Synthesis of a β-Lactam Related to the Cephalosporins

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An improved synthesis of pyrrolidine-2,4-dione (tetramic acid) is described. The addition of its 3-diazo-derivative to dibenzyl acetylenedicarboxylate gave a mixture of (*E*)- and (*Z*)-adducts. U.v. irradiation of the mixture promoted both the photolytic Wolff rearrangement and the isomerisation of the (*Z*)- to the (*E*)-isomer, so that in the presence of t-butyl carbazate a single β-lactam (8) was obtained. Transformation of the t-butoxycarbonylhydrazide into a phenylacetamido side-chain was accomplished by a previously used procedure, and catalytic hydrogenolysis of the benzyl ester groups gave 2-(2-oxo-3-phenylacetamidoazetidin-1-yl)maleic acid (3; R = H).

THE antibacterial properties of the penicillins (1) and the cephalosporins (2) stem from their ability to inhibit the transpeptidase responsible for cross-linking the peptidoglycan chains used in bacterial cell wall synthesis. Elucidation of the structure and biosynthesis of bacterial cell walls and their peptidoglycan precursors led to the suggestion that the acyldipeptide function of the antibiotics resembles features of the peptidoglycan chains by which the transpeptidase recognises its natural substrate. When the antibiotic binds to the enzyme, the β-lactam irreversibly inhibits it.[1] The β-lactam however needs to be especially reactive and in the penicillins (1) this is achieved largely by ring strain induced by fusion with the thiazolidine ring. In the cephalosporins (2) the β-lactam appears to be activated electronically. The ineffectiveness of dethiopenicillin [2] and Δ^2-cephalosporins [3] support this hypothesis, as does the crystallographic evidence.[4] In order to see if electronic activation of a suitably substituted β-lactam is sufficient to generate a molecule with antibacterial properties, the β-lactam (3; R = H) was synthesised. The route adopted for this synthesis incorporated the recently developed photolytic Wolff rearrangement for the generation of the β-lactam.[5]

Ethyl glycinate and ethyl hydrogen malonate were coupled with dicyclohexylcarbodi-imide and the product was cyclised (and transesterified) with sodium methoxide in benzene. Base-catalysed demethoxycarbonylation of the product by the method of Isowa and Ohta [6] was, however, unsatisfactory in our hands, as condensation products were obtained. Instead the desired reaction could be effected in refluxing xylene solution in a manner similar to the removal of a t-butoxycarbonyl group from a tetramic acid.[5] In dimethyl sulphoxide at 50° about 50% of the methyl ester was demethoxycarbonylated in 1 h as shown by the n.m.r. spectrum. The best preparative method, however, was to suspend the ester in acetonitrile and heat under reflux until the mixture became clear (about 2 h). Pyrrolidine-2,4-dione (tetramic acid) (4) was isolated quantitatively from this solution. This remarkably ready transformation allowed the overall conversion of ethyl glycinate into the pyrrolidine-2,4-dione (4) to be achieved in 65% yield. A similar de-ethoxycarbonylation was observed by Harris *et al.* when 5-s-butyltetramic acid was obtained rather than the expected 3-ethoxycarbonyl-5-s-butyltetramic acid from the base-catalysed cyclisation of *N*-ethoxycarbonylacetylisoleucine ethyl ester.[7]

Pyrrolidine-2,4-dione (4) was readily converted into its 3-diazo-derivative (5) by base-catalysed diazo-exchange with methanesulphonyl azide (*cf.* ref. 8). Treatment of the diazo-compound (5) with sodium hydride and dibenzyl acetylenedicarboxylate gave the (*E*)- and (*Z*)-adducts, which were separated chromatographically.

