressure using 5% palladium on charcoal (1 g.) as catalyst. The catalyst was filtered and washed with ethanol, the combined solutions were evaporated to dryness and the residue was recrystallized from ethanol-ethyl acetate to yield 2.25 g. (100%), m.p. 183-185° dec.; nmr (DMSO-d$_6$): δ 10.38 (broad s, 2H 2-COOH), 7.32 (s, 5H -Ph), 2.53 (s, -CH$_2$CH$_2$-).

Anal. Calcd. for $C_{12}H_{11}NO_5$: C, 57.83; H, 4.45; N, 5.62. Found: C, 57.57; H, 4.46; N, 5.58.

2-Carbobenzoxymethyl-5-phenyl-2,5-diazaspiro[3,4]octane-1,3,6-trione (XII).

The malonic acid derivative XI (4.9 g., 0.02 mole) and phosphorus pentachloride (8.32 g., 0.04 mole) were stirred for 20 hours at 20°. Phosphorus oxychloride which was formed during the reaction was then distilled off. The crude acid chloride was dissolved in dry dioxane (100 ml.) and benzyl glycinate-*p*-toluenesulfonate (6.74 g., 0.02 mole) was added to it in one portion. After stirring the mixture for 30 minutes, a solution of triethylamine (10 ml.) in dry dioxane (50 ml.) was added dropwise during 3 hours, and the mixture then refluxed for 2 hours. The reaction mixture was cooled, filtered and the solution evaporated to dryness. Chromatography over silica gel using chloroform as eluant yielded 1.1 g. (15%) of XII, m.p. 166-167° (methylcyclohexane-benzene); ir (nujol) 1890 cm^{-1} (malonimide); nmr (deuteriochloroform): δ 7.5-7.1 (m, 15H 3-Ph), 5.13 (s, 2H -OC*H*$_2$Ph), 4.01 (s, 2H -NCH$_2$CO-), 2.64 (m, 4H, -COCH$_2$CH$_2$C-).

Anal. Calcd. for $C_{21}H_{18}N_2O_5$: C, 66.66; H, 4.79; N, 7.40. Found: C, 66.57; H, 4.85; N, 7.32.

2-Carboxymethyl-5-phenyl-2,5-diazaspiro[3,4]octane-1,3,6-trione (XIII).

Hydrogenolysis of XII (0.9 g., 0.00235 mole) in ethyl acetate (45 ml.) was carried out for 1 hour at room temperature and atmospheric pressure using 5% palladium on charcoal (0.5 g.) as a catalyst. The filtered solution was evaporated to dryness, and the oily residue solidified when triturated with methylcyclohexane. Recrystallization from methylcyclohexane-ethyl acetate afforded 0.62 g. (90%) of XIII, m.p. 181° (sintering at 163°). On repeated recrystallization the m.p. remain unchanged; ir (nujol) 1900 cm^{-1} (malonimide); nmr (DMSO-d$_6$): δ 7.6-7.1 (m, 5H -NPh), 4.12 (s, 2H -NC*H*$_2$COOH), 2.61 (s, -COCH$_2$CH$_2$C-).

Anal. Calcd. for $C_{14}H_{12}N_2O_5$: C, 58.33; H, 4.20; N, 9.72. Found: C, 58.46; H, 4.26; N, 9.69.

Diethyl *N*-(2-Phenylacryloyl)aminomalonate (XVII).

A solution of β-bromo-α-phenylpropionic acid (10) (13.8 g., 0.06 mole) in dry ether (75 ml.) was added dropwise to a stirred and cooled (5-10°) solution of *N*,*N*′-dicyclohexylcarbodiimide (6.7 g., 0.033 mole) in dry ether (75 ml.). The mixture was stirred overnight at 20° and then filtered. The filtrate was evaporated to dryness to yield 12.8 g. of crude XV.