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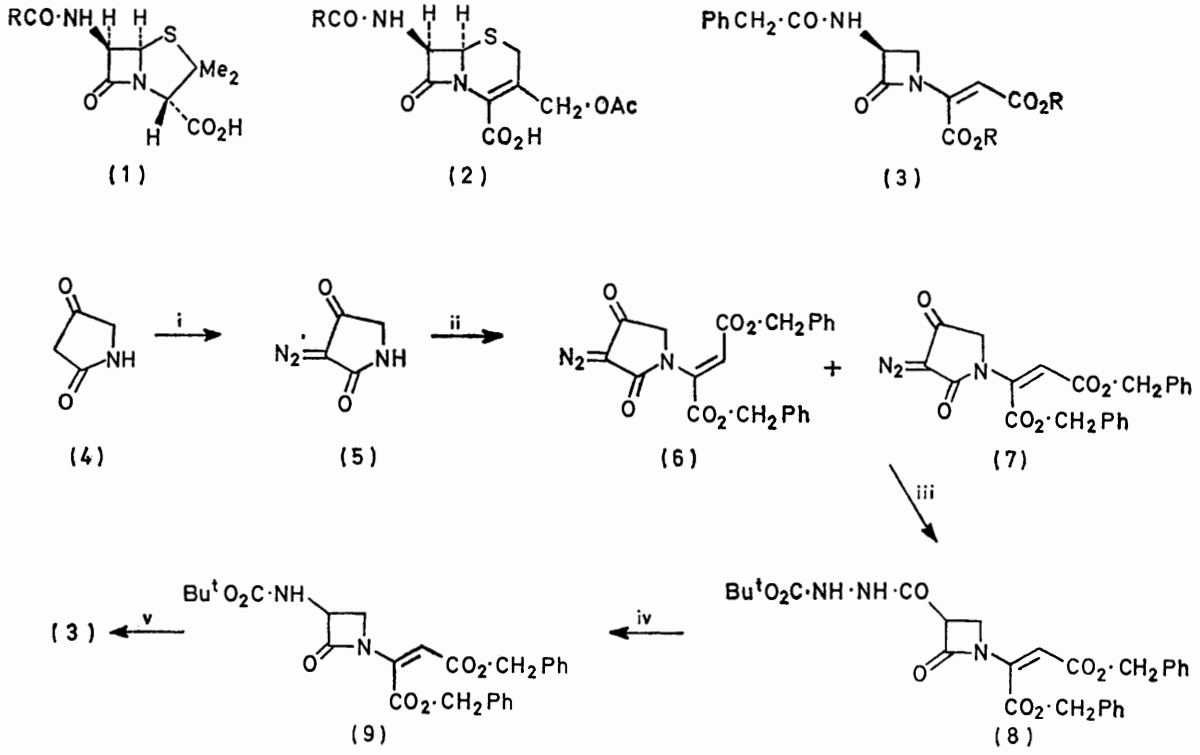
The major adduct was the (*Z*)-isomer (6), identified on the basis of its u.v. absorption spectrum [$\lambda_{max.}$ 297 nm (ε 13,000)]; the minor (*E*)-adduct (7) showed $\lambda_{max.}$ 282 nm (ε 31,100).[9] The higher extinction coefficients of these absorption bands compared with those of the 5-methyl analogues, indicates that the 5-methyl group is involved in non-bonded interaction with the chromophoric system.[5] The isomer assignments were supported by the chemical shifts of the olefinic protons [(*Z*)-isomer (6) τ 3·30; (*E*)-isomer (7) τ 4·80]. The chemical shift of the olefinic proton in substituted or unsubstituted maleate esters appears always to be at higher field than in the corresponding fumarate esters.[10]

The 5-methyl analogue of (6) has been shown to undergo photoinduced isomerisation about the double bond during photolysis of the diazo-group.[5] The mixture of (*Z*)- and (*E*)-adducts (6) and (7) was therefore irradiated in the presence of t-butyl carbazate in benzene solution. As expected only one product was isolated, the (*E*)-adduct (8).

The transformation of the t-butoxycarbonylhydrazide into the phenylacetamido side-chain was accomplished by the procedure similar to that used in previous syntheses.[11] Treatment with trifluoroacetic acid gave the unprotected hydrazide, which was converted into the acid azide by treatment with nitrous acid at 0—5° in a two-phase system. The acid azide was refluxed in dry benzene for 45 min to effect the Curtius rearrangement and the product, without isolation, was converted into the urethane (9) by refluxing with t-butyl alcohol for a further 3 h.

Deprotection of the urethane (9) with trifluoroacetic acid and phenylacetylation of the amine so formed gave the phenylacetamido-derivative (3; R = CH_2Ph). This β-lactam had an i.r. absorption band at 1790 cm^{-1}, which is in accordance with our expectation that the β-lactam should be electronically activated. Hydrogenolysis over palladium black removed the benzyl groups to give the dicarboxylic acid (3; R = H), which was characterised as its methyl ester (3; R = Me). The acid in sodium phosphate buffer at pH 7·0 showed no inhibition of the growth of *Staphylococcus aureus* (Oxford), *Alcaligines faecalis* (Bristol), or *Salmonella typhi* (Felix and Pitt) at 1 mg ml^{-1}.

A qualitative kinetic study has shown that in spite of the high frequency absorption of the β-lactam carbonyl group in the diesters (3; R = CH_2Ph or R = Me), the dicarboxylic acid (3; R = H) is not readily attacked by nucleophiles. The fact that an additional negative charge is placed on the molecule at neutral pH could be



Reagents: i, $MeSO_2N_3$–NEt_3; ii, NaH–$PhCH_2·O_2C·C{\equiv}C·CO_2·CH_2Ph$; iii, *hν*–$Bu^tO_2C·NH·NH_2$; iv, (a) $CF_3·CO_2H$, (b) $NaNO_2$–HCl, (c) heat in benzene, (d) heat with Bu^tOH; v, (a) $CF_3·CO_2H$, (b) $PhCH_2·COCl$–NEt_3, (c) Pd–H_2.

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responsible for this low susceptibility to nucleophilic attack and also the lack of antibacterial activity, but it may also be necessary for antibacterial activity that the enamine group is prevented from becoming coplanar with the β-lactam system by being constrained within a ring. Synthetic studies which should resolve this point are in progress.

EXPERIMENTAL

M.p.s were determined with a Kofler hot-stage apparatus. I.r. spectra were measured on a Perkin-Elmer 257 grating spectrometer. ^{1}H N.m.r. spectra were recorded on Perkin-Elmer R10 and R14 instruments (operating at 60 and 100 MHz, respectively) and mass spectra on an A.E.I. MS9 instrument. Microanalyses were carried out by Dr. Strauss and his staff in this laboratory. Harrington's M60 silica gel was used for column chromatography. Adsorbents used in t.l.c. and preparative layer chromatography (p.l.c.) were $HF_{254+366}$ and $PF_{254+366}$ silica gel (Merck), respectively. Anhydrous sodium sulphate was used to dry organic solutions, unless otherwise stated. Light petroleum refers to the fraction of b.p. 40—60°.

N-(*Ethoxycarbonylacetyl*)*glycine Ethyl Ester*.—To a stirred solution of glycine ethyl ester hydrochloride (35 g) in dichloromethane (500 ml) at 0 °C was added triethylamine (26 g). To this solution were added ethyl hydrogen malonate (33 g) in dichloromethane (200 ml) and dicyclohexylcarbodi-imide (52 g) in dichloromethane (100 ml). The mixture was stirred at 0 °C for 15 min. and then for 2 h at room temperature. The suspension was filtered and the precipitate washed with dichloromethane. The combined filtrates were washed with water, dried, and evaporated to give a solid mass which was dissolved in acetone; the solution was filtered and concentrated to about 100 ml. Addition of light petroleum afforded *N*-(ethoxycarbonylacetyl)-glycine ethyl ester as white needles (42 g, 76%), m.p. 71·5—72° (lit.,[6] 64—65°), $\nu_{max.}$ (CCl_4) 3440 (amide), 1685 (amide), and 1740 cm^{-1} (ester); τ (CCl_4) 2·60br (1H, s, NH), 5·82 (4H, q, *J* 8 Hz, ·O·C*H*$_2$·CH$_3$), 6·05 (2H, d, *J* 6 Hz, ·NH·C*H*$_2$), 6·78 (2H, s, ·CO·CH$_2$·CO), and 8·71 (6H, t, *J* 8 Hz, ·O·CH$_2$·C*H*$_3$) (Found: C, 49·5; H, 6·9; N, 6·4. Calc. for $C_9H_{15}NO_5$: C, 49·7; H, 6·9; N, 6·4%).

Methyl 2,4-*Dioxopyrrolidine*-3-*carboxylate*.—To a solution of sodium methoxide [from sodium (0·877 g) in dry methanol (30 ml)] was added a solution of the foregoing ester (8·27 g) in dry benzene (200 ml). The mixture was refluxed for 6 h under nitrogen, cooled, and diluted with water, and the two phases were separated. The organic layer was washed twice with water. The combined aqueous layers were carefully acidified with conc. hydrochloric acid and the dioxopyrrolidine ester slowly precipitated as a white powder (5·10 g, 86%), m.p. >360°; $\nu_{max.}$ (Nujol) 3318 (amide NH), 1690 (β-keto-ester), and 1560—1650br cm^{-1} (β-diketone and amide); τ [$(CD_3)_2SO$] 6·19 (2H, s, NH·C*H*$_2$·CO) 6·39 (3H, s, OCH$_3$), and 6·87 (1H, s, C*H*·CO$_2$·CH$_3$); $\lambda_{max.}$ (H_2O) 228 (ε 25,300) and 262 nm (24,200) (Found: C, 45·8; H, 4·6; N, 8·8. Calc. for $C_6H_7NO_4$: C, 45·9; H, 4·5; N, 8·9%).