A solution of diethyl aminomalonate (4.8 g., 0.0275 mole) and of XV (12.8 g.) in dry benzene (100 ml.) was refluxed for 2 hours. Triethylamine (8 ml.) was added dropwise with stirring at 10° during 1 hour and the mixture was left overnight. The filtered solution was washed successively with 5% sodium carbonate and with water, dried over magnesium sulfate and evaporated to dryness. The residue was distilled and the fraction boiling at 175-185° (0.2 mm) was redistilled at 160-164° 0.1 mm to yield 5.5 g. (65%) of XVII, b.p. 160-164° (0.1 mm); ir (neat) 1750 cm^{-1} (esters), 1670 cm^{-1} (unsaturated amide); nmr (carbon tetrachloride): δ 7.5-7.1 (m, 5H -Ph), 6.66 (broad d J = 7, 1H -CO*NH*CH- which disappeared when treated with deuterium oxide and deuterium chloride), 6.02 (d J = 1 H–C = C–CO), 5.60 (d J = 1 1H H–C = C–Ph), 5.08 (d J = 7, -NHC*H*- turned to s when treated with deuterium oxide and deuterium chloride), 4.18 (q, J = 7, 4H 2-OC*H*$_2$CH$_3$), 1.22 (t, J = 7, 6H 2-OCH$_2$C*H*$_3$).

Anal. Calcd. for $C_{16}H_{19}NO_5$: C, 62.94; H, 6.27; N, 4.59. Found: C, 62.45; H, 6.15; N, 4.62.

3-Phenyl-5,5-carboethoxy-2-pyrrolidone (XVIII).

Methyl β-bromo-α-phenylpropionate (XX) (b.p. 80° 0.15 mm) was prepared in 95% yield by diazomethane-esterification of the corresponding acid. Diethyl aminomalonate hydrochloride (XIX) (3.165 g., 0.015 mole) was added in one portion to a cold solution of sodium (0.59 g., 0.03 g.-atom) in dry ethanol (20 ml.), and the mixture was then stirred thoroughly. A solution of XX (3.65 g., 0.015 mole) in dry ethanol (10 ml.) was added dropwise to the stirred reaction mixture during 15 minutes, which was subsequently refluxed for 3 hours. The cold mixture was evaporated to dryness, treated with water and ethyl acetate and the organic layer was dried over magnesium sulfate and evaporated. The fraction collected at 170-185° 0.1 mm from the distillation of the residue was recrystallized from cyclohexane to yield 1.7 g. (40%) of XVIII, m.p. 89-90°; nmr (carbon tetrachloride): δ 8.24 (s, 1H -CO*NH*C-, disappeared when treated with deuterium oxide), 7.22 (s, 5H -Ph), 4.21 (q, J = 7, 2H -O*CH*$_2$CH$_3$), 4.19 (q, J = 7, 2H -O*CH*$_2$CH$_3$), 1.23 (t, J = 7, 3H -OCH$_2$*CH*$_3$), 1.21 (t, J = 7, 3H -OCH$_2$*CH*$_3$), 3.92-2.30 (ABX pattern 3H -CHCH$_2$-).

Anal. Calcd. for $C_{16}H_{19}NO_5$: C, 62.94; H, 6.27; N, 4.59. Found: C, 63.15; H, 6.20; H, 4.40.

3-Phenyl-5,5-dicarboxy-2-pyrrolidone (XXI).

Compound XVIII (0.61 g., 0.002 mole) was added to a solution of barium hydroxide (0.63 g., 0.002 mole) in water (10 ml.) and the mixture was stirred at 90-100° for 2 hours. A solution of sulfuric acid (0.2 g., 0.002 mole) in water (5 ml.) was added dropwise to the stirred and cooled (5-10°) reaction mixture during 15 minutes. After filtration from the precipitated barium sulfate, the solution was evaporated to dryness. The solid residue was treated with methylcyclohexane and filtered. The product XXI was dissolved in a mixture of ethyl acetate and ethanol, filtered from some impurities and crystallized out when the solvents were partly evaporated. This procedure was repeated three times to raise the m.p. of the compound from 144-146° to 155-156° dec. and to yield 0.39 g. (78%).

Anal. Calcd. for $C_{12}H_{11}NO_5$: C, 57.83; H, 4.45; N, 5.62. Found: C, 57.65; H, 4.47; N, 5.65.

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