Pyrrolidine-2,4-*dione* (4).—The foregoing dioxo-ester (3·39 g) was dispersed in acetonitrile (2·5 l) and the mixture was refluxed for 2 h. The solid dissolved gradually to give a clear solution. The solvent was removed and pyrrolidine-2,4-dione (4) (2·16 g, 100%) was obtained as a pale yellow solid, m.p. >360°; $\nu_{max.}$ (Nujol) 3230 (amide NH) and 1640—1550br cm^{-1}; τ [$(CD_3)_2SO$] 1·80br (s, NH), 2·95br (s, NH), 5·24 (s, C=CH), 6·22 (s, HN·C*H*$_2$·CO), 6·26 (s, HN·C*H*$_2$·C=C), and 7·08 (s, CO·C*H*$_2$·CO) (34% enol form); $\lambda_{max.}$ (H_2O) 240br (ε 65,300), 257br (6160), and 320br nm (3880); $\lambda_{max.}$ (0·1N-NaOH) 258·7 (ε 37,500) and 312 nm (1940) (Found: C, 48·7; H, 5·2; N, 14·1. Calc. for $C_4H_5NO_2$: C, 48·5; H, 5·1; N, 14·1%).

3-*Diazopyrrolidine*-2,4-*dione* (5).—Methyl 2,4-dioxopyrrolidine-3-carboxylate (4·64 g) was dispersed in acetonitrile (2·6 l). The mixture was refluxed for 2 h, cooled, and concentrated to *ca.* 500 ml. To this rapidly stirred solution under nitrogen at 0 °C were added methanesulphonyl azide (4·5 g) in acetonitrile (100 ml) and triethylamine (3·5 g) in acetonitrile (50 ml). The mixture was stirred at 0° for 15 min and then for 2 h at room temperature. The solvent was removed and the residue, a deep red gum, was dissolved in water (50 ml) and continuously extracted with ethyl acetate (4 × 200 ml) for 4 days. The combined extracts were evaporated to dryness and the residue, a pale yellow solid, was recrystallised in acetone–ether to give light yellow needles of 3-*diazopyrrolidine*-2,4-*dione* (3·07 g, 81%), which sublimed appreciably on the hot-stage above 150° without showing a definitive m.p.; $\nu_{max.}$ ($CHCl_3$) 3446 (amide NH), 2143 (diazo), and 1690 cm^{-1} (α-diazocarbonyl); τ ($CDCl_3$) 3·65br (1H, s, NH) and 6·05 (2H, s, NH·C*H*$_2$·CO); $\lambda_{max.}$ (EtOH) 210 (ε 34,700) and 240 nm (36,600); $\lambda_{infl.}$ 290 nm (ε 6580) (Found: C, 38·4; H, 2·5; N, 33·6. $C_4H_3N_3O_2$ requires C, 38·4; H, 2·4; N, 33·6%).

Adducts (6) *and* (7) *from* 3-*Diazopyrrolidine*-2,4-*dione* (5) *and Dibenzyl Acetylenedicarboxylate*.—(a) *With sodium hydride in benzene at room temperature*. To a suspension of sodium hydride (50% oil dispersion; 73 mg) and dibenzyl acetylenedicarboxylate (1·02 g) in benzene (10 ml) was added 3-diazopyrrolidine-2,4-dione (5) (189 mg) and the suspension was stirred under nitrogen at room temperature for 44 h. The mixture was poured into ice-cold 1% sulphuric acid and chloroform and the aqueous layer was re-extracted three times with chloroform. The combined organic layers were dried and evaporated and the residue was subjected to p.l.c. with 20% acetone–light petroleum as developing solvent. After three elutions, three dark bands were evident on examination of the plate with u.v. light (λ 254 nm). The most polar band was unchanged diazo-compound (5) (12 mg).

The least polar fraction (48 mg), a pale yellow oil, was the (*Z*)-adduct (6), $\nu_{max.}$ (CCl_4) 2130 (diazo), 1740—1700 (ester and α-diazocarbonyl), and 1640 cm^{-1} (alkene); τ (CCl_4) 2·70 (10H, s, ArH), 3·30 (1H, s, C=CH), 4·83 and 4·91 (each 2H, s, C*H*$_2$Ph), and 5·93 (2H, s, CO·CH$_2$N); $\lambda_{infl.}$ (EtOH) 297 nm (ε 13,000).

The middle fraction (30 mg), a pale yellow oil, was the (*E*)-adduct (7); $\nu_{max.}$ (CCl_4) 2140 (diazo), 1750—1700 (ester and α-diazocarbonyl), and 1615 cm (alkene); τ (CCl_4) 2·74, (10H, s, ArH), 4·80 (1H, s, C=CH), 4·91 and 4·98 (each 2H, s, C*H*$_2$Ph), and 6·20 (2H, s, CO·CH$_2$·N); $\lambda_{max.}$ (EtOH) 282 nm (ε 31,100).

(b) *With sodium hydride in benzene–*1,2-*dimethoxyethane* (1 : 1 v/v) *at* 80°. To a suspension of sodium hydride (50% oil dispersion; 0·53 g) in benzene–1,2-dimethoxyethane (1 : 1 v/v; 100 ml) was added the diazo-compound (5) (1·25 g) in benzene–1,2-dimethoxyethane (1 : 1 v/v; 600 ml). The mixture was stirred under nitrogen until evolution of hydrogen was no longer observed. To this solution was added dibenzyl acetylenedicarboxylate (5·88 g) in benzene–1,2-dimethoxyethane (1 : 1 v/v; 200 ml) and the mixture

was refluxed under nitrogen for 24 h. The solution was concentrated to *ca.* 100 ml and then poured into ice-cold aqueous 5% citric acid and ether. The aqueous solution was re-extracted with ether and the ethereal solutions were dried and evaporated. The residue was chromatographed on silica gel (400 g) to give a mixture of the (*Z*)-adduct (6) and (*E*)-adduct (7) (1·28 g, 40%).

The aqueous solution was continuously extracted with ethyl acetate. The extract was evaporated to dryness and the residue recrystallised in acetone–ether to give unchanged diazo-compound (5) (0·29 g).

Dibenzyl 2-[2-*Oxo*-3-(3-*t*-*butoxycarbonylcarbazoyl*)*azetidin*-1-*yl*]*maleate* (8).—A solution of the mixture of diazo-compounds (6) and (7) (670 mg) and t-butyl carbazate (264 mg) in dry benzene (450 ml) was irradiated in a Pyrex vessel under nitrogen for 1·5 h. The solvent was evaporated off and the residual oil showed only one major product on t.l.c. A sample (100 mg) of the crude product was subjected to p.l.c. with 30% acetone–light petroleum as developing solvent. The fraction of R_F 0·23 (62 mg) was the t-butoxycarbonylhydrazide (8), $\nu_{max.}$ (CCl_4) 1780 (β-lactam), 1750 (ester), 1710 and 1695sh (urethane and hydrazide), and 1625 cm^{-1} (alkene); τ (CCl_4) 2·75 (10H, s, ArH), 4·55 (1H, s, C=CH), 4·89 and 4·99 (each 2H, s, CH_2Ph), 5·88 6·25, and 6·55 (3H, multiplets, three ring protons), and 8·64 (9H, s, Bu^t); $\lambda_{max.}$ (EtOH) 272 nm (ε 32,400).

Dibenzyl 2-(2-*Oxo*-3-*t*-*butoxycarbonylaminoazetidin*-1-*yl*)-*maleate* (9).—The t-butoxycarbonylhydrazide (8) (0·504 g) (purified on a silica gel column by eluting with ether) was treated with trifluoroacetic acid (1 ml) for 1 h at 20°. The trifluoroacetic acid was removed and the residual gum was dissolved in ether (20 ml). The ethereal solution of hydrazide was added to 10M-hydrochloric acid (10 ml) and cracked ice (20 g). The stirred solution was maintained below 5°, while a solution of sodium nitrite (1·2 g) in water (5 ml) was added, and the mixture was stirred for a further 5 min. The ether layer was separated and the aqueous layer was extracted twice with chilled ether. The combined ethereal extracts were dried and evaporated. The residue in dry benzene (20 ml) was refluxed for 45 min. t-Butyl alcohol (5 ml) was added and the solution was refluxed for a further 3 h. The solvent was removed and the product was purified by p.l.c. Elution with ether–light petroleum (7 : 3) gave the *urethane* (9) (R_F 0·3; 0·171 g, 37%). Crystallisation from ethyl acetate–light petroleum gave white prisms, m.p. 118—120°, $\nu_{max.}$ (CCl_4) 3440 (NH), 1790 (β-lactam), 1740 (ester), 1715 (urethane), and 1630 cm^{-1} (alkene); τ (CCl_4) 2·70 (10-, s, ArH), 4·32 (1H, d, J 9 Hz, N*H*·CH), 4·5 (1H, s, C=CH), 4·85 and 4·92 (each 2H, s, CH_2·Ph), 5·10 (1H, m, C*H*–CH_2), 6·50 (2H, m, CH–C*H*$_2$), and 8·60 (9H, s, Bu^t); $\lambda_{max.}$ (EtOH) 274 nm (ε 45,400) (Found: C, 65·1; H, 6·0; N, 5·8. $C_{26}H_{28}N_2O_7$ requires C, 65·0; H, 5·9; N, 5·8%).

Dibenzyl 2-(2-*Oxo*-3-*phenylacetamidoazetidin*-1-*yl*)*maleate* (3; R = CH_2Ph).—The urethane (9) (120 mg) was treated for 1 h at 20° with trifluoroacetic acid (1 ml). The solvent was removed, the residue was dissolved in dichloromethane (10 ml), and triethylamine (80 mg) and phenylacetyl chloride (200 mg) were added. The mixture was stirred for 2 h at 5°, diluted with dichloromethane, and washed with 2M-hydrochloric acid, water, and brine. The dried solution was concentrated and the residue was purified by p.l.c. Elution with 30% acetone–light petroleum gave the *phenylacetamido-derivative* (3; R = CH_2Ph) (R_F *ca.* 0·2; 75 mg, 60%). Recrystallisation from ethyl acetate–light petroleum gave white prisms, m.p. 114—115°, $\nu_{max.}$ (CCl_4) 3400 (NH), 1790 (β-lactam), 1750 (ester), 1690 (amide), and 1625 cm^{-1} (alkene); τ ($CDCl_3$) 2·82 (10H, s, ArH), 3·95 (1H, d, *J* 8 Hz, NH), 4·40 (1H, s, C=CH), 4·78 and 4·87 (each 2H, s, CH_2Ph), 5·02 (1H, m, $J_{NH,CH}$ 7, J_{cis} 5, J_{trans} 2 Hz, NH·C*H*·CH_2), 6·39 (2H, s, PhCH_2·CONH), 6·27 (1H, dd, *J* 5 and 7 Hz, CH·CH_α), and 6·55 (1H, dd, *J* 2 and 7 Hz, CH·CH_β); $\lambda_{max.}$ (EtOH) 274 nm (ε 45,500) (Found: C, 69·5; H, 5·5; N, 5·6. $C_{29}H_{26}N_2O_6$ requires C, 69·8; H, 5·3; N, 5·6%).

2-(2-*Oxo*-3-*phenylacetamidoazetidin*-1-*yl*)*maleic Acid* (3; R = H).—The phenylacetamido-derivative (3; R = CH_2Ph) (46 mg) dissolved in absolute ethanol (10 ml) was stirred with palladium black (12 mg) under hydrogen at 22° and 1 atm for 5 h, after which time no ester remained (t.l.c.). The catalyst was filtered off and washed with ethanol. Removal of the solvent from the filtrate gave the diacid (3; R = H) as a viscous gum (all attempts to crystallise the compound were unsuccessful), $\lambda_{max.}$ 272 nm (ε *ca.* 35,000), $\nu_{max.}$ (film) 3600—2500 (CO_2H and NH), 1780—1690 (CO_2H and CONH), and 1630—1590 cm^{-1} (Ph and alkene).

To a stirred solution of the diacid (11 mg) in methanol (2 ml) at 0° was added diazomethane [from *N*-nitrosomethylurea (11 mg)] in ether (8 ml). The mixture was stirred at 0° for 20 min. The solution was filtered and the solvent removed to give the dimethyl ester (8 mg), R_F *ca.* 0·30 on t.l.c. (elution with 30% acetone–light petroleum), $\lambda_{max.}$ (EtOH) 272 nm (ε 43,000); $\nu_{max.}$ ($CHCl_3$) 1790 (β-lactam), 1750 (CO_2Me), and 1625 cm^{-1} (alkene).

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